

**An analysis of the *Pseudocordylus melanotus* complex  
(Sauria: Cordylidae)**

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## **DECLARATION**

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and has not previously been submitted in its entirety or in part at any university for a degree.

Signature:

Date: 5 March 2007

## ABSTRACT

The taxonomic status of southern Africa's rupicolous crag lizards (genus *Pseudocordylus*) was investigated. As considerable confusion exists in the literature regarding the type specimens and type localities of the various taxa, resolution of these problems were considered the starting point of the study. Examination of museum specimens allowed for the designation of lectotypes, alloparalectotypes and/or paralectotypes. Of particular relevance to this study was the re-discovery of Andrew Smith's type specimens of *P. m. melanotus* and *P. m. subviridis*. Restriction of the type locality of *P. m. subviridis*, based on entries in Smith's diary and journal, allowed for the confirmation of previous interpretations and definitions of the two taxa. The geographical distribution of the various taxa and populations was determined using an extensive locality database.

Two kinds of molecular markers, namely allozymes and mitochondrial DNA, were used in an attempt to resolve taxon boundaries within the *P. melanotus* species complex. The allozyme analysis indicated that *P. m. melanotus* might be polyphyletic and comprised of two unrelated lineages. Furthermore, fixed allelic differences between parapatric populations of *P. m. melanotus* and *P. m. subviridis*, and between sympatric populations of *P. m. subviridis* and *P. langi*, suggested that all three forms might be considered full species, with the possibility of more cryptic species present in the complex. *Pseudocordylus transvaalensis* differed from most other populations by 1-3 fixed allelic differences, but was indistinguishable from the Nkandhla district (central KwaZulu-Natal) population of *P. m. melanotus*. There were no heterozygous individuals in a sample from Monontsha Pass (Qwa-Qwa), a population reportedly comprising *P. m. melanotus* and *P. m. subviridis*, as well as intermediates, and all specimens were assignable to *P. m. subviridis*. The allozyme study was, however, based on phenetic principles and for further taxonomic resolution a cladistic approach was required. An mtDNA analysis (16S rRNA gene) using Maximum Parsimony, Maximum Likelihood and Bayesian analyses was therefore conducted to determine phylogenetic relationships among species and subspecies and to re-assess the taxonomic status of forms in the *P. melanotus* species complex. The mtDNA analysis corroborated most of the results obtained in the allozyme analysis. Firstly, *P. langi* was again found to be basal. With the addition of *P. microlepidotus* and *P. spinosus* to the ingroup, it is now apparent that *P. langi* is the basal species in the genus. (Recent studies have indicated that *P. capensis* and *P. nebulosus* are not congeneric with *Pseudocordylus*.) Secondly, the 16S rRNA results confirm that *P. m. melanotus*, as presently construed, is comprised of two clades that are not sister groups. The northern populations of *P. m. melanotus* (Sabie and Lochiel) form a fairly deeply divergent clade that may represent a separate species. The Nkandla population was,

however, found to cluster with the other southern *P. m. melanotus* populations and not with *P. transvaalensis* as was the case in the allozyme electrophoretic analysis. However, the most surprising result of the 16S rRNA analysis was the finding that both *P. microlepidotus* and *P. spinosus* are embedded within *P. m. subviridis*. This suggests that these two species evolved from within *P. m. subviridis* and may have been separated only recently, with rapid morphological divergence occurring, but with limited genetic differentiation. It is suggested that all of the above three taxa be provisionally treated as full species.

There was also morphological support for the uniqueness of all groupings indicated by the mtDNA analysis. *Pseudocordylus transvaalensis* is characterized by its large size, unique dorsal and gular (black) colour patterns, as many as three horizontal rows of lateral temporal scales, a series of small scales posterior to the interparietal scale, and usually two subocular scales behind the median subocular on either side of the head. The various populations currently classified under the name *P. melanotus* are more difficult to separate, but *P. m. melanotus* and *P. m. subviridis* usually differ as follows: frontonasal divided in *P. m. melanotus*, undivided in *P. m. subviridis* (and most Northern *melanotus*); lateral temporals in two rows, upper more elongate versus single row of much elongated scales; longitudinal rows of dorsolaterals closely-set versus widely separated; femoral pores of females pit-like versus deep with secretory plug. Northern *melanotus* differs from Southern *melanotus* in usually having an undivided frontonasal scale and seldom having a small scale present behind the frontonasal. *Pseudocordylus langi* has unique dorsal and gular colour patterns (including a series of blue spots on the flanks), granular dorsals with 6-9 paravertebral rows of enlarged flat scales, high total numbers of femoral pores (25-34) and usually only five (smooth not keeled or ridged) infralabial scales on either side of the head. *Pseudocordylus spinosus* also has unique dorsal and gular colour patterns, spinose lateral scales, frontonasal longer than wide and excluded from the loreal scales, low total femoral pore counts (6-9), and females (not only males) have differentiated femoral scales. Both Principal Components Analysis (PCA) and Canonical Discriminant Analysis (CDA) distinguished four groups, namely *P. transvaalensis*, *P. langi*, *P. spinosus* and a *P. melanotus/subviridis/microlepidotus* cluster. A separate CDA of all *P. melanotus* populations partly distinguished between Southern *melanotus* and *P. m. subviridis*, and largely separated Northern *melanotus*; whereas a CDA of *P. transvaalensis* showed that all three allopatric populations are 100% distinguishable in morphological space.

A Nested Clade Analysis indicated that fragmentation as well as range expansion played a role in the distribution of the *P. melanotus* species complex. This may be explained by climatic oscillations (high-low temperatures and wet-dry cycles) during the Cenozoic that caused habitat expansion and contraction. Based on the topology of the mtDNA phylogram it is apparent that

the genus *Pseudocordylus* originated along the eastern escarpment. A *P. langi*-like ancestor may have had an extensive range along the eastern escarpment, with the Maloti-Drakensberg forming the southern limit of its range. During a subsequent rise in global temperatures, range contraction and fragmentation took place, leaving an isolated population in the south and one in the north. The southern population survived unchanged in the Maloti-Drakensberg refugium, but the northern population was forced to adapt to the warmer conditions. Thereafter, the northern form expanded its range again, but during a subsequent cooler period, range contraction occurred, resulting in an isolated north-eastern population in the Sabie-Lochiel area in Mpumalanga (Northern *melanotus*) and a western population. Relationships in the latter clade are not sufficiently resolved to allow further reconstruction of biogeographic history, but it is clear that a *P. m. subviridis*-like form became isolated in the south where it eventually came into contact with *P. langi* at high elevations. *Pseudocordylus m. subviridis* eventually extended its range south-westwards into the inland mountains of the Eastern Cape and Cape Fold Mountains to give rise to the *P. microlepidotus* complex. This cycle of range expansion and contraction may also account for the isolated populations at Suikerbosrand, Nkandhla district, and in the Amatole-Great Winterberg mountain region. Furthermore, it is suggested that *P. spinosus* originated from a *P. m. subviridis*-like ancestral population that became isolated on the lower slopes of the Drakensberg where terrestrial predation pressure resulted in a quick shift in morphology from fairly smooth body scales to a more spiny morphology.

## UITTREKSEL

Die taksonomiese status van suidelike Afrika se rotsbewonende krans-akkedis (genus *Pseudocordylus*) is ondersoek. Omdat daar aansienlike verwarring in die literatuur bestaan met betrekking tot die tipe monsters en die tipe lokaliteite van die verskillende taksa, is die oplossing van hierdie probleme as die beginpunt van hierdie studie geneem. Die bestudering van akkedis-monsters in museums het dit moontlik gemaak om lektotipes, alloparalektotipes en/of paralektotipes aan te wys. Van besondere belang vir hierdie studie is die herontdekking van Andrew Smith se tipe monsters van *P. m. melanotus* en *P. m. subviridis*. Die beperking van die tipe lokaliteit van *P. m. subviridis*, gebaseer op inskrywings in Smith se dagboek en joernaal, het dit moontlik gemaak om vorige interpretasies en definisies van die twee taksa te bevestig. Die geografiese verspreiding van die verskillende taksa en bevolkings is bepaal deur middel van 'n omvattende lokaliteit databasis.

Twee soorte molekulêre merkers, naamlik allosieme en mitokondriale DNS, is gebruik in 'n poging om uitsluitel te verkry oor die takson-grense binne die *P. melanotus*-spesiekompleks. Die allosiem-analise het daarop gedui dat *P. m. melanotus* moontlik polifileties mag wees en uit twee onverwante stamboom-vertakkings kan bestaan. Verder het vaste alleliese verskille tussen parapatriese bevolkings van *P. m. melanotus* en *P. m. subviridis*, en tussen simpatriese bevolkings van *P. m. subviridis* en *P. langi*, daarop gedui dat al drie vorme as volledige spesies beskou kan word, met die moontlikheid dat meer kriptiese spesies in die kompleks teenwoordig kan wees. *Pseudocordylus transvaalensis* het van die meeste ander bevolkings verskil met 1-3 vaste alleliese verskille, maar was ononderskeibaar van die bevolking van *P. m. melanotus* van die Nkandhla distrik (sentraal KwaZulu-Natal). Daar was slegs homosigote individue in 'n steekproef van Monontsha Pas (Qwa-Qwa), 'n bevolking wat volgens die literatuur *P. m. melanotus* en *P. m. subviridis*, sowel as intermediêre omvat, en alle monsters was toekenbaar aan *P. m. subviridis*. Die allosiemstudie is egter gebaseer op fenetiese beginsels en vir verdere taksonomiese oplossing is 'n kladistiese benadering vereis. 'n Mitokondriale DNS-analise (16S rRNS geen) wat gebruik maak van Maksimum Parsimonie-, Maksimum Waarskynlikheids- en Bayes-analises is daarom uitgevoer om die filogenetiese verwantskappe tussen spesies en subspecies te bepaal en om die taksonomiese status van vorme in die *P. melanotus*-spesiekompleks te herondersoek. Die mtDNS-analise het die meeste van die resultate van die allosiem-analise bevestig. Eerstens, *P. langi* is weer bevind om basaal te wees. Met die byvoeging van *P. microlepidotus* en *P. spinosus* tot die binne-groep het dit nou duidelik geword dat *P. langi* die basale spesie in die genus is. (Onlangse studies het aangedui dat *P. capensis* en *P. nebulosus* nie kongeneries met *Pseudocordylus* is nie.) Tweedens, die 16S rRNS resultate bevestig dat *P. m. melanotus*, soos

tans vasgestel, saamgestel is uit twee klade wat nie sustergroepe is nie. Die noordelike bevolkings van *P. m. melanotus* (Sabie en Lochiel) vorm 'n redelik diep divergente klade wat 'n afsonderlike spesie mag verteenwoordig. Dit is egter bevind dat die Nkandla bevolking saamgegroepeer het met die ander suidelike *P. m. melanotus*-bevolkings en nie met *P. transvaalensis* soos wat die geval was in die allosiem-elektroforetiese analise nie. Die mees verbasende resultaat van die 16S rRNS-analise was egter die bevinding dat beide *P. microlepidotus* en *P. spinosus* genestel was binne *P. m. subviridis*. Dit dui daarop dat hierdie twee spesies kon ontwikkel het vanuit *P. m. subviridis* en slegs onlangs van mekaar geskei het, toe vinnige morfologiese splitsing voorgekom het, maar met beperkte genetiese differensiasie. Dit word voorgestel dat al drie die bogenoemde taksa voorlopig as volledige spesies beskou word.

Daar was ook morfologiese steun vir die uniekheid van al die groeperings wat die mtDNS-analise uitgewys het. *Pseudocordylus transvaalensis* kan uitgeken word aan sy bogemiddelde grootte, unieke dorsale en (swart) kleurpatrone op die keel, so veel as drie horisontale rye lateraal-temporale skubbe, 'n reeks klein skubbe agter die interpariëtale skub, en gewoonlik twee subokulêre skubbe agter die middelste subokulêre skub op beide kante van die kop. Die verskillende bevolkings wat tans geklassifiseer word as *P. melanotus* is moeiliker om van mekaar te skei, maar *P. m. melanotus* en *P. m. subviridis* verskil gewoonlik soos volg: frontonasale skub in twee gedeeltes in *P. m. melanotus*, heel in *P. m. subviridis* (en in die meeste Noordelike *melanotus*); lateraal-temporale skubbe in twee rye, die boonste ry met verlengde skubbe teenoor 'n enkele ry verlengde skubbe; longitudinale rye van dorsolaterale skubbe naby aan mekaar teenoor ver uit mekaar; femorale porieë van wyfies klein en vlak teenoor diep met sekreterende proppe. Noordelike *melanotus* verskil van Suidelike *melanotus* deurdat hulle gewoonlik 'n heel frontonasale skub het en daar selde 'n klein skub teenwoordig is agter die frontonasale skub. *Pseudocordylus langi* het unieke dorsale en keel-kleurpatrone (wat 'n reeks blou kolle op die sye insluit), granulêre dorsale skubbe met 6-9 rye vergrote plat skubbe langs die rugsteen, 'n groot totale aantal femorale porieë (25-34), en gewoonlik net vyf (glad, ongerif) infralabiale skubbe op elke kant van die kop. *Pseudocordylus spinosus* het ook unieke dorsale en keel-kleurpatrone, skerp laterale skubbe, frontonasale skub langer as wyd en nie in kontak met die loreale skubbe nie, klein totale aantal femorale porieë (6-9), en wyfies (nie net mannetjies nie) het gedifferensieerde femorale skubbe. Die Hoof-komponent Analise (HKA) en die Kanonieke Diskriminant Analise (KDA) het albei vier groepe geïdentifiseer, naamlik *P. transvaalensis*, *P. langi*, *P. spinosus* en 'n *P. melanotus/subviridis/microlepidotus* groepering. 'n Aparte KDA van alle *P. melanotus* bevolkings het gedeeltelik onderskei tussen Suidelike *melanotus* en *P. m. subviridis*, en die Noordelike *melanotus* is grootliks van die ander onderskei; terwyl 'n KDA van *P. transvaalensis* daarop gedui het dat al drie allopatriese bevolkings 100% onderskeibaar in morfologiese ruimte is.

’n Genestelde Klaad-Analise het aangedui dat fragmentasie, sowel as gebiedsuitbreiding, ’n rol gespeel het in die verspreiding van die *P. melanotus*-spesiekompleks. Dit kan moontlik verklaar word deur die klimaatwisselinge (hoë-lae temperature en nat-droë siklusse) gedurende die Senosoikum wat habitat-uitbreiding en –verkleining veroorsaak het. Gebaseer op die topologie van die mtDNS filogram is dit duidelik dat die genus *Pseudocordylus* al langs die oostelike platorand ontstaan het. ’n Voorouer soortgelyk aan *P. langi* kon ’n uitgebreide gebied al langs die oostelike platorand gehad het, met die Maloti-Drakensberg wat die suidelike limiet van hierdie gebied gevorm het. Gedurende ’n daaropvolgende toename in globale temperature het gebiedsverkleining en fragmentasie plaasgevind, wat ’n geïsoleerde bevolking in die suide en een in die noorde tot gevolg gehad het. Die suidelike bevolking het onveranderd oorleef in die Maloti-Drakensberg skuilplek (“refugium”), maar die noordelike bevolking is geforseer om aan te pas in die warmer toestande. Daarna het die noordelike vorm se gebied weer uitgebrei, maar gedurende ’n daaropvolgende koeler periode het gebiedsverkleining weer plaasgevind, met die gevolg dat daar ’n geïsoleerde noord-oostelike bevolking in die Sabie-Lochiel-area in Mpumalanga (Noordelike *melanotus*) en ’n bevolking in die weste was. Verwantskappe in die laasgenoemde klaad is nie voldoende opgelos om verdere rekonstruksie van die biogeografiese geskiedenis moontlik te maak nie, maar dit is duidelik dat ’n vorm soortgelyk aan *P. m. subviridis* geïsoleer geraak het in die suide waar dit eindelijk op hoë liggings in kontak gekom het met *P. langi*. Die gebied van *P. m. subviridis* is ook later suidweswaarts uitgebrei tot in die binnelandse berge van die Oos-Kaap en Kaapse Plooiberge om tot die ontstaan van die *P. microlepidotus*-kompleks aanleiding te gee. Hierdie siklus van gebiedsuitbreiding en verkleining kan ook ’n verklaring bied vir die geïsoleerde bevolkings by Suikerbosrand, Nkandhla distrik, en in die Amatole-Groot Winterberg-streek. Verder word voorgestel dat *P. spinosus* ontstaan het uit ’n voorouerlike bevolking soortgelyk aan *P. m. subviridis* wat geïsoleerd geraak het op die laer hange van die Drakensberg waar die druk van aardsbewonende roofdiere tot ’n vinnige verandering in morfologie vanaf redelik gladde liggaamskubbe tot ’n meer skerppuntige morfologie gelei het.

## **DEDICATION**

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## CHAPTER 1

### General Introduction

#### 1.1 Status of reptile taxonomy in southern Africa

The vast majority of taxonomic studies in the field of herpetology have been based primarily or entirely on morphological characters. Species and subspecies were usually separated on the basis of fixed or near-fixed morphological traits (*e.g.* presence or absence of particular scales), differences in meristic characters (*e.g.* numbers of supralabials), and size and colouration (*e.g.* Branch 1999; Broadley 2000; Broadley & Branch 2002). However, these kinds of characters may be susceptible to environmental plasticity (*e.g.* generation gland counts in *Cordylus Laurenti*, 1768 – Du Toit, Mouton, Flemming & Van Niekerk 2004) and studies based on morphology alone may fail to distinguish cryptic species.

According to Branch (2006: 2) the current rate of reptile species descriptions for southern Africa “shows little indication of reaching a plateau”. The number of recognized species in the region increased from 397 in 1988 to 480 in 1998 and there are now over 520 species (Branch 2006), most of which are lizards. In fact, most of the new reptile species described in the past 25 years are lizards. One of the reasons for the increase in the number of recognized species is the adoption of phylogenetic or evolutionary species concepts (see section 1.7) by most southern African herpetologists (*e.g.* Branch 1998, 2006; Broadley & Branch 2002). This has resulted in the recognition of several species previously considered subspecies because they were defined on the basis of limited numbers of scale differences. According to these species concepts, limited but significant scale differentiation, together with allopatry, is regarded as an indicator of separate species status of populations. The use of genetic markers has now made it possible to gain further insight into inter- and intra-specific relationships, and has resulted in the discovery of several new species (see section 1.4 – allozymes; section 1.5 - mtDNA).

There are still unresolved taxonomic problems in 50 genera of southern African reptiles (Branch 2006). This is due in part to a high degree of morphological conservatism in some lizard genera, with a paucity of characters useful in traditional taxonomic approaches. Morphologically cryptic or near-cryptic species are therefore likely to occur in several genera (*e.g. Nucras, Pedioplanis, Afroedura, Agama, Bradypodion*). Their taxonomy will be resolved only once molecular analyses have been conducted. For the most part, herpetology in southern African has reached the point where molecular markers have become an essential tool in systematics. At a workshop held in Cape Town in February 2006, taxonomically problematic genera and species complexes were identified, and a plan formulated to encourage and financially support phylogenetic studies on the reptiles of South African, Lesotho and Swaziland (Branch, Tolley, Cunningham, Bauer, Alexander, Harrison, Turner & Bates 2006). The emphasis was on mtDNA analyses, but the importance of seeking concordant morphological support was also recognized.

Allozymes were used for reptiles with increasing frequency from about 1970 (Soule, Yang, Weiler & Gorman 1973; Murphy *et al.* 1996), whereas mtDNA became the preferred molecular marker from about 1990 (Hillis, Mable, Larson *et al.* 1996). Other kinds of molecular markers are now also being used (section 1.3 below). For southern African reptiles the first allozyme study appears to be that of Brody, Mouton & Grant (1993) on the *Cordylus cordylus* species complex, whereas the first mtDNA study is that of Lamb & Bauer (2000) on the *Pachydactylus rugosus* species complex.

## 1.2 Status of lizards in the *Pseudocordylus melanotus* species complex

There are currently 10 species and subspecies of *Pseudocordylus*, all of which are diurnal and insectivorous, and restricted to South Africa, Lesotho and Swaziland, where they occupy mountainous areas or rocky outcrops with narrow, deep crevices in which to shelter (FitzSimons 1943; De Waal 1978; Jacobsen 1989; Branch 1998; McConnachie, Alexander & Whiting 2004). All of these taxa are communal, with the exception of *P. transvaalensis*, which is almost always found singly in rock outcrops (Jacobsen 1989; Branch 1998; pers. obs.). The taxonomic status of taxa currently known by the names *Pseudocordylus melanotus melanotus* (A. Smith, 1838), *P. melanotus subviridis* (A.

Smith, 1838) and *P. transvaalensis* FitzSimons, 1943 is controversial and remains unresolved. These taxa, together with *P. langi* and *P. spinosus*, both previously confused with *P. m. subviridis*, are here considered to comprise the *P. melanotus* species complex. Although *P. transvaalensis* was, until recently, regarded as a subspecies of *P. melanotus*, no objective reasons were given by Jacobsen (1989) or Branch (1998) for raising it to species rank.

Previous attempts to separate species and subspecies of *Pseudocordylus* using morphological characters (*e.g.* scales, size, colour) resulted in different and often incompatible taxonomic arrangements (*e.g.* FitzSimons 1943; Loveridge 1944; Broadley 1964; De Waal 1978; Jacobsen 1989). This was at least partially the result of inappropriate methods of evaluation, such as placing too much emphasis on particular (sometimes subjective) characters, or summarizing variation in scale characters in such a way that any differences between particular populations were subsumed within the total range of variation. Both Branch (1985) and Mouton (1997) indicated that the *P. melanotus* species complex was in need of revision. It was evident that in addition to a detailed morphological analysis, a molecular approach was required to resolve the confused relationships of populations in the *P. melanotus* species complex.

An examination of the literature indicated considerable confusion regarding the type specimens and type localities of several taxa in both the *P. melanotus* and the closely related *P. microlepidotus* species complexes. As it is important to know which names to assign to which morphotypes and geographical populations, the first aim of this study was to identify type specimens and where necessary, restrict type localities (Chapter 2). Because of confusion regarding the identification of the various forms, their geographical distribution ranges have been confused. An attempt was therefore also made to determine distribution ranges after the compilation of an extensive database of museum and literature records (Chapter 2; Appendix 2.1).

The main goal of this study was to produce a molecular phylogeny for the *P. melanotus* species complex and attempt to find concordant morphological support (Chapter 5) for the main genetic assemblages determined by the analyses (Chapters 3 and 4). Strong congruence between molecular and morphological data provides good evidence that the underlying historical pattern has been discovered (*e.g.* Hillis 1987).

### 1.3 Molecular markers available for inferring phylogeny

Apart from allozymes and mitochondrial DNA (see below), a variety of molecular markers are now available and several are regularly used in phylogeny reconstruction. For example, variation in the number, size or conformation of DNA fragments provides a measure of sequence variation. Fragment analysis does not always provide the same level of resolution as nucleotide sequencing, but it is nevertheless a cost-effective alternative when large samples or large segments of a genome are to be screened, especially for specific changes in sequence (Dowling, Moritz, Palmer & Rieseberg 1996). Variations in fragment pattern that are evident after digestion by restriction enzymes are called Restriction Fragment Length Polymorphisms (RFLPs). However, many molecular systematists turned instead to Randomly Amplified Polymorphic DNA (RAPD) markers as they revealed higher levels of polymorphism and were far less expensive (Robinson & Harris 1999). Whereas RFLP involves changes within a specific, targeted segment of DNA, RAPD detects sequence changes within PCR priming sites (Dowling *et al.* 1996). Hypervariable minisatellite sequences and their use in DNA fingerprinting brought about a revolution in the analysis of population-level variation. However, several technical and statistical problems are apparent using this method (Dowling *et al.* 1996). Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs; also known as microsatellites) appear to have supplanted RAPD analyses. AFLPs are fragments of DNA amplified using directed primers from restriction-digested genomic DNA. This technique tends to generate large numbers of polymorphisms and is useful even for differentiating individuals in a population (Robinson & Harris 1999). Microsatellites have been widely used in population genetics during the last 10 years, largely because of their high variability and ability to score co-dominant genotypes with exact allele sizes (Dowling *et al.* 1996). For example, microsatellites were used by Laube & Kuehn (2006) to analyse genetic variability and assess social structure in the lacertid lizard *Lacerta viridis*. Recently, Single Nucleotide Polymorphisms (SNPs) has become a popular tool for use in population genetics (*e.g.* Rosenblum, Belfiore & Moritz 2006: lizard *Sceloporus undulatus*). SNP variation occurs when a single nucleotide replaces another.

Although the variety of molecular markers now available has resulted in a taxonomic revolution of sorts, progress is often slow because “as we build up information on the

history of a taxon using different markers, we often find not one history but many” (Baird 2006: 81). Nevertheless, the approach in the current study was to examine several loci using nuclear markers (allozymes) so as to gain insight on male and female gene flow, and to detect potential fixed allelic differences among populations for the purpose of species/group identification (section 1.4); and also to examine a mitochondrial gene (16S rRNA) with the main aim of generating a species phylogeny (section 1.5).

#### 1.4 Allozyme studies

Although most genetic studies on animals now involve mitochondrial or nuclear DNA sequence data, recent allozyme work includes Nishikawa, Matsui & Tanabe’s (2005) phylogenetic study of *Hynobius* salamanders, and Gabor, Ryan & Morizot’s (2005) attempt at finding correlations between allozymes and behaviour in sailfin mollies (*Poecilia*). In recent systematic studies allozymes have been used in combination with DNA sequence data (Busack & Lawson 2006, *Psammodromus* lacertids) and morphology (Parra-Olea, Garcia-Paris, Papenfuss & Wake 2005, *Pseudoeurycea* salamanders). Busack, Lawson & Arjo (2005) used mtDNA, allozymes and morphology in their phylogeographic and taxonomic study of the (lacertid) *Podarcis vaucheri* species complex. They noted that while sex-limited mitochondrial markers (e.g. mtDNA) probably reflected deep phylogenetic history, bi-parentally inherited allozymic markers probably accurately reflected recent movement and assembly. In a recent study on two species of freshwater mussels, the analysis of allozymes revealed distinct geographical structuring, whereas mtDNA sequence data provided more variable results (Berg, Elderkin, Christian, Metcalfe-Smith, Vaughn & Guttman 2002), thus indicating the value of using both kinds of markers in studies of genetic variation. For the purposes of taxonomic identification and determination of the geographical ranges of two species of salamanders, Wagner, Millet & Haig (2006) used both mtDNA and allozymes, but restricted the use of allozymes to loci that were diagnostic for each species within a particular region, based on a previous study.

Allozyme electrophoresis is a relatively cost-effective method for investigating genetic phenomena at molecular level involving the migration of proteins in a gel under the influence of an electric field. In most studies several single-copy nuclear gene loci are

screened. Sliced gels are then stained and the resultant bands scored. Allozymes – a subset of isozymes – are variants of polypeptides that represent different allelic alternatives of the same gene locus (Murphy, Sites, Buth & Haufler 1996). These variants reflect independent Mendelian polymorphisms at various loci in the genome. Differences in mobility of enzymes – due to differences in electrical charge, shape or size – are interpreted as reflecting changes in the encoding DNA sequence; and differences are considered to be genetically based and heritable. Enzyme expression is (largely) co-dominant – all alleles at a particular locus are expressed – and interpretation of banding patterns depends on the number of subunits in the enzyme (Murphy *et al.* 1996). This co-dominance allows for the discrimination of heterozygous (*e.g.* hybrids) and homozygous individuals. Protein electrophoresis is most useful for the identification of species that diverged less than 50 million years ago (Murphy *et al.* 1996).

Limitations related to the use of allozymes include the fact that only a certain number of loci can be visualized using available histochemical staining techniques. Although over 300 loci can now be stained for, this represents only a small fraction of the total genome (see Murphy *et al.* 1996). Also, Thorpe (1982) reported that limited amounts of allelic variation are detectable because only 20 to 30% of amino acid substitutions cause changes in electromorph mobility. For some species complexes there is a definite taxonomic limit to the resolving power of protein electrophoresis - allozymes may not be variable enough in some organisms, meaning that other molecular methods such as mtDNA RFLP studies may be more useful (Murphy *et al.* 1996). There may, for example, be significant differences in spatial and temporal heterogeneity of mtDNA haplotypes in the absence of allozyme divergence. Also, there may be more restriction site markers in both mtDNA and nuclear ribosomal DNA than in allozymes in some species (see Murphy *et al.* 1996). Some of the other restrictions of allozyme electrophoretic studies are: two different gene loci may encode for enzymes of exactly the same electrophoretic mobility; electrophoresis detects only amino acid substitutions that affect electrophoretic mobility; and electrophoretic techniques are largely restricted to water-soluble proteins encoded by structural genes (Avisé 1974). In addition, the scoring of gels is susceptible to subjective interpretation; and bands appearing at the same level may not be homologous. Allozymes are nevertheless particularly useful for studying population processes and for testing hybridization in sympatry.

One or more fixed allelic differences between populations in sympatry is usually considered evidence for the existence of two species, but the criteria for assessing the status of allopatric populations are more problematic (Baverstock & Moritz 1996). Factors used have included the level of genetic divergence (controversial; *e.g.* allele frequency differences); comparing genetic divergence between populations suspected of representing distinct species with that between similarly separated populations within each form; and the proportion of fixed or near-fixed allelic differences between samples as a measure of genetic divergence (Baverstock & Moritz 1996). Clearly the greater the number of loci screened the better the chance of detecting differences.

Many allozyme studies have indicated “discordant geographical patterns between levels of genetic divergence and taxonomic boundaries inferred from morphological data, especially for geologically old and morphologically conservative radiations” (Murphy *et al.* 1996: 58). In other words cryptic, or morphologically very similar, species may be distinguished by, for example, fixed allelic differences, although conversely, morphologically distinct taxa sometimes display little or no genetic divergence. Allozyme electrophoresis is particularly useful for detection of morphologically cryptic taxa in (widespread) polymorphic species (Hillis, Mable & Moritz 1996). Once species boundaries are indicated by allozymes, diagnostic morphological features should be looked for and may be discovered.

Murphy *et al.* (1996) noted that allozyme data could be of particular use as diagnostic markers (*e.g.* fixed allelic differences) for a priori identification of taxa or groups. This is especially relevant “in view of the potential for over-splitting taxa defined exclusively by rapidly evolving portions of the animal mitochondrial genome” (Murphy *et al.* 1996: 58). Rapidly evolving sequences (*e.g.* mtDNA) can be used for resolving relationships within groups (Hillis, Mable & Moritz 1996). In most current studies on reptiles, molecular phylogenies are based on mtDNA data. Compared to some other markers, allozymes may exhibit low levels of variability, but are still useful nuclear markers for indicating male and female gene flow, and for detecting potential fixed allelic differences among populations for the purpose of species identification.

According to Hillis, Mable & Moritz (1996), molecular techniques can, and often should, be used in combination. For example, effectiveness can be maximized by using high

resolution techniques (*e.g.* mtDNA nucleotide sequence data) together with techniques like allozyme electrophoresis that provide broad coverage of individuals and/or loci.

### 1.5 Mitochondrial DNA analyses

While allozyme electrophoresis has been popular in zoology since the 1960s, other advanced molecular approaches are now being used. The current tendency in systematics is to test traditional species-level taxonomies based on morphology against haplotype phylogenies based on DNA sequence data. Since about 1990 there has been rapid growth in phylogenetic systematics due to the use of nucleotide sequence data. Nucleotides are the basic units of information encoded in organisms. Comparisons of DNA sequences of various genes between different organisms provide a great deal of information about relationships that cannot be inferred using morphology. According to Hillis, Mable & Moritz (1996: 521) “all heritable information is potentially accessible to DNA sequencing, whereas only subsets of this information are accessible to the other techniques” (*e.g.* allozyme electrophoresis). Genomes evolve by gradual accumulation of mutations in the reproductive cells of organisms. The amount of nucleotide sequence difference between a pair of genomes from different organisms should therefore provide an indication of how recently these genomes shared a common ancestor. If two genomes diverged only recently they should exhibit fewer differences than genomes with an older common ancestor.

According to Moritz & Hillis (1996: 5) studies that combine sequence and allozyme analyses “provide an approach for linking allelic phylogeny to genetic analyses of populations or species”. The molecular approach to phylogeny is considered particularly illuminating in cases where morphological variation is limited. They also noted that: “studies that incorporate both molecular and morphological data will provide much better descriptions and interpretations of biological diversity than those that focus on just one approach”. DNA sequence data allow for the generation of gene trees, and from these, inferences can be made concerning the relationships among populations and species (*i.e.* species trees).

Because of the resolution power (high information content) of nucleic acid sequencing, this technique has become one of the most popular molecular approaches for inferring phylogenetic history (Hillis, Mable, Larson, Davis & Zimmer 1996). The latter authors referred to the fact that even at that time, sequencing had been used in about half of all molecular systematic studies and one-quarter of phylogenetic studies. Comparative nucleic acid sequencing has many applications in systematics, including tracing allelic genealogies within species, studies of geographic variation, gene flow, hybridization, and construction of species phylogenies to allow evaluation of macroevolutionary patterns and processes (Hillis, Mable, Larson *et al.* 1996). Sequencing is no longer expensive and time consuming, but, in studies requiring the examination of multiple loci (*e.g.* geographical variation studies), techniques such as allozyme electrophoresis may still be preferable. Nevertheless, Hillis, Mable, Larson *et al.* (1996) noted that for phylogeny reconstruction of ancient lineages (older than 50 million years), appropriate nucleotide sequence data represents the most informative molecular technique.

Amplification and sequencing of animal mitochondrial DNA can be used to characterize the haplotypes present in a population and to reconstruct the gene phylogeny that relates them (Hillis, Mable, Larson *et al.* 1996). These authors also note (p. 336) that “because animal mtDNA is maternally transmitted (at least most of the time in most species) and non-recombining, all parts of the molecule share the same historical pattern of common descent”; and (p. 337) “the use of these gene phylogenies of mtDNA together with geographic information on the populations sampled provides a means for evaluating the genetic structure of populations” (*i.e.* intraspecific phylogeography). Uniparentally inherited loci (*e.g.* mtDNA) usually display lower levels of variation within populations and more between populations than biparentally inherited loci (*e.g.* autosomal nuclear loci) (Moritz & Hillis 1996). Nuclear and mitochondrial genes that encode ribosomal RNA are especially useful for inferring species phylogenies because they are easy to access, collectively demonstrate a wide range of evolutionary rates, and can potentially provide resolution across a large time scale (Hillis, Mable, Larson *et al.* 1996). The best studied and most commonly used mtDNA sequences are ribosomal RNA genes 12S and 16S, cytochrome oxidase I and II, cytochrome b, and control region (Hillis, Mable, Larson *et al.* 1996).

The success of molecular phylogenetic and phylogeographic studies depends largely on whether or not an appropriate marker has been used. Therefore, it is important to choose the best gene for inferring the mitochondrial gene tree. Mueller (2006: 289) found that “slower rate of evolution and longer gene length both increased the probability that a gene would perform well phylogenetically”. She determined that in salamanders, estimated rates of molecular evolution varied 84-fold among different mitochondrial genes and different lineages, while mean rates of evolution among genes varied 15-fold. Differences in rates of molecular evolution were considered as probably being due, at least in part, to differences in numbers of possible synonymous nucleotide substitutions among genes. The genes with the fastest mean rates of nucleotide substitution and the highest rates of evolution were the cytochrome oxidases (*cox1*, *cox2*, *cox3*) and *cob*, whereas the slowest rates of nucleotide substitution were for *rrnS* (12S rRNA) and *rrnL* (16S rRNA) respectively. The greatest variation in evolutionary rates was also attributable to the cytochrome oxidases and *cob*. According to Mueller (2006) the gene that performed the best phylogenetically was 16S rRNA, followed by *nad4* and *nad2*, with 12S rRNA ranked seventh and *cox1* ranked eighth.

Nucleotide substitution in the mitochondrial genome occurs at a rapid rate (providing a rich source of variable characters), but this, combined with no more than four character states, a strong base compositional bias, and functional constraints, contributes to high levels of homoplasy (see Engstrom, Shaffer & McCord 2004). Nuclear protein-coding genes and introns (absent in mtDNA) evolve at a slower rate, which means that they are less prone to excessive homoplasy (see Engstrom *et al.* 2004). Also, nuclear introns “have the further advantage of being free from many of the evolutionary constraints imposed on protein-coding sequences, resulting in phylogenetic markers, which, in vertebrates, usually show little base compositional bias, relatively low transition-transversion ratio, and little among-site rate heterogeneity” (see Engstrom *et al.* 2004). The slow rate of evolution of nuclear DNA does, however, mean there is often a lack of variation on shorter time scales (see Engstrom *et al.* 2004).

It is always preferable to screen at least two different genes, especially for apparently closely related taxa (*e.g.* *Pseudocordylus transvaalensis* FitzSimons, 1943 and the two subspecies of *P. melanotus* [A. Smith, 1838]), as a one gene-based tree may differ from the species tree because of retained ancestral polymorphisms (Baverstock & Moritz

1996). Other problems with sequence data include the occurrence of pseudogenes and rate heterogeneity within genes.

Errors may occur when inferring species phylogenies from molecular sequence data if there is sufficient random or systematic error, and because of deep coalescence, gene duplication and horizontal gene transfer (see Slowinski & Page 1999). However, as a result of intra- and inter-chromosomal recombination, the nuclear genome comprises several historically linked sets of nucleotides with different histories referred to as linkage partitions, *i.e.* independent estimators of the overlying species phylogeny (Slowinski & Page 1999). Each member of the partition is a sequence of contiguous nucleotides and sequences are hierarchically divided from ancestral sequences. The above-mentioned authors added that separate gene trees should be inferred for each linkage partition and the species phylogeny inferred from the set of trees. In other words, nucleotides from genes with different histories should not be combined for phylogenetic analysis. Nucleotides should be considered as characters of gene trees, while gene trees should be considered as characters of species trees. There are three problems associated with previous approaches to phylogeny inference using sequence data. Simultaneous analysis of sequence data concatenates all available nucleotides for a set of taxa into a single matrix for analysis, effectively collapsing two levels of analysis into one (Slowinski & Page 1999). Every nucleotide is therefore erroneously treated as an independent estimator of the overlying species phylogeny; also, the distinction between homoplasy and gene tree/species tree conflict is ignored; and sequence polymorphism is not accommodated (Slowinski & Page 1999). Because recombination usually does not occur in mitochondrial genomes, its nucleotides form a series of historically linked characters which define a single linkage partition (Slowinski & Page 1999). These authors added that a nuclear gene sequence, even if it has not undergone recombination or experienced a similar history, is considered as a separate linkage partition, *i.e.* an independent estimator of species phylogeny. Rather than simultaneously analyzing nucleotides from different genes, Slowinski & Page (1999) therefore proposed simultaneous analysis of all gene trees based on different linkage partitions.

Species delimitation should not be based entirely on mtDNA sequence data. Mitochondrial genes are inherited as a single linkage group, which means that “any mismatch between gene and population histories caused by ancestral polymorphism or

gene flow between species will simultaneously affect all mitochondrial genes” (Wiens & Penkrot 2002: 70). In addition, mtDNA is maternally inherited and therefore any resultant phylogenies will reflect only female gene flow patterns which may differ considerably from those of males. Tolley & Burger (2004) noted that in the case of chameleons of the genus *Bradypodion* Fitzinger, 1843, mtDNA may only indicate historical isolation of lineages, and that nuclear DNA should also be examined, together with a full morphological analysis. There is in fact a tendency of late to include at least one nuclear gene in molecular systematics analyses (e.g. Matthee, Tilbury & Townsend 2004).

However, a major advantage of using mtDNA is that “the smaller effective population size ( $N_e$ ) of the mitochondrial genome will cause mtDNA haplotypes of a particular species to coalesce (*i.e.*, become ‘monophyletic’) four times more quickly than will nuclear markers (given some assumptions)” (Wiens & Penkrot 2002: 70). Therefore, newly-formed species should become distinct in their mtDNA haplotype phylogenies long before doing so in nuclear-based markers (*i.e.* nuclear genes, allozymes, morphology). Analysis of mtDNA should therefore allow resolution of species limits in groups that are too recently diverged to resolve using nuclear-based markers; and should do so more efficiently and with a greater probability of success (Wiens & Penkrot 2002). Speciation may have occurred so rapidly that no diagnostic morphological features have evolved and in such cases mtDNA haplotype phylogenies will be especially useful because of rapid species differentiation (Wiens & Penkrot 2002).

Baverstock & Moritz (1996) discuss various situations in which morphological data alone is not sufficient for defining species boundaries. These include situations similar to that between *P. transvaalensis* and *P. melanotus melanotus* where two allopatric populations are morphologically different but their status as biological species is questionable; and between *P. melanotus melanotus* and *P. melanotus subviridis* [A. Smith, 1838], although in this case morphological variation alone suggests hybridization at one locality.

Molecular studies should be followed by a search for morphological features diagnostic of the species uncovered (Baverstock & Moritz 1996). This is particular relevant in view of the fact that discordance in species boundaries determined using different data sets (e.g. molecular versus morphological) is reportedly common in reptiles (e.g. *Sceloporus*,

Wiens & Penkrot 2002; see also references in Balakrishnan 2005). Mitochondrial DNA sequences may not always accurately reflect species boundaries and species histories (see references in Balakrishnan 2005). Relationships indicated by mtDNA data may, for example, be in contradiction to those determined using morphology (see Engstrom *et al.* 2004).

Wiens & Penkrot (2002) noted that although tree-based species delimitation may be attempted using a combination of DNA and morphological data, they expected a strong intraspecific phylogenetic signal from DNA data and a weak intraspecific signal from morphological data. In other words, a combination of the two kinds of data will simply result in the DNA haplotype phylogram or something similar, as they found to be the case in their study of *Sceloporus* lizards. Therefore, there was no real advantage to using a combined analysis.

Relatively few phylogenetic analyses have combined molecular and morphological data sets. In such combined analyses there is often morphological data available for all taxa, but molecular data for only some. In such cases the “incomplete” taxa (those lacking molecular data) are excluded from the analysis. However, Wiens & Reeder (1995) have argued that incomplete taxa can be informative in phylogenetic analyses of combined data sets (*i.e.* it is better to have an hypothesis that is mostly right rather than having no hypothesis at all).

Nested Clade Analysis (NCA), as used in the present study, provides a phylogeographic framework allowing differentiation – in both space and time – of recurrent events such as gene flow or system of mating, from historical events such as fragmentation or range expansion. This form of analysis combines the four types of information in allele trees, namely topology, branch length, allele frequency and geographical distribution of alleles, and evaluates the observed patterns by comparing this with randomised distributions (Templeton, Routman & Phillips 1995; Templeton 1998, 2004). The technique efficiently distinguishes various forms of phylogeographic structure as well as historical processes.

However, NCA has been criticized. For example, Knowles & Maddison (2002: 2623) noted that this form of analysis “does not assess error in its inferences about historical

processes or contemporary gene flow”. They added (p. 2623) that “NCA did not identify the processes used to simulate the data, confusing among deterministic processes and the stochastic sorting of gene lineages.” Knowles & Maddison (2002) concluded that there is not enough justification of the technique’s ability to accurately infer or distinguish between alternative processes. Templeton (2004: 798) noted that although Knowles & Maddison (2002) objected to “the a posteriori use of the inference key to make biological interpretations from statistically significant geographical associations”, their methods also had an implicit inference key, although it was generated a priori. Templeton (2004: 798) added that both Knowles & Maddison’s method and NCA “distinguish among alternative interpretations by finding a statistic or set of statistics that deviate significantly from some well-defined model coupled with an interpretative key.” The major difference between approaches is that the interpretative key is applied a priori and implicitly by Knowles & Maddison (2002) versus a posteriori and explicitly by Templeton *et al.* (1995). Without strong prior knowledge of all possibilities, or when it is suspected that processes or occurrences other than those with prior knowledge are also happening, then NCA, which considers a greater variety of possibilities, is more appropriate (Templeton 2004). In conclusion, Templeton (2004) argued that both a priori and a posteriori interpretative frameworks have a role in statistical phylogeography.

## 1.6 Concordance between genetics and morphology

Wiens & Penkrot (2002) noted that when isolated for a sufficiently long time, distinct species should: have exclusive DNA haplotype phylogenies relative to other species; possess one or more diagnostic morphological characters (either fixed or at high frequency); and form strongly supported clades of populations based on morphology. Species isolated for an intermediate period should: become exclusive in their mtDNA haplotype phylogenies long before becoming exclusive in morphology-based phylogenies and before acquiring diagnostic morphological characters; whereas species separated very recently should: have non-exclusive haplotype phylogenies (*i.e.* individuals or populations will be paraphyletic or polyphyletic relative to one or more other species), lack diagnostic morphological characters, and exhibit non-exclusive population-level phylogenies based on morphology (Wiens & Penkrot 2002; section 6.3). In the present

study an attempt will be made to match morphology with clades determined by the genetic analyses.

Apart from comparing individual morphological characters (quantitative and qualitative scale characters, and external body measurements) between populations and taxa (character-based delimitation), two forms of multivariate analyses will be used in this study, namely Principal Components Analysis (PCA) and Canonical Discriminant Analysis (CDA). These analyses evaluate the extent to which individuals of a putative species cluster together. PCA partitions total variation among specimens without reference to pre-defined groups. Discriminant function analyses are based on a posteriori classification of individuals into groups using the distinguishing characters determined by the analysis. These latter analyses have been used in studies of geographical variation as well as morphological introgression.

### 1.7 Species concepts

According to Winston (1999: 44) species concepts are “models of the patterns brought about by the way the evolutionary process works under various conditions”; and are “attempts to explain how phenetic variation is compartmentalized”. The first species concept used by biologists was the Aristotelian typological species concept. Mayr (1942) later defined species as “groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups”. This became known as the Biological Species Concept (BSC) and was, to a large extent, embraced by subsequent generations of biologists. Coyne & Orr (2004) still support this concept, although they accept that limited gene exchange may occur between different species, *i.e.* complete reproductive isolation is not necessary. They emphasize (p. 30) that: “the process of speciation involves acquiring reproductive barriers, and that this process yields intermediate stages when species status is more or less irresolvable”. Criticisms of the BSC include the fact that it does not provide a series of operations by which a biological species can be identified; and it infers biological characters on the basis of phenetic evidence only (see Wiley 1981). According to Frost, Kluge & Hillis (1992) the main concern phylogeneticists have with the BSC is that it is non-dimensional rather than

historical, and classifications based on reproductive compatibility are often inconsistent with the recovered history of evolution.

Considerable debate has raged with regard to which species concept is most appropriate in zoology, resulting in numerous proposals, definitions and summaries (*e.g.* De Queiroz 1998, 2005; Coyne & Orr 2004). The three primary schools of taxonomic thought, namely evolutionary, phenetic and cladistic have played a major role in influencing the various proposals (Lazell 1992). Despite considerable disagreement among biologists, De Queiroz (1998: 60) noted that modern species definitions “explicitly or implicitly equate species with segments of population level evolutionary lineages”. Therefore, according to his General Lineage Concept, “species are segments of population level evolutionary lineages” (p. 63). A lineage (unbranched) is a population extended through time, while a population is a short segment or cross-section of a lineage (De Queiroz 1998). The main differences between definitions relate to species criteria – *i.e.* standards for judging whether an entity qualifies as a member of the species category. De Queiroz (1999: 79) noted that although most biologists now foster the same general concept of species, disagreements result from the interpretation of certain contingent properties of lineages as necessary properties of species (*i.e.* species criteria), leading to “species definitions that are incompatible both in theory (because they are based on different necessary properties) and in practice (because they result in the recognition of different species taxa)”. Frost *et al.* (1992) noted that although most phylogeneticists now agree that species are lineages, there is still disagreement as to how inclusive the recovered entities (lineages) should be.

According to De Queiroz (1998) the term Phylogenetic Species Concept (PSC) accurately describes all modern species definitions, *i.e.* those that equate species with branches. He discusses three different groups of phylogenetic systematic species definitions. In the first, speciation is equated with cladogenesis and ancestral species are no longer thought to exist after giving rise to descendants (*e.g.* Hennig 1966; Ridley 1989). In this regard Wiley (1981) stated that: “Ancestral species may become extinct during speciation events if they are subdivided in such a way that neither daughter species has the same fate and tendencies as the ancestral species”. The second group is characterized by the monophyly criterion that implies that ancestral lineages cannot be species, only terminal lineages – or sub-lineages if descendants are ignored - can (*e.g.* Bremer & Wanntorp 1979). Species

recognition is based on shared derived characters (synapomorphies) that define monophyletic groups. Finally, the third group is characterized by the idea of diagnosability, *i.e.* unique combinations of primitive and derived characters (*e.g.* Cracraft 1983). For example, Eldredge & Cracraft (1980: 92) defined a species as “a diagnosable cluster of individuals within which there is a parental pattern of ancestry and descent, beyond which there is not, and which exhibits a pattern of phylogenetic ancestry and descent among units of like kind”. Followers of the latter approach might consider any trait (apomorphy) as diagnostic of a new species – *e.g.* minor differences in plumage colour, a single fixed allelic difference, or a single nucleotide difference in a DNA sequence. However, such an approach may distort evolutionary history because species diagnosis is based on simple diagnostic features rather than shared derived traits (Coyne & Orr 2004). Baum & Donoghue (1995) also considered their Genealogical Species Concept to be a phylogenetic species concept.

According to Echelle (1990) the PSC of Cracraft (1987) is preferable to the Evolutionary Species Concept (ESC; see below) (*e.g.* Wiley 1978, 1981; Frost & Hillis 1990) because species names are assigned to objectively delimited evolutionary units, *i.e.* diagnosable groups of organisms (= species). There is no need to interpret intraspecific variation in taxonomic terms - subspecies are not recognized as real entities. Although one of Frost & Hillis's (1990) main complaints against the PSC was that transitory components of populations (demes) may be regarded as separate species, Echelle (1990) noted that this would be the case only if these populations exhibited one or more fixed character differences. Echelle (1990: 110) added that “two populations fixed for different traits represent only one species if a third population is polymorphic for the traits” and this meant names would not generally be assigned to ephemeral entities. However, he did admit that De-oxyribose Nucleic Acid (DNA) sequencing studies (*e.g.* mtDNA) “have the potential to reveal many phylogenetic species among small, isolated populations” (p. 110).

Wiley (1978) defined an evolutionary species as “a single lineage of ancestor-descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate”. By lineage he meant “one or a series of demes that share a common history of descent not shared by other demes” (Wiley 1981). Frost & Hillis (1990) noted that evolutionary species are the largest evolving entities, or

largest lineages on single phylogenetic trajectories, whereas phylogenetic species are the smallest detectable supra-organismal systems.

The ESC may be considered more applicable than the BSC because it is logically consistent with both sexual and asexual taxa, and can deal with species as spatial, temporal, genetic, epigenetic, ecological, physiological, phenetic and behavioural entities (Wiley 1981; Frost & Hillis 1990). However, Frost & Hillis (1990) disagreed that Wiley's ESC was also applicable to non-Mendelian species (*e.g.* hybridogens). According to Echelle (1990: 111) the ESC is in fact applicable to non-Mendelian forms "if one accepts 'phenotypic cohesion' as a manifestation of developmental, genetic and ecological constraints that can hold a species together despite the lack of gene flow between lineages". The PSC is also applicable to non-Mendelian forms "if the diagnosability criterion is not extended to mutants at the molecular level" (Echelle 1990: 111).

Wiley (1978, 1981) discussed four logical corollaries derived from his definition of evolutionary species:

1. All organisms, past and present, belong to some evolutionary species.
2. Species must be reproductively isolated from each other to the extent that this is required for maintaining their separate identities, tendencies and fates.

In this regard, Simpson (1961) noted that: "the important question is not whether two species hybridize, but whether two species do or do not lose their distinct ecological and evolutionary roles. If, despite some hybridization, they do not merge, then they remain separate species in the evolutionary perspective".

3. Evolutionary species may or may not exhibit recognizable phenetic differences, thus any investigator may overestimate or underestimate the actual number of existing independent lineages in a study.

Detailed analyses often show that apparently homogeneous species actually consist of several distinct lineages (sibling species) (Wiley 1981). Also, when only morphological characters are used, cryptic species may go undetected. On the other hand, if data is inadequate (*e.g.* poor or sparse sampling), this may lead to overestimates of the number of species.

4. No presumed separate, single, evolutionary lineage may be subdivided into a series of ancestral and descendant species.

This is mainly in reference to some palaeontologists who more-or-less arbitrarily subdivide a single lineage into a number of species for populations undergoing anagenesis. Such species are referred to as paleospecies, successive species or chronospecies (see Wiley 1981).

Brooks & McLennan (2002) regarded the ESC as the fundamental ontological species concept for evolutionary biology. They noted, however, that it lacked operationality – it did not provide discovery modes or evaluation criteria. These authors then discuss three distinct categories of historical species concepts: two forms of the Phylogenetic Species Concept (PSC) and the Composite Species Concept. According to Brooks & McLennan (2002) the form of the PSC requiring evidence of both lineage splitting and character evolution is the operational surrogate of the ESC. These authors point out that together the ESC and its surrogate the PSC bridges the conceptual gap between the process of evolution and what has evolved.

There are in fact many similarities between the BSC and ESC. Coyne & Orr (2004) point out that in many ways the ESC is in fact equivalent to the BSC, particularly with regard to sympatric species. In this regard Wiley (1978) noted that: “Separate evolutionary lineages (species) must be reproductively isolated from one another to the extent that this is required for maintaining their separate identities, tendencies and historical fates”. With regard to allopatric populations, Wiley (1978) stated that if there was no corroboration that a geographic event leads to separate evolutionary paths there was also no reason to recognize two separate species. Recognition of two evolutionary species would require significant evolutionary divergence. Wiley (1978) was not explicit in what he meant by this, but if such divergence prevented populations exchanging genes if they became sympatric then, as indicated by Coyne & Orr (2004), the ESC becomes the BSC. Wiley (1981: 36) even referred to the BSC as “a special case of the evolutionary species concept”, but applied exclusively to bisexual species. In practice, however, morphological divergence of allopatric populations may be considered as an indication of evolutionary divergence.

According to Coyne & Orr (2004) the ESC is unique in being able to deal with a single lineage evolving through time. Such a lineage is understood to comprise a single species as long as it does not branch, irrespective of the extent of evolutionary (character) change

it undergoes. This approach avoids the arbitrary and subjective naming of slices of the same lineage, even though it means that the same species name may be used for organisms that differ substantially (*e.g.* successional species of *Homo*).

De Queiroz (2005) recently proposed the Unified Species Concept. This new species concept differs from his earlier General Lineage Concept (De Queiroz 1998) in that, although it retains the idea that species are separately evolving segments of population level lineages, it contends that this is the only necessary and defining property of species. Therefore, lineages need not be reproductively isolated, morphologically distinguishable, diagnosable, monophyletic, ecologically divergent or conform to any other secondary species criteria to be considered species. The primary factor is that they (species) are evolving separately from other lineages. In this sense it is similar to the ESC. According to De Queiroz (2005: 196) secondary species criteria can be used as “lines of evidence relevant to assessing lineage separation or as properties that define different subcategories of the species category (*e.g.*, reproductively isolated species, monophyletic species, diagnosable species)”. Implications of the Unified Species Concept are the following: undifferentiated and undiagnosable lineages are species (*e.g.* morphologically indistinguishable, but genetically distinct), species can fuse, they can be nested within other species, the species category is not a taxonomic rank, and new taxonomic practices and conventions are required to accommodate these conclusions. This new concept is probably most applicable when species are indicated (as clades in phylograms) in molecular systematics.

In the present study I follow the ESC as defined by Wiley (1978) and refined by Frost & Hillis (1990) and Frost *et al.* (1992). I recognize modern approaches that consider distinct genetic and/or morphological differentiation (objective criteria), together with allopatry (indicative of reproductive isolation), as evidence of separate species status within an evolutionary species concept. In some cases the current sympatric (*P. langi* and *P. m. subviridis*) or parapatric (*P. m. subviridis* and *P. m. melanotus*) distributions of taxa mask the possibility that they were at some time separated (allopatric). It is recognized that even when distinct genetic and/or morphological differences are apparent, and there is evidence of allopatry, there is still an element of subjectivity when deciding whether or not a population should be recognized as a separate species.

## 1.8 Subspecies

According to Mayr (1963) a subspecies is “an aggregate of local populations of a species inhabiting a geographic subdivision of the range of the species, and differing taxonomically from other populations of the species”. Wiley (1981) noted that one problem regarding the use of the interbreeding community criterion is that some researchers consider speciation incomplete until sympatry has occurred. Also, if hybridization occurs speciation is considered incomplete. Differentiated parapatric or allopatric populations that should be considered good evolutionary species might then be treated as polytypic species comprising two or more subspecies. Wiley (1981: 28) concluded that: “the ‘subspecies’ as an evolutionary lineage will be confounded with the subspecies as a category of convenience – a variant population of an evolutionary species”.

Frost & Hillis (1990: 90) noted that: “allopatric lineages, whose component organisms are mutually apomorphic but which share reproductive compatibility”, would be considered distinct species under the Wiley criterion. These authors propose a concept similar to that of Wiley (*e.g.* 1981). They point out (p. 92) that application of an evolutionary (or phylogenetic) species concept would do away with clinal subspecies, although the subspecies category “could be used theoretically for sublineages not incontrovertibly removed from the possibility of interaction with other sublineages, but the use of this category would necessarily follow recovery of the historical relationships of the subpopulations”.

Frost & Hillis (1990) noted that allopatric and clearly diagnosable populations should be considered species, not subspecies. They added (p. 93) that: “if one thinks that allopatric populations are likely to interact, or be interacting in time, and wants to join them under a single binomial, one should demonstrate that these populations are reproductively compatible (not merely gametically compatible) and together form a monophyletic group”. Also (p. 93): “If the organisms among populations have already diverged strongly, we assume that it is less likely that the populations are influencing each other via immigration or that they will ultimately reconstitute a single population”. They also point out that any decision to treat a monophyletic group of populations as “a single

interacting lineage (one species) or as several distinct species is not operational and comes down to traditional inanities of ‘lumping’ or ‘splitting’”.

According to Frost *et al.* (1992) it is better to use conservative classifications (species only) rather than those making claims of relationship (subspecies). In other words they preferred (p. 48) not to base classifications on “predictions of the future of evolution (*i.e.* that differentiated populations will reconnect)”. They added (p. 48) that “if ‘subspecies’ in the ‘biological species’ sense really had anything to do with that concept of species, taxonomists trying to apply the concept of ‘biological’ species would require that subspecies be recognized on the basis of developing reproductive incompatibility, *i.e.*, that the populations designated ‘subspecies’ were demonstrably undergoing ‘speciation’”. If subspecies are incipient biological species then partial reproductive isolation needs to be demonstrated, but this is seldom the case (Frost *et al.* 1992).

According to Frost *et al.* (1992) the validity of the subspecies category depends on whether subspecies are historically discoverable items (temporarily isolated lineages). Within the context of phylogenetic inference a subspecies is a temporarily isolated sublineage. Its use is greatly restricted because (p. 48) “identifying a sublineage requires the same kind of evidence for recognizing a lineage, not less, and also requires the additional assumption that the sublineages will reconnect in the future to reconstitute the lineage”. Even within the context of the BSC, Mayr (1982: 594) indicated that subspecies “was not a concept of evolutionary biology but simply a handle of convenience for the clerical work of the museum curator. The subspecies was likewise found deficient when studied as the adaptive response to local environmental conditions. During the study of clines, workers found the more-or-less arbitrarily determined subspecies borders to be often more of a hindrance than a help”.

However, Montanucci (1992) noted that because distributional boundaries are dynamic there is always the possibility of interactions (exchange of genes) occurring between disjunct populations through time. Van Deventer, Lowe, McCrystal & Lawler (1992) questioned the concept of raising weakly differentiated subspecies of montane isolates to species rank if there was a reasonable chance that they would be joined following the next glacial. They added (p. 12) that much information on evolutionary variation is already

provided in the subspecies framework and naming of populations with “discontinuous clinal or allopatric variation inherently expresses inferred relationships”.

In a paper dealing with conservation genetics, Ryder (1986: 9) stated that it was difficult to determine “which subspecies actually represent populations possessing genetic attributes significant for present and future generations of the species”. He then introduced the concept of Evolutionarily Significant Units (ESUs), referring to subspecific populations that represented significant adaptive variation. Identification of such ESUs required the use of various kinds of data, such as morphometry, allozymes and mtDNA. However, it was suggested that there should be concordance between sets of data obtained using different techniques, *e.g.* allopatry combined with a measure of genetic distance (Ryder 1986). Most subsequent definitions suggested that an ESU should be geographically discrete and that there should be concordant divergence for both molecular and non-molecular traits (see Moritz 1994). Moritz (1994: 373), with particular emphasis on molecular population genetics, noted that the main purpose of defining ESUs was “to ensure that evolutionary heritage is recognized and protected and that evolutionary potential inherent across the set of ESUs is maintained”. He was of the opinion that ESUs “should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci.” It was the pattern, rather than the extent, of sequence divergence, that was important. Moritz (1994: 374) then commented that: “Populations that do not show reciprocal monophyly for mtDNA alleles, yet have diverged in allele frequency, are significant for conservation in that they represent populations connected by such low levels of gene flow that they are functionally independent.” He referred to these populations as Management Units (MUs), defined as “populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles” (p. 374). Moritz (1994) concluded that it was important to distinguish between ESUs, dealing with historical population structure, mtDNA phylogeny and long-term conservation needs; and MUs, dealing with current population structure, allele frequencies and short-term management issues. In section 6.4 various *Pseudocordylus* populations are referred to the categories ESU and MU. Genetically differentiated and allopatric populations may differentiate further over time and will therefore be less likely to interbreed and re-constitute. It is important to recognize their potential as unique faunal units that should be afforded conservation protection.

## 1.9 Speciation

Eldridge & Cracraft (1980) noted that speciation is often a result of geographical separation of populations, resulting in character variation and eventually attainment of reproductive isolation. This reproductive isolation is thought to be an accidental by-product of the physical separation of populations, brought about by selection and genetic drift. These authors then refer to Bush's models of allopatric, parapatric and sympatric speciation. Two allopatric modes of speciation are discussed. In the first, a large population is divided into two similar sized parts, each of which undergoes divergence. This results in two populations that are diagnosably different from the common ancestor and each is treated as a separate and new species. The second mode involves a peripheral isolate that attains autapomorphies and is then considered a new species. However, in this case the ancestral species persists, such that there is only a single descendant species. Apart from cases of reduction speciation and speciation by hybridization, speciation events couple lineage splitting with differentiation, resulting in two or more species from one species (Wiley 1981).

According to Wiley (1981), Wallace, in 1855, noted that closest relatives often occupy separate but contiguous geographical regions, and sympatric species resembled one another less than allopatric species. This appears to be the case with members of the *Pseudocordylus melanotus* (A. Smith, 1838) complex (see Chapters 2 and 5).

Bremer & Wanntorp (1979) point out that the branching points in phylograms do not indicate the sequence of speciation (species splitting), but rather the sequence of geographical separation. Also, populations may remain isolated for considerable periods of time, acquiring apomorphic characters, but without reproductive barriers forming. They then noted that geographical separation of populations represents the initiation of speciation, while biological species are delimited by development of reproductive barriers representing the completion of speciation. To overcome this incongruity, Bremer & Wanntorp (1979) believed that what was required was a definition recognizing morphologically distinct allopatric populations as species. This definition, they believed, could be made under Wiley's (1978) ESC.

Bremer & Wanntorp (1979) point out that apart from bifurcation, multifurcation (multiple splitting) of parental populations also occurs in nature. This may result from climatic changes that lead to multiple pocketing of populations in the remaining favourable sites, or inundation of land by sea or fresh water resulting in isolated island populations.

With regard to reticulate evolution, Bremer & Wanntorp (1979) mention that there are several examples of polytypic species with morphologically distinct but reproductively undifferentiated populations. The disappearance of geographic barriers within such species complexes may result in adjacent populations merging and even sharing synapomorphies with two other species.

### 1.10 Key questions

The following key questions will be addressed:

- i) What are the phylogenetic relationships among populations?
- ii) How many evolutionary species are identifiable in the *P. melanotus* complex?
- iii) Are the various isolated populations taxonomically distinguishable?
- iv) What is the taxonomic status of the population at Monontsha Pass? Is the area a possible contact zone between the two subspecies of *P. melanotus*?
- v) What are the morphological features that distinguish the various taxa?
- vi) How are the various taxa distributed geographically?
- vii) What possible models can be hypothesized to explain the biogeographical distribution of taxa?

## CHAPTER 2

### **Taxonomic history and geographical distribution of the *Pseudocordylus melanotus* (Smith, 1838) and *P. microlepidotus* (Cuvier, 1829) species complexes (Sauria: Cordylidae)**

#### **2.1 Introduction**

The monophyly of the scincomorph lizard clade Cordyliformes Fitzinger, 1826 is generally accepted (*e.g.* Lang 1991; references in Lamb *et al.* 2003). There is, however, some disagreement amongst authors as to whether the Cordyliformes comprises a single family, namely Cordylidae Gray, 1837 (*e.g.* Odierna, Canapa, Andreone, Aprea, Barucca, Capriglione & Olmo 2002 - molecular and karyological data), two families, namely Cordylidae and Gerrhosauridae Fitzinger, 1843 (*e.g.* FitzSimons 1943; Lang 1991 – both using morphological data) or one family with two subfamilies, namely Cordylinae and Gerrhosaurinae (*e.g.* Wermuth 1968). Odierna *et al.* (2002) and Lamb *et al.* (2003) provided additional references to papers dealing with cordyliform relationships. At this time it seems most appropriate to recognize two families (Frost, Janies, Mouton & Titus 2001; Lamb *et al.* 2003).

Most authors (*e.g.* FitzSimons 1943; Lang 1991; Branch 1998) recognize four genera in the sub-Saharan African family Cordylidae, namely *Cordylus* Laurenti, 1768; *Pseudocordylus* Smith, 1838; *Chamaesaura* Schneider, 1799; and *Platysaurus* Smith, 1844. Lang (1991) subdivided Cordylidae into the subfamilies Chamaesaurinae (genus *Chamaesaura*) – the earliest diverging taxon – and Cordylinae. The latter was further subdivided into the tribes Cordylini (*Cordylus*) and Pseudocordylini (*Pseudocordylus* and *Platysaurus*). However, the molecular data of Frost *et al.* (2001) suggested that only two genera should be recognized, namely *Platysaurus* and *Cordylus*, the latter including *Pseudocordylus* and *Chamaesaura* (but see comments below).

According to Lang (1991) the family Gerrhosauridae contains two subfamilies, namely a sub-Saharan African Gerrhosaurinae and a Madagascan Zonosaurinae Lang, 1991. He

subdivided the Gerrhosaurinae into two tribes, the Angolosaurini (genus *Angolosaurus* FitzSimons, 1953) and Gerrhosaurini (*Gerrhosaurus* Wiegmann, 1828 and the sister genera *Cordylus* Gray, 1865 and *Tetradactylus* Merrem, 1820). However, the molecular study of Lamb *et al.* (2003) determined that *Angolosaurus* (comprising *A. skoogi* [Andersson, 1916]) should be transferred to *Gerrhosaurus*, such that Lang's tribes fall away. Lang's (1991) subfamily Zonosaurinae comprises two genera, namely *Zonosaurus* Boulenger, 1887 and *Trachyloptychus* Peters, 1854. The mtDNA analysis of Odierna *et al.* (2002), using 12S and 16S rRNA, indicated that the morphologically distinct *Trachyloptychus madagascariensis* Peters, 1854 was nested in one of two *Zonosaurus* (five species analyzed) clades, suggesting that all Madagascan gerrhosaurids belong in a single genus. However, in a recent molecular study using the cytochrome *b* gene, based on 12 species of *Zonosaurus* and both known species of *Trachyloptychus*, the two genera were determined to be reciprocally monophyletic (Yoder, Olson, Hanley, Heckman, Rasoloarison, Russell, Ranivo, Soarimalala, Karanth, Raselimanana & Goodman 2005).

In 1838 Andrew Smith described nine species of *Cordylus*, eight of which were new to science. He erected three subgenera (*Cordylus*, *Hemicordylus*, *Pseudocordylus*) to accommodate them. Smith noted that dorsal scales in the genus *Cordylus* were arranged in transverse rows. In the subgenus *Cordylus* these scales were contiguous or overlapping, whereas those ("of each row") of *Pseudocordylus* were "more or less separated by the intervention of small granular scales" (Smith 1838: 32). The mid-dorsal scales in *Hemicordylus* were similar to those of *Cordylus*, but the flanks were covered in small tubercular or granular scales. According to Smith (1838) the subgenus *Cordylus* also had "projecting spinous scales" (p. 31) on the sides of the neck, whereas those of *Hemicordylus* were granular. According to Branch (1998) *Pseudocordylus* differs from *Cordylus* in having granular scales on the neck and back (in addition to enlarged ones), the body scales lack osteoderms, and the tail is not as heavily spined.

*Pseudocordylus* was subsequently not recognized as a subgenus by Smith (1843), although he did retain *Hemicordylus* for *Cordylus capensis* A. Smith, 1838. Smith (1843) did not indicate why he continued to use the subgenus name *Hemicordylus*, but by default this means that all of the other *Cordylus* he mentioned were referable to the subgenus *Cordylus*. Gray (1845) treated both *Hemicordylus* and *Pseudocordylus* as full genera.

The latter genus was retained by subsequent authors to accommodate species in Smith's (1838) subgenus *Pseudocordylus* and, later on, a few additional taxa. However, *C. (H.) capensis* had been transferred to the genus *Zonurus* Merrem, 1820 by Duméril & Bibron (1839) and simply referred to as *Zonurus capensis*, an arrangement followed by most subsequent authors. *Zonurus robertsi* was described by Van Dam (1921).

Stejneger (1936) revived the name *Cordylus*. He noted that *Cordylus verus* Laurenti, 1768 was a synonym of *Lacerta cordylus* Linnaeus, 1758, the genotype of *Cordylus*. Therefore, Merrem's (1820) monotypic *Zonurus* (*Z. cordylus*) is a junior synonym of *Cordylus*.

FitzSimons (1943) treated *Zonurus capensis* and *Z. robertsi* as subspecies of *C. capensis*. Loveridge (1944), however, treated them as distinct species of *Pseudocordylus*, together with a new species named *P. langi*. He justified this change by noting that *P. capensis* and *P. robertsi* were similar to other *Pseudocordylus* in having the neck covered with granules instead of scales. Two more species of *Pseudocordylus* were later described, namely *P. spinosus* FitzSimons, 1947 and *P. nebulosus* Mouton & Van Wyk 1995. However, the status of this genus remains controversial and some authors (*e.g.* Branch 1981) have suggested that it might be congeneric with *Cordylus*. Herselman (1991) conducted a cladistic analysis of the family Cordylidae and suggested that *Cordylus coeruleopunctatus* (Methuen & Hewitt, 1913) be transferred to the genus *Pseudocordylus*. Although Branch (1998) noted that *C. coeruleopunctatus* was closely related to *P. capensis* and *P. nebulosus*, and should perhaps be transferred to *Pseudocordylus*, he retained it in the genus *Cordylus*. *Cordylus coeruleopunctatus* is similar to *Pseudocordylus* in that it also possesses, *inter alia*, granular scales on the sides of the neck.

In recent years two studies have been conducted on the phylogeny of the family Cordylidae using mitochondrial DNA sequencing (Frost *et al.* 2001; J. Melville, unpublished data). Frost *et al.* (2001) determined that *Cordylus* is paraphyletic with respect to both *Pseudocordylus* and *Chamaesaura*. These authors also stated that *Pseudocordylus* is dubiously monophyletic and suggested that *Pseudocordylus* and *Chamaesaura* be considered junior synonyms of *Cordylus*. However, their study was based on limited taxa (15 species of *Cordylus*, five species and subspecies of

*Pseudocordylus*, two species of *Platysaurus* A. Smith, 1844, one species of *Chamaesaura*), resulting in several unresolved polytomies. Two *Pseudocordylus* clades were recognized: one comprising *P. capensis* and *P. nebulosus*, and the other comprising *P. m. microlepidotus* (Cuvier, 1829), *P. m. namaquensis* Hewitt, 1927 and *P. melanotus* (subspecies not named). In the latter clade, as expected, the two subspecies of *P. microlepidotus* were most closely related.

J. Melville (unpublished data) also found that there was no evidence for the monophyly of *Pseudocordylus*. As in the study by Frost *et al.* (2001), two main *Pseudocordylus* clades were recognized. One comprised *P. capensis* and *P. nebulosus*, while the other comprised *P. melanotus melanotus* (A. Smith, 1838), *P. melanotus subviridis* (A. Smith, 1838), *P. microlepidotus* (subspecies not named) and *P. langi*. *Pseudocordylus m. subviridis* and *P. microlepidotus* were, perhaps surprisingly, found to be the sister group to *P. m. melanotus*. In addition, neither of the above-mentioned studies found evidence of a close relationship between *Pseudocordylus* and *Platysaurus*. The status of generic boundaries within Cordylidae thus remains unresolved. *Pseudocordylus* is therefore provisionally still treated as a valid genus, distinct from *Cordylus*.

The taxonomy of the various species and subspecies of *Pseudocordylus* has been controversial for some time. Branch (1981) treated *P. robertsi* as a subspecies of *P. capensis*, noting that specimens from the Cedarberg were morphologically intermediate between these two taxa. He also stated that specimens from the Kammanassieberg had characters in common with both taxa. The status of these taxa was later resolved by Herselman, Mouton & Van Wyk (1992) who referred *Z. robertsi* to the synonymy of *P. capensis*. While *P. nebulosus* is still recognized as a distinct taxon (*e.g.* Branch 1998), its recent transfer to the genus *Cordylus* by Frost *et al.* (2001) is problematic as the new name is pre-occupied by *Cordylus nebulosus* A. Smith, 1838, a junior synonym of *Cordylus cataphractus* Boie, 1828. However, *P. capensis* and *P. nebulosus* - considered sister species (Frost *et al.* 2001) - may be placed in a separate genus (*Hemicordylus* Smith is available) in the near future and a new name is therefore not considered necessary (P. Mouton, pers. comm., 2005).

The taxonomic status of taxa currently known by the names *Pseudocordylus melanotus melanotus* (A. Smith, 1838), *P. melanotus subviridis* (A. Smith, 1838) and *P.*

*transvaalensis* FitzSimons, 1943 is still unresolved. These taxa, together with *P. langi* and *P. spinosus*, both previously confused with *P. m. subviridis*, are here considered to comprise the *P. melanotus* species complex. The status of the three subspecies of *P. microlepidotus*, namely *P. microlepidotus microlepidotus*, *P. microlepidotus fasciatus* (A. Smith, 1838) and *P. microlepidotus namaquensis*, as well as a *fasciatus* population from Transkei currently considered an undescribed subspecies of *P. microlepidotus* by Branch (1998), is also unresolved. The latter taxa are hereafter referred to as the *P. microlepidotus* species complex. The other currently recognized taxa in the genus are *P. capensis* and *P. nebulosus*. There are thus 10 currently recognized species and subspecies of *Pseudocordylus*, all of which are rupicolous, with the genus endemic to South Africa, Lesotho and Swaziland (Branch 1998; Fig. 2.1).

The most recent revisions of *Pseudocordylus* resulted in dissimilar classifications. FitzSimons (1943) recognized three subspecies of *P. microlepidotus*, namely *P. m. microlepidotus* (with *C. melanotus* as a synonym), *P. m. fasciatus* and *P. m. namaquensis*. He also recognized *P. subviridis* and described a new subspecies, namely *P. s. transvaalensis*. Loveridge (1944) recognized the same three subspecies of *P. microlepidotus*, but treated *C. melanotus* as a fourth subspecies of *P. microlepidotus* - with *subviridis* and *transvaalensis* as junior synonyms - and also described *P. langi*. Wermuth (1968) and Welch (1982) later followed Loveridge's classification of *Pseudocordylus*, but added *P. spinosus*, described in 1947. Loveridge (1944: 76) also noted that "the present disposition must be regarded only as tentative" and added that "The precise status and ranges of the forms of this difficult group [*Pseudocordylus*] will not be settled until some South African herpetologist is able and willing to assemble all the material from the South African museums and subject them to intensive comparative study."

Broadley (1964) revised the genus *Pseudocordylus* in KwaZulu-Natal, but noted that much remained to be done, especially with regard to the Cape forms *P. microlepidotus fasciatus* and *P. m. namaquensis*. Branch (1985) also noted that the taxonomy of both the *P. melanotus* and *P. microlepidotus* species complexes was in need of revision. Jacobsen (1989) subsequently evaluated the status of *Pseudocordylus* in the former Transvaal province (comprising provinces currently known by the names Limpopo, Mpumalanga, Gauteng and [eastern] North-West). Unfortunately his study was, like that of Broadley

(1964) and De Waal (1978), restricted to political boundaries (provinces) and thus excluded populations from large parts of the range of the *P. melanotus* species complex. Branch & Bauer (1995) later commented that the status of the various subspecies and geographical isolates of *P. microlepidotus* was in need of detailed analysis. In an overview on the status of the family Cordylidae, Mouton (1997: 21) noted that: “the most pressing problem in the genus [*Pseudocordylus*] is the status of the races of *microlepidotus* and of *melanotus*”. Branch (1988a,b; 1998) pointed out the uncertain status of an apparently isolated population in the Transkei that he tentatively considered an undescribed subspecies of *P. microlepidotus*.

In this chapter the taxonomic and nomenclatural history of both the *P. melanotus* and *P. microlepidotus* species complexes is discussed based on a critical review of the literature and the examination of selected museum specimens, including all available types. Type localities are also discussed and in some cases restricted, and type specimens designated where appropriate. The geographical distribution of all taxa is mapped (Fig. 2.1) and discussed, and a detailed list of localities provided (Appendix 2.1). This chapter, in slightly modified form, was published by Bates (2005).

## 2.2 Materials and Methods

### 2.2.1 Source of distribution data and identification of specimens

A thorough revision of the literature yielded numerous records and to these were added an even larger number of additional records obtained from museums and private collections in South Africa, Zimbabwe, United Kingdom and the United States (Appendix 2.1). Several of these specimens had been examined as part of previous studies (Free State: De Waal 1978; Bates 1992a, 1996; Limpopo, Mpumalanga and Gauteng provinces: Jacobsen 1989; KwaZulu-Natal: Bourquin 2004), while additional specimens, including large samples in the *P. microlepidotus* species complex, were examined during the course of this study to confirm taxonomic status (catalogue numbers marked by an asterisk in Appendix 2.1). Characters used to separate taxa are discussed below. The remaining specimens were identified either by collectors or museum workers. As crag lizards in the *P. melanotus* and *P. microlepidotus* species complexes have a distinct appearance, most

of the latter identifications were probably correct at least at genus level, but in cases where the documented taxonomic status of specimens was considered questionable on geographical grounds, or because of confusion with regard to names, I have assigned them to what I considered the most likely species or subspecies on the basis of geographical distribution (Fig. 2.1). A few records were obtained from the Virtual Museum section of the Southern African Reptile Conservation Assessment (SARCA) project ([www.saherps.net](http://www.saherps.net)). Photographs of specimens were identified by the author and at least one additional member of SARCA's Experts Panel (selected individuals in southern Africa with specialist knowledge of local reptiles). Figure 2.1 is therefore most probably a fair representation of the true geographical distribution of populations and known taxa in the two species complexes.

### 2.2.2 Validation and documentation of distribution data

The co-ordinates and spelling of localities and other place names in most South African provinces were checked on the 1: 250 000 topocadastral map series published by the Chief Director of Surveys and Mapping (Mowbray). However, localities in the Free State and adjacent areas on the Drakensberg escarpment were checked using the 1: 50 000 topocadastral map series and index of (Orange) Free State farms. For Lesotho, the 1: 50 000 topocadastral map series (1979-1982) published by the Government of the United Kingdom (Directorate of Overseas Surveys) for the Government of Lesotho, was used. Leistner & Morris's (1976) *Southern African Place Names* and the 1: 250 000 *Map of Lesotho* published by the Lesotho Government (1994) were also used.

If a record was available in the form of a locality name (*e.g.* farm) only, the co-ordinates for the center of the area were determined, as was the range of elevations for the entire area. When exact collection localities (degrees, minutes and seconds) and (often) altitudes (meters above sea level) were determined this is indicated in Appendix 2.1 using an asterisk after the co-ordinates. When the elevations were provided on museum documentation or in the literature, or when it was possible using 1: 50 000 maps to determine an altitudinal range (usually within 200 m), this is also listed in Appendix 2.1. In cases where a different elevation applies to the same locality, this is indicated after the museum catalogue number. Elevations given in feet above sea level were converted (x

0.305) to the nearest 1 m. Localities presented as distances from towns or villages refer to straight-line displacement.

In Appendix 2.1 a single catalogue number refers to one specimen unless otherwise indicated. Catalogue numbers listed as (for example) NMB R2415-8 refer to all specimens from 2415 to 2418.

### 2.2.3 Mapping of distribution data

Localities in Figure 2.1 were plotted using the quarter-degree grid and locus code method, but when possible, smaller scale eighth-degree locus codes were provided in Appendix 2.1 (see De Waal 1978; Bates 1992b – but, *e.g.* 29°30'S, 26°30'E = 2926Da1, not 2926Ad4).

### 2.2.4 Morphological features examined

Some of the head shields discussed below are illustrated in FitzSimons (1943, figs 371 & 372). Scallation details are also discussed in sections 5.2 and 5.3 (Chapter 5) and Appendix 5.2. Numbers of scales were the same on either side of the head unless otherwise indicated. The most posterior supraciliary is a small scale above and slightly behind the eye, in contact with the most posterior supraocular. It lies behind what FitzSimons (1943, fig. 372) considered the most posterior supraciliary, sometimes separated from it by one or more small granules. The keeled posterior infralabial is at least partially in contact with the corner of the mouth; and the posterior sublabial is largely in contact with the posterior infralabial. Transverse dorsal rows are counted from the first row behind the posterior insertion of the forelimb to the row anterior to the vent; counted on the right side of the body; incomplete rows not counted. Longitudinal dorsal rows consist of enlarged scales counted at the widest part of the body about midway between fore- and hindlimbs, but including the reduced paravertebral scales; small or granular dorsals less than half the size of adjacent enlarged scales were not counted. Longitudinal rows of ventrals were counted in the same region as described above; lateral ventral plates were smooth, flattened and at least one-third the size of adjacent ventrals. Lamellae under fourth finger and toe were counted from the first scale entirely or largely (>60%) anterior to the junction between 3<sup>rd</sup> and 4<sup>th</sup> digits, excluding incomplete lamellae.

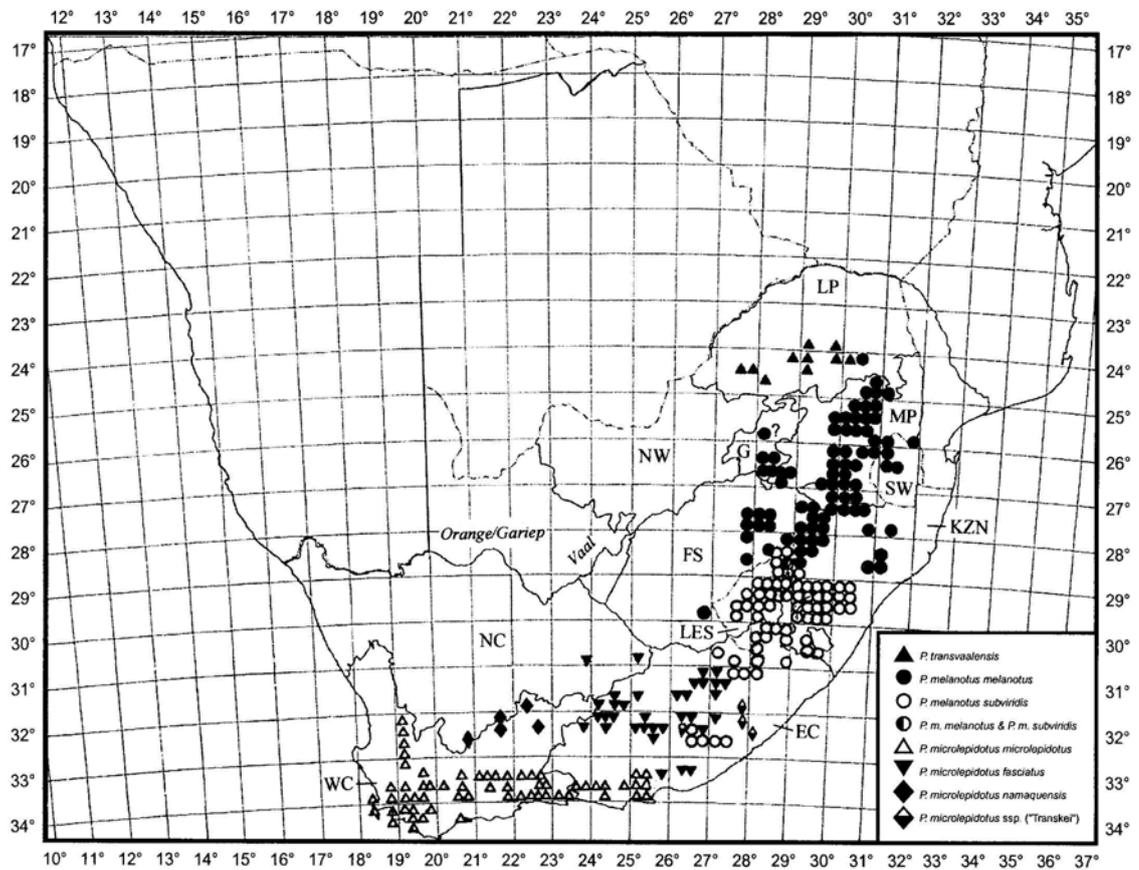
Other morphological characters are described in the text below. Measurements were performed using vernier calipers (0.02 mm); and values presented were rounded to the nearest 0.1 mm. Head width was measured at the widest part of the head, excluding the temporal spines.

### 2.2.5 Museum abbreviations

Museum abbreviations for specimens examined (see Appendix 2.1) or referred to in the text denote the institutions below.

AM	Albany Museum (Grahamstown) – incorporated into the PEM collection
AMNH	American Museum of Natural History (New York)
AJL	Herpetological Collection of A.J.L. Lambiris (Hillcrest)
BMNH	(The) Natural History Museum (London)
CAS	California Academy of Sciences (San Francisco)
CDNEC	(Western) Cape Department of Nature and Environmental Conservation (Jonkershoek, Stellenbosch)
DNSM	Durban Natural Science Museum (Durban)
JV	John Visser private herpetological collection (Jeffrey’s Bay)
MCZ	Museum of Comparative Zoology, Harvard (Cambridge)
MNHN	Muséum National d’Histoire Naturelle (Paris)
MMK	McGregor Museum (Kimberley)
NMB	National Museum (Bloemfontein)
NML	National Museum of Lesotho (Maseru)
NMSA	Natal Museum (Pietermaritzburg)
NMSZ	National Museums of Scotland (Edinburgh)
NMWN	National Museum of Namibia (Windhoek)
NMZB	Natural History Museum of Zimbabwe (Bulawayo)
NUM	University of KwaZulu-Natal museum (Pietermaritzburg)
PEM	Port Elizabeth Museum (Bayworld) (Port Elizabeth)
RMNH	National Museum of Natural History (Leiden)
SAM	Iziko South African Museum (Cape Town)
TM	Transvaal Museum (Pretoria)
UKNHM	University of Kansas Natural History Museum (Lawrence)

USEC	University of Stellenbosch Ellerman Collection (Stellenbosch)
USNM	National Museum of Natural History, Smithsonian Institution (Washington)
ZMA	Zoologisch Museum, University of Amsterdam (Amsterdam)



**Figure 2.1:** Geographical distribution of the *Pseudocordylus melanotus* and *P. microlepidotus* species complexes (see Appendix 2.1 for details). The ranges of two species in the *P. melanotus* complex are not shown on the map: *P. langi* has been recorded from only four quarter-degree units, namely 2929AA, 2829CC, 2828DB & DD; *P. spinosus* occurs in the latter three units as well as 2929AB & AD & BA and 3030AA. South African provinces: LP = Limpopo Province, MP = Mpumalanga Province, G = Gauteng, NW = North-West, FS = Free State, KZN = KwaZulu-Natal, NC = Northern Cape, EC = Eastern Cape, WC = Western Cape; Swaziland: SW; Lesotho: LES.

## 2.3 Status of taxa in the *Pseudocordylus microlepidotus* species complex

### 2.3.1 *Pseudocordylus microlepidotus microlepidotus* (Cuvier, 1829)

*Cordylus microlepidotus* Cuvier, 1829, *Le Règne Animal*, ed. 2, 2, p. 33 (Type locality: “Cape of Good Hope”).

*Zonurus microlepidotus* Gray, 1831, in *Griffith's Animal Kingdom*, IX, Syn., p. 63.

*Zonurus Wittii* Schlegel, 1834, *Tijdschr. Nat. Gesch. Phys.*, I, p. 207, pl. vii, figs 1a-c (but text refers to *Zonurus microlepidotus* from “southern tip of Africa”).

? *Zonurus Davyi* Gray, 1838, *Ann. Nat. Hist.*, 1, p. 388 (No type locality given).

*Cordylus (Pseudocordylus) montanus* A. Smith, 1838, *Mag. Nat. Hist.* 2(2), p. 32 (Type locality: “South Africa”; restricted to: “Table Mountain, and the hills near Cape Town” by Smith, 1843, *Ill. Zool. S. Afr. Rept.*).

*Cordylus (Pseudocordylus) algoensis* A. Smith, 1838, *Mag. Nat. Hist.* 2(2), p. 32 (Type locality: “South Africa”; restricted to: “rocky precipices at and around Algoa Bay” by Smith, 1843, *Ill. Zool. S. Afr. Rept.*).

*Pseudocordylus microlepidotus* Gray, 1845, *Cat. Liz. Br. Mus.*, p. 51.

*Pseudocordylus montanus* Hewitt, 1927, *Rec. Alb. Mus.* 3, p. 390.

*Pseudocordylus microlepidotus microlepidotus* FitzSimons, 1943, *Mem. Transvaal Mus.* 1, p. 464.

*Pseudocordylus algoensis* Branch, 1981, *Ann. Cape Prov. Mus.* 13(11), p. 160.

*Cordylus microlepidotus microlepidotus* Frost *et al.*, 2001, *Am. Mus. Nov.* 3310, App. 1, p. 9.

*Cordylus microlepidotus* was described, in a footnote in George Cuvier’s (1829) *Le Règne Animal*, as “the *Cordylus* with the small scales on the back”. The lack of a proper diagnosis or reference to a figure led Brygoo (1985) to question the nomenclatural validity of Cuvier’s name. However, while the description is obviously extremely vague, it does - as suggested by Brygoo (1985) - contain a diagnostic element and should therefore be accepted. It draws attention to the tiny granular dorsal scales that, in combination with the larger and often keeled scales, are typical of this species. This character may have been considered sufficient for diagnostic purposes as at least three of the other four *Cordylus* species described in Cuvier’s (1829) footnote lack granular dorsals, *i.e.* *C. griseus* (synonym of *C. cordylus*) and *C. niger* - both based on paintings in Albertus Seba’s (1735) *Thesaurus* - and *C. dorsalis* (syn. *C. cordylus*). The fourth taxon, namely

*C. laevigatus* (not *C. laevigatus* [FitzSimons, 1933]) – described only as having almost no spines on the body and tail - cannot be associated with any known species.

Brygoo (1985), with reference to Cuvier (1817, 1829), has established that the type locality of *C. microlepidotus* is indeed “Cape of Good Hope” as noted by FitzSimons (1943: 464). The latter term refers to the finger-like projection of land from Cape Town southwards to Cape Point – also called the Cape Peninsula - representing the southwesternmost portion of Africa. Cuvier (1817), in the first edition of his *Le Règne Animal*, noted that the only species of *Cordylus*, known at that time as *Lacerta cordylus* (represented by figs 3 & 4, pl. 84, vol. 1 [= *C. griseus*, see below] and fig. 5, pl. 62, vol. 2 [= *C. niger*, see below] in Seba 1735), came from “Cape of Good Hope”. In his second edition Cuvier (1829: 32-33) stated that “The Cape of Good Hope produced many of them [*Cordylus*] for a long time identified under the name of *Lacerta cordylus*, L.”. Finally, Cuvier’s (1829: 33) footnote, associated with the sentence above, reads as follows: “We have four of these species: the grey *Cord.* (*Cord. griseus*), Nob., Seb. I, LXXXIV, 4; - the black *C.* (*C. niger*), with the soft-ended scales, Seb. II, LXII, 5; - the *C.* with a yellow dorsal line (*C. dorsalis*); - the *C.* with small scales on the back (*C. microlepidotus*). In the Cape there is also a cordyle of which the scales, also on the tail, have almost no spines (*C. laevigatus*, Nob.)”.

According to Loveridge (1944), Cuvier (1829) based his description of *C. microlepidotus* on Seba’s (1735) “*Lacerta, Africana, elegantissima*” (*i.e.* elegant African lizard), illustrated as fig. 6 on pl. 62. However, the latter is an iguanine lizard with black and blue bands on the body. On the same plate - as fig. 5 - is an illustration of a stout black cordylid described by Seba (1735) as “*Lacerta nigra, Africana*” (*i.e.* African black lizard), but this is the specimen on which Cuvier (1829) based his description of *Cordylus niger*. In order to demonstrate that Loveridge (1944) was wrong, Brygoo (1985) correctly noted that Duméril & Bibron (1839) - in their account of the reptiles in the Natural History Museum (Paris) - did not refer to Seba, but to fig. 1 on pl. 6 of Guérin-Méneville’s (1829-1844) collection of illustrations and captions depicting many of the animal species described by Cuvier. Wermuth (1968) listed the type locality of *C. microlepidotus* as “Africa”, with reference to Loveridge (1944).

In vol. 2 of Guérin-Méneville (1829-1844), *C. microlepidotus* is illustrated by means of a small lateral view in colour (fig. 1, pl. 6) and a line drawing (about 3.0 x 2.3 cm) showing the scalation of the dorsal aspect of the head (fig. 1a, pl. 6) (J.C. Poynton, pers. comm., 7 June and 29 July 2003). Unfortunately the back was not illustrated and it was therefore not possible to confirm or refute any reference to minute scales. Nevertheless, a tracing of the original of fig. 1a was prepared by Poynton (*op. cit.*) and sent to the author. It was compared to FitzSimons' (1943) fig. 371 showing the dorsal aspect of the head of a (presumably typical) *P. m. microlepidotus* (TM 13601) from Table Mountain, Cape Town. The two figures are in general agreement, but the Guérin-Méneville head differs most notably from that of FitzSimons as follows: supranasals in very narrow contact, not clearly separated by the frontonasal; prefrontals more elongated; posterior parietals not paired, forming a single plate.

Brygoo (1985) considered six specimens in the Muséum National d'Histoire Naturelle (Paris), all collected prior to 1829, to be the syntypes of *C. microlepidotus*. These are MNHN 8023 (150 mm snout-vent length [SVL] + 125<sup>+</sup> mm tail length [<sup>+</sup> = incomplete or regenerated]), a mounted specimen lacking collector's details; MNHN 8369 (120 + 105<sup>+</sup>), mounted specimen collected by Pierre Antoine Delalande (in 1818: [www.en.wikipedia.org](http://www.en.wikipedia.org)); and four specimens donated by Jean-René Constant Quoy and Joseph Paul Gaimard (collected 1822-5: [www.en.wikipedia.org](http://www.en.wikipedia.org)): MNHN 2802 (103 + 92<sup>+</sup>), MNHN 2803 (120 + 70<sup>+</sup>), MNHN 2804 (130 + 105<sup>+</sup>) and MNHN 2804A (122 + 110<sup>+</sup>). Digital colour images of the six specimens (photographed by N. Pruvost) were obtained from I. Ineich (Natural History Museum, Paris) for examination. All specimens have longitudinal rows of large (smooth or obtusely keeled) scales on the back, with small granular scales between them; there is a distinct longitudinal furrow along the vertebral region of the back in all except MNHN 8369; the tail consists of whorls of strongly keeled scales; dorsal pattern similar to *P. m. microlepidotus* as illustrated in fig. 1, pl. 72 in Branch (1998), e.g. back dark brown with narrow cream-yellow bands, although poorly marked in MNHN 2802 and more-or-less uniform brown in MNHN 8023 and 8369; lateral temporals in more-or-less 3-4 rows horizontally, the uppermost row with the smallest and shortest scales. There is some variation in the relative positions of the rostral, supranasal and frontonasal scales: supranasals in narrow contact in MNHN 8023 and apparently also MNHN 2802 and 2803; in MNHN 2804 the supranasals are separated by the frontonasal which is therefore in contact with the rostral (which has a

short longitudinal groove medially at its base); in MNHN 2804A there is a small squarish scale separating supranasals, rostral and frontonasal; while the anterior region of the head of MNHN 8369 is damaged or fragmented. None of the six specimens perfectly matches the figure in Guérin-Méneville (1829-1844), but the latter may be a composite of two or more syntypes. The undivided posterior parietal in the latter figure may have been in error, as a pair of posterior parietals is present in all six syntypes.

I hereby designate MNHN 2804 as lectotype of *Cordylus microlepidotus*, whereas MNHN 2802, 2803, 2804A, 8023 and 8369 become paralectotypes.

In accordance with Recommendation 74C of the 1999 Code I hereby list the following data, in addition to that above, pertaining to the lectotype MNHN 2804, sex unknown (variation in paralectotypes is indicated in parentheses): Lateral temporals in approximately three rows horizontally on right side of head, the scales of the middle row the longest (3-4 rows in paralectotypes, the middle or lowest row with the most elongate scales); five (4 in MNHN 2802, 5 in 2803, ? in others) supralabials anterior to median subocular (right side of head); frontonasal with short groove posteriorly (undivided in paralectotypes), as long as it is wide, in contact with loreals (? in MNHN 8023, 8369); anterior and posterior parietals undivided, posterior about 1.5 times larger than anterior; no small scales posterior to interparietal; dorsolaterals the largest, followed by the laterals, the medians being the smallest.

Gray (1831) transferred *C. microlepidotus* to the genus *Zonurus*. A few years later Schlegel (1834) discussed in detail a cordylid from the “southern tip of Africa” (p. 217) – possibly also meaning “Cape of Good Hope” as discussed above – that he named “*Zonurus Wittii*” in a plate depicting the upper, lower and side views of the head. The single type (holotype) specimen of *Zonurus Wittii* (RMNH 3600) at the National Museum of Natural History (Leiden, The Netherlands) is labeled as having being collected in the “Cape” (J.W. Arntzen, pers. comm., 6 April 2005). On page 206 Schlegel (1834) noted that he “readily recognized” that his new lizard was referable to *Z. microlepidotus* after consulting an illustration in Guérin-Méneville (1829-44). Then, on p. 207, he added that he had “previously” named this lizard *Z. Wittii*. Prior to describing the specimen [as *Z. microlepidotus*] Schlegel (1834: 217) noted that he “named this species after him [i.e. Mr

De Witt from Bedford], which name also appears on our plate [pl. 7, fig. 1a-c], but must now be changed to the name given earlier by Cuvier [i.e. *microlepidotus*]. In Schlegel's fig. 1b the frontonasal is in contact with the rostral, as is typical of *P. m. microlepidotus* (see Fitzsimons 1943). Gray (1845), Boulenger (1885), FitzSimons (1943) and Loveridge (1944) all referred *Z. wittii* to the synonymy of *P. m. microlepidotus*.

In 1838 Gray described *Zonurus Davyi* from "Cape of Good Hope" (p. 388). Apart from characters shared with other congeners, he described it (p. 388) as: "Black ? Temporal scales large, smooth, many-sided; three pairs of preanal plates, hinder largest." Gray also attempted to distinguish *Z. davyi* from *Z. microlepidotus* on account of the "keeled" versus "slightly keeled" dorsolateral scales respectively. Both Gray (1845) and Boulenger (1885) later placed *Z. davyi* in the synonymy of *P. microlepidotus*, but the name was preceded by a question mark, suggesting that they did not have a specimen at hand. According to C. McCarthy (pers. comm., 8 June 2004) there is no record at the British Museum of any type material of *Z. davyi*. FitzSimons (1943) also questionably treated *Z. davyi* as a junior synonym of *P. m. microlepidotus*, but Loveridge (1944) did not mention it.

Also in 1838, Smith re-instated the genus *Cordylus*, dividing it into three subgenera as discussed above. He provided brief and rather inadequate descriptions of several cordylids, including *Cordylus (Pseudocordylus) montanus*, *C. (P.) fasciatus*, *C. (P.) melanotus*, *C. (P.) Algoensis* and *C. (P.) sub-viridis* respectively. Later, in *Illustrations of the Zoology of South Africa*, Smith (1843) abandoned the use of the subgenus *Pseudocordylus*, but retained *Hemicordylus* for *Cordylus capensis*. He relegated most of the above-mentioned species to the synonymy of *C. microlepidotus* - although he still described them as (un-named) varieties - but continued to treat *C. fasciatus* as a full species.

The type locality of all *Cordylus* species described by Smith (1838) is "South Africa" (see also FitzSimons 1943; Wermuth 1968). This is derived from Smith's opening statement (p. 30), his only reference to a locality: "Whilst lately engaged in examining the saurian reptiles of South Africa ...". In Smith's time "South Africa" probably applied to all of southern Africa south of 23° latitude. In fact, Smith's journeys were conducted mainly within the boundaries of the former Cape Colony, Orange Free State and Transvaal - i.e.

present-day South Africa - although they did include western Lesotho, a portion of south-eastern Botswana and southern Namibia in the vicinity of the Richtersveld (Kirby 1940, 1965; Lye 1975).

According to a footnote in Smith (1843) the specimens illustrated in fig. 1, pl. 24 (*montanus*), fig. 2, pl. 24 (*algoensis*), fig. A, pl. 25 (male *melanotus*), fig. A, pl. 26 (male *subviridis*) and fig. 1, pl. 27 (*fasciatus*) are the same specimens used for the line drawings on pl. 30. However, as will be discussed below, the paintings do not match the line drawings, although they are very similar in the case of *C. fasciatus*. This discrepancy between plates and figures was also noted by Hewitt (1927) for *C. montanus*, and Broadley (1964) and De Waal (1978) for *C. subviridis*.

Apart from the colour paintings, Smith (1843) also described the colour patterns of *C. fasciatus* and the different “varieties” of *C. microlepidotus* in some detail. For the varieties referable to *melanotus* and *subviridis* he described the colouration of males and females separately. Although his discussion of “form” (including scalation characteristics) did not distinguish between varieties, he did refer to plate 30, a collection of diagrams showing head scalation, and the femoral region of all except *C. montanus*.

FitzSimons (1937: 260) noted that: “It is apparent that in many of his original descriptions, Smith had more than one specimen before him, and although at a later date these species were figured, there is no guarantee that he actually figured one of his original specimens.” FitzSimons also suggested (p. 260) that some of the descriptions and even figures in Smith’s (1849a) *Illustrations* were composite - *i.e.* based on more than one specimen - and in such cases “definite localizing of the type is impossible”. For *Pseudocordylus* Smith (1838) does not clearly indicate how many specimens he examined and his descriptions are so vague that they cannot be associated with any known museum specimens. It is therefore quite possible that several specimens were examined for at least some taxa and that many or even all of the *Pseudocordylus* specimens collected during his expeditions and now housed at the Natural History Museum and National Museums of Scotland (see below) represent syntypes. It should also be noted that Smith’s (1843) descriptions and comments post-date his original descriptions and may not have involved all the specimens examined for his 1838 paper.

Alternatively, he may have examined larger samples for the 1843 treatise, including or excluding specimens used for the 1838 paper.

Smith's (1838) brief descriptions are so vague and lacking in detail that it is in fact often difficult to decide which names correspond to the varieties he later described under *C. microlepidotus* (Smith 1843).

Smith (1838: 32) described *Cordylus* (*Pseudocordylus*) *montanus* as follows:

“Scales forming the transverse rows small, somewhat ovate and faintly carinated; those on the sides largest; scales of tail with moderate sized spines. Colour above, brown or blackish brown, and transversely divided at nearly equal distances by 7 or 8 interrupted yellowish bands; below, yellow or orange, with tints of red; legs variegated by transverse yellow bands; tail irregularly marked, black and yellow. Femoral pores 8 in the last, and 4 or 5 in the first row. Length, from 10 to 13 inches.”

For *C. (P.) montanus* the description of the black and yellow colouration on the back, limbs and tail is decisive as this is quite distinct in Smith's (1843) illustration (fig. 1, pl. 24) and is also mentioned in the text. The “faintly carinated” dorsal scales are also evident in pl. 24. Smith's (1838) reference to the number of femoral pores/scales cannot be used because, for some reason, he failed to provide an illustration of the femoral region of *montanus* in his 1843 paper. It should be noted here that although Smith's (1843) *montanus* is labeled as fig. 1 on pl. 24 (bottom illustration), it is referred to as “fig. A” in his species account. That the variety discussed in the species account does in fact refer to fig. 1 (and not fig. 2) in pl. 24 is confirmed by Smith's (1843) text reference to a “pale reddish orange colour over each eye”. The latter colouration is evident only in fig. 1, pl. 24 and not on any other cordylids illustrated in Smith (1843).

Despite Smith's (1843) comments to the contrary, the illustrations of *C. montanus* on plates 24 and 30 do not appear to be of the same specimen. In plate 24 the rostral and frontonasal are clearly in contact, whereas in plate 30 they are clearly separated by a pair of supranasals.

FitzSimons (1937) was unable to locate type specimens of any of Smith's *Pseudocordylus* at either the British Museum of Natural History (now the Natural History Museum) in

London or the Royal Scottish Museum (now National Museums of Scotland) in Edinburgh. According to catalogue copies provided by C. McCarthy (pers. comm., 1999) the Natural History Museum houses only a few *Pseudocordylus* donated by Andrew Smith. These are *P. m. microlepidotus* (BMNH 65.5.4.16, see below; BMNH 64.2.21.27, skeleton) and the “type” of *C. [P.] algoensis* (BM 1946.8.8.49, see below). In addition, the Earl of Derby donated a specimen of *P. m. fasciatus* (BMNH V.6a, see below) originally given to him by Smith (see also FitzSimons 1937).

In 1859 Smith donated 1010 lizards to the University of Edinburgh (Sprackland & Swinney 1997). This collection later became part of the National Museums of Scotland (NMS) (registered as NMSZ 1859.13) and comprised material (including types) from all over the world. However, the collection is generally poorly labeled and most specimens are without locality data (Sprackland & Swinney 1997). According to the NMS catalogue the specimens were received on 5 May 1859. Included were specimens currently identified (by R. Sprackland) in the catalogue as *Pseudocordylus melanotus transvaalensis* (NMSZ 1859.13.751 [one specimen], NMSZ 1859.13.X65 [two specimens]) and *Pseudocordylus subviridis* (NMSZ 1859.13.756 [four specimens], NMSZ 1859.13.X66 [one specimen]), but none are accompanied by locality data (G. Swinney, pers. comm., 19 April 2005). These specimens were all examined (although X65 is a single specimen) and comprise three taxa, namely *P. microlepidotus fasciatus*, *P. melanotus melanotus* and *P. melanotus subviridis* (see below).

Because Smith (1838) noted that length varied from 10 to 13 inches, and Smith (1843) mentioned “some specimens”, there were probably at least two, possibly three or more, syntypes of *C. montanus*. However, no known type specimens of this species could be found at the Natural History Museum in London (C. McCarthy, pers. comm., 12 July 2004). Nevertheless, BMNH 65.5.4.16 from “S. Africa” presented to the British Museum by Smith is referable to *P. microlepidotus microlepidotus*, although its head scalation differs from Smith’s (1843) *montanus* illustrated as fig. 1, pl. 30 (e.g. the frontonasal is not in contact with the rostral as in Smith’s figure; anterior parietals are not divided diagonally; the median occipital is much enlarged and in contact with an elongate scale also in contact with the interparietal; an extranumery scale – in contact with the supraciliaries - is present between the 2<sup>nd</sup> and 3<sup>rd</sup> supraoculars on both sides of the head).

Smith (1843) restricted the type locality of *C. montanus* to “Table Mountain, and the hills near Cape Town”.

Smith (1838: 32) described *Cordylus (Pseudocordylus) algoensis* as follows:

“Scales forming the transverse rows, sub-ovate, each with an elevated disc, and a faint carina; those towards the dorsal line smallest. Colour above, reddish brown, crossed by some imperfect yellow bands in the male, and by 6 or 7 rows of yellow spots in the female; sides and belly orange yellow, tinted with vermilion red; two large black spots on each side of the neck. From 7 to 9 femoral pores in the last row, and 4 in the first. Length, from 14 to 16 inches.”

Smith’s (1838) description of the dorsal colouration of male *C. (P.) algoensis* is similar to the specimen illustrated in fig. 2 on plate 24 (Smith 1843), although the base colour is brown, not reddish- or orange-brown. In his text description of colour pattern, Smith (1843) does not mention gender. Smith’s (1838) description of femoral pores/scales is similar to fig. 2b on pl. 30, showing nine femoral pores and four differentiated femoral scales on the right thigh.

Head scalation of the *C. algoensis* specimen depicted in Smith’s (1843) fig. 2, pl. 24 is similar to that of fig. 2, pl. 30, but the lateral temporals of the former figure differ from fig. 2a, pl. 30. In fig. 2, pl. 30 the interparietal of *algoensis* is shown as narrowing anteriorly but extending forward to separate the anterior parietals, whereas the interparietal does not fully separate the anterior parietals in *C. fasciatus* (fig. 5, pl. 30). There are also greater numbers of enlarged lateral temporals in the figure of *algoensis*.

In his description of *C. algoensis* Smith (1838) mentions at least two specimens (*i.e.* types), namely a male and female. Smith (1843) later restricted the type locality of *algoensis* to “rocky precipices at and around Algoa Bay”. He added that specimens measure 14-16 inches (= 356–406 mm) in length. According to the British Museum catalogue at least one “type” of *algoensis* is preserved - as a skin (BMNH 1946.8.8.49 [57.6.13.88]). The latter specimen may in fact be one of two syntypes. However, it certainly does not correspond with fig. 2 on pl. 30 (Smith 1843), differing with regard to at least four head shield characters: frontonasal narrowly separated from rostral by a pair of supranasals (BMNH 1946.8.8.49) versus frontonasal in very narrow contact with, or very narrowly separated from, rostral (pl. 30; see also pl. 24); anterior part of frontal like

a shallow “W” versus like an inverted “V”; interparietal does not separate anterior parietals versus completely separates anterior parietals; and median occipital large, separating about half the length of the posterior parietals versus median occipital small, barely separating the posterior parietals. The frontonasal-rostral condition is intermediate between typical *microlepidotus* and the other two subspecies (see FitzSimons 1943). Although the vent area of BMNH 1946.8.8.49 is damaged, it has an estimated SVL of 169 mm and tail length of at least 137 mm (but tip missing), *i.e.* total length of about 306 mm, and therefore does not fit the size range given by Smith (1838).

According to Matschie (1891: 606) the Museum für Naturkunde (Berlin) also possessed a specimen of *P. microlepidotus* from “Algoa-Bay”. On the basis of Smith’s descriptions, Hewitt (1927: 391) stated that “perhaps” *algoensis* should be considered a junior synonym of *P. fasciatus*. FitzSimons (1937) did not find any type specimens of *algoensis*. Subsequently, neither FitzSimons (1943) nor Loveridge (1944) examined type material and both authors referred *algoensis* to the synonymy of *P. m. fasciatus* without providing reasons. However, Loveridge (1944: 81) noted that: “It is possible that *algoensis* (including Matschie, 1891a) may prove to be distinct and have to be removed from the synonymy [of *fasciatus*]”. According to Branch (1981) specimens from the Port Elizabeth–Suurberg mountain area may be referable to *Pseudocordylus algoensis*. However, Branch (1988a, 1998) later treated populations from this area as *P. m. microlepidotus*.

Neither Smith’s (1838) earlier descriptions nor any of the illustrations or text in Smith (1843) provide any reasonable evidence that *C. algoensis* is more similar to *C. fasciatus* than it is to *C. montanus* (= *P. m. microlepidotus*). *Cordylus algoensis* is therefore provisionally referred to the synonymy of *P. m. microlepidotus* on the basis of its geographical affinity to eastern populations currently classified under this name (see Branch 1998).

In the *Catalogue of the Lizards in the British Museum*, Gray (1845) resurrected *Pseudocordylus*, but this time as a full genus. He treated *Z. wittii*, *Z. davyi* and all of Smith’s (1838) species in the subgenus *Pseudocordylus* as junior synonyms of *P. microlepidotus*. In a later edition of this catalogue Boulenger (1885) followed the same

arrangement, but erroneously listed Smith's (1843) *C. fasciatus* as "*C. (P.) fasciatus*" in his synonymy.

For over 80 years, subsequent to Smith (1843) and prior to Hewitt (1927), all specimens in the *P. microlepidotus* and *P. melanotus* species complexes were referred to as *P. microlepidotus*. This includes Boulenger's (1903) material from Deelfontein in the Richmond district of the Northern Cape Province (transferred to *P. microlepidotus fasciatus* by FitzSimons 1943, and to *P. m. namaquensis* by Loveridge 1944), Boulenger's (1905) material from Wakkerstroom in Mpumalanga Province (transferred to *P. subviridis subviridis* by FitzSimons 1943, and *P. microlepidotus melanotus* by Loveridge 1944; material from same locality referred to as *P. m. melanotus* by Jacobsen 1989), Boulenger's (1908) material from Balgowan in KwaZulu-Natal (transferred to *P. s. subviridis* by FitzSimons 1943, and *P. microlepidotus melanotus* by Loveridge 1944), Hewitt's (1918) material from Albany District in Eastern Cape Province (referable to *P. microlepidotus fasciatus* according to his description of colour pattern), and Essex's (1927) specimens from Amatola Mountains (including Hogsback) in the Eastern Cape Province and Drakensberg Range (including Mont-aux-Sources [listed under *P. langi* by Loveridge 1944]) in KwaZulu-Natal (all referable to *P. melanotus subviridis*, see Branch 1998), and Grahamstown and Tembuland in the Eastern Cape Province (referable to *P. microlepidotus fasciatus*, see Branch 1998).

Hewitt (1909: 37) included, under the name *P. microlepidotus*, specimens from "coastal districts of south and east Cape Colony" (*i.e.* *P. m. microlepidotus* and *P. m. fasciatus*), Richmond District in the Northern Cape (*P. m. fasciatus* or *P. m. namaquensis*), KwaZulu-Natal (*P. melanotus melanotus* and/or *P. m. subviridis*), Free State (*P. m. melanotus* and/or *P. m. subviridis*) and the former Transvaal (Wakkerstroom and Pretoria District [*P. m. melanotus*] and Zoutpansberg District [*P. transvaalensis*, see discussion below]).

Hewitt (1927: 390) later re-considered the status of species described by Smith (1838) in the subgenus *Pseudocordylus* and was satisfied that "several are indeed worthy of subspecific rank at least". However, he recognized *P. microlepidotus*, *P. fasciatus* and *P. subviridis*, all as full species, and described a new subspecies, namely *P. microlepidotus namaquensis*. Hewitt (1927: 391) discussed the name *P. montanus*, based on Smith's

(1843) fig. 1 on pl. 24, but noted that it is “probably the true *microlepidotus* of Cuvier”. He also used the name *P. microlepidotus* in the caption to his fig. 3, pl. 23 (wrongly listed as pl. 22, *i.e.* plate numbers 22 and 23 are transposed, in the Explanation of Plates) illustrating a “Typical form from Capetown” (p. 415).

Hewitt (1927) placed particular emphasis on certain characters mentioned or illustrated in Smith (1843), namely barring on the flanks, size and shape of lateral temporals, appearance and relative size of dorsal scales, markings on the throat, shape of frontonasal, and whether or not the frontonasal and rostral were in contact. For example, he noted that in his two new specimens from Cape Town the frontonasal was about as long as wide and in contact with the rostral. Hewitt used one or more of the above-mentioned characters to distinguish between the various forms of *Pseudocordylus* (see discussion below). Later workers also used some or all of these characters (Appendix 2.2).

FitzSimons (1943) used various characters to separate the three subspecies of *P. microlepidotus* (see Appendix 2.2). According to him *P. m. microlepidotus* usually had the frontonasal and rostral in contact, whereas in both *P. m. fasciatus* and *P. m. namaquensis* these scales were usually separated by a pair of supranasals. Also, *fasciatus* differed from *namaquensis* on account of its mostly smooth dorsals versus dorsals with raised centres, ribbed towards the edges, respectively. Whereas Hewitt (1927) and Loveridge (1944) knew *P. m. microlepidotus* as occurring only in the vicinity of Cape Town, FitzSimons (1943) documented it from several localities in the present-day Western Cape Province. Both Duméril & Bibron (1839) and De Rochebrune (1884) erroneously stated that *P. microlepidotus* also occurs in Sierra Leone in West Africa.

Loveridge (1944) distinguished between the three subspecies of *P. microlepidotus* mainly on the basis of the appearance and number of lateral temporals, and the relative shape of median versus lateral gular (throat) scales. According to him, temporals of the upper row were enlarged and vertically elongate in *namaquensis* versus relatively small and polygonal - with 0-2 vertically elongate - in *microlepidotus* and *fasciatus*. There were 8-11 enlarged temporals in *microlepidotus* versus 16-17 in *fasciatus*. The median gulars were slightly elongated like the laterals in *microlepidotus* versus more-or-less squarish, not even slightly elongate like the laterals, in *fasciatus* and *namaquensis*. In a table on

page 69, Loveridge (1944) also listed 34-38 transverse ventral rows in *microlepidotus* versus 41 rows in *fasciatus*.

Broadley (1964) erroneously stated that Loveridge (1944) transposed the captions for his fig. 2, pl. 10 (*C. montanus*) and fig. 1, pl. 11 (*C. algoensis*). Broadley (1964) may have been confused by the fact that Smith (1843) labeled the top illustration in pl. 24 as fig. 2 (*C. algoensis*) and the bottom illustration as fig. 1 (*C. montanus*), whereas Loveridge (1944) numbered the reproductions of Smith's two illustrations in the opposite way in his plates. It should also be noted that in his text description Smith (1843) referred to *C. montanus* as fig. A and *C. algoensis* as fig. B.

### 2.3.2 *Pseudocordylus microlepidotus fasciatus* (A. Smith, 1838)

*Cordylus (Pseudocordylus) fasciatus* A. Smith, 1838, *Mag. Nat. Hist.* 2(2), p. 32 (Type locality: "South Africa").

*Cordylus fasciatus* A. Smith, 1843, *Ill. Zool. S. Afr. Rept.*, pl. 24, fig. 2; pl. 27, fig. 1 & pl. 30, figs 2 & 3 (Type locality restricted to: "rocky hills in the neighbourhood of Graham's Town [= Grahamstown]").

*Pseudocordylus fasciatus* Hewitt, 1927, *Rec. Alb. Mus.*, 3, p. 391.

*Pseudocordylus microlepidotus fasciatus* FitzSimons, 1937, *Ann. Transvaal Mus.*, 27, p. 266.

Smith (1838: 32) described *Cordylus (Pseudocordylus) fasciatus* as follows:

"Scales forming the transverse rows rather closely set, somewhat circular, and with elevated discs. Anterior margin of ear concealed by three projecting horny scales, the lowest being largest. Colour above, brown-black, variegated by 7 or 8 transverse rows of dirty white spots, 2 of which rows cross the back of the neck; beneath, light livid brown. Seven femoral pores in the last row, and 4 or 5 in the first. Length, from 8 to 10 inches."

Smith's (1838) description of *C. (P.) fasciatus* corresponds with Smith's (1843) fig. 1 on pl. 27 and the text description with regard to colour pattern, and is similar to fig. 5b on pl. 30 with regard to femoral pores/scales. Smith's (1838: 32) statement concerning "seven femoral pores in the last row" and "4 or 5 in the first" is apparently in reference to the numbers of femoral pores and differentiated femoral scales respectively. In Smith's (1843) fig. 5b on pl. 30 there are eight femoral pores and five additional differentiated femoral scales (right thigh depicted). Smith (1838: 32) also noted that in *fasciatus* the

anterior margin of the ear opening is “concealed by three projecting horny scales, the lowest being largest”. However, this apparently also applies equally to both *C. montanus* (fig. 1, pl. 24) and *C. algoensis* (fig. 2, pl. 24), although such scales appear to be absent in the illustrations of both *melanotus* (pl. 25) and *subviridis* (pl. 26) (Smith 1843).

In his description of *C. fasciatus*, Smith (1838: 32) noted that length varied from “8 to 10 inches”, thus suggesting that more than one type specimen existed. Smith’s (1843) description concludes with “The largest specimen which I have seen – the one described – measured nine inches and a half in length.” This is apparently in reference to his detailed written description, fig. 1 on pl. 27, and figs 5, 5a and 5b on pl. 30. However, the specimen illustrated in pl. 27 does not match the specimen in pl. 30 with regard to the position of the frontonasal (separated from rostral by supranasals in pl. 27; in narrow contact with rostral in pl. 30) and the arrangement of lateral temporal scales (in three rows of mostly slightly elongated scales in pl. 27, but only the scales of the middle of three rows distinctly elongated in pl. 30). However, in most regards, fig. 1 on pl. 27 and fig. 5 on pl. 30 are remarkably similar.

In a footnote Smith (1843) restricted the type locality of *C. (P.) fasciatus* by stating: “Two of the three specimens I have examined were obtained on the rocky hills in the neighbourhood of Graham’s Town [= Grahamstown], and the third, which is in the Museum at Fort Pitt, was, I believe, obtained from the same locality.” FitzSimons (1937: 260) noted that a large portion of Smith’s collections “went first to the Army Medical College Museum at Fort Pitt, Chatham, and from there to Nettley, where it was broken up, the British Museum taking over what it desired and the remainder, which was badly preserved, perishing.”

From the above it is therefore not clear whether the illustrations in Smith (1843) are based on a single type, or a combination of two or more types. However, it seems that Smith’s (1838) vague description was based on the three Grahamstown (650 m a.s.l.) specimens mentioned above, while Smith’s (1843) description was based on only one specimen.

One of the specimens listed by Gray (1845) under *P. microlepidotus* is a spirit-preserved adult from South Africa presented to the British Museum by the Earl of Derby. It is accompanied by the following: “*C. fasciatus*, A. Smith, Ill. Zool. S. Afr. t. 27, f. 1, t. 30, f.

5” (Gray 1845: 51). The latter may suggest that this is the specimen used by Smith (1843) to illustrate (and describe) *fasciatus*. Specimen BMNH V.6a (examined: female with ovaries) is a spirit-preserved adult from South Africa presented by the Earl of Derby, but it is not accompanied by a type label (C. McCarthy, pers. comm., 30 May 2003). Nevertheless, it is probably the same specimen referred to by Gray (1845). However, it differs from Smith’s (1843) pl. 27 in having a large portion of regenerated tail, rather than a complete, original tail, and the right side lateral temporal areas do not correspond. Also, a close up of the head (digital image) shows that the frontonasal is narrowly separated from the rostral by a pair of supranasals (*i.e.* *fasciatus*-like, see Appendix 2), whereas fig. 5 in pl. 30 shows the frontonasal to be in very narrow contact with the rostral; the shape of the anterior part of the frontal scale also differs from pl. 30, while the posterior part of the frontal is fragmented in pl. 30; and in pl. 30 there is an extranumery scale on the left side of the head between the second and third supraoculars. According to Smith (1838) *fasciatus* measures 8-10 inches (203–254 mm) in length. BMNH V.6a measures 135.5 mm SVL with a tail length of 115.3 mm (regenerated part = 57.5 mm), *i.e.* 249.8 mm (9.8 inches) total length, thus falling within Smith’s (1838) size range and approximating the nine-and-a-half-inch-long (241 mm) specimen of Smith (1843).

One of the seven *Pseudocordylus* specimens in the National Museums of Scotland (NMSZ 1859.13.751; male, left testis examined), as mentioned above, bears a series of generation glands paravertebrally on the back and is referable to *P. microlepidotus*, while the others are *P. melanotus* (see below). The head shield arrangement of NMSZ 1859.13.751 is very similar to Smith’s (1843) fig. 5 on pl. 20, depicting *fasciatus*. As indicated in fig. 5, the frontonasal is divided anteriorly and posteriorly but appears to be fused medially; the frontal is unusual in being fragmented; while the posterior parietals are unusual in being divided diagonally. Lateral temporals are somewhat dissimilar to fig. 5, being in three distinct rows on the left (scales of the middle and lower rows of similar size), but in three somewhat less distinct (almost assymetrical) rows on the right. Also, there is an extranumery scale between the second and third supraoculars on the right, while this arrangement is shown on the left side of the head in fig. 5. Although Smith (1843) describes a dorsolateral scale as having “a small horny tubercle near its center”, most dorsolaterals examined were largely smooth (and almost in contact on the sides). Ventrals are in 14 longitudinal rows as stated by Smith (1843), but the arrangement of femoral scales differs slightly. In Smith’s fig. 5b, pl. 30, depicting the

right thigh, there is an anterior row of five, and posterior row of eight, pore-like scales (femoral pores and differentiated femoral scales not differentiated). In NMSZ 1859.13.751 there are five pore-bearing scales on the left thigh, six on the right; and seven differentiated femoral scales (generation glands) on the left thigh and eight on the right. Using Smith's terminology, these were arranged in two rows on the left thigh, consisting of an anterior row of five scales and a posterior row of seven scales, and in three rows on the right thigh (1: 4: 9). Smith (1843) also noted that there were six supralabials and six infralabials, but the specimen examined differs in having seven infralabials on the right side of the head. There is also a pair of dark, parallel stripes medially on the throat, a feature not mentioned by Smith (1838, 1843). The specimen is an adult with a total length of about 235 mm (111.8 mm SVL; 123 mm tail length – could not be straightened, measurement thus not accurate), similar to Smith's (1843) 241 mm specimen. In conclusion, it can be stated that NMSZ 1859.13.751 bears a strong resemblance to the specimen described by Smith (1843), which was almost certainly one of two or three specimens before him when he described *fasciatus* in 1838.

If it is accepted that the specimens described in detail by Smith (1843) formed part of the series available to him in 1838 for his original descriptions, then, in consideration of the above-mentioned factors, both BMNH V.6a and NMSZ 1859.13.751 are syntypes of *Cordylus (Pseudocordylus) fasciatus*. As NMSZ 1859.13.751 closely approximates Smith's (1843) illustrations, I hereby designate this specimen as lectotype of *Cordylus (Pseudocordylus) fasciatus*, whereas BMNH V.6a is designated as alloparalectotype. These two specimens were probably collected during Smith's stay in Grahamstown, from 3 September 1821 to early 1825 (Branch & Bauer 2005).

In accordance with Recommendation 74C of the 1999 Code I hereby list the following data pertaining to the lectotype (NMSZ 1859.13.751, male) housed in the collection of the National Museums of Scotland, Edinburgh (variation in the alloparalectotype BMNH V.6a [female] is indicated in parentheses): Type locality as above. SVL 111.8 mm (135.5 mm); tail length 123 mm, original (115.3 mm, regenerated); head width 24.6 mm, *i.e.* 22.0% SVL (27.7 mm, 20.5%). Lateral temporals in 3 rows horizontally, in lectotype less distinct on the left side, right side consisting of 6 elongate scales in the upper row, 5 mostly hexagonal scales in middle row and 4 similar scales in the lower row; supraoculars 4; supraciliaries 5 (6); suboculars 4, two posterior to median; 4 (5) supralabials anterior to

median subocular; infralabials 6 left, 8 right (6 on both sides in V.6a); sublabials 5; dorsals in 49 (50) transverse and 42 (48) longitudinal rows; ventrals in 14 longitudinal rows; 17 (16) lamellae under 4<sup>th</sup> finger and 22 (17) under 4<sup>th</sup> toe; femoral pores 5 left, 6 right (5 on both sides in V.6a); differentiated glandular femoral scales 7 left, 8 right (3 on both sides in V.6a); frontonasal distinctly divided anteriorly and posteriorly, but more-or-less fused medially (undivided in V.6a), as long as it is wide, in contact with loreals and very narrowly separating supranasals (not separating supranasals in V.6a); no additional scales between frontal and frontonasal; anterior parietals undivided, but posterior parietals divided diagonally (posterior parietals undivided in V.6a); no small scales posterior to interparietal; dorsals scales in contact on the sides or very slightly separated (spaces between longitudinal rows of dorsolaterals <0.25 width of adjacent dorsolaterals); dorsolaterals the largest, followed by the laterals, the medians being the smallest; dorsolaterals smooth or with a slight caruncle with weakly ribbed edges; throat pale with a pair of dark, parallel stripes medially (apparently unmarked in V.6a); gular scales for the most part distinctly elongated (variable in V.6a); posterior infralabial keeled; lowermost enlarged temporal spine distinctly flattened and triangular, but feebly projecting (moderately projecting in V.6a); colour pattern faded (in V.6a: back brown with about six pale cream crossbands starting from the occipital region; flanks pale with a few dark vertical bands that do not reach as far as the ventral plates).

Smith (1843) recognized *C. fasciatus* as a full species. While noting the similarity of *C. fasciatus* to *C. microlepidotus*, he was of the opinion that “when the scales of the neck and centre of the back are examined, and contrasted with those on the same parts of the species just named [*C. microlepidotus*], sufficient differences are observable to justify my regarding them at present as probably distinct”. Exactly what Smith meant by this is unclear as no obvious differences in these characters are apparent in the paintings of the various forms.

Hewitt (1927: 391) noted that *P. fasciatus* was “a well marked subspecies, or even a good species, although near to the typical form”. His material included near-topotypes from Grahamstown. With reference to these specimens he noted that the frontonasal scale is usually well separated from the rostral by the supranasals (as in Smith’s 1843 fig. 1, pl. 27), but in narrow contact in one specimen (as in Smith’s fig. 5, pl. 30). Hewitt (1937) later recorded *P. fasciatus* from several localities in the Eastern Cape Province. I have

examined three *fasciatus* from Grahamstown (TM 175-7), all of which have the frontonasal and rostral well separated by a pair of supranasals.

FitzSimons (1937) considered *P. fasciatus* a subspecies of *P. microlepidotus*, but did not comment or offer reasons for this opinion. Subsequently, FitzSimons (1943), Loveridge (1944) and other authors (e.g. Branch 1988a, 1998) also used this combination.

Hewitt (1927), FitzSimons (1943) and Loveridge (1944) all recorded *P. m. fasciatus* from various localities in the present-day Eastern Cape Province, including the Transkei (Butterworth and Tsomo areas). However, different characters were used to distinguish *fasciatus* from other *P. microlepidotus* taxa (Appendix 2.2). Neither FitzSimons (1943) nor Loveridge (1944) examined Boulenger's (1903) "Deelfontein" material (also listed as *P. microlepidotus* by Hewitt [1909]), but this record was referred to *P. m. fasciatus* by FitzSimons and to *P. m. namaquensis* by Loveridge.

Boulenger's (1903) isolated "Deelfontein" record (3023DD) for *P. microlepidotus* is questionable. All taxa in the *P. microlepidotus* species complex are known to be strictly rupicolous (Branch 1998), yet Boulenger (1903: 215) noted that Deelfontein "is situated in the middle of a barren region extending for miles in every direction, with nothing but brushwood and thorns". The specimens may have been collected elsewhere, or perhaps there was in fact some suitable, isolated rocky habitat at Deelfontein. Two specimens from this locality - almost certainly the same ones examined by Boulenger - in the collection of the Natural History Museum in London (BMNH 1903.4.27.32-3) have been examined. In both specimens the frontonasal is separated from the rostral by a pair of supranasals; the lowermost temporal spine is feebly to moderately projecting (*i.e.* *fasciatus*-like); and the throat is not darkly coloured. Geographically the Deelfontein specimens are best referred to *P. m. fasciatus* (see Fig. 2.1).

Hewitt (1927) was the first author to suggest that the Transkei population of crag lizards differed from other *P. microlepidotus*. He noted (p. 391) that specimens from near Butterworth had "quite smooth dorsal scales" (similar to *fasciatus*), but the dorsal pattern differed. It consisted of pale crossbands that were "less distinctly composed of isolated spots" and the banding extended slightly onto the lateral surfaces. Branch (1988a,b; 1998) later treated the Transkei population as an undescribed subspecies of *P.*

*microlepidotus*. A colour photograph of a specimen from this population is illustrated as fig. 5, pl. 72 in Branch (1988a, 1998). The back is dark brown with several narrow, often incomplete, cream coloured crossbands. Figure 2 on the same plate shows that the (grey) belly of Transkei specimens differs from that of the three known subspecies of *P. microlepidotus*.

### 2.3.3 *Pseudocordylus microlepidotus namaquensis* Hewitt, 1927

*Pseudocordylus microlepidotus namaquensis* Hewitt, 1927, *Rec. Alb. Mus.*, 3, p. 392, pl. 23, fig. 1 (Type locality: “Namaqualand”).

*Cordylus microlepidotus namaquensis* Frost *et al.*, 2001, *Am. Mus. Nov.* 3310, App. 1, p. 10.

Hewitt (1927: 392) described *Pseudocordylus microlepidotus namaquensis* as follows:

“Frontonasal and rostral well separated, the former broader than long: scales immediately behind occiput small but not sub-granular: dorsal scales not simply keeled, but with slightly raised centres and finely ribbed, stellate fashion, towards the periphery: temporal scales rather few – about 8 – referable to two rows, those of upper row enlarged and somewhat elongated vertically: two or three prominent enlarged scales on the anterior boundary of the ear, which scales may project strongly outwards: small scales along mesial region of throat not elongated but more or less rounded or squarish – in true *microlepidotus* they are mostly elongated like the scales lateral to them. Colour pattern not easily made out in the specimens, but the throat is without infuscation. Length from snout to vent 127 mm.”

In his description Hewitt (1927: 392) stated that “Three specimens in the collection of the South African Museum, labeled ‘Namaqualand’, seem to represent a fourth form now described as *Pseudocordylus microlepidotus namaquensis*.” This statement was followed by a brief description (see above) and the comment: “Colour pattern not easily made out in the specimens”. The use of the plural “specimens” thus implies that all three specimens were used in formulating the description (at least with regard to colour pattern) and all are therefore part of the type series. Hewitt (1927: 393) then named “No. 872” in the collection of the South African Museum (SAM) as the “Type”. The latter specimen (SAM 872) is therefore the holotype whereas the other two are paratypes. Hewitt (*op. cit.*) also noted that: “An old specimen from Beaufort West in the same collection can also be referred to *namaquensis*.” This specimen, however, has no nomenclatural

standing because it is mentioned separately which therefore expressly excludes it from the type series (Article 72.4.6 of the 1999 Code).

Unfortunately the holotype (SAM 872) cannot be located at the SAM and is presumed lost (D. Drinkrow, pers. comm., 25 April 2003). According to the SAM catalogue the holotype and three additional specimens (SAM 859, 130.1 mm SVL; SAM 864, 122.4 mm; SAM 873, 121.5 mm: all examined) in the SAM collection from “Namaqualand” were all accessioned “7/9/1896”, although none of the latter are marked as types. However, the SAM catalogue also lists six “*Pseudocordylus microlepidotus*” (SAM 1135, 1147-50, 18357; no longer in SAM collection) from Beaufort West. It may be that either SAM 859, 864 or 873 was erroneously assigned the locality “Namaqualand” subsequent to Hewitt’s description, but it is also possible (but less likely) that although there were four specimens from “Namaqualand”, Hewitt (1927) examined only three when preparing his description. Therefore, while it seems likely that at least two of the three “Namaqualand” specimens are in fact paratypes of *P. m. namaquensis*, it cannot be stated with any certainty which is and which is not. The non-type specimen is likely to be the one from Beaufort West.

What is certain is that none of the three “Namaqualand” specimens examined matches Hewitt’s (1927) photograph (fig. 1, pl. 22) illustrating the dorsal aspect of the head, neck, anterior part of the back and part of the forelimbs. In all three specimens examined the enlarged spinose scales at the anterior borders of the ears, as well as the parietal region, differ from the illustrated specimen (fig. 1). In addition, the frontonasal in SAM 859 is much wider than that of Hewitt’s (1927) illustrated specimen, while the frontonasal and rostral of SAM 873 are separated by a small granule rather than by the supranasals. Finally, only SAM 859 (130.1 mm SVL) is similar in size to the 127 mm SVL (presumably for the holotype) mentioned in Hewitt’s (1927) description. Three character states mentioned in the text of Hewitt’s (1927) description can be checked against - and match - the illustration, namely: frontonasal and rostral well separated by supranasals, frontonasal broader than long, and two or three strongly outward-projecting scales on the anterior border of each ear opening. The caption for fig. 1, pl. 22 (erroneously printed under pl. 23; the caption of “fig. 1, pl. 22” refers to “*Pachydactylus capensis oculatus*”) states: “Head of type specimen from Namaqualand”. This, together with the facts

mentioned above, indicate that the specimen illustrated as fig. 1, pl. 22 is indeed the holotype (SAM 872) of *Pseudocordylus microlepidotus namaquensis*.

From Hewitt's (1927) description of scale characters it is not clear whether or not he presented data for the holotype only, all three type specimens, or possibly the three types and the Beaufort West specimen. Both SAM 859 and 864 do in fact closely match Hewitt's written description, but SAM 873 differs in at least four ways. It has a granular scale between the frontonasal and rostral; the frontonasal is slightly (1.04 times) longer than it is wide; the lateral temporal scales are somewhat asymmetrically arranged, certainly not referable to a vertically elongated upper row and smaller lower row; and the median scales on the throat are definitely not "rounded or squarish", but rather rectangular and slightly elongated. This suggests that SAM 873 may in fact be the old Beaufort West specimen, while the other two are paratypes. However, it can also be noted, with regard to Hewitt's reference to the "old" Beaufort West specimen, that in both SAM 859 and 864, most of the scales on the back are missing. Although SAM 864 also has virtually all of its head shields missing, it is not obvious which is the longest-preserved lizard.

Despite Hewitt's (1927: 392) comment that "the throat is without infuscation [= darkness]", SAM 864 clearly has a longitudinal pair of dark stripes in the middle of the throat, whereas indications of such markings are also present in both SAM 859 and 873.

Hewitt's (1927) vague type locality "Namaqualand" deserves further comment. The specimens mentioned by Hewitt were collected by L. Peringuey, who was Assistant Director of the South African Museum at that time. They were accessioned on 7 September 1896 after the arrival of W. Sclater, the new Director. According to Branch & Bauer (1994), Peringuey did not keep a written record of specimens collected, many of which were accessioned several years later. This may therefore have resulted in erroneous or vague localities being assigned to particular specimens. As noted by Branch & Bauer (1994) the term "Namaqualand" had a broader connotation in the latter part of the 19<sup>th</sup> century than it does now and probably referred to the area from Walvis Bay in Namibia, south to Clanwilliam in the Western Cape, extending several hundred kilometres inland, possibly including Beaufort West. In his list of localities for *P. m. namaquensis*, FitzSimons (1943) considered "Namaqualand" to mean "Little

Namaqualand”. Loveridge (1944: 78) listed “Namaqualand” but added “whether Little or Great not known”. As currently understood Namaqualand extends from Namibia in the north to the Northern Cape in the south and from the Namib Desert in the west to the Kalahari Desert in the east; and is divided by the Orange River into Great Namaqualand (Namibia) and Little Namaqualand (Northern Cape, possibly extending peripherally into the Western Cape) (Anon 2003).

Subsequent to Hewitt (1927), FitzSimons (1943: 466) recorded only one additional locality, namely “Btwn. Beaufort West and Rhenosterkop”. Loveridge (1944) did not list the latter locality, but included, on geographical grounds, Boulenger’s (1903) “Deelfontein, Richmond District” record for *P. microlepidotus* (see discussion under *P. m. fasciatus*). Loveridge (1944: 78) also added: “Whether *namaquensis* deserves recognition is uncertain though geographically probable”. According to Branch (1988a, 1998) this subspecies is restricted to the Nuweveldberg mountains from Sutherland to Beaufort West. Therefore, in light of our current knowledge on the distribution of crag lizards (Branch 1998; Fig. 2.1), “Little Namaqualand” as defined above would certainly be a reasonable restriction of the type locality of *namaquensis*. According to the Transvaal Museum catalogue *P. m. microlepidotus* was collected in the Cederberg mountains (TM 79652; 3219AA) near Clanwilliam, an area that falls within the broad definition of Little Namaqualand. Nevertheless, considering the discussion above, I hereby restrict the type locality of *Pseudocordylus microlepidotus namaquensis* to the Great Escarpment (Roggeveldberg, Komsberg and Nuweveldberg mountains) and vicinity in the Northern and Western Cape Provinces in the area bounded by latitudes 31°30’S and 32°45’S, and longitudes 19°30’E and 23°E.

As discussed above and indicated in Appendix 2.2, *namaquensis* is poorly differentiated from *fasciatus*. FitzSimons (1943) and Loveridge (1944) used different characters to separate the two taxa. Anon (2002) considered *P. m. namaquensis* a junior synonym of *C. fasciatus*. If the former is found to be a valid species in the genus *Cordylus* (see Frost *et al.* 2001) it will require a new name, being pre-occupied by *Cordylus namaquensis* (Methuen & Hewitt, 1914).

#### 2.4 Morphological differentiation in the *Pseudocordylus microlepidotus* species complex

At least 10 characters have been used in the past to distinguish between two or all three of the currently recognized subspecies of *P. microlepidotus* (Appendix 2.2). Smith's (1838) original descriptions are extremely vague and do not provide any decisive differentiating characters between *montanus* (= *microlepidotus*) and *fasciatus*, although the lithographs and head diagrams in Smith (1843) provide more details. Subsequently, Hewitt (1927), FitzSimons (1943) and Loveridge (1944) all attempted to distinguish between the three taxa but not always using the same characters. Loveridge (1944) also used the number of enlarged lateral temporals to distinguish between *P. m. microlepidotus* (8-11) and *P. m. fasciatus* (16-17), but as no indication was given as to what comprised an "enlarged temporal", this character is of dubious value. Clearly the status of the three currently recognized subspecies of *P. microlepidotus* requires detailed investigation, using both morphology and genetics. M. Cunningham (pers. comm.; 2004) is currently undertaking a phylogeographical analysis of the *P. microlepidotus* species complex.

From the discussion above it is clear that there is some confusion as to which characters are appropriate for separating the three taxa. According to the literature only a few characters seem to be useful (Appendix 2.2). Perhaps the most consistently reported is the position of the frontonasal in relation to the rostral shield. This character was examined in a sample of 140 specimens referable to the *P. microlepidotus* species complex (see catalogue numbers indicated by asterisks in Appendix 2.1, locality numbers F3, 14, 16-17, 24-25, 27, 30, 33-34, 36-37, 40-45, 57, 59, 61, 64, 67, 80, 89, 90, 116, 117; G1, 2, 7, 12, 15-16, 18-25, 29-32, 34-36, 40, 48, 52-56; H1-2, 6, 8, 13; I1-6). In *P. m. microlepidotus* ( $N = 41$ ) the frontonasal was in contact with the rostral in 71% of specimens, it was separated by the supranasals in 20%, separated by one or more granules in 7%, and fragmented in 2%; in *P. m. fasciatus* ( $N = 62$ ) the scores were: 16%, 77%, 6%, none fragmented; *P. microlepidotus* ssp. (Transkei) population ( $N = 22$ ): 27%, 73%, none separated by granules or fragmented; *P. m. namaquensis* ( $N = 15$ ): 20%, 53%, 27%, none fragmented. The above data support the finding that *microlepidotus* usually has the frontonasal and rostral in contact, whereas in *fasciatus*, *namaquensis* and "Transkei" these scales are usually separated, in most cases by the suture of a pair of supranasals.

Two additional distinguishing characters - presence (one or more, usually several) or absence of differentiated femoral scales (generation glands), and the type of femoral pores present (distinct with secretions or pit-like without secretions) - were examined in a sample of 95 adults (see catalogue numbers indicated by asterisks in Appendix 2.1 [but excluding unsexed adults SAM ZR859, 864, 873, 2020-1, 18306a & d, 18621a & b], locality numbers F3, 14, 16, 17, 24, 25, 27, 30, 33, 34, 36, 37, 40-45, 57, 59, 61, 64, 80, 89, 116, 117; G2, 7 [femoral pores in TM 175 and 176 damaged, not scored], 12, 15, 18-23, 25, 32, 34-36, 40, 48, 54, 56; H1, 6, 8; I1-6) and were found to differentiate between the various taxa. Adults were identified by the presence of one or both testes or ovaries, or enlarged post-oviductal follicles; and measured at least 110 mm SVL. Whereas all males had differentiated femoral scales and distinct femoral pores with secretions (*microlepidotus*  $N = 22$ , *fasciatus*  $N = 16$ , "Transkei"  $N = 7$ , *namaquensis*  $N = 3$ ), this differed amongst females. In *microlepidotus* ( $N = 15$ ) only 7% of females (only PEM R3533, Matroosberg) had differentiated femoral scales, while 13% had distinct femoral pores with secretions (PEM R3533; JV 1501, Rooiberg); and in the Transkei population ( $N = 7$ ) 29% had differentiated scales and 29% had distinct pores. However, the situation was reversed in both *fasciatus* (67%,  $N = 18$ ; and 88% respectively;  $N = 16$ ) and *namaquensis* (100% for both characters;  $N = 7$ ). However, the taxonomic value of differentiated femoral scales in *P. microlepidotus* requires further investigation as Mouton, Gagiano & Sachse (2005) found that in female *P. m. microlepidotus* these glands were either present or absent, and Du Toit, Mouton, Flemming & Van Niekerk (2004) found that in *Cordylus* their presence or absence was influenced by climatic variables.

In *P. m. microlepidotus* the dark dorsal bands extend onto the flanks and almost reach the belly, whereas in the other two subspecies these bands extend only partly onto the flanks (Appendix 2.2). The throat of both *microlepidotus* and *fasciatus* is reportedly uniformly dark (bluish or black), but in *namaquensis* it is immaculate or bears an elongate 8-shaped dark bluish marking (Appendix 2). However, although *microlepidotus* specimens examined sometimes had black throats, the throats of *fasciatus* and *namaquensis* almost always had a medial pair of dark longitudinal stripes and were never uniformly dark (unpublished data). According to Branch (1998), gular pattern and colouration vary amongst the different populations of *P. microlepidotus*.

The limited morphological character differentiation discussed above indicates that although *P. m. microlepidotus* and *P. m. fasciatus* could be considered good subspecies pending a more detailed evaluation, the status of *P. m. namaquensis* is questionable. In fact, according to the literature, it appears that *namaquensis* differs from *fasciatus* only by virtue of its strongly (versus feebly) projecting lowermost temporal spine (a rather subjective character) and by its gular markings (but see comments above) (Appendix 2.2), but both characters are likely to be variable.

## 2.5 Status of taxa in the *Pseudocordylus melanotus* species complex

### 2.5.1 *Pseudocordylus melanotus melanotus* (Smith, 1838)

*Cordylus (Pseudocordylus) melanotus* A. Smith, 1838, *Mag. Nat. Hist.* 2, p. 32 (Type locality: “South Africa”; restricted to “hills between the principal branches of the Orange River, to the eastward of Phillopolis” [= Philippolis] by Smith, 1843, *Ill. Zool. S. Afr. Rept.*; and “Ficksburg [administrative] district” by De Waal, 1978, *Mem. nas. Mus., Bloemfontein* 11, p. 59 & 61).

*Pseudocordylus microlepidotus melanotus* Loveridge, 1944, *Bull. Mus. comp. Zool.* 95(1), p. 75.

*Pseudocordylus melanotus melanotus* De Waal, 1978, *Mem. nas. Mus., Bloemfontein* 11, p. 59.

*Cordylus melanotus* Anon, 2002, Report of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, 12<sup>th</sup> meeting, p. 6.

*Cordylus melanotus melanotus* Bourquin, 2004, *Durban Mus. Novit.* 29: 57-103.

Smith (1838) described *Cordylus (Pseudocordylus) melanotus* as follows:

“Scales circular and small along the middle of the back, on the sides larger and somewhat ovate, each with a faint carina, ending in a rudimentary spine. Colour above, black, sides and belly orange yellow, tinted with vermilion red. On each side of the neck two large black spots. Ten femoral pores in the last row, and 8 in the first. Length, from 12 to 14 inches. The female has the back freely variegated with short yellowish stripes.”

The second sentence of Smith’s (1838) description above is largely in agreement with the male depicted in fig. A, pl. 25 (Smith 1843), although the flanks are depicted as dull orange-brown with scattered groups of black scales. Smith’s (1838) description of the female (last sentence above), although vague, can be said to match fig. B, pl. 25, although fig. B on pl. 26 - depicting the female of *C. (P.) subviridis* - is similar (Smith 1843).

Also, Smith's (1838) description of femoral pores/scales matches fig. 3b on pl. 30 (left thigh) in Smith (1843), *i.e.* 10 femoral pores and eight differentiated femoral scales.

Smith (1843) noted that the male depicted as fig. A, pl. 25 (*i.e. melanotus*) is the same specimen used for the line drawings on pl. 30 (fig. 3 & 3a). However, the occipitals in pl. 25 are much larger than in pl. 30 and the arrangement of lateral temporals differs. The specimen depicted in pl. 30 also does not match the female depicted as fig. B, pl. 25. In the latter illustration the occipitals are of moderate size, but in fig. 3, pl. 30 they are much smaller.

There appears to be some confusion in the literature with regard to the type locality of *melanotus*. As mentioned earlier, the type locality for all *Cordylus* species described by Smith (1838) is South Africa. However, both Loveridge (1944: 75) and Broadley (1964: 103) incorrectly reported that Smith (1838) used "Cape of Good Hope" as type locality for both *melanotus* and *subviridis*. They probably derived this from Smith's (1838: 32) comment about a specimen of *Cordylus capensis* that was "sent" from the "Cape of Good of Hope" to the "Museum of the Army Medical Department". De Waal (1978) and Mouton (1997) later erred in stating that Smith (1838) omitted to give type localities for both *melanotus* and *subviridis*.

Smith (1843) restricted the type localities of all species described previously by him under the subgenus *Pseudocordylus*. The type locality of *melanotus* (Smith's plate 25) was restricted to "hills between the principal branches of the Orange River, to the eastward of Phillopolis". Broadley (1964) understood the latter to mean the Rouxville-Zastron area of the south-eastern Free State, presumably interpreting "between the principal branches of the Orange [= Gariiep] River" to mean between the upper "Orange" (= Senqu) and lower Caledon Rivers. This area comprises primarily the Wepener, Smithfield, Zastron and Rouxville administrative districts. However, De Waal (1978) later considered the principal branches of the Orange River to be the Vaal and upper Orange Rivers and restricted the type locality to Ficksburg administrative district in the eastern Free State. He noted that Smith had in fact traveled through this area in November 1834 (2827DD, see Kirby 1940) and *P. m. melanotus* definitely occurs there now (*e.g.* 2827DB, De Waal 1978; Fig. 2.1). In the Free State Smith travelled as far north as the Witteberg range, south-west of Bethlehem (Kirby 1940).

Broadley's (1964) interpretation seems more accurate: it should be noted that Ficksburg district is north-east, not east, of Philippolis. In fact, De Waal (1978) even recorded *P. m. melanotus* from the farm Ceylon (2926DD) in the Wepener district. Bates (1992) confirmed the identity of the two specimens from the latter locality. However, it is possible that De Waal (1978) was dubious about this locality as it appeared isolated from other *P. m. melanotus* populations. Recent attempts by the author to collect *P. melanotus* from this area proved fruitless and it therefore seems possible that De Waal's (1978) Ceylon specimens were in fact wrongly labeled. Nevertheless, it should be noted that according to Kirby's (1940) map, Smith did in fact also journey through the south-eastern Free State in September and October 1834, passing within about 50 km south-east of where the farm Ceylon is situated. Also, the scalation of a huge male (NMSA 551a, examined) from Herschel first reported on by Broadley (1964) is *melanotus*-like: divided frontonasal; lateral temporals on left side of head in two rows (right side aberrant/scarred), those of the upper row elongate; dorsolaterals closely set, almost touching; and 10 differentiated femoral scales on each thigh. Branch (1981) suggested that the Herschel specimens were referable to *P. m. melanotus*, but it is not clear why he was of this opinion or whether he even examined the specimens. These specimens are probably referable to *subviridis* (see below).

De Waal (1978) noted that according to FitzSimons (1937) the types of both *melanotus* and *subviridis* were lost. FitzSimons (1937) did not, in fact, mention *melanotus* by name, but was unable to locate type specimens of any of Smith's *Pseudocordylus* (see above). However, of the seven specimens at the National Museums of Scotland (see above), at least two (NMSZ 1859.13.X65 & X66, both males - testes examined) are referable to *P. melanotus melanotus*. They have the frontonasal divided longitudinally, lateral temporals in two rows (upper row with elongate scales) and longitudinal rows of dorsolaterals almost in contact (spaces between are less than one-quarter the width of dorsolaterals) (see Smith 1843). It is quite possible that the above-mentioned specimens were used in Smith's (1838, 1843) descriptions of *melanotus* and may thus be considered syntypes, as his route (Kirby 1940; Lye 1975) definitely included several parts of the known range of this taxon, including the Ficksburg area (De Waal 1978; Bates 1996; Fig. 2.1). The scalation of NMSZ 1859.13.X65 is similar to Smith's (1843) fig. A, pl. 25 and the specimen has five (usually 4, Bates in prep.) supralabials anterior to the median subocular (on both sides of the head) as shown in fig. 3a, pl. 30 (Smith 1843). NMSZ 1859.13.X65

is therefore designated as lectotype of *Cordylus (Pseudocordylus) melanotus*, whereas NMSZ 1859.13.X66 becomes paralectotype.

In accordance with Recommendation 74C of the 1999 Code I hereby list the following data pertaining to the lectotype (NMSZ 1859.13.X65, male – left testis examined) housed in the collection of the National Museums of Scotland, Edinburgh (variation in the paralectotype NMSZ 1859.13.X66 [male - testes examined] is indicated in parentheses): Type locality as above. SVL 118.9 mm (88.3 mm); tail length (tip regenerated) about 156 mm - could not be straightened (tail broken in X66); head width 28.3 mm, *i.e.* 23.8% SVL (18.4 mm, 20.8%). Lateral temporals in two rows horizontally, the upper row consisting of 6 (left side) or 7 (right side), elongated scales, lower row with 5 (both sides) distinctly enlarged scales (X66: 5/3 on left side; three rows on right side - 4 scales in top row, 5 elongate scales in middle row, 3 scales in lowermost row); supraoculars 4; supraciliaries 5; suboculars 3 left, 4 right - two posterior to median (X66: 4 left – 2 posterior to median, 5 right – 3 posterior to median); supralabials anterior to median subocular 5 (4); infralabials 6; sublabials 6 left, 5 right (5 left, 6 right in X66); dorsals in 49 (45) transverse and 44 (37) longitudinal rows; ventrals in 12 longitudinal rows; 16 (13) lamellae under 4<sup>th</sup> finger and 20 (18) under 4<sup>th</sup> toe; femoral pores 10 left, 9 right (7 on either side in X66); differentiated glandular femoral scales 11 left, 9 right (0 in X66); frontonasal divided longitudinally, 1.5 times wider than long, in contact with loreals on either side, not separating supranasals; no additional scales between frontal and frontonasal; both anterior and posterior parietals undivided; no small scales posterior to interparietal; spaces between longitudinal rows of dorsolaterals 0.25-0.50 size of adjacent scales (scales in contact or spaces <0.25 size of adjacent scales in X66); dorsolaterals the largest, followed by the laterals, the medians being the smallest; dorsolaterals smooth or with a slight caruncle (distinctly keeled in X66) with weakly ribbed edges; throat pale with a pair of dark, parallel stripes medially; gular scales for the most part distinctly elongated; posterior infralabial keeled; lowermost enlarged temporal spine not distinctly flattened (except on the right side in X66), triangular and strongly projecting (feebly projecting in X66); colour pattern faded (X66: back grey with black stipples and blotches).

Although NMSZ 1859.13.756a & b may have been part of Smith's (1838, 1843) type series, they are morphologically intermediate between *melanotus* and *subviridis*. Because

they cannot be confidently assigned to either taxon, they cannot at this time be assigned as syntypes of either. Although they are most similar to *melanotus* (e.g. spacing of dorsolaterals, pit-like femoral pores; see below) and both specimens have two rows of lateral temporals on either side of the head (i.e. *melanotus*), the upper row of temporals consists of very elongate scales similar to *subviridis*. Also, 756b has the frontonasal divided anteriorly only.

NMSZ 1859.13.756a & b differ from the lectotype of *melanotus* as follows (values for 756b in parentheses): SVL 90.3 mm (101.6 mm); tail broken (118.4 mm, original tail); head width 19.3 mm, i.e. 21.4% SVL (20.4 mm, 20.1%); upper row of lateral temporals with 4 (5 on right side of 756b) elongated scales, lower row with 4 (3) left, 3 (2) right distinctly enlarged scales; suboculars 4 left - two posterior to median, 3 right (3 on both sides in 756b); median subocular of 756a divided transversely into two scales; supralabials anterior to median subocular 4; sublabials 5; dorsals in 43 transverse and 38 (40) longitudinal rows; 18 (16) lamellae under 4<sup>th</sup> finger, and 20 (19) lamellae under 4<sup>th</sup> toe; femoral pores 9 (6) left and 8 right, pit-like without secretory plugs; no differentiated femoral scales; frontonasal divided (only anterior half divided in 756b); 1.6 times wider than long; spaces between longitudinal rows of dorsolaterals <0.25 size of adjacent dorsolaterals; lowermost enlarged temporal spine distinctly flattened and strongly projecting.

*Cordylus (Pseudocordylus) melanotus* is the only one of Smith's (1838) *Pseudocordylus* taxa not mentioned by Hewitt (1927). Surprisingly, FitzSimons (1943) considered *melanotus* a junior synonym of *P. microlepidotus microlepidotus*, but did not give reasons for this action. However, Loveridge (1944) subsequently revived *melanotus* as a subspecies of *P. microlepidotus*, but with both *subviridis* and *transvaalensis* as junior synonyms.

Loveridge (1944) argued that FitzSimons (1943) was wrong to place *melanotus* in the synonymy of *P. m. microlepidotus* because Smith's (1843) figure of *melanotus* (pl. 30, fig. 3a; not "3b" as given by Loveridge) shows it with vertically elongated temporals like *subviridis* (pl. 30, fig 4a; not "4b" as given by Loveridge), whereas *microlepidotus* has small temporals. Furthermore, Loveridge (1944) concluded that what FitzSimons (1943) called *P. s. subviridis* was in fact a composite of *melanotus*, *subviridis* and the newly

described *P. langi*. Judging by the localities listed by FitzSimons under *subviridis*, and the character states mentioned, Loveridge was probably correct at least as far as *melanotus* and *subviridis* were concerned. For example, FitzSimons (1943: 468) mentioned: “frontonasals often bisected longitudinally”. This is the typical condition in *P. m. melanotus* (see De Waal 1978; chapter 5).

Loveridge (1944: 77) also noted that *subviridis* “may be separable as a southeast race on the basis of the almost contiguous, vertical (not horizontal) juxtaposition of the lateral scales”. It is not clear what Loveridge (1944) meant, but he may in fact have been referring to the spacing between longitudinal rows of dorsals. He added that: “In the northern form (*melanotus* + *transvaalensis*) these scales are separated both vertically and horizontally by granules and with or without small, scattered, subcircular scales” and “Where the two forms [presumably northern *melanotus* + *transvaalensis* and southern *subviridis*] merge it is impossible for me to say, and instead of speculating I prefer to treat both as *melanotus* for the difference may not prove to be constant when a large series is studied.” Although it is not especially clear in Smith’s (1843) plates 25 and 26, the female *subviridis*, at least, does appear to have the enlarged, obtusely keeled dorsolateral scales more widely spaced (typical *subviridis*) than the *melanotus* female. Loveridge (1944) also noted that the characters used by FitzSimons (1943) to separate *subviridis* and *transvaalensis* did not separate the *Pseudocordylus* material he examined according to the supposed geographical ranges.

Broadley (1964) considered *melanotus* a junior synonym of *P. subviridis subviridis* and was of the opinion that Loveridge’s (1944) revival of *melanotus* was “most unfortunate” (p. 104) as it would otherwise have been treated as a *nomen oblitum* (= forgotten name) under the International Code of Zoological Nomenclature. Broadley (1964: 104-105) added that in Smith’s (1843) plate 30, neither *melanotus* (fig. 3) nor *subviridis* (fig. 4) are depicted with elongate lateral temporals typical of the “common Basutoland-Natal Drakensberg form”. However, he added that Smith’s plate 26 (*subviridis*) illustrated typical *P. s. subviridis*, with elongate temporals and a uniform black back in the male, but that plate 25 (*melanotus*) showed a male with typical *P. s. transvaalensis* temporal arrangement and a female with elongate temporals. However, the lateral temporal region of the latter female is not clearly represented in pl. 25 and there are in fact indications of both elongate upper, as well as small lower, temporals. Broadley (1964) examined the

type series of *P. s. transvaalensis* but appears to have been biased towards FitzSimons' (1943: 469) statement: "two distinct rows of temporals, the upper of which are larger and vertically elongate, the lower smaller and hexagonal". However, most type specimens in fact have three horizontal rows of lateral temporals. Variation in temporal shield arrangement in Smith's (1843) figures of *melanotus* and *subviridis* may in fact be due to Smith's illustrations being based on more than one specimen (see FitzSimons 1937).

De Waal (1978) disagreed with Broadley's (1964) interpretation, as discussed above, noting that the temporal scale arrangement of both male and female *melanotus*, as figured by Smith (1843), can be reproduced in the range of variation of this form in the Free State. He added that Smith's (1843) figures of *melanotus* (figs A & B, pl. 25) showed a divided frontonasal, whereas that of *subviridis* was undivided.

De Waal (1978: 61) then noted that, according to pl. 25, figs A & B and pl. 30, figs 3 & 3a, Smith (1843) appears to consider *melanotus* as a form with a divided frontonasal (a character overlooked by previous workers); and a temporal scale arrangement "consisting of an upper vertical elongate row and a smaller lower row", *i.e.* similar to FitzSimons' (1943) description of *transvaalensis*. An examination of Smith's (1843) plates and line drawings confirms De Waal's (1978) findings. De Waal (1978) noted that the name *melanotus* has page priority over *subviridis* and therefore used, for the first time, the combinations *P. melanotus melanotus* and *P. m. subviridis*.

Broadley (1964) also noted that although no material was available from what he considered the type locality of *P. melanotus* – *i.e.* the Rouxville-Zastron area – he was able to report on three specimens from Herschel, about 35 km SSE of Zastron. Of these he referred two "females" to *P. s. subviridis* but noted that the third was a massive male (145 mm SVL, 170 + mm tail length). The temporal arrangement on one side of the latter specimen was apparently *transvaalensis*-like, but the other side was scarred over. According to Broadley this male was most similar to *P. microlepidotus fasciatus* and identifiable with Smith's (1843) fig. A, pl. 25 (referred to as the "male cotype" [= syntype] of *melanotus* by Broadley). He therefore restricted the name *C. melanotus* to the "male cotype" and relegated it to the synonymy of *P. microlepidotus fasciatus*, and treated the "female cotype" (fig. B, pl. 25) as *P. s. subviridis* on the basis of what he interpreted to be elongate temporals. Broadley (1964) also noted that the specimen

depicted in fig 3, pl. 30 has five (not four) supralabials anterior to the subocular, a typical arrangement in *P. microlepidotus*. However, five such scales are occasionally present in both *melanotus* and *subviridis* (De Waal 1978; chapter 5).

I have examined the three Natal Museum specimens from Herschel bearing the number NMSA 551. The large specimen (NMSA 551a) is indeed a male (testes), but whereas one of the smaller specimens (NMSA 551c) proves to be a female (ovaries), the other (NMSA 551b) is a young male (testes) with typically female colour pattern. As mentioned above, the large male has the scutellation features of *P. m. melanotus*, but the smaller specimens are clearly referable to *P. m. subviridis* (frontonasal undivided, lateral temporals in a single row of elongated scales, longitudinal rows of dorsolaterals widely spaced – separated by a distance equal to or larger than the adjacent scales, femoral pores distinct and with secretions) as noted by Broadley (1964). However, their geographical location suggests that all three Herschel lizards are referable to *subviridis*.

Broadley's (1964) relegation of the male "cotype" of *C. melanotus* to the synonymy of *P. microlepidotus fasciatus* is incorrect as the colour pattern of this specimen is typical of male *melanotus* (and *subviridis*) and adult males of all three subspecies of *P. microlepidotus* usually have generation glands on the back (Van Wyk & Mouton 1992; Mouton *et al.* 2005; see below), or at least a distinct longitudinal furrow along the vertebral region, neither of which are present in the Herschel specimens or any other members of the *P. melanotus* species complex (see plates in Smith 1843; section 2.7 below).

Broadley's (1964) use of the term "cotype" (= syntype) for the male illustrated as fig. 3 on pl. 30 requires comment. Firstly, *melanotus* was described in Smith's (1838) earlier paper that did not include illustrations, and secondly, neither the original description nor the detailed description or line drawings in Smith (1843) were necessarily based only on the particular male and female illustrated. As noted by FitzSimons (1937), some of the illustrations in Smith (1843) may be based on more than one specimen.

Loveridge (1944) reproduced most of Smith's (1843) illustrations of cordylids. However, as pointed out by Broadley (1964), Loveridge's captions to fig. 3, pl. 8 (male *melanotus*) and fig. 2, pl. 9 (male *subviridis*) are transposed.

### 2.5.2 *Pseudocordylus melanotus subviridis* (A. Smith, 1838)

*Cordylus (Pseudocordylus) sub-viridis* A. Smith, 1838, *Mag. Nat. Hist.* 2, p. 33 (Type locality: “South Africa”; restricted to: “top of the high mountainous range, which extends behind Kafferland and the country of Natal” by Smith, 1843, *Ill. Zool. S. Afr. Rept.*; interpreted as “Drakensberg, from Kaffirland to Natal” by FitzSimons 1943, *Transvaal Mus. Mem.* 1, p. 467).

*Pseudocordylus subviridis* Hewitt, 1927, *Rec. Albany Mus.* 3, p. 392.

*Pseudocordylus microlepidotus subviridis* FitzSimons, 1937, *Ann. Transvaal Mus.* 17(4), p. 266.

*Pseudocordylus subviridis subviridis* FitzSimons, 1943, *Transvaal Mus. Mem.* 1, p. 467.

*Pseudocordylus melanotus subviridis* De Waal, 1978, *Mem. nas. Mus., Bloemfontein* 11, p. 61.

*Cordylus subviridis* Anon, 2002, Report of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, 12<sup>th</sup> meeting, p. 9.

*Cordylus melanotus subviridis* Bourquin, 2004, *Durban Mus. Novit.* 29: 57-103.

Smith (1838) described *Cordylus (Pseudocordylus) subviridis* as follows:

“Scales of transverse rows smallest towards the dorsal line, where they are of a somewhat circular form; on the sides they are larger, and inclined to a triangular shape, with elevated discs, and each faintly carinated. Colour above, blue green, the back freely variegated with faint longitudinal short whitish streaks, beneath greenish brown. Length 10 inches.”

Smith’s (1838) description of dorsal colouration allows a match with the specimens illustrated on plate 26 in Smith (1843). The “blue green” on the flanks of the male (fig. A) and juvenile (fig. C) is unmistakable, although not evident in the female (fig. B) (Smith 1843) which has a greenish brown back “freely variegated with faint longitudinal short whitish streaks”. However, the male colouration described above is apparently uncommon, as Mouton & Van Wyk (1993: 1717) did not mention any blue-green specimens. However, they did report that 22 (13%) out of a total of 165 males from the Katse Dam catchment area in Lesotho had “light lemon” flanks. The colouration on the flanks of Smith’s juvenile is apparently in error (based on male colour) as juveniles are dull in colour, similar to typical females (Mouton & Van Wyk 1993; pers. obs.). Smith’s (1838) description of colour does not refer to gender. He appears to have confused male and female colour patterns as males may have “blue green” on the flanks, but usually have a dark central band on the back with few or no pale markings, whereas females typically have greyish flanks and a grey or dark brown back “freely variegated” with pale

streaks (*e.g.* pl. 73, Branch 1998; chapter 5). The dorsal pattern and colouration of female *melanotus* (fig. B, pl. 25) and *subviridis* (fig. B, pl. 26) are indistinguishable.

The male *subviridis* depicted as fig. A, pl. 26 does not match the head figures in pl. 30. In pl. 26 the rostral and frontonasal are clearly in contact, whereas in fig. 4, pl. 30 they are distinctly separated by a pair of supranasals. In addition, the frontonasal in the two figures differs in shape. In pl. 26 there are five lateral temporals and only the posterior one is accompanied below by a smaller temporal scale, whereas in fig. 4a, pl. 30 the lateral temporals are arranged in an upper row of elongated scales and a lower row of small, non-elongate scales (similar to typical *melanotus*). The figures in pl. 30 also do not match either the female or juvenile in pl. 26. There are five elongate lateral temporals only in both the female (fig. B) and juvenile (fig. C) *subviridis* (pl. 26). Unlike in fig. 4, pl. 30, the juvenile in pl. 26 has a small scale between the supranasals, frontal and frontonasal; and the frontoparietal and frontal differ in shape between the two plates.

Smith's (1838: 30) type locality for *C. (P.) subviridis* is "South Africa". However, he later restricted it to "top of the high mountainous range, which extends behind Kafferland and the country of Natal" (Smith 1843). This is apparently in reference to the interior plateau of southern Africa. "Kafferland" refers to the area "immediately beyond the eastern frontiers of the [Cape] colony" (Lye 1975: 48), or as Smith (1849) put it, "a district of country lying along the sea coast to the eastward of the [Cape] colony [*i.e.* Eastern Cape Province]". Smith's (1843) restricted type locality was later interpreted as "Drakensberg, from Kaffirland to Natal" (FitzSimons 1943: 467), "Obviously the Drakensberg" (FitzSimons 1948: 75) and "Drakensberg Range" (De Waal 1978: 61). These authors may have been influenced by Smith's (1844) restricted type locality for *Cordylus giganteus* A. Smith, 1844, namely: "interior districts of Southern Africa, and is not unfrequently seen on the rocky pinnacles of the Quathlamba mountains, which separate the country of the south-east coast, from that of the interior". While this is almost certainly a mistake, as *C. giganteus* is a terrestrial, grassland species that takes refuge in self-excavated burrows (De Waal 1978), Smith (1844) may in fact have confused the habitat of *C. giganteus* with that of *P. m. subviridis*. "Quathlamba" means barrier, or battlement, of spears and is the Zulu name for the Drakensberg range. Smith probably considered both the Drakensberg (of KwaZulu-Natal and adjacent regions) and Maloti Mountains (of central Lesotho, c. 2000-2500 m) as being part of the Quathlamba

Mountains. The Maloti Mountains, when viewed from KwaZulu-Natal, are sometimes referred to as the Drakensberg (Ambrose, Talukdar & Pomela 2000).

According to the maps and other information in Kirby (1940; 1965) and Lye (1975), Smith did not journey into the area currently referred to as “Drakensberg”. This area is now known to include the north-eastern part of the Eastern Cape Province, western KwaZulu-Natal and the Free State/KwaZulu-Natal border, extending northwards into Mpumalanga and Limpopo provinces where it is often referred to as the Transvaal Drakensberg. In 1834 Smith traveled through the south-eastern Free State into western Lesotho and the eastern Free State, reaching as far north as the Ficksburg area in the vicinity of the Witteberg mountains. *Pseudocordylus m. subviridis* has not been recorded along Smith’s route within the Free State, which has been well surveyed for reptiles (De Waal 1978; Bates 1996), but it does occur in at least one area along Smith’s route in western Lesotho, namely Morija (2927DA, FitzSimons 1943). However, western Lesotho is relatively low lying (1500-1800 m) and although there are occasional hills (2000-2100 m), no part of it can reasonably be considered as representing the “top” of a “high mountainous range”. Another nearby locality for this subspecies in Lesotho, namely Maseru (2927BC, UKNHM 209729-45, lateral temporals consisting of a single row of elongated scales, and frontonasal entire), is questionable as these lizards have apparently never been found there by any other collectors, including Gordon Setaro (pers. comm., October 2004), who conducted detailed searches in the area. In any case, the Maseru area, like Morija, is not a high mountainous area.

After returning from the Ficksburg area Smith traveled southwards into north-western Lesotho. On 14 November 1834 he reached the vicinity of current-day Mapoteng and then proceeded east. His diary entry for 18 November reads: “At daylight 8 of our party started to ascend the mountain range and by 1/2 past twelve reached one of the highest points where water boiled at 190 of Fah.” (Kirby 1939: 139). The boiling point of water decreases by 1°F (= 0.47°C) for every 152.5 m ascended, indicating that Smith must have reached an elevation of about 3355 m a.s.l. (certainly an over-estimate). This suggests the vicinity (top) of Menyameng Pass (about 3100 m). In his Journal entry for the same day, however, Smith noted that on this summit water boiled at 187°F (Lye 1975), *i.e.* about 3813 m - an even greater exaggeration. The next day Smith noted that his party were to remain in the area “in consequence of its being desirable to have representations of

several lizards, frogs and snakes procured on the mountains previous to death” (Kirby 1939: 139). In his Journal entry for 18 November Smith again discusses the climb to the plateau and ends by stating “... and besides possessing three examples of a new species of lizard of the genus *Cordylus* ... all of which were obtained high in the mountains” (Lye 1975: 101).

*Pseudocordylus melanotus subviridis* has been recorded about 20 km to the east of Menyameng Pass in both the upper Bokong River valley and near the Mokhoulane River (both 2928AB) and is common in the vicinity of the nearby Katse Dam (Mouton & Van Wyk 1996). It is the only cordylid known from this area (De Waal 1978; Bates 1996; Branch 1998). In a randomly selected sample of five specimens from the Upper Bokong River valley (locality C104 in Appendix 2.1; USEC-H2513, 2600 [both adult males], 2602 [small male], 2599, 2601 [both adult females]) and five specimens from a tributary of the Mokhoulane River (locality C114 in Appendix 2.1; USEC-H2593 [adult male], 2408, 2409, 2461, 2474 [all adult females]) the frontonasal was always undivided, lateral temporals were arranged as a single row of elongated scales (one scale was divided laterally in USEC-H2600) and the space between longitudinal rows of dorsolaterals was as wide or wider than the scales on either side (D. du Toit, pers. comm., 8 April 2005). These specimens (gender determined by examination of reproductive organs – D. du Toit, pers. comm., 24 July 2006) were therefore similar to Smith’s (1843, pls 26 & 30) illustrations of specimens referable to *subviridis*, apparently collected in the same vicinity. In addition, the femoral pores of the USEC females discussed above were small but contained yellowish secretions (D. du Toit *op. cit.*) typical of *subviridis* (De Waal 1978; Chapter 5).

On the basis of the above argument I therefore restrict the type locality of *Cordylus* (*Pseudocordylus*) *subviridis* to near, or at, the top of Menyameng (sometimes spelled “Monyameng”) Pass, Front Range, western part of the Maloti Mountains, north-western Lesotho (about 3100 m a.s.l.; 29°08’30”S, 28°13’30”E; 2928Aa4).

Confirmation that Smith did journey through, or at least reach the vicinity of, the Maloti Mountains is provided by the caption to one of Bell’s paintings published in Lye (1975), entitled “Bushmen of the Maluti Mountains, Lesotho”.

While in the Mapoteng area (c. 2928AA) on 22 November, on route back to the Free State, Smith noted that: “Botha shot a fine specimen of *Zonurus* amongst the rocks” (Kirby 1939: 142). The latter statement was probably also in reference to *subviridis*.

The coloured lithographs of Smith’s (1843, 1844) cordylids were the work of George Ford (Kirby 1965: 264). In his Preface in the Mammalia volume of *Illustrations of the Zoology of South Africa*, Smith (1849b) put great trust in Ford’s accuracy as an illustrator of animals, noting that: “A cursory survey of the plates will, I think, convince any one that they are the production of a master’s hand – a hand that depicts nature so closely as to render the representation nearly, if not equally, as valuable as the actual specimen.” The plates in Smith (1843) are therefore likely to be good representations of the true appearance and colour of live *subviridis*. However, according to Kirby (1965: 263), while most specimens were illustrated during the expedition, a few were done later on in Cape Town. Smith (1849b) also noted that most of the illustrations were by Ford, “from specimens either living or recently dead”.

Although FitzSimons (1937) claimed not to have found any *subviridis* at the museums in London and Edinburgh, at least two of the seven specimens at the National Museums of Scotland (NMSZ 1859.13.756c & d) are referable to *subviridis* and may have been used for Smith’s (1838, 1843) descriptions. They have the frontonasal undivided, lateral temporals in a single row of elongated scales, and longitudinal rows of dorsolaterals widely separated (spaces as wide as adjacent dorsolaterals). NMSZ 1859.13.756d has a small scale present between rostral and frontonasal, separating the supranasals. This condition - very rare in *subviridis* (Chapter 5) – is also apparent in the juvenile illustrated as fig. C, pl. 26 (Smith 1843). The general scalation of Smith’s juvenile is also similar to 756d, which may in fact be the same specimen. NMSZ 1859.13.756c is here designated as lectotype of *Cordylus (Pseudocordylus) subviridis*, whereas NMSZ 1859.13.756d becomes paralectotype. Unfortunately the hinder portion of the back of the lectotype is damaged.

In accordance with Recommendation 74C of the 1999 Code I hereby list the following data pertaining to the lectotype (NMSZ 1859.13.756c, female – ovary examined) housed in the collection of the National Museums of Scotland, Edinburgh (variation in the paralectotype NMSZ 1859.13.756d [juvenile] is indicated in parentheses): Type locality

as above. SVL 87.2 mm (65.3 mm); tail broken (tail twisted not measured); head width 16.9 mm, i.e. 19.4% SVL (14.7 mm, 22.5%). Lateral temporals consist of a single row of much elongated scales, 4 on left side, 5 on right (5 on both sides in 756d); supraoculars 4; supraciliaries 5 left, 6 right (5 on both sides in 756d); suboculars 3 left, 4 right - two posterior to median (3 on both sides in 756d); 4 supralabials anterior to median subocular; infralabials 6; sublabials 5 (2<sup>nd</sup> sublabial on right side divided longitudinally in 756d); dorsals in about 50 (47) transverse rows (hinder part of back of lectotype damaged), and 36 (35) longitudinal rows; ventrals in 12 (14) longitudinal rows; 15 (16) lamellae under 4<sup>th</sup> finger and 19 (21) under 4<sup>th</sup> toe; femoral pores 7 (9) left, 8 right, pores distinct with secretory plugs; differentiated glandular femoral scales 0 (13/12); frontonasal undivided (with fold on right side anteriorly, and ridge posteriorly in the center, in lectotype), 1.4 (1.5) times wider than long, in contact with loreals on right but narrowly separated on left (in contact on both sides in 756d), not separating supranasals (supranasals separated by a small squarish scale that is in contact with both rostral and frontonasal in 756d); no additional scales between frontal and frontonasal; both anterior and posterior parietals undivided; no small scales posterior to interparietal; spaces between longitudinal rows of dorsolaterals equal to size of adjacent scales (*i.e.* widely separated); dorsolaterals, laterals and medians all of similar size; dorsolaterals keeled with weakly ribbed edges; throat pale with a pair of dark, parallel stripes medially; gular scales for the most part distinctly elongated; posterior infralabial keeled; lowermost enlarged temporal spine distinctly flattened and moderately projecting.

Hewitt (1927: 392) considered *P. subviridis* to be a “very distinct form” found in the Drakensberg Mountains. He observed the vertically elongated lateral temporals - as illustrated in Smith’s (1843) plate 26 - in a sample of 40 specimens of various sizes collected at the “summit” of Mont-aux-Sources and in Lesotho, and considered this character state diagnostic of the species. He included as *P. subviridis* material from Ugie and the Amatole Mountains, both in the Eastern Cape Province. However, his material from near Belfast in Mpumalanga is referable to *P. m. melanotus* (see Jacobsen 1989).

FitzSimons (1937) used the combination *Pseudocordylus microlepidotus subviridis* but did not comment on the taxonomy or nomenclature of the genus. He later (1943) treated *subviridis* as a full species.

As mentioned earlier, Loveridge (1944) noted that *subviridis* may be distinguishable as a south-eastern subspecies of *P. microlepidotus* on the basis of the almost contiguous, vertical (not horizontal) juxtaposition of the lateral scales.

Regarding the arrangement of lateral temporal scales, De Waal (1978) noted that the male, female and juvenile in Smith's (1843) pl. 26 all have a single row of vertically elongate temporals, but fig. 4a on pl. 30, "which should show the scale arrangement of plate 26, figure A [male], in fact shows only two median vertically elongate temporal scales surrounded by smaller ones." De Waal (1978: 61) then reasoned that because the three lizards on pl. 26 all had a single row of elongate temporals, fig. 4a on pl. 30 was incorrect, and "it can nevertheless be concluded that Smith regarded *subviridis* as a form with an undivided frontonasal and a single row of vertical elongate temporals (plate 26)". An examination of fig. 4 shows that, although the frontonasal is virtually undivided, it does in fact have what appears to be a small longitudinal suture posteriorly.

Based on variability in the condition of the frontonasal (entire or divided) in *Pseudocordylus* from Mpumalanga, Gauteng and Limpopo provinces, Jacobsen (1989) was of the opinion that *subviridis* was of doubtful validity. Indeed most of the northernmost *melanotus* material examined by Jacobsen was found to have undivided frontonasals (Bates, in prep.). Jacobsen (1989) then listed De Waal's (1978) *P. m. subviridis* in the synonymy of *P. m. transvaalensis*.

### 2.5.3 *Pseudocordylus transvaalensis* FitzSimons, 1943

*Pseudocordylus subviridis transvaalensis* FitzSimons, 1943, *Mem. Transvaal Mus.* 1, p. 469 (Type locality: "Woodbush, Pietersburg District, N. Tvl. [= Northern Transvaal]").

*Pseudocordylus melanotus transvaalensis* De Waal, 1978, *Mem. nas. Mus., Bloemfontein* 11, p. 61.

*Pseudocordylus transvaalensis* Branch, 1998, *Field Guide to Snakes and other Reptiles of Southern Africa*, p. 207, pl. 73(5).

*Cordylus transvaalensis* Anon, 2002, Report of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, 12<sup>th</sup> meeting, p. 9.

FitzSimons (1943) provided a fairly detailed description of *Pseudocordylus subviridis transvaalensis* based on eight adults and subadults from Woodbush (Forestry Station) in Limpopo Province. The type series was collected by Dr L.H. Gough in December 1907 (1908 according to the Transvaal Museum catalogue). However, FitzSimons also referred specimens from the following localities to this form: Haenertsburg - with reference to Matschie (1891) and Selati (both Limpopo Province); Carolina, Lydenburg, Mariepskop, Maribashoek, Sabie and Lochiel (Mpumalanga Province); and Forbes Reef (northern Swaziland).

FitzSimons (1943: 470) listed TM 1695 as the largest male (151 mm SVL, 176 mm tail length) and TM 1699 as the largest female (134 mm + 171 mm), describing both as “cotypes” of *P. subviridis transvaalensis*. These were the only type specimens referred to by catalogue number, but FitzSimons (1943) did not actually designate a holotype or allotype. All of the types, including the named “cotypes”, are thus merely syntypes (Article 73.2.1 of the 1999 Code). De Waal (1978: 61) later examined the “holotype and cotypes” (TM 1695, 1697, 1699-701, 1954-5) of *transvaalensis*, but did not state which were considered which. Nevertheless, according to the bottle label and catalogue at the Transvaal Museum, TM 1695 is listed as holotype, TM 1699 as allotype and five others (TM 1697, 1699-701, 1954-5) are listed as paratypes (M. Burger, pers. comm., 3 August 2004).

There are five labels affixed to TM 1695: a narrow label with the word “Holotype”, an old label bearing only the number “1695”, another old label (my commas except after “Dec.”): “No. [presumably left blank for the museum accession number] Woodbush., Dec., 1907., Gough”, another old label: “Liz. of S. Afr” (used for any TM specimen referred to or quoted by catalogue number in FitzSimons’ 1943 monograph according to W.D. Haacke [pers. comm., 30 August 2005]), and a larger (apparently more recent) white label bearing the following (my commas): “Type, *Pseudocordylus subviridis Transvaalensis*, Woodbush, Soutpansberg dist., N. Tvl., 1907, L.H. Gough”. TM 1699 has four labels: a narrow “Allotype” label, an old label bearing only the number “1699”, a label with “No. ... Woodbush., Dec., 1907., Gough” and one with “Liz. of S. Africa”. The “Holotype” and “Allotype” labels, as well as the “Liz. of S. Afr” labels, were prepared by the hand of V.F.M. FitzSimons himself according to W.D. Haacke (*op. cit.*), a past curator of the Transvaal Museum who worked with FitzSimons in the late 1960s

and early 1970s. However, the mere mention of the term “type” or an equivalent expression by another author (*e.g.* De Waal 1978), or its use in a catalogue or on a specimen label (as explained above), “is not necessarily evidence that a specimen is or is fixed as any of the kinds of types referred to in this Chapter” (Article 72.4.7 of the 1999 Code).

Four type specimens (TM 1697, 1700, 1954-5) bear only two old labels, one with a catalogue number and the other with “No. Woodbush., Dec., 1907., *Gough*”. TM 1696 - represented only by a skull - is listed in the catalogue as possibly being a “paratype” (M. Burger pers. comm., 2 August 2004; Haacke *op. cit.*). This is most likely the last of the eight type specimens as it was collected at the same time, at the same place, and by the same collector, as the other types. Although it can no longer be located and the body may have been discarded after preparing the skull (Haacke *op. cit.*), this specimen should also be considered a syntype. TM 1698 was listed in the TM catalogue as being part of the series of specimens later treated as “cotypes” of *transvaalensis*, but it was donated to Normal College, Johannesburg, possibly before FitzSimons’ (1943) description (Haacke, *op. cit.*). It is thus not considered a syntype.

According to my measurements TM 1695 is indeed the largest male (150.2 mm SVL), but TM 1955 is the largest female (144.6 mm SVL). In terms of size and scalation both TM 1695 and TM 1699 are typical *transvaalensis* (although the occipital and gular regions of the former are damaged) and it seems unnecessary to designate any other specimens as lectotype or alloparalectotype. I therefore hereby formally designate TM 1695 as lectotype of *Pseudocordylus subviridis transvaalensis* and the others (TM 1696-7, 1699-701, 1954-5) thus become paralectotypes. In addition, TM 1699 - the second largest female syntype (my SVL measurement = 136.8 mm) - is hereby designated as alloparalectotype. One of the paralectotypes, *viz.* TM 1701, is a misidentified *P. melanotus melanotus* that was probably assigned the wrong locality (see below).

In accordance with Recommendation 74C of the 1999 Code, I hereby list the following data pertaining to the lectotype (TM 1695 – male, left testis examined) housed in the collection of the Transvaal Museum, Pretoria (variation in the alloparalectotype TM 1699 [female – post-ovulatory follicles] is indicated in parentheses):- Type locality: Woodbush (in reference to the Forestry Station, approxim. 23°49’S, 29°59’30”E; about 1600-1800 m

a.s.l.); December 1907; Snout-vent length 150.2 mm (136.8 mm), tail length 177.5 mm - tail detached, tied to body (tail broken in TM 1699 but 171 mm according to FitzSimons 1943); collected by L.H. Gough. Lateral temporals on left side of head asymmetrically arranged, in three rows horizontally on right side upper row with elongate scales, scales of middle row mostly larger than those below (2 rows on left side, 3 on right in TM 1699); supraciliaries 6 left side, 5 right (5 on either side in TM 1699); suboculars 4 (two posterior to median); 4 supralabials anterior to median subocular (5 on left side, 4 on right in TM 1699); infralabials 6 left, 7 right (7/6 in TM 1699); sublabials 6 left, 5 right (7/6 in TM 1699); dorsals in 41 (43) transverse and 43 (44) longitudinal rows; ventrals in 14 (12) longitudinal rows; 15 (16) lamellae under 4<sup>th</sup> finger and 19 (18) under 4<sup>th</sup> toe; femoral pores 8 left, 7 right (6/7 in TM 1699); differentiated glandular femoral scales 9 on either side (0 in TM 1699); frontonasal undivided except for a small suture posteriorly – *i.e.* posterior one-quarter divided, slightly wider than long, in contact with loreals (separated on right side in TM 1699) and separating supranasals (supranasals in contact in five of the six available paralectotypes, including TM 1699); large scale between frontal and frontonasal; anterior parietals undivided; 5 (4) small scales posterior to interparietal; spaces between longitudinal rows of dorsolaterals equal to, or about half the width of, adjacent scales; posterior infralabial keeled; throat black.

Loveridge (1944: 77) erroneously referred to a specimen from “Selati” – in the collection of the Museum of Comparative Zoology (Harvard) - as being a “paratype” of *transvaalensis*. FitzSimons (1943) had referred Transvaal Museum material [non-types] from this locality to *transvaalensis*. Five specimens from this locality in the Transvaal Museum collection were examined and are in fact referable to *P. melanotus melanotus* (see below).

In his key FitzSimons (1943) distinguished between *P. s. subviridis* and *P. s. transvaalensis* as follows:

*P. s. subviridis*: A single row of large vertically elongate temporals; lowermost temporal spine moderately projecting in males.

*P. s. transvaalensis*: Two rows of temporals, the upper vertically elongate and much larger than the subhexagonal lower; lowermost temporal spine feebly projecting and only bluntly pointed.

Loveridge (1944) noted that the characters used by FitzSimons (1943) to separate the two forms did not separate the *Pseudocordylus* material he examined according to the supposed geographical ranges. He added (p. 77) that, with regard to temporal shield arrangement, some individuals from the same locality could be assigned to *subviridis*, while others to *transvaalensis*; and temporals on one side of the head of an individual may correspond to *subviridis*, while on the other side to *transvaalensis*. Also, the bluntness of the temporal spine (= lowest ante-auricular scale) is apparently affected by age and wear, rendering it of dubious value. However, Loveridge (1944) did note that according to FitzSimons' (1943) measurements the extreme northern form (*transvaalensis*) was much larger than the southern form (*subviridis*). FitzSimons' (1943) largest males of *transvaalensis* and *subviridis* had SVLs of 151 mm versus 110 mm respectively, while females measured 134 mm versus 85 mm respectively.

With reference to FitzSimons' (1943) key, Broadley (1964: 106) writes as follows: "Actually there is an *average* difference in the temporal arrangement of northern and southern populations of *subviridis* and it may be possible to plot a character gradient, but the nature of the dorsolateral scalation provides a more stable character on which to base a northern race." Loveridge (1944: 77) had noted that dorsolateral scales in both *melanotus* and *transvaalensis* were similarly arranged. In his key, Broadley (1964: 102) separated the two subspecies on the basis of the "lateral" (probably meaning dorsolateral) scales being smaller than the vertical interspaces between them in *subviridis* and larger than these interspaces in *transvaalensis*. Broadley was probably referring to the spaces between longitudinal rows of dorsolaterals.

Broadley (1964) treated as *P. s. transvaalensis* material from several localities in KwaZulu-Natal, from Pietermaritzburg northwards to the midlands and even the north-western parts of that province. He also included the north-eastern Free State, western Swaziland and Mpumalanga Escarpment in the range of *transvaalensis*, apparently based on some of the localities included by FitzSimons (1943) under *P. subviridis subviridis* and by Loveridge (1944) under *P. microlepidotus melanotus*.

De Waal (1978: 61) examined the "holotype" (not specified) and six "paratypes" (excluding TM 1696) of *transvaalensis* and expressed his opinion as follows: "I am convinced that *transvaalensis* is closely related to, if not synonymous with, *melanotus*,

except for the undivided frontonasal (divided in one specimen [TM 1701, actually partly divided]) and the presence of three or four small scales posterior to the interparietal. As in *melanotus*, the females of *transvaalensis* also show only pits and no developed femoral pores.” De Waal (1978) was probably also influenced by the fact that TM 1701 is in fact a *P. m. melanotus* (see below).

Apart from differences in male colour pattern, Jacobsen (1989) separated “Transvaal” *P. m. melanotus* (Mpumalanga and Gauteng provinces) and *P. m. transvaalensis* (Limpopo Province) as follows:

*P. m. melanotus*: Frontonasal usually divided; lateral temporals usually in a single row; dorsals in 32-47 longitudinal rows; largest male 143 mm SVL, largest female 136 mm SVL.

*P. m. transvaalensis*: Frontonasal divided in 63% of specimens, undivided in 37%; lateral temporals usually in two or more rows; dorsals in 39-58 (mostly 43-50) longitudinal rows; largest male 151 mm SVL, largest female 155 mm SVL.

As shown on Jacobsen’s (1989) map, *P. transvaalensis* occurs in three allopatric populations. There are morphological differences between these populations (Jacobsen 1989; chapter 5) that explain, in part, why Jacobsen’s key (see above) is so confusing.

Jacobsen (1989) felt that colour pattern was the most consistent factor separating *melanotus* and *transvaalensis*. He continues (p. 632) as follows: “It is also my opinion that these forms are not likely to hybridise if contact between them should ever become likely again. With this in mind, and considering the large range of variation within the morphological characters, it is suggested that this species be given specific status, *P. transvaalensis* FitzSimons.”

Material from only two of FitzSimons’ (1943) original localities – “Woodbush” and “Selati” - were assigned to *transvaalensis* by Jacobsen (1989). However, the Selati specimens (TM 168, 171-4) have been examined and are in fact referable to *P. m. melanotus* (lateral temporals in 1-3 [usually two] rows horizontally; frontonasal entire in two, divided in two and partly divided in one, specimen; throat pale with a pair of dark, parallel stripes medially; differentiated femoral scales in male six left, five right; femoral pores in females pit-like without secretions; dorsal pattern typical of female *P. melanotus*

[see Branch 1998, p. 207 & fig. 3, pl. 73]; no small scales posterior to interparietal). All remaining localities for this species as given by FitzSimons (1943) are referable to *P. m. melanotus* (see map in Jacobsen 1989; Appendix 2.1; chapter 5).

While most of Broadley's (1964) *P. s. transvaalensis* material is apparently referable to *P. m. subviridis*, a few are referable to *P. m. melanotus*: Qudeni Forest (NMSA 997a-e: lateral temporals in 1-2 rows, the uppermost – or single – row with distinctly elongated scales; frontonasal fully divided longitudinally in three specimens, but divided anteriorly only in two; three specimens [?females, with narrow heads] with pit-like femoral pores lacking secretions); Van Reenen; Muller's Pass (NMSA 898a-h: lateral temporals in two rows, the uppermost with scales distinctly elongated; frontonasal divided, but anteriorly only in 898f; all five females with pit-like femoral pores); Botha's Pass (Fig. 2.1).

Branch (1988a) considered *transvaalensis* to be a northern subspecies of *P. melanotus*, extending from the Mpumalanga Escarpment through western Swaziland and southwards into the KwaZulu-Natal midlands as far south as Pietermaritzburg. Jacobsen (1989) did not comment on the status of material previously assigned to *transvaalensis*, including that from KwaZulu-Natal (see Broadley 1964). Nevertheless, Branch (1998) later accepted Jacobsen's (1989) proposal that *P. transvaalensis* is a full species restricted to Limpopo Province.

Matschie (1891) recorded two juvenile *P. microlepidotus* from Mphome Mission Station at "Hanertsburg in District Zoutpansberg, north of Maraba's Stadt". It is recorded that this mission station was also known as Kratzenstein Mission (<http://www.sahistory.org.za/pages/mainframe.htm>), the ruins of which are to be found within 50 km of Haenertsburg ([www.marzinfo-cape.co.za/infos/tzan\\_e.htm](http://www.marzinfo-cape.co.za/infos/tzan_e.htm)). According to Leistner & Morris (1976) it was situated at locus code 2329DD. Hewitt (1909) later recorded "Zoutpansberg District" as a locality for *P. microlepidotus*, almost certainly in reference to Matschie (1891). FitzSimons (1943) included Matschie's (1891) record (as Haenertsburg) and Hewitt's (1909) reference to it (as Zoutpansberg District) under *P. s. transvaalensis*. Loveridge (1944) listed this locality (as Zoutpansberg District) under *P. microlepidotus melanotus*. In the late 19<sup>th</sup> and early 20<sup>th</sup> centuries the Haenertsburg area may have formed part of "Zoutpansberg District", but it is currently within the Pietersburg district. Neither Jacobsen (1989) nor any other workers (see Branch 1998)

have collected either *transvaalensis* or *melanotus* in the vicinity of the present-day Soutpansberg 1 or Soutpansberg 2 districts of Limpopo Province. A specimen of *P. m. melanotus* from “Soutpansberg Mountain”, housed at the Transvaal Museum (TM 47225) was, according to the donator (W.R. Branch, pers. comm., 20 February 2002), probably incorrectly labeled.

The identity of *P. transvaalensis* is further confused by the fact that one of the types, namely TM 1701, is in fact referable to *P. m. melanotus* (lateral temporals in two rows, the uppermost with distinctly elongated scales; only posterior part of frontonasal divided longitudinally; differentiated femoral scales seven left, nine right; only one small scale behind interparietal; throat pale with a pair of dark, parallel, median stripes; typical male *melanotus* dorsal pattern [see Branch 1998]: dark central band, flanks paler). Although the Selati record for *melanotus* suggests that the two species occur at least parapatrically in the Wolkberg area (see below; Fig. 2.1), sympatry in the Woodbush area seems unlikely. During the present study only *transvaalensis* was collected in the Haenertsburg-Wodbush area (Appendix 2.1: A23-26). Also, the two species were not found together at any localities during Jacobsen’s (1989) intensive reptile survey of the former Transvaal province. It seems more likely that TM 1701 was collected elsewhere and incorrectly labeled, or assigned the wrong locality. It is noteworthy that TM 1701 is the only one of the seven ethanol-preserved type specimens (*i.e.* excluding TM 1696 – skull only) without the old label (*i.e.* “No. ... Woodbush., Dec., 1907., Gough”), suggesting that it may not have been collected with the other type specimens. The inclusion of this specimen as a type is probably the reason for FitzSimons’ (1943) confused description of colour pattern. TM 1701 was probably the only type specimen considered by De Waal (1978) to have a “fully” (actually partly) divided frontonasal. Also, it has only a single small scale posterior to the interparietal, not “three or four” as noted by De Waal (1978: 61) for all type specimens of *transvaalensis*.

#### 2.5.4 *Pseudocordylus langi* Loveridge, 1944

*Pseudocordylus langi* Loveridge, 1944, *Bull. Mus. comp. Zool., Harvard*, 95(1), p. 73 (Type locality: “Mont-aux-Sources, Drakensberg, Basutoland [= Lesotho]”).

*Cordylus langi* Anon, 2002, Report of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, 12<sup>th</sup> meeting, p. 6; Bourquin 2004, *Durban Mus. Novit.* 29, p. 97.

Loveridge (1944) provided a fairly detailed description of *Pseudocordylus langi* based on a single adult male (MCZ 46835) from Mont-aux-Sources on the Lesotho side of the Drakensberg. Although this was the only specimen he examined, Loveridge included data from other specimens (probably all *P. m. subviridis*) in his table on p. 69. He also listed eight paratypes from the Drakensberg, collected from the same general area as the holotype as well as from near Underberg and near Kokstad, that were included “on the basis of information kindly supplied by Mr. V. FitzSimons” (p. 74) of the Transvaal Museum.

Loveridge then referred to FitzSimons’ comments to him that with regard to the condition of scales on the flanks, specimens intermediate between *langi* and *subviridis* (but assignable to the latter) also occur lower down at “7000 ft” (= 2134 m) in the Mont-aux-Sources area. Loveridge (1944: 74) listed additional localities in Lesotho and the Eastern Cape Province under *langi*, but in a footnote stated that these were taken from the literature and should be “regarded with reserve”. Material from these latter localities is probably referable to *subviridis* (Appendix 2.1; Chapter 5). A second footnote reads: “Unless referable to *P. m. [= microlepidotus] melanotus*, the specimens from Doornkop, near Belfast, Transvaal, mentioned by Hewitt [1927], should be added.” These specimens are indeed referable to *P. melanotus melanotus* (Jacobsen 1989). Loveridge (1944: 73) regarded *P. langi* as being most closely related to *P. capensis* and *P. robertsi*, from which it differed in “the feeble development of enlarged dorsal scales which are confined to the vertebral region”. *Pseudocordylus langi* and *P. capensis* differ from all other *Pseudocordylus* in having the flanks and dorsolateral regions covered by homogeneous granules (Branch 1998).

FitzSimons (1948) later re-examined all (four) *P. langi* paratypes from Mont-aux-Sources, together with a new series of 12 specimens from the summit of Mont-aux-Sources, referring all of them to *P. s. subviridis*. He found that rather than having the flanks covered only by homogeneous granules, they were, in all cases, covered by granules as well as longitudinal rows of enlarged, widely spaced tubercles, the latter varying considerably in size and shape. I have examined all *P. langi* paratypes (TM 13846-7, 13849-50: Mont-aux-Sources; TM 2531, 2533: Drakensberg on Basutoland [= Lesotho] side; TM 20992: Drakensberg near Underberg; TM 21063: Drakensberg near Kokstad) and agree with FitzSimons' (1948) comments. These specimens are also assignable to *subviridis* with regard to the diagnostic characters mentioned by De Waal (1978). FitzSimons (1948: 76) then stated: "It would thus appear that the single specimen examined and described by Loveridge as new, under the name *langi*, represents merely an extreme stage in the reduction of the lateral [probably meaning dorsolateral] scales or tubercles, and cannot thus be regarded as distinct". Unfortunately he did not examine Loveridge's (1944) holotype. FitzSimons (1948) incorrectly listed Loveridge's (1944) *P. langi* as "*Cordylus langi*" in his synonymy.

Broadley (1964) re-validated *P. langi* on the basis of 16 specimens from Organ Pipes Pass in the Cathedral Peak area. Although he did not examine the holotype, Broadley sent a specimen identified as *langi* - from Organ Pipes Pass - to the Museum of Comparative Zoology in Harvard for comparison with the holotype. The two specimens agreed in all diagnostic characters. Apart from the subuniform granules on the flanks, Broadley (1964) distinguished *langi* from other *Pseudocordylus* in KwaZulu-Natal by its lower infralabial count (five versus usually six in other taxa) and greater number of femoral pores on each thigh (11-17 versus 3-10 in other taxa).

Examination of digital colour images of the holotype of *P. langi* sent by J. Rosado (Museum of Comparative Zoology, Harvard) revealed that this specimen is similar to other *langi* examined (Appendix 2.1; Chapter 5), including most of Broadley's (1964) specimens from Organ Pipes Pass (*e.g.* frontonasal entire; single row of vertically elongated lateral temporals; dorsal scales granular except for a few rows of flat, smooth scales paravertebrally).

Bourquin & Channing (1980) added Giant's Castle Game Reserve as a locality for *P. langi*, with reference to material in the Transvaal Museum. However, the latter museum does not have any records of this species from that locality. According to records at the KwaZulu-Natal Nature Conservation Service (J. Craigie, pers. comm., 23 December 1998) the specimens referred to by Bourquin & Channing (1980) are TM 2532 (listed as *P. m. subviridis* - collected on 11 December 1914 in "Giants Castle area" – according to the Transvaal Museum catalogue; this specimen can no longer be located [L. Mashinini, pers. comm., 2005]) and TM 2533 (listed as a paratype of *P. langi* - from "Drakensberg on Basutoland side" - by Loveridge 1944; examined and determined to be *subviridis*, see above).

Further confusion regarding the species limits of this taxon resulted from the publication of Visser's (1984) distribution map where the range includes not only the above-mentioned areas, but also an isolated locality at locus 3029AD. Branch's (1988c) map differs slightly, but includes locus 3029AD as well as 2929CC, thus suggesting that the species may occur in high-lying areas from Mont-aux-Sources and adjacent northern Lesotho, southwards along the Drakensberg escarpment to as far south as Kokstad. No author has contested Broadley's (1964) concept of *P. langi* and it thus appears as if the two maps are partly incorrect, possibly having included some of Loveridge's (1944: 74) additional *langi* localities that are almost certainly all referable to *subviridis* (possibly excluding "Great Winterberg" = *P. melanotus subviridis* or *P. microlepidotus fasciatus*). For example, locus 3029AD represents the Kokstad area, a locality listed by Loveridge (as "Drakensberg near Kokstad") under *langi*. Branch's (1988c) 2929CC record, represented by Port Elizabeth Museum material from Sehlabathebe National Park in Lesotho (Appendix 2.1), is referable to *subviridis* (P. le F.N. Mouton, pers. comm., 1998). The shaded maps in Branch (1988a, 1998) illustrate a range similar to that in Branch (1988c). Bourquin's (2004) plotted records for this species could not be verified. His records at 2828Db3 and 2828Db4 probably refer to the Mont-aux-Sources area, 2929Ab1 probably refers to Organ Pipes Pass, whereas 2929Ad2 is apparently in reference to Giant's Castle (unacceptable as discussed above). Finally, his plotted locality at locus 2929Cb1 refers to the Sani Pass area and may be in reference to TM 29050-9 and 30067, all collected in December 1963 and identified as *P. melanotus subviridis* in the Transvaal Museum catalogue (see also C62, 151), or PEM R4723-33 (C151 in Appendix 2.1, specimens examined marked with an asterisk: frontonasal undivided, lateral temporals a

single row of elongated scales, spaces between longitudinal rows of dorsolaterals wider than adjacent dorsolaterals) marked on their tags as *P. cf. langi* but here identified as *P. melanotus subviridis*.

*Pseudocordylus langi* has been confirmed as occurring in only two main areas, namely Mont-aux-Sources (Loveridge 1944) and Organ Pipes Pass (Broadley 1964). It should be noted that early references to Mont-aux-Sources probably referred to the general area around, but not necessarily at, the actual peak known by this name. In the case of *langi* the actual collection localities were probably on the summit or at the escarpment edge at elevations of at least 2800 m. Several specimens have been collected at Organ Pipes Pass (Broadley 1964; Appendix 2.1; chapter 5) and one specimen was collected nearby at Cleft Peak in Lesotho (NMZB-UM 2421). Apart from the holotype, several additional museum specimens are now also available from the Chain Ladder and Nemahadi Pass, both in the vicinity of the type locality (Appendix 2.1). All of the specimens mentioned above (Appendix 2.1) were examined and identified as *langi* according to the key in Broadley (1964).

#### 2.5.5 *Pseudocordylus spinosus* FitzSimons, 1947

*Pseudocordylus spinosus* FitzSimons 1947, *Ann. Natal Museum* 11(1), p. 116, fig. 1; pl. 1, figs 5-6 (Type locality: “Cathkin Peak area, Drakensberg, Natal”).

*Cordylus spinosus* Anon, 2002, Report of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, 12<sup>th</sup> meeting, p. 9; Bourquin 2004, *Durban Mus. Novit.* 29, p. 97.

FitzSimons (1947) provided a detailed description of *P. spinosus*. He named a holotype (TM 21267) from Cathkin Peak area and 10 paratypes (TM 21262-5 and NMSA 647 [three specimens] from the type locality; TM 2521 from Giant’s Castle area; NMSA 550 and 555 from Giant’s Castle). Paratypes TM 2521, 21262, 21264 and 21265 have been examined and agree with the character states given by FitzSimons (1947). It should be noted that according to the old Natal Museum catalogue (D. Jennings, pers. comm., 2 & 4 March 2004), NMSA 550 and 555 are from “Giant’s Castle Game Reserve” and the three specimens labeled NMSA 647 are from “Little Tugela [River] Valley” (below about 1200 m, probably locus 2929BA or 2829DC). NMSA 648, a specimen of the frog

*Phrynobatrachus natalensis* (A. Smith, 1849), is from “Cathkin Peak” (Jennings, *op. cit.*) and it appears as if the locality for this specimen was confused with that of NMSA 647.

*Pseudocordylus spinosus* is easily distinguished from other members of the genus by the combination of closely set, keeled dorsolaterals, spinose laterals, and a frontonasal that is usually longer than wide and separated from the loreals (Chapter 5).

FitzSimons (1947) indicated that he had included some *P. spinosus* in his earlier (1943) account of *P. subviridis*. Broadley (1964) added a few additional *spinosus* localities in the Drakensberg (Dooley Ridge in Royal Natal National Park; Cathedral Peak; Champagne Castle), while De Waal (1978) recorded this species from “Sentinel” (2439 m; *i.e.* probably along the Sentinel Road in the vicinity of Witzieshoek Mountain Resort) in the Drakensberg of the eastern Free State. It appears to be restricted to the lower and middle slopes of the Drakensberg range (900–2517 m) (Visser 1984; Branch 1998; Bourquin 2004; Appendix 2.1), although Branch (1988d) also mapped an isolated sub-population at locus 3030AA. I have examined two specimens from the latter area (Farm: Eersteling [1370], Ixopo district: TM 55302-3) and both are indeed referable to *spinosus* according to the key in Broadley (1964) (although the frontonasal in TM 55302 is as long as wide, not longer). Bourquin’s (2004) plotted record at locus 3030Aa4 is almost certainly based on the Eersteling locality. He also recorded *spinosus* nearby at 2929Dd2 (near Polela). However, even though this latter locality appears to be situated in suitable habitat and would bridge the gap between the main Drakensberg population and the Eersteling locality, it cannot be associated with any known museum specimens (see Appendix 2.1). Bourquin’s (2004) isolated record at 2729Dc2 probably refers to two specimens (TM 80077-8, examined) from “Ncandu Forest Reserve” listed as *P. spinosus* in the Transvaal Museum catalogue, but here identified as *P. m. melanotus* (lateral temporals in two rows, the upper row with elongate scales; frontonasals fully or partly divided; frontonasal wider than long and in contact with loreals; longitudinal rows of enlarged dorsolaterals slightly separated – by a distance of less than one-quarter the width of an adjacent dorsolateral; laterals non-spinose). Although Bourquin’s (2004) records could not be individually verified, his plotted record at 2828Db4 refers to the Sentinel - Mont-aux-Sources area, his records at loci 2829Cc4 and 2829Cd3 are referable to the Cathedral Peak area, while loci 2929Ab4, Ac2, Ba3 and Bc1 are located in the Champagne Castle, Cathkin Peak and Giant’s Castle areas.

It should be noted that most of the earlier records of *P. spinosus* appear to be somewhat vague, referring to areas rather than exact places. Localities such as Cathedral Peak, Cathkin Peak, Champagne Castle and Giant's Castle all refer to peaks at altitudes in excess of 3000 m, well above those usually associated with this species (see Bourquin 2004). These localities were probably in reference to areas in the vicinity of these peaks, rather than the peaks themselves. In fact, FitzSimons (1947) noted that his types were collected at altitudes of 5000-8000 ft (1524-2438 m). Therefore, several *spinosus* localities in Appendix 2.1 are accompanied only by eighth- or quarter-degree grid references rather than co-ordinates. The highest confirmed elevation at which *spinosus* has been collected is 2517 m (locality E19, Appendix 2.1).

## 2.6 Morphological differentiation in the *Pseudocordylus melanotus* species complex

According to De Waal (1978) the two subspecies of *P. melanotus* can be distinguished using five characters: frontonasal usually divided in *melanotus*, entire in *subviridis*; femoral pores are shallow pits in female *melanotus*, but distinct pores in female *subviridis*; differentiated femoral scales in males 1-17 in *melanotus*, usually 19-34 in *subviridis*; dorsolateral scales closely spaced or in contact in *melanotus*, well separated in *subviridis*; lateral temporals usually in two rows - the upper row consisting of elongated scales - in *melanotus*, usually in a single row of much elongated scales in *subviridis*. A combination of the frontonasal and lateral temporal characters usually separated examined specimens of the two taxa (Appendix 2.1; Chapter 5).

According to Jacobsen (1989) *P. m. melanotus* in Mpumalanga and Gauteng provinces usually has a divided frontonasal [often undivided in the northern-most populations indicated as B1-59 in Appendix 2.1] and the lateral temporals are irregularly arranged or in one or two rows (occasionally three), the uppermost being dorso-ventrally elongate (Chapter 5). Jacobsen separated *transvaalensis* and *melanotus* mainly on the basis of what appeared to be distinct differences in colour pattern, and 2-3 rows of lateral temporals in *transvaalensis* versus 1-2 such rows in *melanotus*. *Pseudocordylus transvaalensis* is characterized by its large size, unique dorsal and gular (black) colour

patterns, usually three rows of horizontal temporals, and a series of small scales posterior to the interparietal (Appendix 2.1; Chapter 5).

Branch (1988a, 1998: 207) appears to follow De Waal (1978) with regard to the two subspecies of *P. melanotus*, but incorrectly states that in *subviridis* the “lateral scales” (probably in reference to the dorsolaterals) are “larger than the spaces between them” (this refers to the typical *melanotus* condition). Branch (1998) appears to follow Jacobsen’s (1989) concept of *P. transvaalensis*, but is wrong in stating that in *transvaalensis* the lateral (probably meaning dorsolateral) scales are smaller than the spaces between them (they are bigger – see Jacobsen 1989; Chapter 5), and that female *transvaalensis* have well developed femoral pores (the latter are shallow pits - paratypes examined, similar to female *melanotus*; Chapter 5). In addition, Branch (1998) did not plot Jacobsen’s (1989) isolated Gauteng sub-population of *P. m. melanotus*, or Broadley’s (1964) somewhat isolated Qudeni Forest record (2830DB) for this subspecies. Collections made during the course of the present study confirm the occurrence of this species in the latter two areas (see Appendix 2.1: B102-106, 165, 167-168).

Both *P. langi* and *P. spinosus* are easily distinguished from other members of the *P. melanotus* species complex (Broadley 1964; Branch 1998; as discussed above; Chapter 5).

## 2.7 Morphological differentiation between the *Pseudocordylus microlepidotus* and *P. melanotus* species complexes

Adult males referable to the two species complexes are readily distinguished by the presence (*P. microlepidotus* species complex) or absence (*P. melanotus* species complex) of generation glands on either side of the backbone (Van Wyk & Mouton 1992; Mouton *et al.* 2005). However, dorsal generation glands are usually fewer in number or absent in females and very young lizards (Mouton *et al.* 2005).

A total of 134 specimens referable to the *P. microlepidotus* species complex were examined for the presence or absence of dorsal generation glands (see catalogue numbers indicated by asterisks in Appendix 2.1 [but excluding unsexed adults SAM ZR859, 864,

873, 2020-1, 18306a & d, 18621a & b], locality numbers F3, 14, 16-17, 24-25, 27, 30, 33-34, 36-37, 40-45, 57, 59, 61, 64, 67, 80, 89, 116-117; G2, 7, 12, 15-16, 18-25, 29-32, 34-36, 40, 48, 52-56; H1-2, 6, 8; I1-6). Adults and juveniles were identified as noted above. Dorsal generation glands were present in *P. m. microlepidotus*: 100% of males ( $N = 23$ ), 13% of females ( $N = 16$ ), 60% of juveniles ( $N = 5$ ); *P. m. fasciatus*: 81% of males ( $N = 16$ ), 28% of females ( $N = 18$ ), 21% of juveniles ( $N = 24$ ); *P. m. namaquensis*: 100% of males ( $N = 3$ ), 43% of females ( $N = 7$ ), 100% of juveniles ( $N = 2$ ); *P. microlepidotus* “Transkei”: 78% of males ( $N = 9$ ), 0% of females ( $N = 7$ ), 17% of juveniles ( $N = 6$ ). A distinct longitudinal vertebral fold (see figs in Smith 1843) was often also present in specimens of *P. microlepidotus*. Dorsal generation glands were absent in all 552 specimens of the *P. melanotus* species complex examined (Appendix 2.1) and there was never a well-developed vertebral fold.

## 2.8 Geographical and altitudinal distribution

The geographical distribution of taxa in the *P. melanotus* and *P. microlepidotus* species complexes (Fig. 2.1) mirrors, to a large extent, the distribution of mountains comprising the Great Escarpment (Fig. 2.2). *Pseudocordylus microlepidotus microlepidotus* is widely distributed in the Western Cape Province and part of the adjacent Eastern Cape Province at elevations of 20-1920 m a.s.l. It occurs in all the main elements of the Cape Fold Mountains, including the Cedarberg, Dutoitskloofberg, Riviersonderendberg, Hexrivierberg, Langeberg, Anysberg, Kammanassieberg, Rooiberg, Swartberg, Outeniqua, Tsitsikama, Langkloof, Baviaanskloofberg, Kouga, Elandsberg, Great Wintershoekberg and Suurberg mountains (Fig. 2.1; Appendix 2.1). The eastern subspecies *P. m. fasciatus* occurs at 440-1900 m in the inland mountains of the Eastern Cape, including the Sneeuberg, Stormberg, Bamboesberg and Winterberg mountains, and Mount Arthur Range, with single known localities in the Northern Cape and Western Cape provinces; whereas *P. m. namaquensis* occurs at around 1600 m in the Nuweveldberg and Komsberg mountains in the Western Cape and Northern Cape provinces (Fig. 2.1; Appendix 2.1). The latter range is more-or-less continuous with the Roggeveldberg where this taxon has yet to be found. These three mountainous ranges are separated from other *Pseudocordylus* localities by large expanses of Karoo.



*Pseudocordylus transvaalensis* occurs in three allopatric populations (1700-2000 m) in Limpopo Province (Fig. 2.1; Appendix 2.1; Jacobsen 1989). The western-most population - in the vicinity of Thabazimbi - is restricted to the Waterberg Mountains and its outliers, namely the Sandriviersberg and Hoekberg mountains. This population is separated from the central population around Mokopane by low-lying areas formed by the Sterk River and its tributaries. The central population occupies mountainous terrain, including the Maribashoekberg, Buffelshoekberg and Highlands Mountains. It appears to be separated from the eastern population - in the Haenertsburg area - by the Chunies River valley (Chuniespoort) south of Polokwane. Further west the Highlands Mountains are also separated from the eastern population by the Nkumpi River and its tributaries. The eastern population occurs in the Strydpoortberg and Wolkberg mountains.

*Pseudocordylus melanotus melanotus* has an extensive and apparently largely continuous distribution from Mariepskop (Mpumalanga Province) in the north southwards through northern Swaziland, into north-western KwaZulu-Natal and north-eastern Free State (Fig. 2.1; Appendix 2.1). Jacobsen (1989) mis-identified *P. m. melanotus* from the locality "Selati" as *transvaalensis* and also plotted this record at locus 2430AB rather than 2430BA (according to his own gazetteer). He was of the opinion that *transvaalensis* and *melanotus* were separated by the dry and hot Olifants River valley that creates a lowland area west of Naphuno 2 district. If so, the Blyde River Canyon and Ohrigstad River valley may also be considered to have played a role in separating populations in this area. As the Selati lizards are referable to *melanotus* (see above), the latter species also occurs north of Mariepskop and only about 26 km SE of the nearest *transvaalensis* locality at Serala (2430AA). The Selati locality is probably referable to the vicinity of Orrie Baragwanath Pass (2430BA; also GaSelati River) in Legalameetse Nature Reserve on the eastern side of the escarpment, rather than Selati Ranch which is situated at altitudes as low as 500-700 m, *i.e.* well below the escarpment. This suggests that *melanotus* and *transvaalensis* occur parapatrically in the Wolkberg area. Neither species is expected to occur in the Lowveld away from the slopes of the escarpment in the north-east, but their absence from the area between Serala and Mariepskop (next nearest *melanotus* locality) - on either side of the Olifants River gap - is inexplicable and probably an artifact of collecting. The area consists of fairly inaccessible mountainous terrain. However, their absence may be due to competition with *Cordylus vandami* (FitzSimons 1930), a similar

sized species that has never been found in microsympatry with either *melanotus* or *transvaalensis* (see Jacobsen 1989).

Jacobsen (1989) alluded to the fact that *melanotus* occurs in three allopatric populations (north, south, west) in Mpumalanga and Gauteng provinces. However, in terms of altitude and habitat, the area between northern and southern populations in Mpumalanga appears to be suitable and new records are now available that at least partially fill the gap, which therefore may be an artifact of collecting. Jacobsen's (1989) northern populations of *melanotus* ("Northern *melanotus*" – see Chapter 4) in northern Mpumalanga include localities within the Mpumalanga Escarpment itself, as well as localities in broken, hilly country referred to as Barberton Mountainland (Bristow 1985) and adjacent areas including northern Swaziland (Figs 2.1 & 2.2). However, there are no clear indications of a break between the northern and southern populations of *melanotus*, nor between the Mpumalanga Escarpment proper and populations in the Lochiel area. The main "Southern *melanotus*" population (1400-2300 m) includes localities in Mpumalanga, north-western KwaZulu-Natal and the Free State. However, the population at Suikerbosrand and nearby areas (1500-1860 m) in the Balfour district of Gauteng does indeed appear to be geographically isolated as it is separated from other *melanotus* populations by Highveld Grassland as well as the Vaal River.

It should be noted that the "Transvaal Drakensberg" is not a northern extension of the "Natal Drakensberg". The two ranges belong to very different ages and geological systems. The "Natal Drakensberg" (generally referred to hereafter as Drakensberg) is the result of geologically recent Karoo deposits that have been continually eroded back, whereas the "Transvaal Drakensberg" (here referred to as Mpumalanga Escarpment) is much older and consists of different rock types (Bristow 1985).

The *P. m. melanotus* population in the Nkandhla district of central KwaZulu-Natal, found at altitudes of 1100-1500 m, also appears to be isolated (Appendix 2.1). While the localities plotted at loci 2730DD and 2731CD may be continuous with the main *melanotus* population – there are areas of 1500-1700 m between the latter populations – the Nkandhla localities appear to form a unit and are separated from the rest by a low-lying area with only occasional higher hills (Fig. 2.1).

The large gap in distribution between *melanotus* and *subviridis* in central KwaZulu-Natal (Fig. 2.1) coincides largely with areas classified physiographically as Basin Plainlands and Low-lying Regions that receive less than 800 mm mean annual rainfall (Bourquin 2004). The eastern-most *subviridis* locality (2.5 km NNE of Mooi River; Dansekop may be closer but cannot be pin-pointed on a map) is separated from the nearest *melanotus* locality (Nkonyane Mountain, Nkandhla district) by about 113 km (Fig. 2.1; Appendix 2.1). Although *subviridis* may occur at a few sites nearer to the Nkandhla population judging by the topography, the two taxa are separated in this area by the Tugela and Sundays River valleys.

The distribution map (Fig. 2.1) also suggests that there is an isolated population around Lindley in the Free State. However, high altitudes - and presumably suitable habitat - are found at loci 2827BD (up to 2003 m), 2828AB (1875 m) and 2828AC (2234 m), suggesting that this area is linked to the main *melanotus* population. The isolated *melanotus* locality in the south-eastern Free State (*i.e.* farm Ceylon, about 1500 m), if valid (see comments above), is hard to explain and is geographically much closer to known *subviridis* localities.

*Pseudocordylus melanotus subviridis* occurs in two allopatric populations, one in the Maloti-Drakensberg and associated areas (Lesotho, Free State, KwaZulu-Natal and Eastern Cape; 1400-3200 m) and another in the Amatole Mountains and vicinity (Eastern Cape; 1400-1600 but probably also higher) (Fig. 2.1; Appendix 2.1). The gap in distribution (about 200 km) between the Drakensberg and Amatole populations appears to be real. There are no literature records of *P. m. subviridis* from this area and intensive collecting by W.R. Branch (*pers. comm.*) during the 1980s failed to turn up any specimens. Although the area between these two populations contains rocky, mountainous habitat (*e.g.* Stormsberg Mountains, Mount Arthur Range, Bamboesberg Mountain), *P. m. subviridis* is replaced here by *P. microlepidotus fasciatus* (recorded at elevations of 900-1900 m in this area). The Amatole population includes localities on Menziesberg, Elandsberg and Xolora Mountains, and Katberg Mountain in the Didima Range. However, there is no obvious separation between the Katberg and Great Winterberg Mountains to the west. In fact, *fasciatus* and *subviridis* occur parapatrically on the farm Finella Falls (3226AD) in the latter area (W.R. Branch, *pers. comm.*; Appendix 2.1). One of the *fasciatus* (PEM R8651) appears to be an adult male, with

generation glands along the middle of the back and differentiated femoral glands, whereas the other (PEM R8652) is a banded juvenile; both have about three horizontal rows of lateral temporals on either side of the head and the dorsolaterals almost in contact. The *subviridis* specimens (PEM R8656-60) have 1-2 rows of lateral temporals (uppermost row with elongate scales) and spaces between longitudinal rows of dorsolaterals 0.5-0.8 times the width of adjacent dorsolaterals. All specimens of both species have undivided frontonasals, except PEM R8659 that has the posterior two-thirds divided.

Although *subviridis* occurs at generally higher elevations in the higher reaches of the Drakensberg compared to *melanotus*, the two taxa also occur at similar altitudes in Qwa-Qwa where they are parapatric (see also Chapters 4 & 5). At one locality, namely Monontsha Pass, specimens have in the past been assigned to *melanotus* and *subviridis*, as well as “intergrades” between the two subspecies (De Waal 1978).

*Pseudocordylus langi* is known from only a small, high elevation area (2805-3048 m) of the Drakensberg in the Mont-aux-Sources - Organ Pipes Pass area (KwaZulu-Natal, Free State, Lesotho; Fig. 2.1) where it is sympatric and even microsympatric with *subviridis* (Broadley 1964; M. Cunningham, pers. comm. 2005; Appendix 2.1). It may, however, occur in a more-or-less continuous band along the rim and summit of the escarpment from the Mont-aux-Sources area to at least the top of Sani Pass in Lesotho. There may be isolated populations of this species on unsampled mountain peaks such as Sentinel and Inner Tower.

*Pseudocordylus spinosus* occurs on the lower (900 m) to middle (2517 m) slopes of the Drakensberg in KwaZulu-Natal and the Free State (Fig. 2.1; Appendix 2.1). Isolated records in southern KwaZulu-Natal require confirmation (see above). It is sympatric, but not known to be microsympatric, with *subviridis* over parts of its range (Fig. 2.1).

## CHAPTER 3

### **An allozyme electrophoretic analysis of the *Pseudocordylus melanotus* (Smith, 1838) species complex (Sauria: Cordylidae)**

#### **3.1 Introduction**

Until recently three subspecies of *Pseudocordylus melanotus* Smith, 1838 were recognized, namely *P. melanotus melanotus*, *P. m. subviridis* Smith, 1838 and *P. m. transvaalensis* FitzSimons, 1943. *Pseudocordylus transvaalensis* is now considered a valid species closely allied to *P. melanotus* (Jacobsen 1989; Branch 1998). Today one of the most pressing taxonomic problems in the genus is the status of taxa in the *P. melanotus* complex, *i.e.* *P. m. melanotus*, *P. m. subviridis*, *P. transvaalensis* and *P. langi*. The geographical distribution of these taxa was discussed in detail in section 2.8 of Chapter 2 and is illustrated in Fig. 2.1.

Previous attempts to separate species and subspecies in the *P. melanotus* species complex on the basis of morphology (*e.g.* scales, size, colour) have resulted in different and often incompatible taxonomic arrangements (*e.g.* FitzSimons 1943; Loveridge 1944; Broadley 1964; De Waal 1978; Jacobsen 1989). It is clear that morphological characters alone are insufficient to evaluate the taxonomic status of the currently recognised forms in the *P. melanotus* complex and that the use of molecular data is required. The use of both morphological and molecular data will result in better descriptions and interpretations of biological diversity (Hillis 1987; Moritz & Hillis 1996). Molecular approaches to analyzing phylogenetic relationships are particularly enlightening in cases of limited morphological variation (Moritz & Hillis 1996). As a first approach, enzyme electrophoresis was used to generate a molecular data set for the *P. melanotus* species complex.

Enzyme electrophoresis is a powerful molecular tool for detection of morphologically cryptic species and as a diagnostic marker for a priori identification of taxa, and has been used with great success in a wide range of animal taxa (Hillis, Mable & Moritz 1996;

Murphy *et al.* 1996). Several allozyme studies have already been conducted to resolve taxonomic uncertainty in southern African reptile taxa, usually involving species groups for which morphological data alone was not sufficient to resolve taxonomic problems. Examples include the study by Brody *et al.* (1993) on the *C. cordylus-oelofseni-niger* complex, several studies in which species were separated by three or more fixed allelic differences, corroborated by morphological differentiation (*Phelsuma* Roux, 1907: Good & Bauer 1995; “*Phyllodactylus*”: Branch, Bauer & Good 1995; Good, Bauer & Branch 1996; Bauer, Good & Branch 1997; large-bodied *Pachydactylus* Wiegmann, 1834: Branch, Bauer & Good 1996; *Rhoptropus* Peters, 1869: Bauer & Good 1996), Flemming’s (1996) analysis of the *Agama atra* Daudin, 1802 species complex which identified two genetic assemblages based on allele frequency differences (no fixed differences), corroborated by morphology and reproductive ecology, and a study by Mouton, Nieuwoudt, Badenhorst & Flemming (2002) that found a total lack of allozyme variation (all 33 loci were monomorphic) in melanistic populations of *Cordylus polyzonus* A. Smith, 1838.

The aims of the allozyme electrophoretic study were, firstly, to evaluate the taxonomic status of taxa within the *P. melanotus* complex, *i.e.* *P. m. melanotus*, *P. m. subviridis*, *P. transvaalensis* and *P. langi*; secondly, to assign morphologically intermediate populations to the correct taxa; and thirdly, to determine whether interbreeding occurs between parapatric populations of *P. m. melanotus* and *P. m. subviridis*.

## 3.2 Materials and Methods

### 3.2.1 Sampling

Because of their unresolved taxonomic status the geographical ranges of the various forms in the *P. melanotus* complex have been confused. Before selecting collecting sites it was therefore imperative to gain a more meaningful insight into the distribution of both the *P. melanotus* species complex and the closely related *P. microlepidotus* species complex. A thorough revision of the literature yielded numerous records and to these were added an even larger number of additional records obtained from museums and private collections (Appendix 2.1). Figure 2.1 is therefore most probably a fair

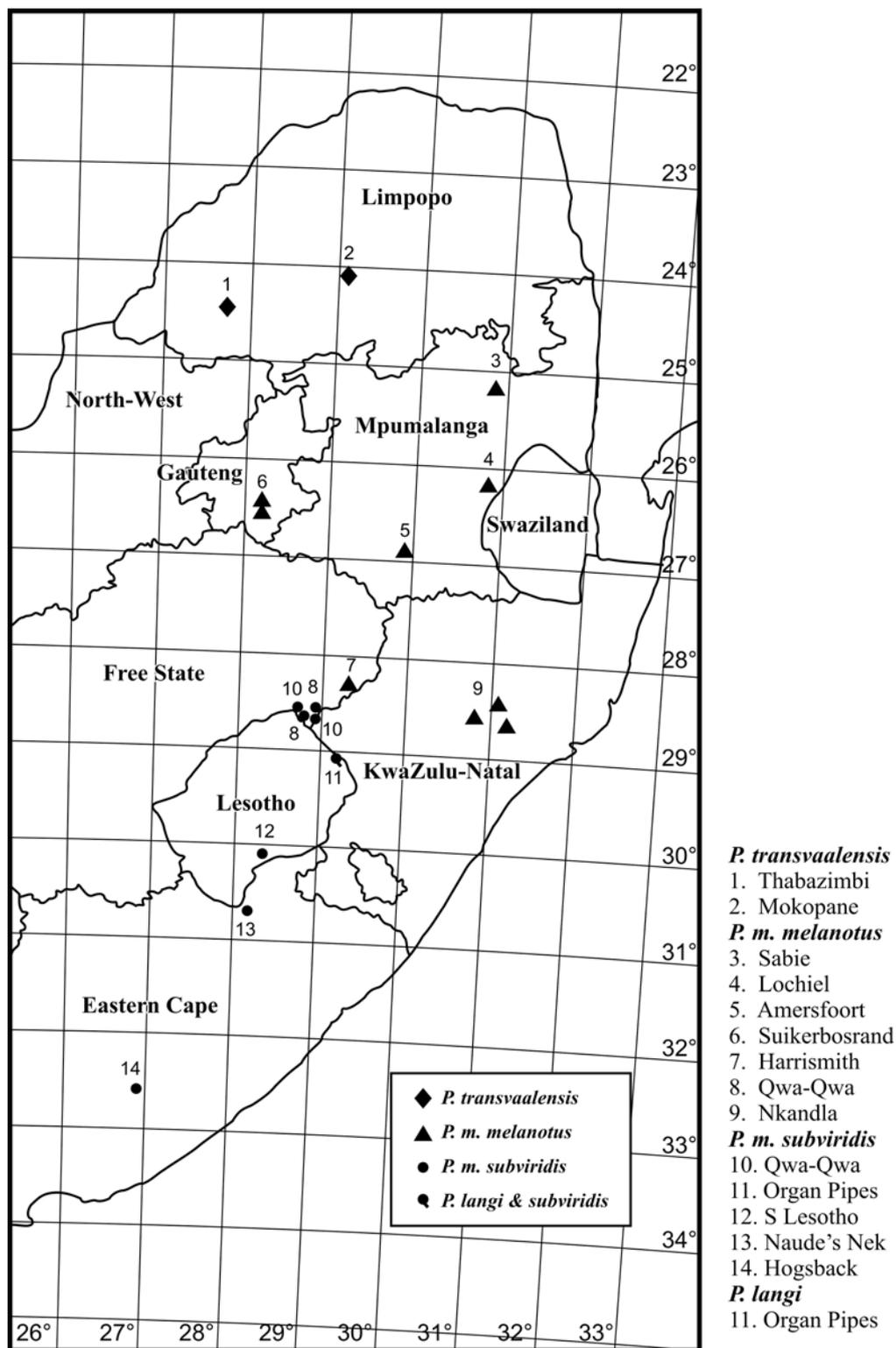
representation of the true geographical distribution of populations and known taxa in the two species complexes. Sampling sites were selected using this map.

A total of 232 lizards were collected from 14 localities from December 1998 to November 2000 (Fig. 3.1; Appendix 3.1). *Pseudocordylus melanotus subviridis* and *P. langi* were collected in sympatry at Organ Pipes Pass. Therefore, a total of 15 populations were sampled. Localities selected were spread across the ranges of the four taxa (*P. transvaalensis*, *P. m. melanotus*, *P. m. subviridis*, *P. langi*) and include the apparently isolated populations of *melanotus* at Suikerbosrand and in Nkandhla district, and the Hogsback population of *P. m. subviridis* isolated in the Amatole and Winterberg Mountains (see Appendix 3.1). Two of the three allopatric *transvaalensis* populations were sampled, namely Western and Central. Morphological character variation for the 15 populations is summarized in Tables 3.1 and 3.2. Definitions of the various characters are provided in Appendix 5.2.

Specimens collected at localities within a supposed contact zone between *P. m. melanotus* and *P. m. subviridis* were sometimes difficult to assign to either taxon. Although most specimens collected at Monontsha Pass were identified morphologically as *subviridis*, some were *melanotus*-like and a few were intermediate (see also Chapter 5). Specimens from Qoqolosing and Thibella in Qwa-Qwa were identified as *melanotus* (and grouped with *melanotus* in the allozyme analysis), but some were difficult to assign based on morphology. Qoqolosing: NMB R8359, 8360 and 8362 were *melanotus*-like: lateral temporals arranged in two rows with the upper row consisting mainly of elongated scales, frontonasal divided, dorsolaterals closely-spaced (less than one-half scale width separating rows), male (NMB R8359) with only 13 differentiated glandular femoral scales on both thighs, female (NMB R8362) with pit-like femoral scales lacking secretions. NMB R8361 (juvenile) was similar but had three rows of temporals (the middle row with the most elongated scales) and the dorsolaterals were slightly more widely separated (spaces about equal to width of adjacent scales). However, while NMB R8363 (male, 13) had most of the characteristics of the male *P. m. melanotus* described above, it had an undivided frontonasal (typical of *subviridis*). NMB R8364 also had an undivided frontonasal and the dorsolaterals were slightly more widely separated as described above, although the temporals were in two rows (see above). Thibella: NMB R8365 (female) has the lateral temporals arranged in a single row of elongated scales,

frontonasal undivided, spaces between rows of dorsolaterals about equal to width of adjacent scales, and femoral pores distinct with secretions. It could be argued that this specimen is more *P. m. subviridis*-like than *P. m. melanotus*, but it groups with other *P. m. melanotus* in the allozyme analysis (see Table 3.3).

Specimens were euthanased by hypodermic injection of sodium pentobarbitone compound to the cardiac region 2-7 days after capture. Whole animals were then stored at  $-70^{\circ}\text{C}$  in an ultra-cold freezer at the University of the Free State (Bloemfontein). They were later de-frosted, dissected and sections of liver and thigh muscle excised, placed in 3.6 or 4.5 ml cryotubes and immersed in liquid nitrogen ( $-196^{\circ}\text{C}$ ). Tissue samples were then transported to the University of Stellenbosch where they were transferred to an ultra-cold freezer ( $-80^{\circ}\text{C}$ ). Dissected lizards were returned to the freezer at the University of the Free State and later transferred to the National Museum (Bloemfontein) where they were accessioned and preserved directly in 70% ethanol.



**Figure 3.1:** Geographical distribution of localities for the allozyme electrophoretic analysis of the *Pseudocordylus melanotus* species complex. *P. melanotus subviridis* and *P. langi* were collected in sympatry at locality 11. Numbers refer to localities listed in detail in Appendix 3.1.

**Table 3.1:** Qualitative characters for 15 populations (seven lineages) in the *Pseudocordylus melanotus* species complex used in allozyme electrophoresis.

Character	Thabazimbi ( <i>P. transvaalensis</i> )	Mokopane ( <i>P. transvaalensis</i> )	Sabie ( <i>P. m. melanotus</i> )	Lochiel ( <i>P. m. melanotus</i> )	Amersfoort ( <i>P. m. melanotus</i> )	Suikerbosrand ( <i>P. m. melanotus</i> )	Harrismith ( <i>P. m. melanotus</i> )	Qwa-Qwa ( <i>P. m. melanotus</i> )	Nkandla ( <i>P. m. melanotus</i> )	Qwa-Qwa ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. m. subviridis</i> )	Naude's Nek ( <i>P. m. subviridis</i> )	Lesotho ( <i>P. m. subviridis</i> )	Hogsback ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. langi</i> )
Lineage	1	1	6	7	4	4	4	4	1	3	3	3	2	2	5
Femoral pores in females Pore-like: Pit-like:	<i>N</i> = 6 100%	<i>N</i> = 6 100%	<i>N</i> = 11 100%	<i>N</i> = 6 100%	<i>N</i> = 5 100%	<i>N</i> = 7 100%	<i>N</i> = 14 100%	<i>N</i> = 2 50% 50%	<i>N</i> = 14 100%	<i>N</i> = 8 88% 13%	<i>N</i> = 9 33% 67%	<i>N</i> = 13 100%	<i>N</i> = 4 100%	<i>N</i> = 9 56% 44%	<i>N</i> = 1 100%
Frontonasal Wider than long: As wide as long: Longer than wide:	<i>N</i> = 15 93% 7%	<i>N</i> = 14 100%	<i>N</i> = 21 57% 29% 14%	<i>N</i> = 11 100%	<i>N</i> = 9 100%	<i>N</i> = 15 100%	<i>N</i> = 20 100%	<i>N</i> = 7 86% 14%	<i>N</i> = 23 100%	<i>N</i> = 24 100%	<i>N</i> = 15 100%	<i>N</i> = 23 96% 4%	<i>N</i> = 10 100%	<i>N</i> = 20 90% 10%	<i>N</i> = 5 100%
Small scale between frontonasal and frontal Present: Absent:	<i>N</i> = 14 43% 57%	<i>N</i> = 14 43% 57%	<i>N</i> = 20 60% 40%	<i>N</i> = 11 18% 82%	<i>N</i> = 9 78% 22%	<i>N</i> = 15 47% 53%	<i>N</i> = 19 16% 84%	<i>N</i> = 7 43% 57%	<i>N</i> = 23 30% 70%	<i>N</i> = 24 4% 96%	<i>N</i> = 15 100%	<i>N</i> = 23 9% 91%	<i>N</i> = 10 100%	<i>N</i> = 20 100%	<i>N</i> = 5 100%
Frontonasal separates supranasals Yes: No:	<i>N</i> = 15 93% 7%	<i>N</i> = 14 71% 29%	<i>N</i> = 21 33% 68%	<i>N</i> = 11 100%	<i>N</i> = 8 38% 63%	<i>N</i> = 15 40% 60%	<i>N</i> = 20 10% 90%	<i>N</i> = 7 100%	<i>N</i> = 23 4% 96%	<i>N</i> = 24 4% 96%	<i>N</i> = 15 7% 93%	<i>N</i> = 23 4% 96%	<i>N</i> = 10 10% 90%	<i>N</i> = 20 100%	<i>N</i> = 5 100%
Frontonasal Divided: Partly-divided: Undivided:	<i>N</i> = 15 87% 7% 7%	<i>N</i> = 14 21% 50% 29%	<i>N</i> = 21 24% 14% 62%	<i>N</i> = 11 18% 9% 73%	<i>N</i> = 9 89% 11%	<i>N</i> = 15 100%	<i>N</i> = 19 95% 5%	<i>N</i> = 7 57% 43%	<i>N</i> = 23 57% 35% 9%	<i>N</i> = 24 4% 96%	<i>N</i> = 14 100%	<i>N</i> = 23 100%	<i>N</i> = 10 100%	<i>N</i> = 20 100%	<i>N</i> = 5 20% 20% 60%
Frontonasal in contact with loreal Yes: No:	<i>N</i> = 15 100%	<i>N</i> = 14 100%	<i>N</i> = 21 100%	<i>N</i> = 11 100%	<i>N</i> = 9 100%	<i>N</i> = 14 100%	<i>N</i> = 18 100%	<i>N</i> = 7 100%	<i>N</i> = 23 100%	<i>N</i> = 23 100%	<i>N</i> = 15 100%	<i>N</i> = 22 91% 9%	<i>N</i> = 9 100%	<i>N</i> = 19 95% 5%	<i>N</i> = 5 100%
Anterior frontal scale Present: Absent:	<i>N</i> = 15 47% 53%	<i>N</i> = 14 79% 21%	<i>N</i> = 21 100%	<i>N</i> = 11 100%	<i>N</i> = 9 33% 67%	<i>N</i> = 15 100%	<i>N</i> = 20 100%	<i>N</i> = 7 100%	<i>N</i> = 22 100%	<i>N</i> = 24 100%	<i>N</i> = 15 100%	<i>N</i> = 23 100%	<i>N</i> = 10 100%	<i>N</i> = 20 100%	<i>N</i> = 5 100%

**Table 3.1** (continued): Qualitative characters for 15 populations (seven lineages) in the *Pseudocordylus melanotus* species complex used in allozyme electrophoresis.

Character	Thabazimbi ( <i>P. transvaalensis</i> )	Mokopane ( <i>P. transvaalensis</i> )	Sabie ( <i>P. m. melanotus</i> )	Lochiel ( <i>P. m. melanotus</i> )	Amersfoort ( <i>P. m. melanotus</i> )	Suikerbosrand ( <i>P. m. melanotus</i> )	Harrismith ( <i>P. m. melanotus</i> )	Qwa-Qwa ( <i>P. m. melanotus</i> )	Nkandla ( <i>P. m. melanotus</i> )	Qwa-Qwa ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. m. subviridis</i> )	Naude's Nek ( <i>P. m. subviridis</i> )	Lesotho ( <i>P. m. subviridis</i> )	Hogsback ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. langi</i> )
Lineage	1	1	6	7	4	4	4	4	1	3	3	3	2	2	5
Anterior parietal scales Both fully divided: Partly or one divided: Both undivided:	<i>N</i> = 15 47% 40% 13%	<i>N</i> = 14 7% 21% 71%	<i>N</i> = 21  100%	<i>N</i> = 11  100%	<i>N</i> = 9  11% 89%	<i>N</i> = 15  100%	<i>N</i> = 20  100%	<i>N</i> = 7  100%	<i>N</i> = 22  100%	<i>N</i> = 24  100%	<i>N</i> = 15  100%	<i>N</i> = 23  100%	<i>N</i> = 10  100%	<i>N</i> = 20  100%	<i>N</i> = 5  100%
Size of median dorsals in relation to dorsolaterals >0.5 ≤0.5	<i>N</i> = 15  100%	<i>N</i> = 14  100%	<i>N</i> = 21  5% 95%	<i>N</i> = 11  100%	<i>N</i> = 9  11% 89%	<i>N</i> = 15  100%	<i>N</i> = 19  100%	<i>N</i> = 7  14% 86%	<i>N</i> = 22  100%	<i>N</i> = 24  92% 8%	<i>N</i> = 15  100%	<i>N</i> = 23  91% 9%	<i>N</i> = 10  100%	<i>N</i> = 20  15% 85%	<i>N</i> = 5  100%
Size of lateral dorsals in relation to dorsolaterals ≥0.75 <0.75	<i>N</i> = 15  100%	<i>N</i> = 14  100%	<i>N</i> = 21  100%	<i>N</i> = 11  9% 91%	<i>N</i> = 9  11% 89%	<i>N</i> = 15  33% 67%	<i>N</i> = 19  21% 79%	<i>N</i> = 7  43% 57%	<i>N</i> = 23  83% 17%	<i>N</i> = 24  96% 4%	<i>N</i> = 15  87% 13%	<i>N</i> = 23  57% 44%	<i>N</i> = 10  60% 40%	<i>N</i> = 20  5% 95%	<i>N</i> = 5  60% 40%
Size of dorsolaterals in relation to median dorsals Larger: Smaller:	<i>N</i> = 15  100%	<i>N</i> = 14  100%	<i>N</i> = 21  100%	<i>N</i> = 11  100%	<i>N</i> = 9  100%	<i>N</i> = 15  100%	<i>N</i> = 19  100%	<i>N</i> = 7  100%	<i>N</i> = 23  100%	<i>N</i> = 24  100%	<i>N</i> = 15  100%	<i>N</i> = 23  100%	<i>N</i> = 10  100%	<i>N</i> = 20  100%	<i>N</i> = 5  100%
Size of horizontal interspaces between dorsolaterals compared to adjacent scales Equal to larger: >0.5 ≤0.5 In contact (granular scales):	<i>N</i> = 15  100%	<i>N</i> = 14  14% 86%	<i>N</i> = 21  71% 29%	<i>N</i> = 11  27% 73%	<i>N</i> = 9  100%	<i>N</i> = 15  100%	<i>N</i> = 20  30% 70%	<i>N</i> = 7  43% 57%	<i>N</i> = 23  4% 96%	<i>N</i> = 24  42% 42% 17%	<i>N</i> = 15  60% 40%	<i>N</i> = 23  22% 78%	<i>N</i> = 10  100%	<i>N</i> = 20  25% 75%	<i>N</i> = 5  100%
Gular colour pattern Parallel pair of dark stripes: Y-shaped dark marking: Black:	<i>N</i> = 15  100%	<i>N</i> = 14  100%	<i>N</i> = 21  95% 5%	<i>N</i> = 11  64% 36%	<i>N</i> = 9  100%	<i>N</i> = 15  100%	<i>N</i> = 20  100%	<i>N</i> = 7  100%	<i>N</i> = 22  100%	<i>N</i> = 24  100%	<i>N</i> = 15  100%	<i>N</i> = 20  100%	<i>N</i> = 10  100%	<i>N</i> = 18  100%	<i>N</i> = 5  100%
Texture posterior infralabial Keel: Ridged: Smooth:	<i>N</i> = 15  100%	<i>N</i> = 14  86% 14%	<i>N</i> = 21  91% 5% 5%	<i>N</i> = 11  100%	<i>N</i> = 8  88% 13%	<i>N</i> = 15  100%	<i>N</i> = 20  75% 25%	<i>N</i> = 7  100%	<i>N</i> = 21  100%	<i>N</i> = 23  96% 4%	<i>N</i> = 14  43% 57%	<i>N</i> = 23  100%	<i>N</i> = 10  100%	<i>N</i> = 19  100%	<i>N</i> = 5  100%

**Table 3.2:** Meristic scale characters for 15 populations (seven lineages) in the *Pseudocordylus melanotus* species complex used in allozyme electrophoresis.

Character	Thabazimbi ( <i>P. transvaalensis</i> )	Mokopane ( <i>P. transvaalensis</i> )	Sabie ( <i>P. m. melanotus</i> )	Lochiel ( <i>P. m. melanotus</i> )	Amersfoort ( <i>P. m. melanotus</i> )	Suikerbosrand ( <i>P. m. melanotus</i> )	Hartismith ( <i>P. m. melanotus</i> )	Qwa-Qwa ( <i>P. m. melanotus</i> )	Nkandla ( <i>P. m. melanotus</i> )	Qwa-Qwa ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. m. subviridis</i> )	Naude's Nek ( <i>P. m. subviridis</i> )	Lesotho ( <i>P. m. subviridis</i> )	Hogsback ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. langi</i> )
Lineage	1	1	6	7	4	4	4	4	1	3	3	3	2	2	5
Upper temporals	5.9 ± 0.26 5-6 (15)	6.00 ± 0.00 6 (14)	6.1 ± 0.44 6-8 (21)	6.0 ± 0.00 6 (11)	6.0 ± 0.00 6 (9)	6.1 ± 0.26 6-7 (15)	6.0 ± 0.00 6 (20)	6.0 ± 0.00 6 (7)	6.0 ± 0.00 6 (23)	6.1 ± 0.41 6-8 (24)	6.00 ± 0.00 6 (15)	6.0 ± 0.00 6 (22)	6.3 ± 0.67 6-8 (10)	6.0 ± 0.00 6 (20)	5.8 ± 0.45 5-6 (5)
Horizontal rows of temporals	6.3 ± 0.70 6-8 (15)	5.3 ± 0.99 4-6 (14)	4.5 ± 0.68 4-6 (21)	4.0 ± 0.00 4 (11)	4.0 ± 0.00 4 (9)	4.0 ± 0.38 3-5 (15)	4.0 ± 0.00 4 (20)	4.3 ± 0.76 4-6 (7)	4.0 ± 0.21 4-5 (23)	3.0 ± 0.86 2-4 (24)	2.9 ± 0.99 2-4 (15)	2.4 ± 0.78 2-4 (23)	2.8 ± 1.03 2-4 (10)	3.4 ± 0.81 2-4 (20)	2.4 ± 0.55 2-3 (5)
Supraoculars	8.0 ± 0.00 8 (15)	8.0 ± 0.00 8 (14)	8.5 ± 0.68 8-10 (21)	8.0 ± 0.00 8 (11)	8.0 ± 0.00 8 (9)	8.0 ± 0.00 8 (15)	8.1 ± 0.31 8-9 (20)	8.0 ± 0.00 8 (7)	8.0 ± 0.00 8 (23)	8.0 ± 0.00 8 (24)	8.0 ± 0.00 8 (15)	8.0 ± 0.00 8 (23)	8.0 ± 0.00 8 (10)	8.0 ± 0.00 8 (20)	8.0 ± 0.00 8 (5)
Supraciliaries	10.2 ± 0.41 10-11 (15)	10.2 ± 0.43 10-11 (14)	10.1 ± 0.44 10-12 (21)	10.1 ± 0.30 10-11 (11)	10.2 ± 0.44 10-11 (9)	10.0 ± 0.38 9-11 (15)	10.0 ± 0.32 9-11 (20)	10.0 ± 0.00 10 (7)	10.0 ± 0.00 10 (23)	10.1 ± 0.28 10-11 (24)	10.4 ± 0.74 10-12 (15)	10.2 ± 0.52 10-12 (23)	10.0 ± 0.00 10 (10)	10.1 ± 0.45 10-12 (20)	10.2 ± 0.45 10-11 (5)
Suboculars anterior to median subocular	2.3 ± 0.72 2-4 (15)	2.0 ± 0.00 2 (14)	2.1 ± 0.36 2-3 (21)	2.5 ± 1.04 2-5 (11)	2.1 ± 0.33 2-3 (9)	2.1 ± 0.27 2-3 (14)	2.0 ± 0.00 2 (20)	2.1 ± 0.38 2-3 (7)	2.1 ± 0.42 2-4 (23)	2.1 ± 0.34 2-3 (24)	2.1 ± 0.52 2-4 (15)	2.1 ± 0.42 2-4 (23)	2.1 ± 0.32 2-3 (10)	2.1 ± 0.31 2-3 (20)	2.0 ± 0.00 2 (5)
Suboculars posterior to median subocular	4.0 ± 0.38 3-5 (15)	4.4 ± 0.94 3-6 (14)	2.5 ± 0.60 2-4 (21)	2.6 ± 0.81 2-4 (11)	2.2 ± 0.44 2-3 (9)	2.0 ± 0.00 2 (14)	2.3 ± 0.55 2-4 (20)	2.0 ± 0.00 2 (7)	2.2 ± 0.52 2-4 (23)	2.4 ± 0.65 2-4 (24)	2.1 ± 0.52 2-4 (15)	2.0 ± 0.00 2 (23)	2.2 ± 0.42 2-3 (10)	2.0 ± 0.00 2 (20)	2.0 ± 0.00 2 (5)
Supralabials	8.6 ± 0.63 8-10 (15)	8.3 ± 0.47 8-9 (14)	8.5 ± 0.75 8-10 (21)	8.8 ± 1.08 8-11 (11)	8.3 ± 0.71 8-10 (9)	8.1 ± 0.59 7-10 (15)	8.2 ± 0.49 8-10 (20)	8.1 ± 0.38 8-9 (7)	8.5 ± 0.67 8-10 (23)	8.5 ± 0.72 8-10 (24)	8.0 ± 0.53 7-9 (15)	8.0 ± 0.47 7-9 (23)	8.2 ± 0.63 8-10 (10)	7.9 ± 0.49 6-8 (20)	8.0 ± 0.00 8 (5)
Infralabials	12.1 ± 0.26 12-13 (15)	12.4 ± 0.63 12-14 (14)	12.0 ± 0.00 12 (21)	12.0 ± 0.00 12 (11)	12.1 ± 0.33 12-13 (9)	11.9 ± 0.35 11-12 (15)	11.9 ± 0.45 10-12 (20)	12.0 ± 0.00 12 (7)	12.0 ± 0.60 11-14 (23)	12.0 ± 0.36 11-13 (24)	12.0 ± 0.38 11-13 (15)	11.9 ± 0.42 10-12 (23)	12.1 ± 0.32 12-13 (10)	12.0 ± 0.56 10-13 (20)	10.0 ± 0.00 10 (5)
Sublabials	10.9 ± 0.96 10-13 (15)	10.8 ± 1.05 10-13 (14)	10.0 ± 0.00 10 (21)	10.0 ± 0.00 10 (11)	10.2 ± 0.67 10-12 (9)	10.0 ± 0.00 10 (15)	10.0 ± 0.00 10 (20)	10.0 ± 0.00 10 (7)	10.0 ± 0.00 10 (23)	10.0 ± 0.20 10-11 (24)	10.0 ± 0.00 10 (15)	10.0 ± 0.00 10 (23)	10.0 ± 0.00 10 (10)	10.0 ± 0.00 10 (20)	9.8 ± 0.45 9-10 (5)
Gulars in contact with anterior sublabials	2.0 ± 0.00 2 (15)	2.1 ± 0.36 2-3 (14)	2.0 ± 0.22 2-3 (21)	2.4 ± 0.67 2-4 (11)	2.8 ± 1.09 2-5 (9)	2.3 ± 0.59 2-4 (15)	2.3 ± 0.45 2-3 (19)	2.4 ± 0.79 2-4 (7)	2.7 ± 0.71 2-4 (23)	2.5 ± 0.78 2-4 (24)	2.3 ± 0.59 2-4 (15)	2.2 ± 0.60 2-4 (23)	2.0 ± 0.00 2 (10)	2.9 ± 1.09 2-5 (20)	2.6 ± 0.89 2-4 (5)
Gulars across throat	32.9 ± 1.96 29-37 (15)	31.2 ± 2.58 27-36 (14)	27.3 ± 2.05 23-32 (21)	24.7 ± 1.83 23-28 (10)	25.2 ± 0.97 24-27 (9)	24.7 ± 1.68 21-28 (15)	25.3 ± 2.5 22-33 (20)	24.7 ± 2.06 21-27 (7)	26.8 ± 2.48 22-32 (23)	28.5 ± 2.41 25-34 (24)	27.3 ± 2.09 25-33 (15)	28.5 ± 2.76 23-32 (23)	30.2 ± 2.90 25-35 (10)	28.4 ± 2.48 23-34 (18)	27.8 ± 3.63 25-34 (5)
Occipitals	8.5 ± 1.13 7-10 (11)	8.0 ± 0.85 7-9 (12)	7.8 ± 1.09 6-10 (21)	10.0 ± 1.10 8-12 (11)	7.0 ± 1.00 6-8 (9)	9.1 ± 1.68 6-12 (15)	8.8 ± 1.32 7-11 (19)	8.7 ± 0.95 7-10 (7)	9.5 ± 1.26 7-12 (22)	7.8 ± 1.59 5-11 (24)	9.3 ± 1.84 7-13 (15)	7.4 ± 1.04 6-10 (23)	7.0 ± 1.25 6-9 (10)	8.0 ± 1.62 6-11 (20)	0.0 ± 0.00 0 (5)
Small scales behind interparietal	7.0 ± 2.42 2-11 (15)	6.9 ± 1.51 5-10 (14)	0.4 ± 0.92 0-4 (21)	0.0 ± 0.00 0 (11)	0.9 ± 1.05 0-2 (9)	0.1 ± 0.26 0-1 (15)	0.2 ± 0.49 0-2 (20)	0.4 ± 1.13 0-3 (7)	0.36 ± 0.45 0-1 (23)	0.4 ± 0.78 0-3 (24)	0.3 ± 0.80 0-3 (15)	0.2 ± 0.42 0-1 (23)	0.0 ± 0.00 0 (10)	0.0 ± 0.00 0 (20)	0.0 ± 0.00 0 (5)
Transverse rows of dorsals	43.7 ± 2.38 40-47 (15)	43.7 ± 2.46 39-48 (14)	50.8 ± 2.76 48-57 (21)	46.9 ± 2.51 43-50 (11)	44.4 ± 2.19 41-47 (9)	42.2 ± 2.04 40-47 (15)	42.9 ± 2.47 37-47 (20)	48.0 ± 3.46 43-53 (7)	44.5 ± 3.16 40-53 (23)	50.0 ± 4.81 42-59 (24)	49.3 ± 3.89 41-56 (15)	48.0 ± 2.64 43-52 (23)	48.6 ± 3.66 43-54 (10)	44.6 ± 2.95 40-50 (20)	0.0 ± 0.00 0 (5)

**Table 3.2** (continued): Meristic scale characters for 15 populations (seven lineages) in the *Pseudocordylus melanotus* species complex used in allozyme electrophoresis.

Character	Thabazimbi ( <i>P. transvaalensis</i> )	Mokopane ( <i>P. transvaalensis</i> )	Sabie ( <i>P. m. melanotus</i> )	Loehiel ( <i>P. m. melanotus</i> )	Amersfoort ( <i>P. m. melanotus</i> )	Suikerbosrand ( <i>P. m. melanotus</i> )	Harrismith ( <i>P. m. melanotus</i> )	Owa-Owa ( <i>P. m. melanotus</i> )	Nkandla ( <i>P. m. melanotus</i> )	Owa-Owa ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. m. subviridis</i> )	Naude's Nek ( <i>P. m. subviridis</i> )	Lesotho ( <i>P. m. subviridis</i> )	Hogsback ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. langi</i> )
Lineage	1	1	6	7	4	4	4	4	1	3	3	3	2	2	5
Longitudinal rows of dorsals	43.2 ± 2.98 40-50 (15)	46.2 ± 3.53 42-53 (14)	44.4 ± 3.76 40-52 (21)	42.6 ± 3.29 37-50 (11)	44.9 ± 1.90 41-47 (9)	37.7 ± 2.43 34-42 (15)	39.2 ± 2.39 36-46(20)	37.3 ± 2.21 34-41-(7)	39.4 ± 2.57 34-44 (23)	35.3 ± 4.51 27-43 (24)	34.7 ± 3.85 30-45 (15)	36.5 ± 2.95 32-44 (23)	36.9 ± 3.11 33-41-(10)	40.7 ± 2.56 36-46-(20)	0.0 ± 0.00 0 (5)
Transverse rows of ventrals	29.9 ± 1.46 28-33 (15)	29.1 ± 0.95 28-31 (14)	29.4 ± 0.81 28-31 (21)	29.1 ± 0.70 28-30 (11)	28.9 ± 0.93 27-30 (9)	29.5 ± 1.25 27-32 (15)	29.5 ± 1.39 26-32 (20)	30.9 ± 0.90 30-32 (7)	29.3 ± 1.06 28-31 (23)	28.8 ± 0.96 26-30 (24)	27.9 ± 1.19 26-30 (15)	28.9 ± 0.90 27-31 (23)	29.4 ± 0.84 28-31 (10)	28.2 ± 1.11 26-30 (20)	30.4 ± 1.34 29-32 (5)
Longitudinal rows of ventrals	13.13 ± 0.99 12-14 (15)	12.3 ± 0.73 12-14 (14)	12.0 ± 0.00 12 (21)	12.0 ± 0.00 12 (11)	12.0 ± 0.00 12 (9)	12.0 ± 0.00 12 (15)	12.2 ± 0.67 11-14 (20)	12.6 ± 0.98 12-14 (7)	12.6 ± 0.94 12-14 (23)	12.6 ± 0.93 12-14 (24)	12.4 ± 0.83 12-14 (15)	12.5 ± 0.90 12-14 (23)	13.4 ± 0.97 12-14 (10)	12.6 ± 0.94 12-14 (20)	12.0 ± 0.00 12 (5)
Lamellae under 4 <sup>th</sup> finger	14.6 ± 1.01 13-16 (14)	14.9 ± 0.95 13-16 (14)	17.7 ± 0.96 15-19 (21)	15.2 ± 1.08 14-17 (11)	15.4 ± 0.88 14-17 (9)	16.6 ± 0.83 15-18 (15)	15.9 ± 0.99 14-18 (20)	15.7 ± 1.60 14-19 (7)	15.7 ± 1.26 13-18 (23)	17.0 ± 1.25 15-19 (24)	17.1 ± 1.10 15-18 (15)	16.3 ± 1.06 14-18 (23)	15.6 ± 0.84 15-17 (10)	15.5 ± 1.00 14-17 (20)	17.8 ± 0.84 17-19 (5)
Lamellae under 4 <sup>th</sup> toe	19.5 ± 0.99 18-22 (15)	19.4 ± 1.15 17-21 (14)	22.4 ± 1.31 19-25 (20)	19.4 ± 1.03 18-21 (11)	18.4 ± 0.73 18-20 (9)	20.2 ± 1.47 18-23 (15)	19.6 ± 1.43 18-23 (20)	19.6 ± 1.72 17-22 (7)	19.7 ± 1.27 18-22 (23)	20.4 ± 2.04 17-25 (24)	20.9 ± 1.58 18-23 (15)	19.7 ± 1.40 18-22 (23)	19.1 ± 1.05 18-21 (9)	19.4 ± 1.18 17-21 (20)	22.6 ± 1.14 21-24 (5)
Femoral pores (all specimens)	14.1 ± 1.10 13-17 (15)	13.1 ± 1.35 10-15 (14)	5.8 ± 0.98 4-8 (29)	5.8 ± 0.98 4-8 (29)	15.8 ± 1.09 15-18 (9)	15.8 ± 1.69 14-20 (13)	17.0 ± 1.51 14-20 (15)	15.7 ± 0.76 15-17 (7)	5.8 ± 0.98 4-8 (29)	16.0 ± 2.18 13-21 (24)	16.3 ± 2.12 13-21 (15)	14.3 ± 1.50 11-17 (23)	13.5 ± 1.65 10-16 (10)	11.3 ± 1.33 10-14 (19)	5.8 ± 0.98 4-8 (29)
Femoral pores (males)	14.0 ± 1.41 13-17 (8)	13.1 ± 1.64 10-15 (8)	13.1 ± 1.27 11-15 (9)	15.5 ± 0.71 15-16 (2)	16.0 ± 1.41 15-18 (4)	16.5 ± 2.17 14-20 (6)	18.0 ± 1.00 17-19 (3)	16.5 ± 0.71 16-17 (2)	15.2 ± 1.17 14-17 (6)	16.9 ± 2.43 13-21 (13)	16.2 ± 1.94 14-19 (6)	14.3 ± 1.60 12-17 (7)	14.2 ± 1.17 13-16 (6)	11.9 ± 1.57 10-14 (7)	29.8 ± 3.77 25-34 (4)
Femoral pores (females)	14.2 ± 0.75 13-15 (6)	13.2 ± 0.98 12-14 (6)	14.2 ± 2.35 12-18 (10)	13.2 ± 1.47 12-15 (6)	15.6 ± 0.89 15-17 (5)	15.1 ± 0.90 14-16 (7)	16.8 ± 1.54 14-20 (12)	15.5 ± 0.71 15-16 (2)	14.7 ± 1.64 12-18 (14)	14.6 ± 1.30 13-17 (8)	16.3 ± 2.35 13-21 (9)	14.2 ± 1.57 11-17 (13)	12.5 ± 1.91 10-14 (4)	11.2 ± 1.09 10-13 (9)	26.0 ± 0.00 26 (1)
Glandular femoral scales in males	21.9 ± 4.42 17-28 (8)	16.6 ± 4.93 8-24 (8)	27.2 ± 7.79 18-40 (9)	23.0 ± 1.41 22-24 (2)	5.8 ± 6.65 0-12 (4)	22.0 ± 3.39 18-27 (5)	14.3 ± 5.77 11-21 (3)	13.0 ± 0.00 13 (2)	15.2 ± 3.25 10-19 (6)	27.8 ± 19.01 0-58 (13)	36.0 ± 20.66 0-54 (6)	12.7 ± 7.39 0-24 (7)	20.8 ± 7.36 11-30 (6)	13.3 ± 2.36 10-16 (7)	12.5 ± 25.00 0-50 (4)
Precloacal pores	0.07 ± 0.26 0-1 (15)	0.43 ± 1.60 0-6 (14)	0.05 ± 0.22 0-1 (21)	0.00 ± 0.00 0 (11)	0.00 ± 0.00 0 (9)	0.00 ± 0.00 0 (15)	0.00 ± 0.00 0 (20)	0.00 ± 0.00 0 (7)	0.00 ± 0.00 0 (22)	0.00 ± 0.00 0 (24)	0.0 ± 0.00 0 (15)	0.04 ± 0.21 0-1 (23)	0.00 ± 0.00 0 (10)	0.00 ± 0.00 0 (20)	0.00 ± 0.00 0 (5)

### 3.2.2 Electrophoretic analysis

Both liver and muscle tissue was homogenised in 0.01 M tris buffer (pH 8.0). Allozyme allelic variation was examined on horizontal starch gels (13% hydrolysed potato starch, Sigma Chemicals) (following Murphy, Sites, Buth & Haufler 1996). Three buffer systems were used: i) TCBL 8.7/8: a discontinuous tris-citrate-borate-lithium hydroxide buffer system with the gel buffer at pH 8.7 and the electrode buffer at pH 8.0 (Ridgeway, Sherburne & Lewis 1970); ii) TBE 8.6: a continuous tris-borate-EDTA buffer system with gel and electrode buffer at pH 8.6 (Markert & Faulhaber 1965); iii) TC 6.9: a continuous tris-citrate buffer system with the gel and electrode buffer at pH 6.9 (Whitt 1970). Control samples representing unique alleles were included on all gels. Transparencies were placed over stained gels so as to mark the positions of bands. These banding patterns were then recorded in diagram form in notebooks for subsequent interpretation. Staining for enzymatic activity followed the protocols of Shaw & Prasad (1970), Harris & Hopkinson (1976) and Murphy *et al.* (1996). Sequential numbering of loci started from the cathodal end of the gel (Shaklee, Allendorf, Morizot & Whitt 1990). The most common allele was assigned a mobility value of 100 and other alleles were scored relative to it.

Thirteen enzymes selected for routine analyses yielded 23 putative loci (aspartate aminotransferase [*AAT-1\** and *AAT-2\**; E.C. 2.6.1.1; liver and muscle: TC 6.9 and TCBL 8.7/8], adenylate kinase [*AK\**; E.C. 2.7.4.3; liver and muscle: TC 6.9], creatine kinase [*CK-1\** and *CK-2\**; E.C. 2.7.3.2; liver and muscle: TCBL 8.7/8], glucose dehydrogenase [*GLDH\**; E.C. 1.1.1.47; liver and muscle: TC 6.9], glucose-6-phosphate isomerase [*GPI\**; E.C. 5.3.1.9; liver and muscle: TCBL 8.7/8], isocitrate dehydrogenase (NADP<sup>+</sup>) [*IDH-1\** and *IDH-2\**; E.C. 1.1.1.42; liver: TC 6.9], lactate dehydrogenase [*LDH-1\** and *LDH-2\**; E.C. 1.1.1.27; liver: TC 6.9, TCBL 8.7/8], malate dehydrogenase [*MDH-1\** and *MDH-2\**; E.C. 1.1.1.37; liver: TC 6.9], malic enzyme (NADP<sup>+</sup>) [*MEP-1\** and *MEP-2\**; E.C. 1.1.1.40; liver: TC 6.9], mannose-6-phosphate isomerase [*MPI\**; E.C. 5.3.1.8; liver and muscle: TBE 8.6], peptidase leucyl-tyrosine dipeptidase [*PEP-LT-1\**, *PEP-LT-2\** and *PEP-LT-3\**; E.C. 3.4.-.-; liver: TBE 8.6], phosphogluconate dehydrogenase [*PGDH-1\** and *PGDH-2\**; E.C. 1.1.1.44; liver: TC 6.9], phosphoglucomutase [*PGM-1\** and *PGM-2\**; E.C. 5.4.2.2; liver and muscle: TCBL 8.7/8]). As it was difficult to assign homology of peptidases used in this study because of multiple substrate affinities (Murphy *et al.*

1996) the term “PEP-LT” is used since leucine-tyrosine was used in the stain. The peptidase involved was probably dipeptidase, peptidase-C or peptidase-S (Harris & Hopkinson 1976).

The first six populations sampled were analyzed for all enzymes selected: *Pseudocordylus transvaalensis* (Mokopane), *P. melanotus melanotus* (Harrismith and Sabie), *P. m. subviridis* (Organ Pipes and Hogsback) and *P. langi* (Organ Pipes). For these populations only four loci varied, namely *AAT-2*, *GLDH*, *GPI* and *PGM-1*. All additional populations were therefore analyzed only for these loci.

### 3.2.3 Genetic analyses

Genetic distance estimates, diversity measures and tests for deviation from Hardy-Weinberg equilibrium were performed using BIOSYS-1 (Swofford & Selander 1981). A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. Average heterozygosity ( $H_{obs}$ ) was calculated according to Nei, Maruyama & Chakraborty (1975). Mean expected heterozygosity ( $H_{exp}$ ) was calculated for each population using Nei's (1978) unbiased estimates. Genetic distances ( $D$ ) among populations were calculated using the method of Nei (1978).  $\chi^2$  analyses with Levene's (1949) correction for small sample size were performed on genotype frequencies for each polymorphic locus for the purpose of estimating deviations from Hardy-Weinberg equilibrium. Deviation from Hardy-Weinberg equilibrium was also tested using exact tests in GENEPOP 1.2 (Raymond & Rousset 1995a,b).

Exact tests were performed to test for heterogeneity of allele frequencies among populations using ARLEQUIN 2.000 (Schneider, Roessli & Excoffier 2000). AMOVA (Excoffier, Smouse & Quattro 1992) was performed to generate  $F_{ST}$  and  $F_{IS}$  and these were tested for significance with permutation tests also using ARLEQUIN 2.000. Four a priori structures were defined. The first structure comprises the four taxa *P. m. melanotus*, *P. m. subviridis*, *P. transvaalensis* and *P. langi*, each consisting of the populations as listed in Appendix 3.1; whereas the second comprises the first three taxa listed above but excludes *P. langi* (sympatric with *subviridis* at Organ Pipes). The third comprises eight geographical regions consisting of the following groups of populations: Thabazimbi and Mokopane (both *P. transvaalensis*); Sabie (*P. m. melanotus*); Lochiel (*P.*

*m. melanotus*); Amersfoort, Suikerbosrand, Harrismith and Qwa-Qwa (all *P. m. melanotus*); Nkandla (*P. m. melanotus*); Qwa Qwa, Organ Pipes, Naude's Nek and S Lesotho (all *P. m. subviridis*); Hogsback (*P. m. subviridis*); Organ Pipes (*P. langi*) (see Appendix 3.1). The fourth structure is like the third but excludes *P. langi*. All populations were included in the structure analysis, with the assumption that loci found to be monomorphic in populations analyzed for all loci were also monomorphic in the remaining populations analyzed for polymorphic loci only. This assumption was also made when reporting genetic distances (Nei 1978) and when using these to construct a neighbour-joining tree (Saitou & Nei 1987) using MEGA 2.1 (Kumar, Tamura, Jakobsen & Nei 2001).

### 3.3 Results

#### 3.3.1 Overall genetic diversity

Allele frequencies for polymorphic loci, mean numbers of alleles per locus, percentage of polymorphic loci and mean expected heterozygosities ( $H_{exp}$ ) are presented in Table 3.3. Observed and expected heterozygosity levels were the same in all cases, except for the Mokopane population of *P. transvaalensis* where there was a small difference (0.012 vs 0.011). Two alleles were observed for *AAT-2*, *GLDH* and *PGM-1*, and three alleles for *GPI*. All the other loci were fixed for the same allele across all populations. No single population had more than two alleles present at a particular locus (see Table 3.3). Nineteen loci were fixed for the same allele in all four taxa among all populations, while two loci (*AAT-2*, *GLDH*) showed fixed allelic differences among lineages. Rare alleles with frequency <0.15 occurred only in *GPI* (*P. m. melanotus* from Qwa-Qwa) and *PGM-1* (*P. transvaalensis* from Mokopane and *P. langi* from Organ Pipes). These were the only loci that were polymorphic within populations (mean number of alleles per locus = 1.04; Table 3.3). Genetic variability within populations was low. Percentage of polymorphic loci was 4.3 in each of the three populations named above. The highest mean expected heterozygosity was 0.011 in both the Mokopane (*P. transvaalensis*) and Organ Pipes (*P. langi*) populations, while this value was 0.006 in the Qwa-Qwa (*P. m. melanotus*) population.

All three cases of within-population polymorphism were in Hardy-Weinberg equilibrium: *GPI*: Qwa-Qwa *P. m. melanotus* ( $\chi^2$ , d.f. = 1, p = 1.000); *PGM-1*: Mokopane *P. transvaalensis* ( $\chi^2$ , d.f. = 1, p = 0.595; and exact test p = 1.000), Organ Pipes *P. langi* ( $\chi^2$ , d.f. = 1, p = 1.000).

**Table 3.3:** Distribution of allele frequencies at four variable loci in 15 populations of the *Pseudocordylus melanotus* species complex. Genetic diversity measures are provided for the six populations analyzed for all enzymes selected. (*N* = sample size; *AL* = mean number of alleles per locus; *PL* = percentage of polymorphic loci; *Hexp* = mean Hardy-Weinberg expected heterozygosity; S.E. = standard error).

Locus		Thabazimbi ( <i>P. transvaalensis</i> )	Mokopane ( <i>P. transvaalensis</i> )	Sabie ( <i>P. m. melanotus</i> )	Lochiel ( <i>P. m. melanotus</i> )	Amersfoort ( <i>P. m. melanotus</i> )	Suikerbosrand ( <i>P. m. melanotus</i> )	Harrismith ( <i>P. m. melanotus</i> )	Qwa-Qwa ( <i>P. m. melanotus</i> )	Nkandhla ( <i>P. m. melanotus</i> )	Qwa-Qwa ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. m. subviridis</i> )	Naude's Nek ( <i>P. m. subviridis</i> )	S Lesotho ( <i>P. m. subviridis</i> )	Hogsback ( <i>P. m. subviridis</i> )	Organ Pipes ( <i>P. langi</i> )
AAT-2	<i>N</i>	15	14	21	11	9	15	20	7	22	24	15	23	10	20	5
	-67	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
GLDH	<i>N</i>	13	14	19	8	8	14	20	7	20	20	15	16	10	11	3
	100	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	0.000	0.000	1.000
	137	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	1.000	0.000
<i>GPI</i>	<i>N</i>	15	14	21	11	9	15	20	7	23	24	15	22	10	20	5
	100	1.000	1.000	0.000	0.000	1.000	1.000	1.000	0.929	1.000	1.000	1.000	1.000	1.000	1.000	0.000
	76	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
	112	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>PGM-1</i>	<i>N</i>	15	14	13	11	9	15	20	7	23	24	15	23	10	12	4
	100	1.000	0.857	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.875
	73	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125
Mean sample size/locus		14.0±0.0	20.6±0.4					20.0±0.0				15.0±0.0			19.3±0.5	4.9±0.1
<i>AL</i> ±S.E.			1.04±0.04	1.00±0.00				1.00±0.00				1.00±0.00			1.00±0.00	1.04±0.04
<i>PL</i>			4.3	0.0				0.0				0.0			0.0	4.3
<i>Hexp</i> ±S.E.			0.011 ±0.011	0.000 ±0.000				0.000 ±0.000				0.000 ±0.000			0.000 ±0.000	0.011 ±0.011

### 3.3.2 Genetic structuring and differentiation

Pair-wise Nei's (1978) unbiased genetic distances and  $F_{ST}$  values among all populations are presented in Table 3.4. Four *P. m. melanotus* populations (Amersfoort, Suikerbosrand, Harrismith, Qwa-Qwa) were genetically indistinguishable based on the allozyme analysis (Tables 3.3 to 3.5). The Qwa-Qwa, Organ Pipes and Naude's Nek populations of *P. m. subviridis* were also indistinguishable. The S Lesotho population is indistinguishable from the geographically isolated Hogsback population. Also, Thabazimbi *P. transvaalensis* and Nkandla *P. m. melanotus* populations were indistinguishable genetically according to the allozyme analysis, even though they differ morphologically (see Chapter 5).

At the *AAT-2* locus there was a fixed allelic difference between the five populations of *P. m. subviridis* and all other populations (Table 3.3). For *GPI* the two northern *P. m. melanotus* populations and *P. langi* (Organ Pipes) were fixed for the 76 allele, while all other populations except Qwa-Qwa *P. m. melanotus* (with a rare 112 allele) were fixed for the 100 allele. However, with regard to *GLDH*, the pattern was not entirely associated with the putative taxa, with the two *P. transvaalensis* populations, two *P. m. melanotus* populations (Sabie, Nkandla) and two *P. m. subviridis* populations (S Lesotho, Hogsback) fixed for the 137 allele and all others fixed for the 100 allele.

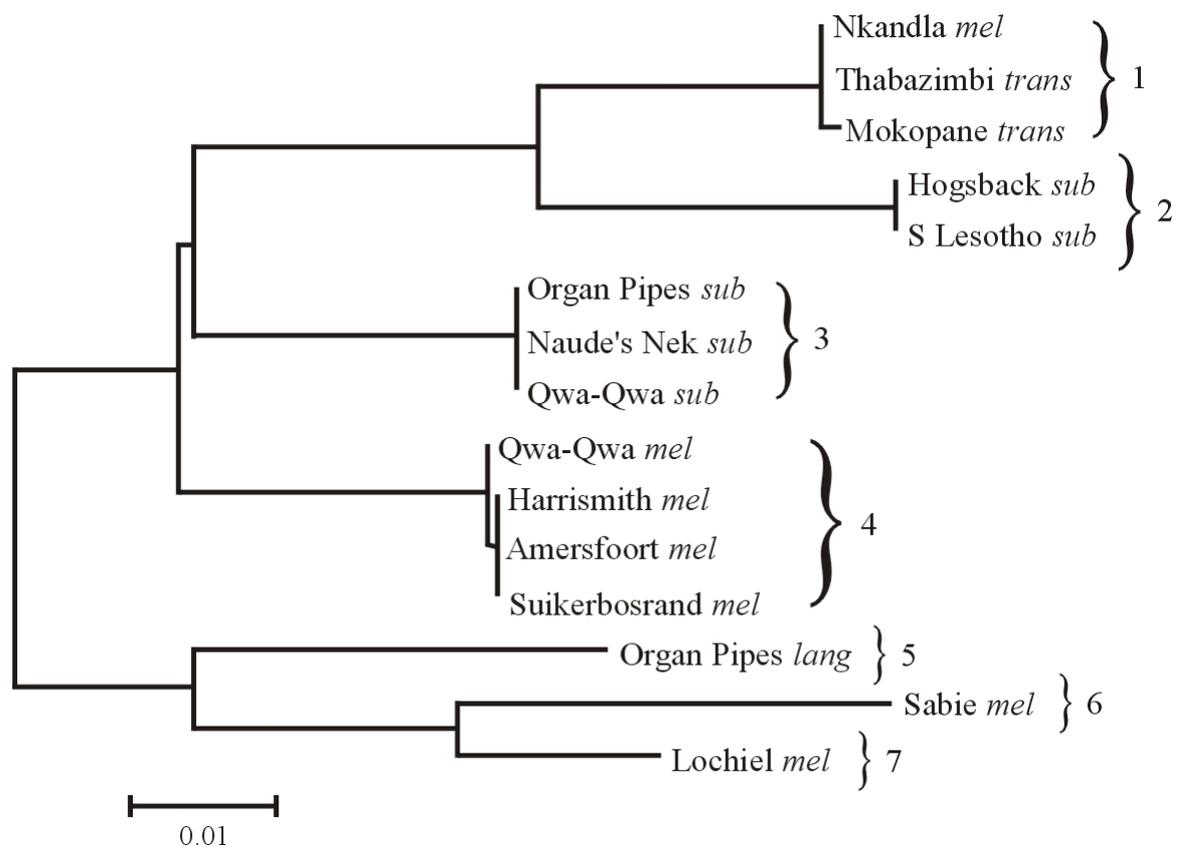
The neighbour-joining tree (Fig. 3.2) illustrates these fixed differences. Seven lineages are distinguishable, namely *P. transvaalensis* (excluding Nkandla *P. m. melanotus*, see below), Hogsback and S Lesotho populations of *P. m. subviridis*, all other populations of *P. m. subviridis*, southern populations of *P. m. melanotus*, Sabie population of *P. m. melanotus*, Lochiel population of *P. m. melanotus*, and *P. langi*.

**Table 3.4:** Nei's (1978) unbiased genetic distance (below diagonal) and pairwise  $F_{ST}$  (above diagonal) for 15 populations in the *Pseudocordylus melanotus* species complex (*tra* = *P. transvaalensis*, *mel* = *P. melanotus melanotus*, *sub* = *P. melanotus subviridis*, *lan* = *P. langi*). Asterisks indicate significant results ( $p < 0.05$ ).

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 Thabazimbi	<i>tra</i>		0.117	1.000*	1.000*	1.000*	1.000*	1.000*	0.948*	0.000	1.000*	1.000*	1.000*	1.000*	1.000*	0.962*
2 Mokopane	<i>tra</i>	0.001		0.897*	0.859*	0.833*	0.870*	0.894*	0.786*	0.157*	0.904*	0.877*	0.902*	0.854*	0.894*	0.775*
3 Sabie	<i>mel</i>	0.044	0.045		1.000*	1.000*	1.000*	1.000*	0.964*	1.000*	1.000*	1.000*	1.000*	1.000*	1.000*	0.971*
4 Lochiel	<i>mel</i>	0.091	0.092	0.044		1.000*	1.000*	1.000*	0.945*	1.000*	1.000*	1.000*	1.000*	1.000*	1.000*	0.952*
5 Amersfoort	<i>mel</i>	0.044	0.045	0.091	0.044		0.000	0.000	0.019*	1.000*	1.000*	1.000*	1.000*	1.000*	1.000*	0.945*
6 Suikerbosrand	<i>mel</i>	0.044	0.045	0.091	0.044	0.000		0.000	0.059*	1.000*	1.000*	1.000*	1.000*	1.000*	1.000*	0.962*
7 Harrismith	<i>mel</i>	0.044	0.045	0.091	0.044	0.000	0.000		0.086	1.000*	1.000*	1.000*	1.000*	1.000*	1.000*	0.970*
8 Qwa-Qwa	<i>mel</i>	0.045	0.046	0.088	0.041	0.000	0.000	0.000		0.962*	0.968*	0.955*	0.967*	0.941*	0.963*	0.852*
9 Nkandla	<i>mel</i>	0.000	0.001	0.044	0.091	0.044	0.044	0.044	0.045		1.000*	1.000*	1.000*	1.000*	1.000*	0.973*
10 Qwa-Qwa	<i>sub</i>	0.091	0.092	0.140	0.091	0.044	0.044	0.044	0.045	0.091		0.000	0.000	1.000*	1.000*	0.974*
11 Organ Pipes	<i>sub</i>	0.091	0.092	0.140	0.091	0.044	0.044	0.044	0.045	0.091	0.000		0.000	1.000*	1.000*	0.962*
12 Naude's Nek	<i>sub</i>	0.091	0.092	0.140	0.091	0.044	0.044	0.044	0.045	0.091	0.000	0.000		1.000*	1.000*	0.972*
13 S Lesotho	<i>sub</i>	0.044	0.045	0.091	0.140	0.091	0.091	0.091	0.091	0.044	0.044	0.044	0.044		0.000	0.949*
14 Hogsback	<i>sub</i>	0.044	0.045	0.091	0.140	0.091	0.091	0.091	0.091	0.044	0.044	0.044	0.044	0.000		0.970*
15 Organ Pipes	<i>lan</i>	0.141	0.140	0.091	0.045	0.091	0.091	0.091	0.088	0.141	0.045	0.045	0.045	0.091	0.091	

**Table 3.5:** Number of fixed allelic differences between 15 populations in the *Pseudocordylus melanotus* species complex (*tra* = *P. transvaalensis*, *mel* = *P. melanotus melanotus*, *sub* = *P. melanotus subviridis*, *lan* = *P. langi*).

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 Thabazimbi	<i>tra</i>														
2 Mokopane	<i>tra</i>	0													
3 Sabie	<i>mel</i>	1	1												
4 Lochiel	<i>mel</i>	2	2	1											
5 Amersfoort	<i>mel</i>	1	1	2	1										
6 Suikerbosrand	<i>mel</i>	1	1	2	1	0									
7 Harrismith	<i>mel</i>	1	1	2	1	0	0								
8 Qwa-Qwa	<i>mel</i>	1	1	2	1	0	0	0							
9 Nkandla	<i>mel</i>	0	0	1	2	1	1	1	1						
10 Qwa-Qwa	<i>sub</i>	2	2	3	2	1	1	1	1	2					
11 Organ Pipes	<i>sub</i>	2	2	3	2	1	1	1	1	2	0				
12 Naude's Nek	<i>sub</i>	2	2	3	2	1	1	1	1	2	0	0			
13 S Lesotho	<i>sub</i>	1	1	2	3	2	2	2	2	1	1	1	1		
14 Hogsback	<i>sub</i>	1	1	2	3	2	2	2	2	1	1	1	1	0	
15 Organ Pipes	<i>lan</i>	3	3	2	1	2	2	2	2	3	1	1	1	2	2



**Figure 3.2:** Neighbour-joining tree based on Nei's (1978) unbiased genetic distances for the *Pseudocordylus melanotus* species complex. Numbers 1 to 7 indicate lineages.

### 3.3.3 Heterogeneity of allele frequencies

Significant heterogeneity ( $p < 0.05$ ) of allele frequencies occurred in 19.0% of pair-wise population comparisons for the polymorphic loci *GPI* and *PGM-1*. For *GPI*, this percentage was 34.3% (several fixed differences between populations), but for *PGM-1* it was only 3.8% (Mokopane *P. transvaalensis* & Harrismith *P. m. melanotus*:  $p = 0.026 \pm 0.001$ ; & Nkandla *P. m. melanotus*:  $p = 0.017 \pm 0.001$ ; & Qwa-Qwa *P. m. subviridis*:  $p = 0.015 \pm 0.001$  & Naude's Nek *P. m. subviridis*:  $p = 0.018 \pm 0.001$ ). Allele frequencies at *PGM-1* were similar for the Mokopane ( $N = 14$ ) and Organ Pipes *P. langi* ( $N = 4$ ) populations as they both share the same two alleles. All other cases of heterogeneity of allele frequencies were the result of fixed or near-fixed allelic differences.

### 3.3.4 Genetic structuring

It was determined that 52.9% ( $p < 0.001$ ) of the variance measured with AMOVA is explained by differentiation between the four taxa ( $F_{ST} = 0.985$ ;  $p < 0.001$ ) (Table 3.6). As much as 45.6% ( $p < 0.001$ ) of variation is attributable to variation among populations within taxa, while as little as 1.5% ( $p < 0.001$ ) is explained by variation within populations. The results are similar when *P. langi* (sympatric with *P. m. subviridis* at Organ Pipes) is excluded (Table 3.6).

In terms of regions, as much as 86.6% ( $p < 0.001$ ) of the variance is explained by differentiation between them ( $F_{ST} = 0.984$ ;  $p < 0.001$ ) (Table 3.6). Only 1.6% ( $p < 0.001$ ) of variance is due to variation within populations, while the rest (11.8%,  $p < 0.001$ ) is attributable to variation among populations within regions. Again, including *P. langi* in the structure analysis does not change the interpretation because similar structuring (1-3 fixed allelic differences) already exists in the species complex. The regions that were defined describe the structuring better than the currently accepted taxa.

**Table 3.6:** AMOVA results for testing a priori structures among populations in the *Pseudocordylus melanotus* species complex. Asterisks indicate significant results ( $p < 0.05$ ).

Source of variation	Variance components			
	All taxa	All taxa excluding <i>P. langi</i>	Regions	Regions excluding <i>P. langi</i>
Among groups	0.442 (52.9%)*	0.432 (52.4%)*	0.669 (86.6%)*	0.658 (86.6%)*
Among populations within groups	0.381 (45.6%)*	0.381 (46.3%)*	0.091 (11.8%)*	0.091 (12.0%)*
Within populations	0.012 (1.49%)*	0.011 (1.28%)*	0.012 (1.61%)*	0.011 (1.39%)*
Total $F_{ST}$	0.985*	0.987*	0.984*	0.986*

### 3.4 Discussion

#### 3.4.1 Lineages within the *Pseudocordylus melanotus* species complex

The allozyme analysis provided information that may be helpful in resolving the taxonomic status of forms in the *P. melanotus* species complex and determining species boundaries. Fixed allelic differences between sympatric and parapatric forms indicate that species status can be awarded. Although the phylogram generated on the basis of genetic distances is phenetic in nature and not a true reflection of phylogenetic relationships, the data suggest that *P. m. melanotus*, as presently construed, may be polyphyletic. Six of the seven *P. melanotus* populations sampled clustered in two distinct groups, namely a northern and a southern one. The remaining population (Nkandla), surprisingly, clustered with the two *P. transvaalensis* populations. The data furthermore suggest that *P. transvaalensis* may be more closely related to *P. m. subviridis* than to *P. m. melanotus*.

The fixed allelic difference between *P. langi* and *P. m. subviridis* in sympatry (*GPI* locus:  $N = 5$  and  $N = 15$  respectively) confirms that they are not conspecific. There are also several morphological differences between the two taxa (see Chapter 5). In a situation similar to that between *P. langi* and *P. m. subviridis* at Organ Pipes Pass, Georges & Adams (1996) regarded a single fixed allelic difference between sympatric (including one case of microsympatric) forms - together with three concordant fixed morphological differences - as sufficient evidence of separate species status for two *Emydura* terrapins. One or more fixed differences in sympatry in what would otherwise be a panmictic population can be taken as evidence of reproductive incompatibility (Georges & Adams 1996). Species identified in this way are reproductively isolated and therefore satisfy the criteria for separate species status according to both the Biological and Evolutionary Species Concepts. While 15 specimens of *P. m. subviridis* were analyzed for allozymes, sample sizes for *P. langi* (a Red Data Book species) were small (3-5 specimens). Nevertheless, there was a complete lack of heterozygotes in *P. m. subviridis*, while *P. langi* shared a rare allele with only the Mokopane *P. transvaalensis* population at one locus (*PGM-1*). Small sample sizes may mask low levels of allelic heterozygosity, but differentiation between *P. langi* and *P. m. subviridis* was confirmed by several morphological differences (Tables 3.1 and 3.2).

Fixed allelic differences, together with differences in external morphology, are often considered sufficient evidence that two parapatric populations represent separate species (see Carlin 1997). Lineages 3 (Drakensberg *P. m. subviridis*) and 4 (Southern *P. m. melanotus*) differed by a single fixed difference at locus *AAT-2*. This also applied to parapatric populations of the two taxa in the Qwa-Qwa region. These two populations shared the same alleles at all other loci, except for a rare allele in the single heterozygous individual of *P. m. melanotus*. This fixed difference, together with morphological differences (Chapter 5), suggests that *P. m. subviridis* should be considered a species distinct from *P. m. melanotus*.

Specimens from the locality Monontsha Pass in this area had previously been assigned, morphologically, to both subspecies of *P. melanotus* as well as the category “intermediates” (De Waal 1978). Hybrids and intermediates are usually recognized by morphological intermediacy. However, this can result in considerable underestimation of intercrossing if individuals from backcrosses are similar to individuals of pure species but still carry foreign genes (Coyne & Orr 2004). There was no evidence of hybridization (introgression) in the allozyme study – no heterozygotes between *P. m. melanotus* and *P. m. subviridis* alleles - and all specimens from Monontsha Pass are referable to *P. m. subviridis*.

A large amount of genetic divergence indicates a long period of isolation, whereas low levels of divergence imply only short periods of isolation (*e.g.* Highton 1997; Thompson & Crother 1998). With regard to allozymes the two allopatric populations of *P. transvaalensis* analyzed are very similar, differing only in that four out of 14 individuals from Mokopane are heterozygous. The latter specimens share an allele with one out of four specimens of *P. langi*. This high genetic similarity suggests that the two populations of *P. transvaalensis* were separated relatively recently and have not had sufficient time to accumulate more allelic differences at structural loci.

High genetic similarity between some populations of *P. m. melanotus* (Qwa-Qwa, Harrismith, Amersfoort) suggests high levels of gene flow, and the same applies to some populations of *P. m. subviridis* (Qwa-Qwa, Organ Pipes, Naude’s Nek) (Table 3.3). The fact that the allopatric Suikerbosrand population of *P. m. melanotus* is indistinguishable from other southern *P. m. melanotus* (“Southern *melanotus*”) on the basis of allozymes

suggests that it was isolated relatively recently. It is possible that the Nkandla population of *P. m. melanotus* became fixed for the same allele as *P. transvaalensis* by chance, rather than due to a recent history of migration.

Apart from the *P. langi* population, the Sabie and Lochiel populations of *P. m. melanotus* (“Northern *melanotus*”) are the only two to share the slowest moving allozyme (76 allele) at the *GPI* locus. However, while the Sabie population also differs from other *P. m. melanotus* at the *GLDH* locus, the Lochiel population shares the same allele. The Sabie population thus differs from the Lochiel, Nkandhla, Thabazimbi and Mokopane populations by only a single fixed allelic difference, but from all other populations by 2-3 fixed differences; whereas the Lochiel population differs from the *P. m. melanotus* populations at Amersfoort, Suikerbosrand, Harrismith and Qwa-Qwa, and the *P. langi* population, by a single fixed difference, but from all others by 2-3 fixed differences. The Sabie population occurs on the Mpumalanga Escarpment, whereas the Lochiel population is situated outside of this range in an area of more patchy rocky habitats known as Barberton Mountainland. The fragmented nature of *P. m. melanotus* in this area may thus explain the fixed difference between these two populations.

Rocky outcrops do not occur uninterruptedly over the southern African landscape. This is of particular relevance to strictly rupicolous animals such as crag lizards, which have never been reported as occurring away from rocks in any other habitat (*e.g.* Branch 1998). During the present study specimens were observed basking near the openings to their crevices and were never found more than a few meters from suitable shelter. Although crag lizards are fast and should be able to move quickly between nearby rocky outcrops to avoid predation and escape the sun’s heat, extensive open areas between outcrops, or areas with limited crevices for shelter, almost certainly represent real barriers to movement. Restricted gene flow with extensive genetic structuring could therefore be expected. Mitochondrial DNA studies have shown this to be the case in at least six other species or species groups of rock-dwelling animals with extensive southern African distributions (rock hyrax, *Procavia capensis* [Pallas, 1766]: Prinsloo & Robinson 1992; rock rabbit, *Pronolagus rupestris* [A. Smith, 1834]: Matthee & Robinson 1996; rock agama, *Agama atra* Daudin, 1802: Matthee & Flemming 2002, and Swart, Matthee & Tolley 2004; sand lizard, *Pedioplanis burchelli* [Duméril & Bibron, 1839]: Makokha,

Tolley & Matthee 2004); crag lizards, *Pseudocordylus microlepidotus* [Cuvier, 1829] and *P. capensis* [A. Smith, 1838]: Cunningham 2004).

Random fixation of alternative alleles may account for the observed diversity among allopatric populations and indicates that isolation has occurred within and between taxa in the *P. melanotus* species complex. Fragmentation of populations is expected of a saxicolous lizard with limited resources (*e.g.* shelter), resulting in inbreeding. For both *P. m. melanotus* and *P. m. subviridis* the genetic structure associated with Figure 3.2 is indicative of taxa with fragmented populations (stepping-stone population structure model – see Baverstock & Moritz 1996). In fact, genetic structuring was best described (explaining nearly 87% of variance) when populations were assigned to geographic regions (as opposed to currently recognized taxa), namely Thabazimbi and Mokopane (both *P. transvaalensis*), Sabie (*P. m. melanotus*), Lochiel (*P. m. melanotus*), Amersfoort, Harrismith, Qwa-Qwa and Suikerbosrand (all *P. m. melanotus*), Nkandla (*P. m. melanotus*), Qwa-Qwa, Organ Pipes, Naude's Nek and S Lesotho (all *P. m. subviridis*), Hogsback (*P. m. subviridis*) and Organ Pipes (*P. langi*).

#### 3.4.2 Taxonomic implications

Both genetic distance and the number of fixed allelic differences have been used to decide on the taxonomic status of populations. According to Murphy & Ottley (1980) a genetic distance of 0.2 or greater is generally considered sufficient to distinguish between species, whereas distances of 0.1-0.2 indicate subspecies, and 0.0-0.1 suggests population level differentiation. However, reported allozyme genetic distances between species of various vertebrate taxa differed considerably ( $D = 0-3$ ; 0-2 in reptiles) (Avice & Aquadro 1982), as did sequence divergence values (0.00-0.26 in reptiles) based on 1800 *cyt b* sequences (Johns & Avice 1998), indicating that there is no reliable predictive value for separating species-level differences from population-level differences (Ferguson 2002). Ferguson (2002: 509) noted that using genetic distance to infer species status “is not parsimonious, its theoretical foundations are not well understood, and it cannot be applied over a wide range of plants and animals.” While genetic divergence measures are useful in population-level analyses and phylogeography, they are not appropriate for identifying separate species (Ferguson 2002). Genetic distance is therefore merely a measure of the degree of genetic divergence between taxa. A better approach for recognizing species

would be to use fixed genetic characters (*e.g.* fixed allelic differences between populations) (Ferguson 2002). These characters imply both genetic differentiation as well as lack of gene flow. Although considerable genetic differentiation may occur over long periods of time, long-term genetic isolation alone does not imply separate species status – the latter would require a speciation event and behavioural and/or ecological changes, resulting in distinct gene pools (Ferguson 2002).

Figure 3.2 indicates that seven lineages - based mainly on fixed allelic differences - are identifiable amongst the 15 evaluated populations in the *P. melanotus* species complex. However, genetic distances between population pairs in the complex are low (0.000 to 0.141; Table 3.4) and the neighbour-joining phylogram is based primarily on small numbers (1-3) of fixed allelic differences (Table 3.5) between population pairs. Using Murphy & Ottley's (1980) criteria the majority of genetic distances obtained in the present study reflect mere population level differentiation, although the *P. transvaalensis* and Nkandla *P. m. melanotus* populations differ from *P. langi* at subspecies level, the *P. m. subviridis* group (lineage) comprised of Qwa-Qwa, Organ Pipes and Naude's Nek populations is a separate subspecies to Sabie *P. m. melanotus*, while the *P. m. subviridis* group comprised of Amatole and S Lesotho populations differs from Lochiel *P. m. melanotus* at subspecies level. In all cases the paired groupings mentioned above differ by three fixed allelic differences and are genetically the most diverse in the complex. However, the phylogram (Fig. 3.2) probably indicates mainly random fixation of alleles resulting from habitat fragmentation and associated separation of gene pools and is therefore a weak approximation of the phylogenetic relationships among the populations studied.

There are several zoological examples in the literature pertaining to the use of both fixed allelic differences and genetic distances in guiding decisions on the species level (*e.g.* Darda 1994; Stanley, Moyle & Schaffer 1995; Stepien & Rosenblatt 1996). Brody *et al.* (1993) studied several species in the *Cordylus cordylus* species complex and recorded low genetic distance values (0.008-0.272) between allopatric population pairs. The highest value (0.272) was between *C. peersi* and one of the *C. cordylus* populations. However, the highest values for any comparison between pairs of *C. niger*, *C. oelofseni* and *C. cordylus* populations was only 0.160. Brody *et al.* (1993) indicated that the two *C. niger* populations were monophyletic but did not consider the fixed allelic difference

between them as indicative of possible separate species status. One of the four *C. oelofseni* populations also differed from the others by a fixed allelic difference (with a second such difference with two of the other three populations). *Cordylus oelofseni* was considered polyphyletic and it was suggested that its species boundaries be re-evaluated.

However, in some studies – usually following a phylogenetic species concept - only fixed allelic differences are used to distinguish species. According to Mink & Sites (1996) even a single fixed difference in allopatry is considered evidence of separate species status (see also Coyne & Orr 2004). Using this criterion can, however, result in the recognition of numerous new species that may in fact merely represent recently isolated populations exhibiting random fixation of particular alleles. Such populations may re-constitute and reproduce freely if and when migration becomes possible (*e.g.* after removal of a physical barrier). Georges & Adams (1996) identified 15 chelid terrapins on the basis of 2-57 (mostly 16 or more) fixed allelic differences in allopatry and one such difference in sympatry. In cases of allopatry they considered two fixed differences sufficient when sample sizes numbered 10 or more, and three fixed differences sufficient when sample sizes were less than 10. Gergus (1998) considered two of the three subspecies of *Bufo microscaphus* to be full species largely because they exhibited two or seven fixed allelic differences in allopatry.

Other studies refer to both fixed allelic differences and morphological differences when making decisions on species level. For example, in the case of geckos of the *Goggia lineata* species complex, Branch, Bauer & Good (1995) and Good, Bauer & Branch (1996) reported 3-11 fixed allelic differences between species pairs as well as various morphological differences. The large-bodied geckos *Pachydactylus kladaroderma* and *P. haackei* were fixed for alternative alleles or allele combinations at 11 loci and also differed morphologically (Branch, Bauer & Good 1996). Green, Kaiser, Sharbel, Kearsley & McAllister (1997) found that although the allopatric frogs *Rana pretiosa* and *R. luteiventris* were fixed for alternate alleles at four loci, morphologically they were distinguishable only by means of a discriminant function analysis of body measurements. In the present study fixed allelic differences, mtDNA data (Chapter 4) and morphological differences (Chapter 5) are used in combination for taking decisions on species level.

According to Wiley (1981) low values of electrophoretic similarity corroborate decisions that two different geographical populations represent different species, whereas high values do not necessarily suggest conspecific status. Nevertheless, as noted by Grant, Dempster & Da Silva (1988), allozyme variation is useful for describing genetic relationships among closely related sibling species, or cryptic species, that exhibit little morphological divergence but nevertheless represent distinct evolutionary lineages.

Although many studies have shown that populations of the same species are generally more similar electrophoretically than populations of different species, some studies have determined that electrophoretic similarity is decoupled from morphological divergence. In other words, while some morphologically distinct species exhibit limited differentiation in terms of their allozymes, some genetically distinct species are not easily distinguished morphologically (Wiley 1981; Hillis 1987). For example, Brody *et al.* (1993) determined a genetic distance of only 0.100 between populations of *Cordylus peersi* and *C. macropholis*, despite the fact that these two taxa are morphologically quite distinct and have very different lifestyles, *i.e.* rupicolous versus terrestrial respectively. There was only one fixed allelic difference between the two species, with a second near-fixed difference. The situation between *C. peersi* and *C. macropholis* can be compared to that of *P. langi* and most other populations in the *P. melanotus* species complex. *Pseudocordylus langi* is morphologically the most distinct taxon (Chapter 5) but exhibits only limited allozyme differentiation.

The separate species status of *P. langi* is supported by allozyme data, including a fixed allelic difference with sympatric *P. m. subviridis* at Organ Pipes Pass. Georges & Adams (1996), as mentioned earlier, regarded a single fixed difference between sympatric forms - together with fixed morphological differences - as sufficient evidence of separate species status. However, Baverstock & Moritz (1996) suggested that, for sympatric species, at least two loci showing fixed differences between individuals might be sufficient evidence of separate species status. Nevertheless, they suggested that an attempt should still be made to find diagnostic morphological features. An apparent lack of heterozygotes at a locus may be the result of ontogenetic variation, or variation may not be under simple genetic control, or there may be strong selection against heterozygotes (see Baverstock & Moritz 1996). Therefore, the more fixed allelic differences between populations (sympatric, parapatric or allopatric), the more likely it is

that at least some of these are indicative of real taxonomic differences (*e.g.* different species). Although the number of fixed differences between populations is the best measure of genetic divergence, very different allele frequencies also indicate strong genetic divergence and are therefore operationally equivalent to fixed differences (Baverstock & Moritz 1996).

The allozyme study showed that *P. m. melanotus* and *P. m. subviridis* differ by a fixed allelic difference. This situation also applied to parapatric populations of the two taxa. Morphologically intermediate specimens from Monontsha Pass (including specimens with *melanotus*- and *subviridis*-like traits) were all referable to *P. m. subviridis* and no heterozygotes were identified which would indicate possible hybridization between *P. m. melanotus* and *P. m. subviridis*. Monontsha Pass and the nearby locality Thibella (see Appendix 3.1) were in fact the only sites where distinctly morphologically intermediate specimens were collected. Most specimens of the two subspecies of *P. melanotus* can be distinguished using the characters provided by De Waal (1978) (see also Chapter 5). Although some individuals of both subspecies of *P. melanotus* are difficult to assign using morphology alone (Chapter 5), both allozyme and morphological data suggest that *P. m. subviridis* be considered a valid species.

Several allozyme studies have resulted in the detection of morphologically cryptic - or nearly indistinguishable - species (see Hillis 1987). This appears to be the case with the Sabie and Lochiel populations of *P. m. melanotus*, which differed from all other populations in the *P. melanotus* species complex (except *P. langi*) at locus *GPI*, but were separated from one another by a fixed allozyme allelic difference at locus *GLDH*. However, the fact that two populations fail to share allozymes at a given locus does not implicitly mean they should be regarded as separate taxa. While the Sabie and Lochiel groups (together referred to as “Northern *melanotus*”) may be allopatric to the rest of *P. m. melanotus*, there are no clear indications that populations representing the two groups are in fact isolated from one another. The fixed allelic difference could therefore be indicative of recent fragmentation and inbreeding, rather than a long period of isolation. This may also explain why the Nkandla population of *P. m. melanotus*, which, morphologically, is undoubtedly referable to this species (see Tables 3.1 and 3.2), groups with *P. transvaalensis* rather than other *P. m. melanotus*. The allozyme data also indicates that *P. transvaalensis* is a valid species.

The allozyme analysis showed that there was a fixed allelic difference between the allopatric Amatole-Winterberg *P. m. subviridis* and the main Maloti-Drakensberg *P. m. subviridis* groups. However, on the basis of the allozyme analysis, S Lesotho *P. m. subviridis* was genetically inseparable from the Hogsback (Amatole-Winterberg) group rather than the rest of the Maloti-Drakensberg group, as might have been expected considering their geographical proximity.

The present analysis indicated that considerable sub-structuring occurs within both *P. m. melanotus* and *P. m. subviridis* as currently diagnosed. Better resolution of the relationships between the various populations of these and other taxa in the *P. melanotus* species complex was obtained using mtDNA sequencing analysis (Chapter 4).

## CHAPTER 4

### **A mitochondrial DNA analysis of the *Pseudocordylus melanotus* (A. Smith, 1838) species complex (Sauria: Cordylidae)**

#### **4.1 Introduction**

The Cordylidae, a small family of lizards endemic to Africa, is currently partitioned into four genera, namely *Chamaesaura*, *Cordylus*, *Pseudocordylus* and *Platysaurus* (Lang 1991). While *Chamaesaura* was previously considered the most basal genus in the family and *Platysaurus* the most advanced (FitzSimons 1943; Loveridge 1944; Lang 1991), Frost *et al.* (2001) demonstrated that *Platysaurus* is in fact the most basal and that both *Pseudocordylus* and *Chamaesaura* are embedded within *Cordylus*. These authors also found that the genus *Pseudocordylus*, as presently construed, is polyphyletic and comprised of two unrelated clades, *P. capensis* and *P. nebulosus* on the one hand, and *P. microlepidotus*, *P. melanotus*, *P. transvaalensis*, *P. langi* and *P. spinosus* on the other hand. While *P. microlepidotus* and *P. spinosus* have always been considered well-defined species, despite the fact that the former is partitioned into three subspecies, species boundaries within the *P. melanotus*-*P. transvaalensis*-*P. langi* complex have always been confused and the taxonomic status of subspecies within *P. melanotus* uncertain. Due to high morphological variability, attempts to resolve the taxonomy of forms in the *P. melanotus* species complex on the basis of morphological characters have been unsuccessful.

The first step in resolving the taxonomic status of forms within the *P. melanotus* complex (Fig. 5.1) was to conduct an enzyme electrophoretic analysis (Chapter 3). This analysis showed that *P. m. melanotus* might be polyphyletic and comprised of two unrelated lineages. Furthermore, fixed allelic differences between parapatric populations of *P. m. melanotus* and *P. m. subviridis*, and between sympatric populations of *P. m. subviridis* and *P. langi* suggest that all three forms may be considered full species, with the possibility of more cryptic species present in the complex. The allozyme study was,

however, based on phenetic principles and for further taxonomic resolution a cladistic approach is required.

In an unpublished study of phylogenetic relationships within the Cordylidae, Melville *et al.* found *P. microlepidotus* to be embedded in the *P. melanotus* complex. This unexpected finding suggests that relationships within *Pseudocordylus* (*i.e.* excluding *P. capensis* and *P. nebulosus*) may be complex and the taxonomic status of forms in the *P. melanotus* complex will remain unresolved unless all species are included in the analysis.

Mitochondrial DNA studies are being used more and more in attempts at resolving confused relationships between morphologically similar reptile taxa or for studying lineages within such taxa. Numerous such studies have been conducted on the southern African lizard fauna in recent years (*e.g.* Lamb & Bauer 2000; Daniels, Heideman, Hendricks & Willson 2002; Matthee & Flemming 2002; Lamb, Meeker, Bauer & Branch 2003; Cunningham 2004; Daniels, Mouton & Du Toit 2004; Makokha, Tolley & Matthee 2004; Swart, Matthee & Tolley 2004; Tolley & Burger 2004; Tolley, Tilbury, Branch & Matthee 2004; Daniels, Heideman, Hendricks, Mokone & Crandall 2005; Tolley, Burger, Turner & Matthee 2006). Congruence between two types of genetic data - in this case multiple nuclear markers (allozymes) and mitochondrial ribosomal gene sequences - serves to strengthen or confirm the outcome of analyses. Molecular approaches to analyzing phylogenetic relationships are considered particularly enlightening in cases of limited morphological variation (Moritz & Hillis 1996), as is the case with the two subspecies of *P. melanotus*.

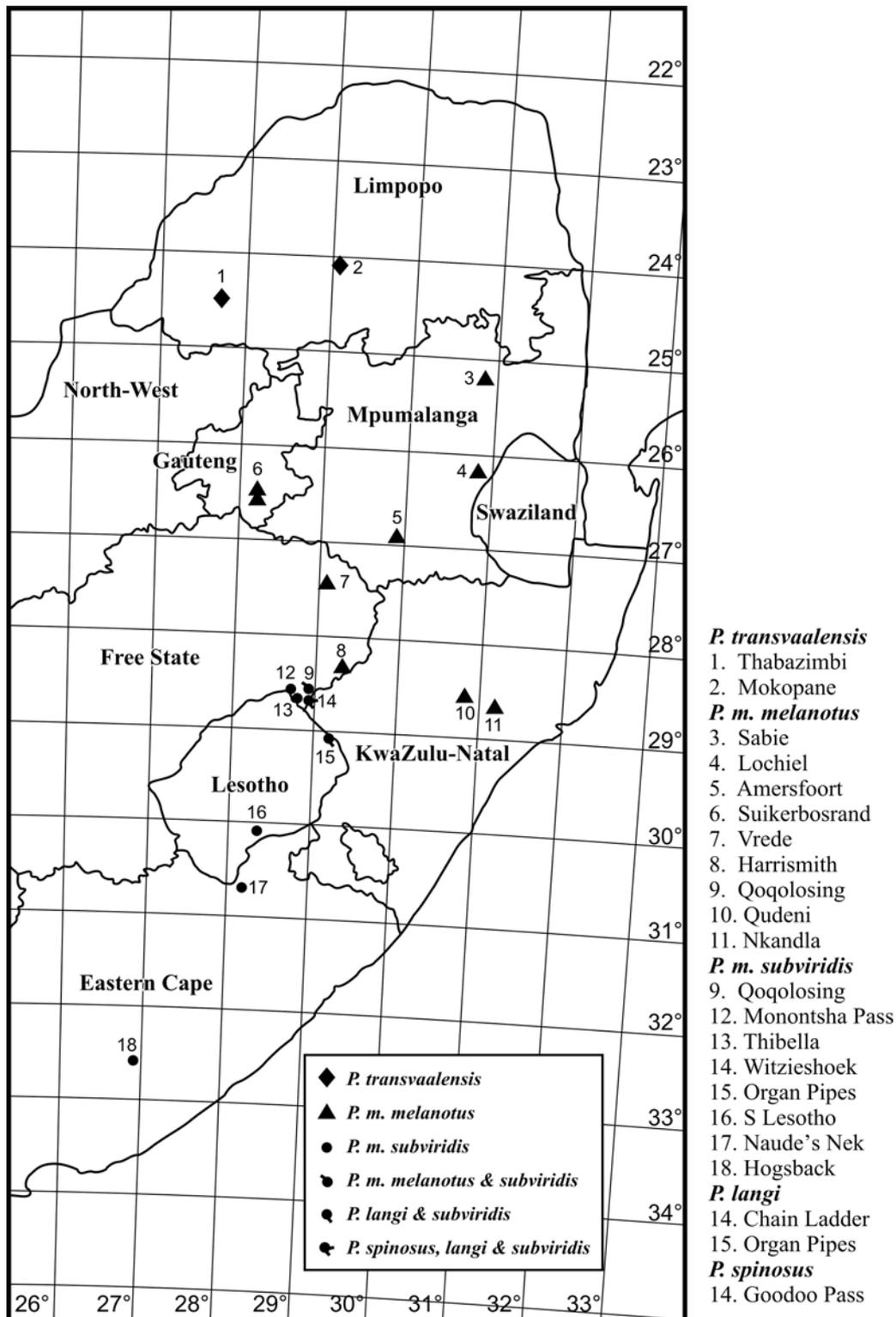
The aims of this study were, firstly, to determine the phylogenetic relationships among species and subspecies in the genus *Pseudocordylus* (excluding *P. capensis* and *P. nebulosus*) using mitochondrial DNA markers, and secondly, to re-assess the taxonomic status of forms in the *P. melanotus* species complex.

## 4.2 Materials and Methods

### 4.2.1 Sampling

Lizards referable to the *P. melanotus* species complex were collected at 18 localities spread throughout the geographical range of the complex (Fig. 4.1; Appendix 4.1). Most formed part of a total of 232 specimens collected from December 1998 to November 2000 for the allozyme analysis (Chapter 3). Localities include the isolated populations of *P. m. melanotus* at Suikerbosrand and in Nkandhla district, *P. m. subviridis* in the Amatole-Winterberg Mountains, and the eastern and central populations of *P. transvaalensis*. Specimens were euthanased by hypodermic injection of sodium pentobarbitone compound to the cardiac region 2-7 days after capture. Whole animals were then stored at  $-70^{\circ}\text{C}$  in an ultra-cold freezer at the University of the Free State (Bloemfontein). They were later de-frosted, dissected and sections of liver and thigh muscle excised, placed in 3.6 or 4.5 ml cryotubes and immersed in liquid nitrogen ( $-196^{\circ}\text{C}$ ). Tissue samples were then transported to the University of Stellenbosch where they were transferred to an ultra-cold freezer ( $-80^{\circ}\text{C}$ ). Dissected lizards were returned to the freezer at the University of the Free State and later transferred to the National Museum (Bloemfontein) where they were accessioned and preserved directly in 70% ethanol. Sections of the tail of these specimens were later removed for sequencing, but in some cases frozen tissues ( $-20$  to  $-80^{\circ}\text{C}$ ) were thawed, placed in 96% ethanol, DNA extracted and then sequenced. Additional specimens, including outgroup taxa, were collected in September 2004, and April and June 2005, and excised tissues (caudal and thigh muscle) stored directly in 96% ethanol. Tissues used for DNA extraction therefore included thigh muscle, caudal muscle and liver.

A total of 84 samples were sequenced, comprising: 10 *P. transvaalensis*, 10 Northern *melanotus*, 23 Southern *melanotus*, 29 *P. m. subviridis*, three *P. langi*, five *P. spinosus* and one specimen each of *P. microlepidotus*, *Platysaurus intermedius intermedius*



**Figure 4.1:** Geographical distribution of localities for the mtDNA analysis of the *Pseudocordylus melanotus* species complex. *Pseudocordylus melanotus melanotus* and *P. melanotus subviridis* were collected in sympatry at locality 9; *P. m. subviridis*, *P. spinosus* and *P. langi* were all collected in the area represented by locality 14; while *P. m. subviridis* and *P. langi* were collected in sympatry at locality 15. All specimens except those from the following localities were also used in the allozyme analysis: locality 7 (*P. m. melanotus*, Vrede), locality 14 (*P. m. subviridis*, one specimen from Witzieshoek; *P. langi*, Chain ladder; *P. spinosus*, Goodoo Pass). Numbers refer to localities listed in detail in Appendix 4.1.

Matschie, 1891, *Cordylus breyeri* (Van Dam, 1921) and *C. vandami* (Appendix 4.1). Specimens of the latter four taxa were identified using FitzSimons (1943) and Branch (1998).

Three species (*P. melanotus subviridis*, *P. spinosus*, *P. langi*) were collected at locality 14, which covers a variety of altitudes, including collection sites at 2000 m (*P. spinosus*) and 3020 m (*P. langi*). However, *P. spinosus* is not known to occur in microsympatry with *P. m. subviridis*, although the latter taxon does occur in sympatry and even microsympatry with *P. langi*. The *P. spinosus* sample was collected in a low rock outcrop in montane grassland. Crevices were near or even at ground level, unlike those of *P. m. subviridis* that are usually much higher up.

#### 4.2.2 DNA sequencing

Tissue samples were first washed in sterile water. Total genomic DNA was then isolated from about 0.5 g of tissue. All samples were digested in sterilized eppendorfs containing 500 µl of DNA lysis buffer (200 ml of 1 X STE [100 mM NaCl, 10 mM Tris HCl, 1mM EDTA] and 30 ml of 10% SDS solution), 20 µl proteinase K at 10 mg/ml and 10 µl RNase at similar concentration, all at 55°C. This mixture was then incubated for either 2 h or overnight, depending on tissue quality and quantity. The DNA was extracted using the phenol/chloroform: isoamyl alcohol method contained in Hillis, Moritz & Mable (1996). A total of 500 µl of Tris buffered phenol and an equal quantity of chloroform:isopropanol was aliquoted into each sample and mixed for 2 min. Subsequently, chloroform was added to the samples. Samples were then centrifuged for 5 min at 13 000 rev/min. The supernatant was removed and cold absolute ethanol was added, together with 45 µl of Ammonia Glutate. Samples were left overnight and centrifuged again to obtain the DNA pellet.

Supernatant was transferred to a new tube and 400 µl of chloroform added. This was then mixed for 5 min and spun for 3 min at 13 000 rev/min. The resulting supernatant was collected and placed in a new tube with 900 µl of ice-cold absolute ethanol. A quantity of 45 µl of 5 M ammonium acetate solution was added. Samples were then incubated for 4-6 h at -80°C or left overnight at -20°C. Each sample was spun for 20 min at 13 000 rev/min. The DNA pellet was washed with 700 µl of 70% ethanol for 5 min and dried in

an oven (35°C) or vacuum dried in a speed vac. Samples were then re-suspended in 50 or 100 µl of water depending on pellet size. Concentrations of DNA were determined using spectrophotometry and the samples diluted to 40 ng/µl. All DNA samples were stored at -20°C until required.

The primers 16Sa (5'-CGC CTG TTT ACT AAA AAC AT-3') and 16Sb (5'-CCG GTC TGA ACT CAG ATC ACG T-3') were used to amplify the 16S gene (see Palumbi *et al.* 1991). For each polymerase chain reaction (PCR) a 25 µl reaction was performed containing 14.9 µl millipore water, 3 µl MgCl<sub>2</sub> (25 mM), 2.5 µl 10 X Mg<sup>2+</sup> free buffer, 0.5 µl dNTP solution (10 mM) and 0.5 µl primer sets (10mM), 0.1 U Hotmaster Taq and 1-3 µl template DNA. PCR temperature regime was 95°C for 2 min, 95°C for 30 s, 50 or 55°C for 40 s, 72°C for 1 min, 32 cycles for the last three steps and finally 72°C for 10 min. Electrophoresis of PCR products was conducted in 1% regular agarose gel containing ethidium bromide for 30 min at 70 V. Ultraviolet light was utilized for visualizing PCR products. The latter were purified using a PCR purification kit (Qiagen). When necessary the products were further purified using a gel purification kit (QIAquick gel extraction Cat. No. 286706). Purified products were then cycle sequenced using standard protocols (3 µl purified PCR product, 4 µl fluorescent-dye terminators with an ABI PRISM Dye Terminator Cycle Sequencing Reaction Kit [Perkin Elmer], and 3 µl primer solution [10 µM] for each primer pair). Unincorporated dideoxynucleotides were removed by gel filtration using Sephadex G-25 (Sigma). Sequencing was conducted on an ABI 3700 automated machine.

#### 4.2.3 Outgroup selection

The first outgroup used was *Platysaurus intermedius intermedius* as the genus *Platysaurus* is one of the most basal taxa in the Cordylidae according to Frost *et al.* (2001) (Fig. 4.2). The latter authors studied the relationships in this family using 12S rRNA, valine tDNA and 16S rRNA. In addition, two representatives of the *Cordylus warreni* (Boulenger, 1908) species complex (see Jacobsen 1989; Branch 1998) were used, namely *C. breyeri* and *C. vandami*. Frost *et al.* (2001) found that *C. warreni* was a sister taxon to the clade containing *Pseudocordylus melanotus* and *P. microlepidotus*. It was decided not to use any other species of *Pseudocordylus* (e.g. *P. capensis*) as outgroups

because this genus is not monophyletic (Frost *et al.* 2001). Also, as the latter authors did not include all known *Pseudocordylus* taxa (*e.g.* *P. langi*, *P. spinosus*) in their study, the relationships of these other taxa to those they used is unknown. Although *P. spinosus* may be considered a likely candidate for outgroup selection on the basis of its morphology, the genetic evidence indicated that it is in fact part of the ingroup (see below).

#### 4.2.4 Phylogenetic analysis

Samples were sequenced in both directions. Aligned forward and reverse sequences were examined for base ambiguity in Sequence Navigator (Applied Biosystems). 16S rRNA sequences were aligned in CLUSTAL X (Thompson, Gibson, Plewniak, Jeanmougin & Higgins 1997) using the default parameters of the program and additionally adjusted by eye in cases where obvious mismatches resulted from computer alignment.

Because of ambiguity in the first 30 bases of the 16S rRNA gene this section was trimmed and excluded from the analysis. Ambiguity in this gene region meant that some bases could not be aligned with confidence and these were thus excluded from the analysis. The 16S rRNA sequences from this study will be deposited in GenBank once the CO1 gene (see section 4.4) has also been analyzed.

Phylogenetic data analyses were conducted in PAUP\*4 version beta 10 (Swofford 2002) using two methods, namely maximum parsimony (MP) and maximum likelihood (ML). For the MP analysis, trees were generated by means of the heuristic search option with TBR branch swapping (100 random replicates) using random taxon addition. For the ML analysis, MODELTEST version 3.06 (Posada & Crandall 1998) was used to calculate the appropriate substitution model using the AIC criteria. Sequence divergence values were determined using uncorrected “p” distances. Phylogenetic confidence in nodes was established from MP as estimated by bootstrapping (Felsenstein 1985). A total of 1000 pseudoreplicates of data sets were analyzed. Because of time constraints only 100 replicates were performed for ML. Bootstrap values <50% were regarded as lacking support, values of 50-75% were considered weakly supported, and values >75% suggested strong support.

Bayesian inference (BI) was used for investigating optimal tree space using MrBayes 3.0b4 (Ronquist & Huelsenbeck 2003). Four Markov chains were run for each analysis. Data sets were run at least four times to test for topological convergence. Each chain started from a random tree and five million generations were generated, sampling every 5000<sup>th</sup> tree. A 50% majority rule consensus tree was generated from retained trees after burn-in trees were discarded using likelihood plots. Posterior probabilities ( $pP$ ) for each node were estimated according to the percentage of time the node was recovered.

#### 4.2.5 Nested Clade Analysis

Nested Clade Analysis (NCA) attempts to identify significant non-random patterns in the geographical dispersion of lineages within a nesting lineage (Templeton *et al.* 1995). To put it differently, NCA will allow an overlay of genetics with geography and provide a better understanding of phylogeographic patterning and potential roots of colonization in the complex. Data used in the analysis comprise the following: co-ordinates for collecting sites, allele abundance within localities, and genealogical relationships among alleles, partitioned into a series of nested clades, each one including both ancestral and descendent lineages (internal versus tips).

The unrooted parsimony strict consensus phylogram (three equivalent trees) was used to identify nested clade structure. Nesting started from the tips of the tree, moving inwards by single mutational steps until all alleles grouped in a single clade at the 22<sup>nd</sup> step level. The species graph (Fig. 4.4) was partitioned into a hierarchy of nested clades according to Templeton *et al.* (1995) and Templeton & Sing (1993). “Tempest”, a computer program developed by M. Cunningham (pers. comm., August 2006) in 2001 (as used by Cunningham 2001), was used for calculating various indices of clade dispersion: clade distance (Dc), nested clade distance (Dn), internal versus tip comparisons (I-TDc, I-TDn), and significance tests from 1000 randomised permutations of each statistic, based on Rolf & Benson’s algorithm as applied by Templeton *et al.* (1995). Permutations were performed for individuals across sites in each nesting clade, while maintaining sample sizes, to assess the significance of observed NCA statistics. Results were shown only when meaningful permutation tests were possible (at least two alleles across two locations in a nesting clade). For significant results, Templeton’s (2004) inference key was used to interpret geographic structure.

For the determination of clade and nested clade distances, the decimal place of latitude and longitude co-ordinates was moved two places to the right (*i.e.* 24.48917S, 27.63278E becomes 2448.917, 2763.278). This is appropriate because, in the study area, a 0.00001 degree change in either latitude or longitude is approximately equal to 1 m (0.01000 ~ 1 km) and the area is not so large that spherical warping of co-ordinates creates significant differences in distances among points. It is therefore not necessary to project these points onto a flat surface to calculate distances among them.

### 4.3 Results

#### 4.3.1 Phylogenetic analysis

A 421 base pair fragment of the 16S rRNA mtDNA gene region was amplified. For the ML analysis the best-fit substitution model was GTR + I + G (-lnL = 1431.75; AIC = 2883.50), with base frequencies of A = 33.28%, C = 23.72%, G = 20.15% and T = 22.84%, while proportion of invariable sites (I) = 0.41 and gamma distribution shape parameter ( $\alpha$ ) = 0.5083. The rate matrix for the substitution model was: R(a) [A-C] = 4.3092, R(b) [A-G] = 14.2500, R(c) [A-T] = 5.2149, R(d) [C-G] = 0.8228, R(e) [C-T] = 22.0070, R(f) [G-T] = 1.0000. Parsimony analysis included 69 informative characters. Six trees were retained with a tree length of 130 steps, CI = 0.71 and RI = 0.94. All trees were nearly identical, differences being confined to swapping among terminal tips at nodes that were not supported. Figure 4.2 presents one of these trees.

The bootstrapped MP tree (Fig. 4.2) was largely congruent with the ML tree, hence the MP tree was selected. In the ML analysis there was no support for the node comprising the ingroup and also no support for the node comprising clades B to G. Nevertheless, there was MP and BI support for these nodes as indicated below. The nodes encompassing clades C to G and clades C to E received support only in the BI analysis. While clades A, B, C and E were well supported (77-100% bootstrap support) in ML, clade F was only weakly supported (69%) and there was no support for clades D (southern *P. m. subviridis*) and G (Southern *melanotus*). In ML there was no clade formation by Southern *melanotus* populations from Harrismith, Amersfoort, Vrede and

Qoqolosing, but 77% bootstrap support (also MP 86% and 1.0 posterior probability in BI) for the isolated Suikerbosrand population. There was also 91% (also MP 95% / 1.0) and 63% support for the Nkandla populations comprising NMB R8366 and 8368, and NMB R8371, 8377 and 8388 respectively. The latter two groups are not monophyletic and appear as distinct lineages. Although clade D was not supported in ML, three specimens (NMB R8348, 8354 and 8358) from Monontsha Pass and Witzieshoek formed a clade with 73% support, and two specimens from Organ Pipes Pass formed a clade with 95% support. For BI, identical topologies were obtained for each of the four runs. Congruence was evident between BI and MP as the same basic topology and well-supported nodes were recovered (Fig. 4.2).

The MP tree indicates that the *P. melanotus*/*P. microlepidotus* complex consists of two major clades, one comprising *P. langi* (clade A, 100% bootstrap support in MP and ML, 1.0 posterior probability in BI) and the other containing all other populations (MP 86% / 1.0) (Fig. 4.2). The latter two groups formed a monophyletic assemblage (MP 92% / 1.0) representative of the *P. melanotus* and *P. microlepidotus* species complexes. The non-*P. langi* group was further subdivided into two main groups, one comprising Northern *melanotus* (clade B, 100% / 98% / 1.0) and the other consisting of all other populations. While Northern *melanotus* represents a distinct lineage, relationships between clades in its sister group were unclear. The topology of the tree indicates that the latter consists of three groups: Southern *melanotus* (clade G, MP 89% / 1.0), *P. transvaalensis* (clade F, 78% / 69% / 1.0) and an assemblage ( $pP = 1.0$ , but no MP or ML support) comprising three strongly supported clades, namely clade C (100% / 99% / 1.0) comprising *P. spinosus* and *P. m. subviridis* (northern populations), clade D (MP 78% / 1.0) comprising *P. m. subviridis* only (northern populations) and clade E (82% / 77% / 1.0) comprising southern populations of *P. m. subviridis* and the single *P. microlepidotus* sequence analysed.



While most groupings indicated by the phylogram are consistent with geography, two of the three clades containing *P. m. subviridis* interdigitated with other taxa. Clade C contains northern populations of *P. m. subviridis* as well as *P. spinosus* (both of which shared the same allele). Also, clades C and D include lizards from the same populations, namely Monontsha Pass, Witzieshoek and Organ Pipes. Clade E consists of three subclades, namely Naude’s Nek-S Lesotho (MP 74% / 1.0), Hogsback (93% / 68% / 1.0) and *P. microlepidotus*. Only the Hogsback sample is considered part of an isolated *P. m. subviridis* population (Amatole-Winterberg).

Uncorrected “p” distances between individuals from the same population were generally low (0-1%), but varied from 0 to 3.59 in the Organ Pipes population of *P. m. subviridis* and 1.93 to 3.82 in the Monontsha Pass population of *P. m. subviridis*. Divergence values between populations of the same group (*transvaalensis*, N and S *melanotus*, *subviridis*, *langi*, *spinosus*) were generally low ( $\leq 1.20\%$ ), but as high as 4.59% in some cross-population comparisons of *P. m. subviridis*. The highest values (2.86-4.59%) were between the Hogsback-S Lesotho-Naude’s Nek clade and other *P. m. subviridis*. The other two *P. m. subviridis* clades are difficult to analyze as they contain specimens from the same populations. Divergence values between *P. transvaalensis*, Northern *melanotus*, Southern *melanotus* and *P. m. subviridis* were moderate (maximum 3.83% between *transvaalensis* and *subviridis*) to small (minimum 1.20% between *transvaalensis* and Southern *melanotus*) (Table 4.1). The most divergent clades were *P. langi* and *P. spinosus* (6.27-7.01%). *Pseudocordylus langi* also differed from all other clades by at least 5.29%, while *P. spinosus* differed by at least 3.11% from all other clades except *P. m. subviridis* (0.00-4.55%)(Table 4.1).

**Table 4.1:** Uncorrected (“p”) sequence divergence values for the 16S rRNA gene among major genetic assemblages (clades/groups) in the *Pseudocordylus melanotus* species complex.

	<i>transvaalensis</i>	N <i>melanotus</i>	S <i>melanotus</i>	<i>subviridis</i>	<i>P. langi</i>
N <i>melanotus</i>	2.41 - 2.90				
S <i>melanotus</i>	1.20 – 3.35	2.65 – 3.61			
<i>P. m. subviridis</i>	1.91 – 3.83	3.35 – 4.61	2.39 – 4.55		
<i>P. langi</i>	5.29 – 5.80	5.77 – 6.01	5.53 – 6.25	5.30 – 6.76	
<i>P. spinosus</i>	3.11 – 4.07	3.85 – 4.80	3.35 – 4.79	0.00 - 4.55	6.27 – 7.01

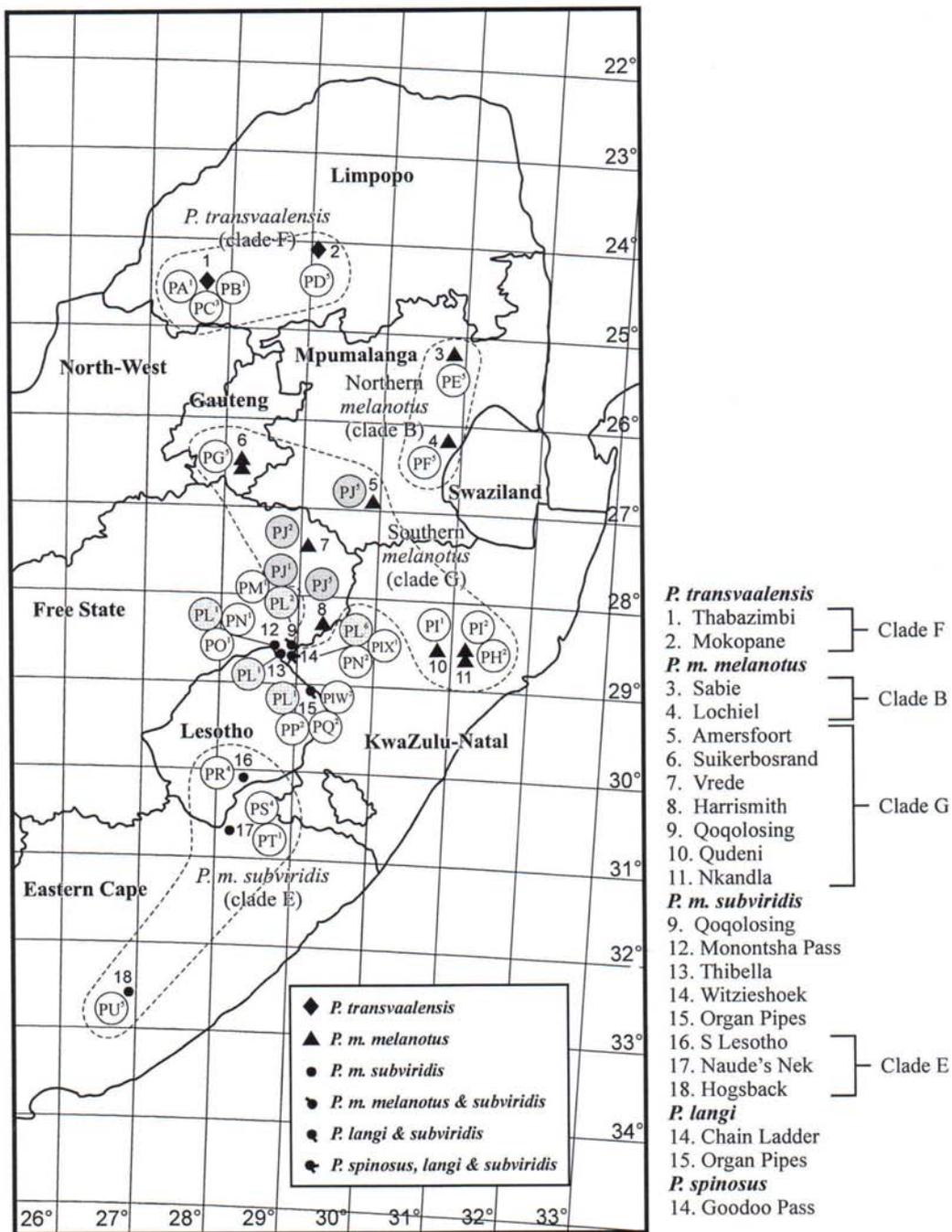
#### 4.3.2 Nested Clade Analysis

Geographical distribution of 22 of the 23 alleles is shown in Figure 4.3. Allele Pmic is restricted to *P. m. microlepidotus* from Vermaakskop (3325CB), a locality not shown on the map. Both *P. m. subviridis* (alleles PL and PM) and Southern *melanotus* (allele PJ) occur at locality 9. Three taxa occur at locality 14, namely *P. m. subviridis* (alleles PL [one] and PN), *P. langi* (allele PIX) and *P. spinosus* (allele PL, five); while two taxa are found at locality 15, namely *P. m. subviridis* (PL, PP and PQ) and *P. langi* (allele PIW). Allele PJ includes samples with missing data at position 121 (Appendix. 4.2). The greatest number of alleles in a single conspecific population is three. This applies to the Thabazimbi population of *P. transvaalensis* (locality 1), and the Monontsha Pass (locality 12) and Organ Pipes Pass (locality 15) populations of *P. m. subviridis* (Fig. 4.3). Four more populations have two alleles each: *P. m. subviridis* - Qoqolosing (locality 9), Witzieshoek (locality 14) and Naude's Nek (locality 17); *P. m. melanotus* - Nkandla (locality 11).

Nesting clades are illustrated in Figure 4.4. Coalescence occurred within one to 22 steps. Full nesting structure and nested clade statistics within each taxon/grouping is presented in Figure 4.5. The latter figure demonstrates a recurrent pattern of significantly small clade distances - particularly for interior clades, some significantly large nested distances for tip clades, with significantly small nested distances for interior clades (resulting in differences between interior and tip clade, and nested distances).

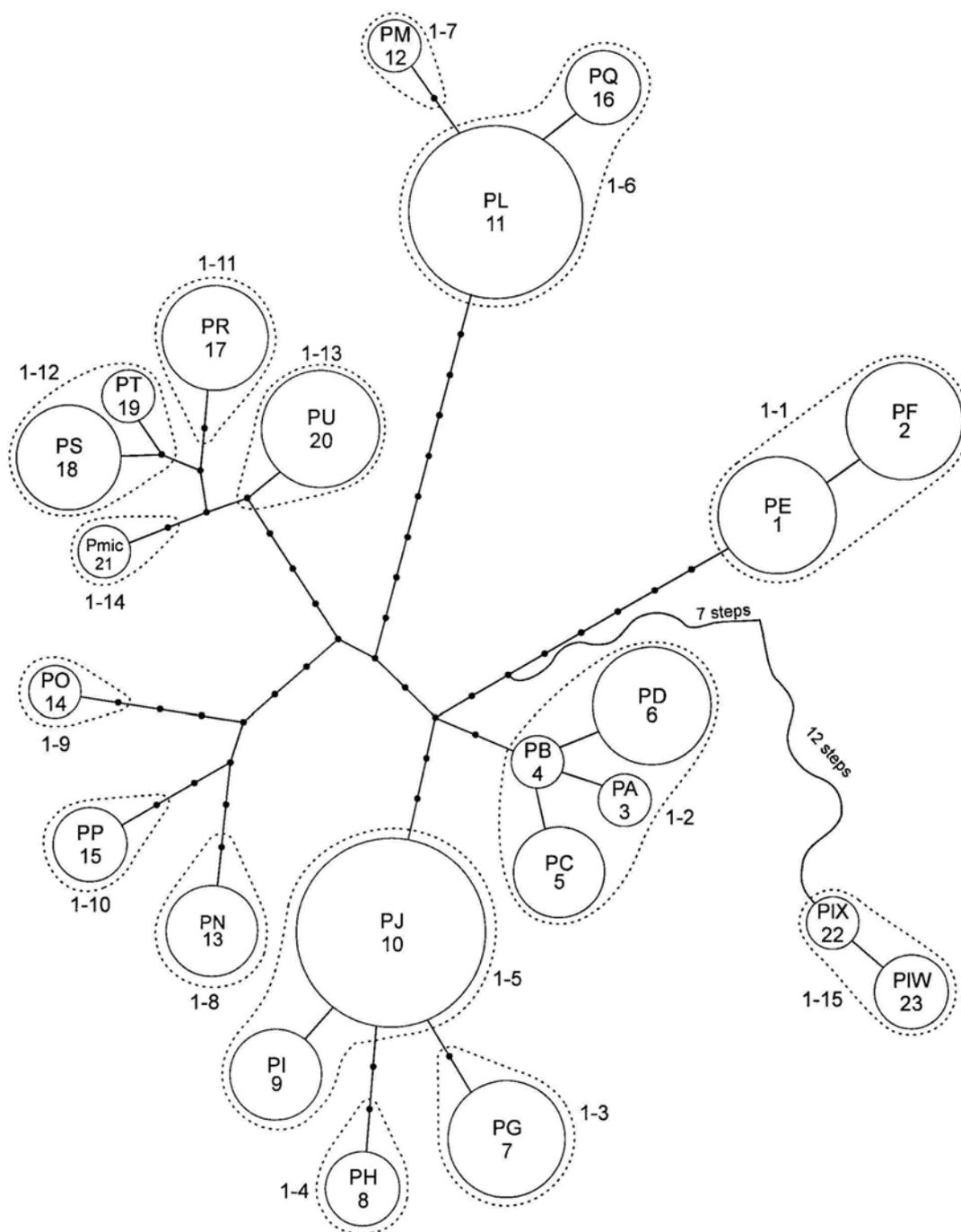
Interpretation of geographic structure is summarised in Table 4.2. Abutting lineage ranges within a clade were interpreted as a continuously distributed group rather than separate areas without intermediates. Allopatric fragmentation was indicated at the 1-step and 2-step levels in Southern *melanotus* (Table 4.2). At the 1-step level in *P. transvaalensis*, genetic structuring is explained by past gene flow followed by the extinction of intermediate populations. Contiguous range expansion was detected at the 1-step level in northern *P. m. subviridis*, 4-step level in *P. subviridis/P. microlepidotus* and 6-step level in Southern *melanotus/P. transvaalensis*. For Southern *melanotus* at the 3-step level, structuring is explained by range expansion/colonization or restricted dispersal/gene flow. Restricted gene flow with isolation by distance is indicated at the 11-step level for the *subviridis/spinosus/microlepidotus* group and at the 13-step level for

the *melanotus/transvaalensis/subviridis/spinosus/microlepidotus* group. Past fragmentation and/or long distance colonization was detected at the 15-step level for the *P. melanotus* species complex (minus *P. langi*). In other cases no historical inferences could be made because of incomplete sampling (Northern *melanotus*) or due to the absence of an interior within a nesting clade.



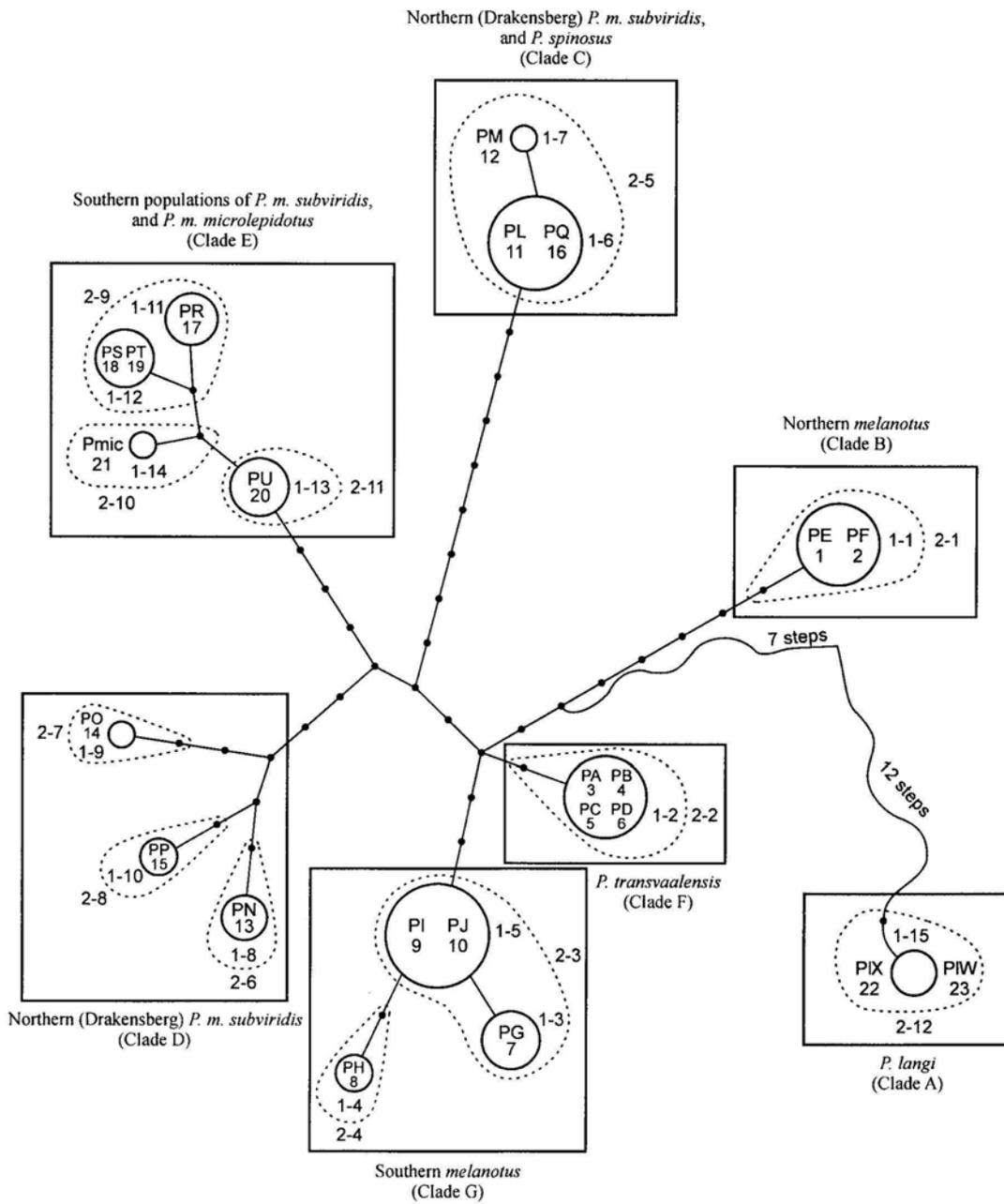
**Figure 4.3:** Geographical distribution of 22 alleles in the *Pseudocordylus melanotus* species complex (allele Pmic for *P. microlepidotus* not shown). Alleles referable to clades B (Northern *melanotus*), E (southern *subviridis*), F (*P. transvaalensis*) and G (Southern *melanotus*) are grouped together. Allele frequency per site is indicated by a superscript. The most common alleles are indicated by grey circles (PJ, 13 specimens) or stippled circles (PL, 11 specimens).

0-step network (1-step nesting)



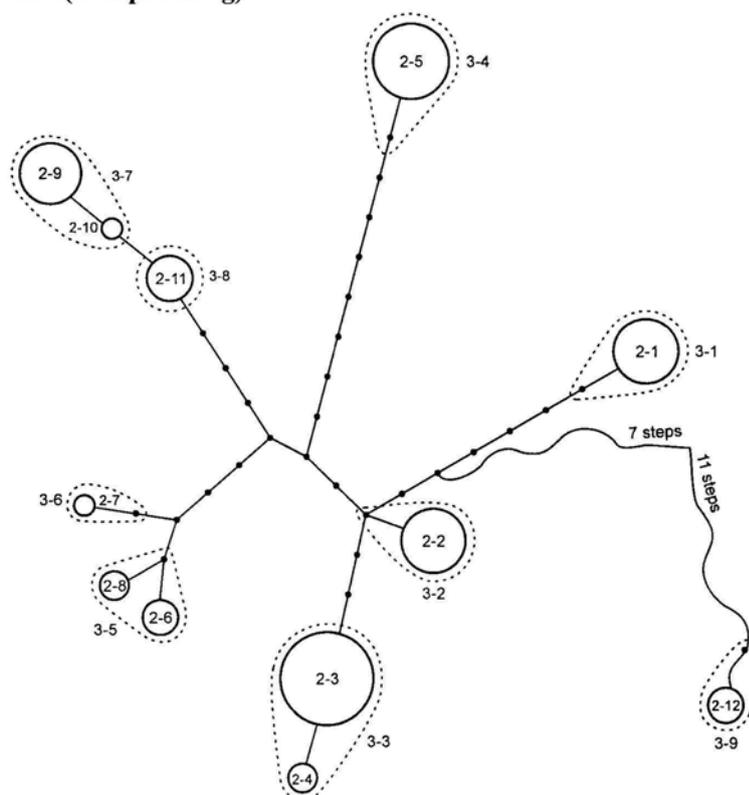
**Figure 4.4:** 16S rRNA phylogeography of the *Pseudocordylus melanotus* species complex. In the unrooted allele tree mutations are indicated as lines, with solid circles indicating missing alleles. In cases where branches lack solid circles there is one mutation along that branch, which has led to the new allele (haplotype) that is one step different. In the case of one solid circle along a branch there have been two mutations, namely the missing one (solid circle) plus the one that led to the new allele. Circles are scaled according to allele frequency.

**1-step network (2-step nesting)**

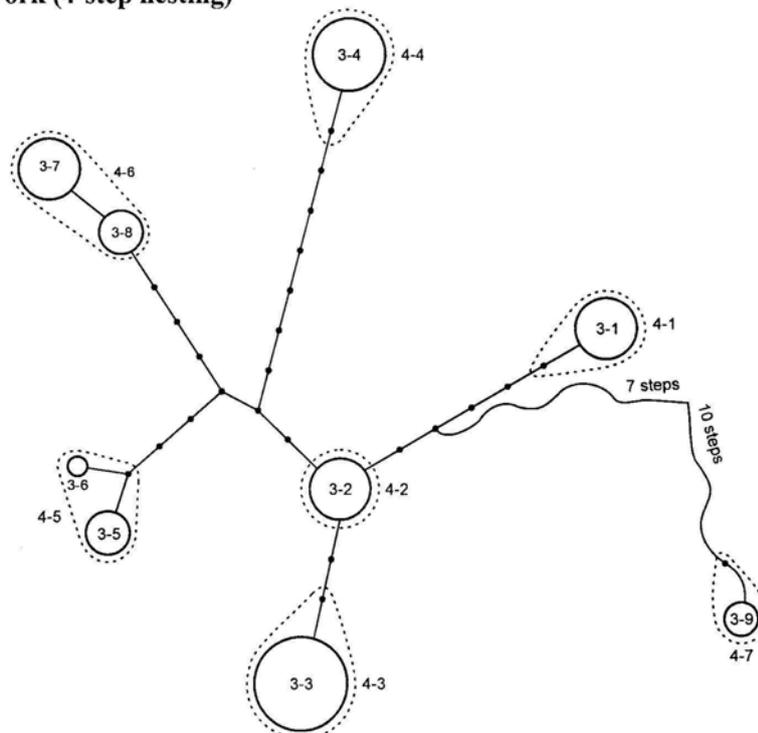


**Figure 4.4 (continued):** 16S rRNA phylogeography of the *Pseudocordylus melanotus* species complex. In the unrooted allele tree mutations are indicated as lines, with solid circles indicating missing alleles. In cases where branches lack solid circles there is one mutation along that branch, which has led to the new allele (haplotype) that is one step different. In the case of one solid circle along a branch there have been two mutations, namely the missing one (solid circle) plus the one that led to the new allele. Circles are scaled according to allele frequency. Alleles referable to clades A to G are shown in boxes.

**2-step network (3-step nesting)**

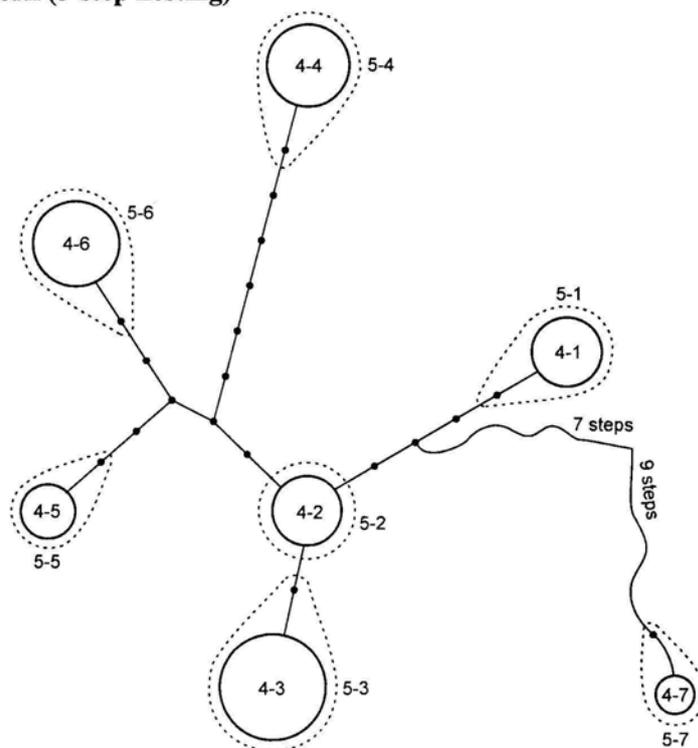


**3-step network (4-step nesting)**

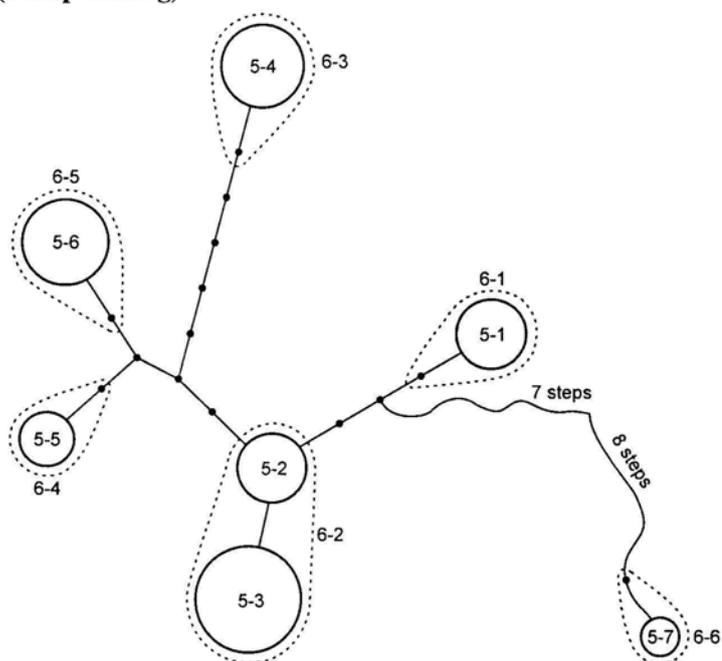


**Figure 4.4 (continued):** 16S rRNA phylogeography of the *Pseudocordylus melanotus* species complex. In the unrooted allele tree mutations are indicated as lines, with solid circles indicating missing alleles. In cases where branches lack solid circles there is one mutation along that branch, which has led to the new allele (haplotype) that is one step different. In the case of one solid circle along a branch there have been two mutations, namely the missing one (solid circle) plus the one that led to the new allele. Circles are scaled according to allele frequency.

**4-step network (5-step nesting)**

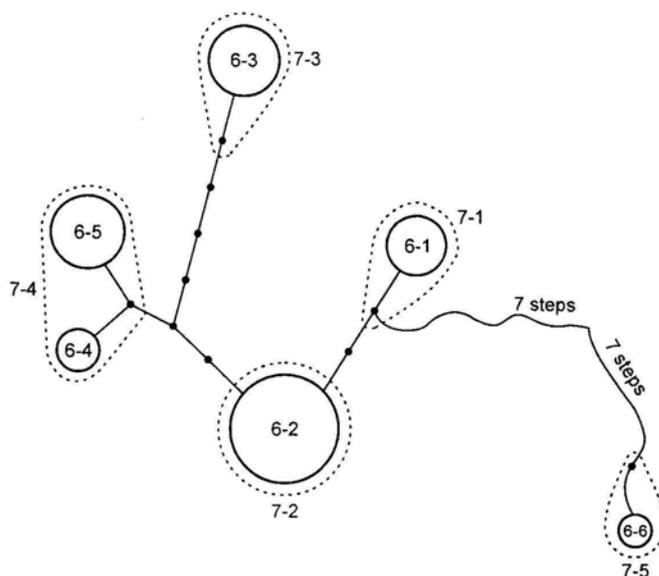


**5-step network (6-step nesting)**

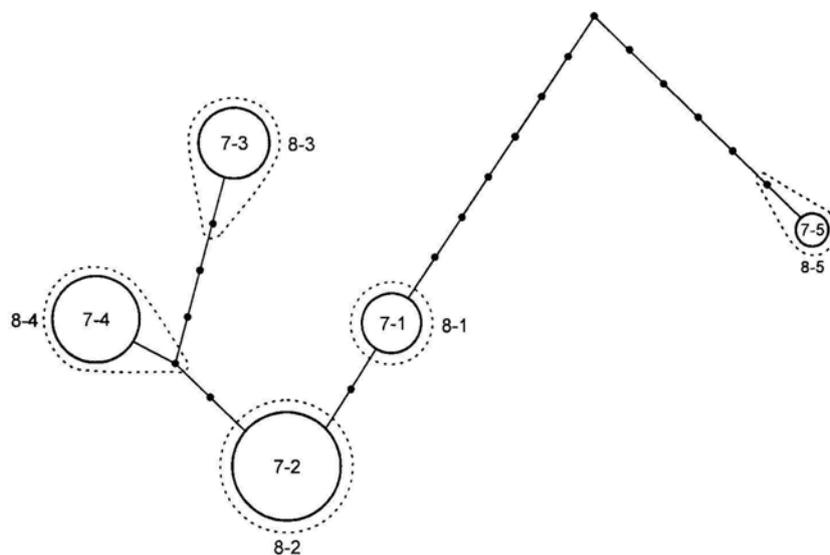


**Figure 4.4 (continued):** 16S rRNA phylogeography of the *Pseudocordylus melanotus* species complex. In the unrooted allele tree mutations are indicated as lines, with solid circles indicating missing alleles. In cases where branches lack solid circles there is one mutation along that branch, which has led to the new allele (haplotype) that is one step different. In the case of one solid circle along a branch there have been two mutations, namely the missing one (solid circle) plus the one that led to the new allele. Circles are scaled according to allele frequency.

**6-step network (7-step nesting)**

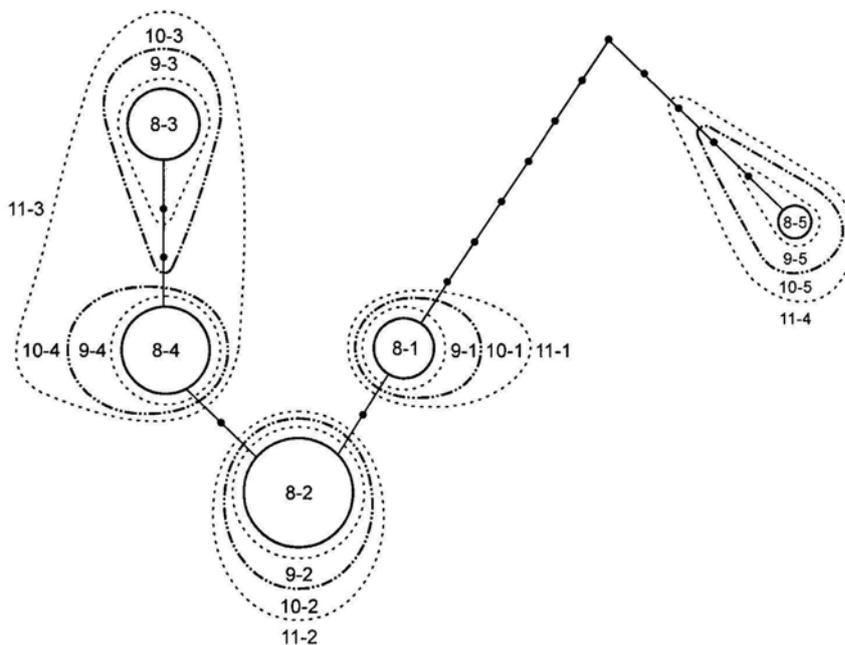


**7-step network (8-step nesting)**

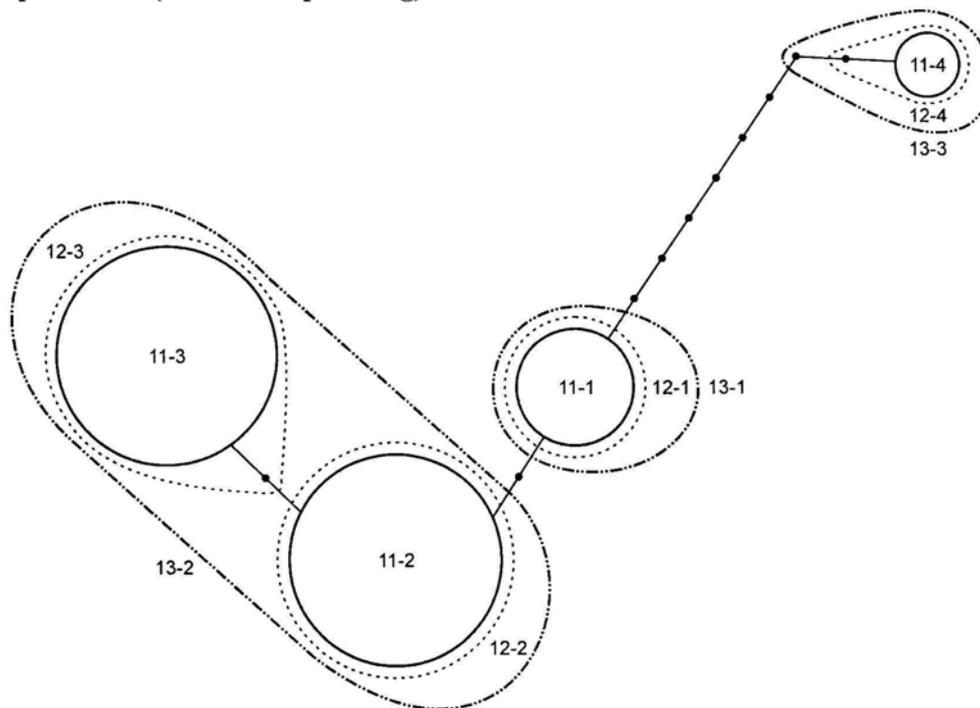


**Figure 4.4 (continued):** 16S rRNA phylogeography of the *Pseudocordylus melanotus* species complex. In the unrooted allele tree mutations are indicated as lines, with solid circles indicating missing alleles. In cases where branches lack solid circles there is one mutation along that branch, which has led to the new allele (haplotype) that is one step different. In the case of one solid circle along a branch there have been two mutations, namely the missing one (solid circle) plus the one that led to the new allele. Circles are scaled according to allele frequency.

**8-step network (9, 10 & 11-step nesting)**

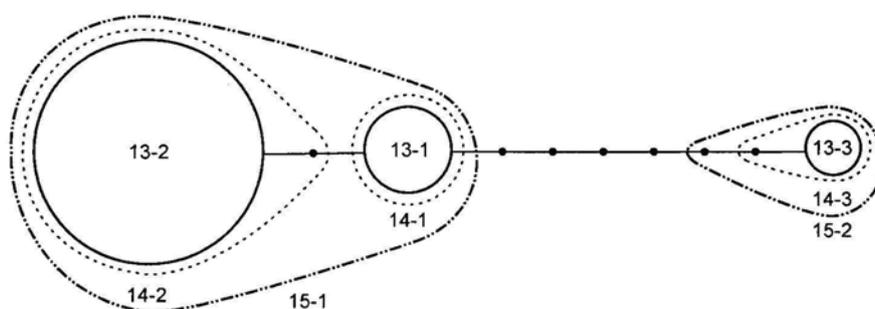


**11-step network (12 & 13-step nesting)**

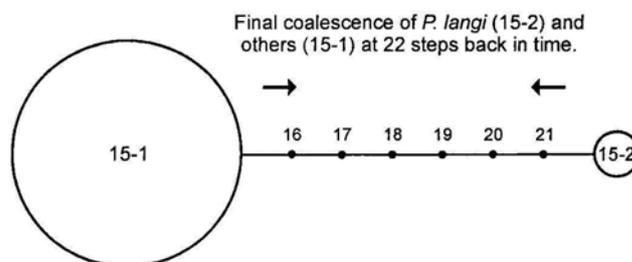


**Figure 4.4 (continued):** 16S rRNA phylogeography of the *Pseudocordylus melanotus* species complex. In the unrooted allele tree mutations are indicated as lines, with solid circles indicating missing alleles. In cases where branches lack solid circles there is one mutation along that branch, which has led to the new allele (haplotype) that is one step different. In the case of one solid circle along a branch there have been two mutations, namely the missing one (solid circle) plus the one that led to the new allele. Circles are scaled according to allele frequency.

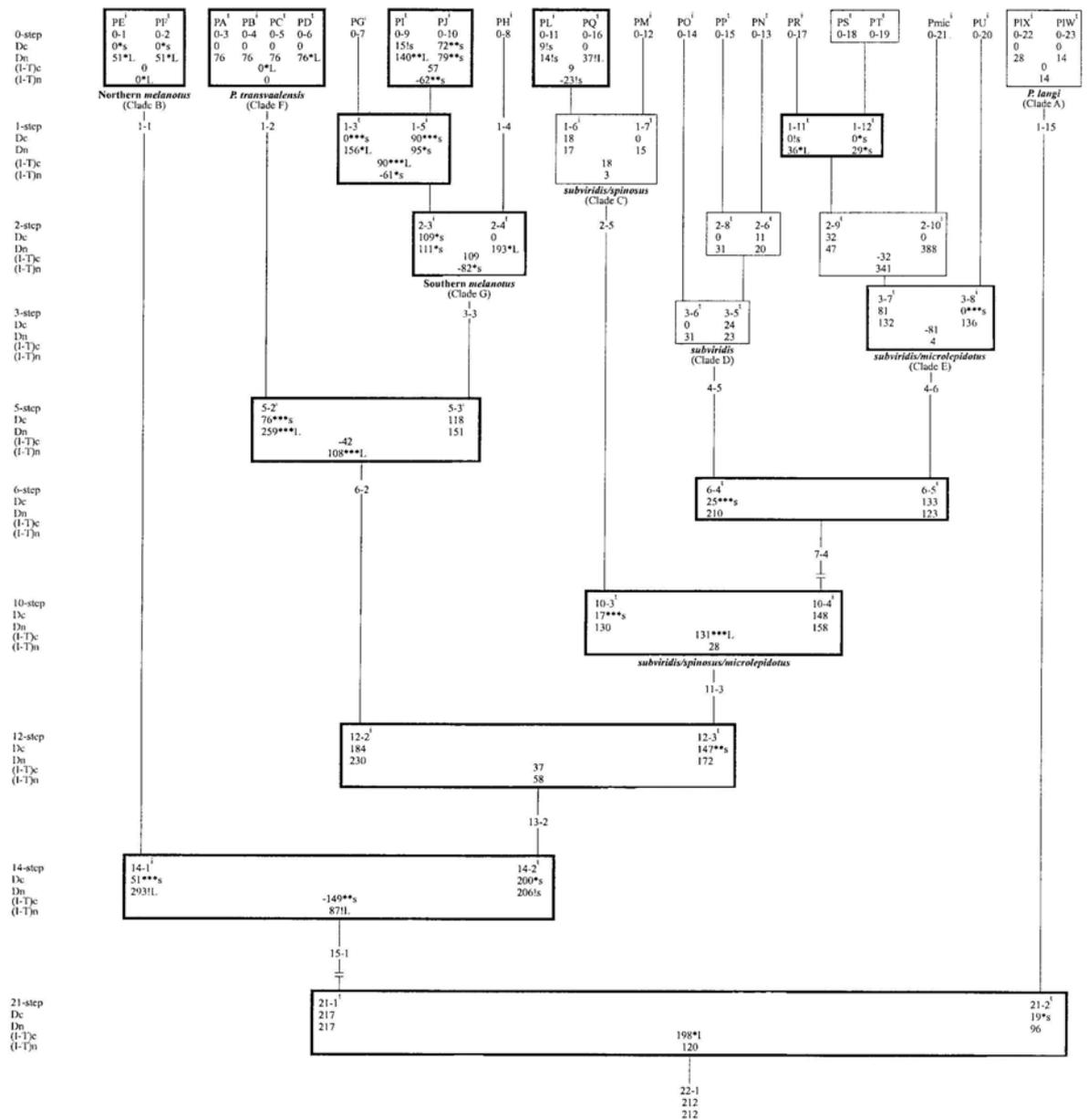
**13-step network (14 & 15-step nesting)**



**15-step network**



**Figure 4.4 (continued):** 16S rRNA phylogeography of the *Pseudocordylus melanotus* species complex. In the unrooted allele tree mutations are indicated as lines, with solid circles indicating missing alleles. In cases where branches lack solid circles there is one mutation along that branch, which has led to the new allele (haplotype) that is one step different. In the case of one solid circle along a branch there have been two mutations, namely the missing one (solid circle) plus the one that led to the new allele. Circles are scaled according to allele frequency.



**Figure 4.5:** Nested clad analysis of the *Pseudocordylus melanotus* species complex. Zero step clades (alleles) are indicated in the top line, <sup>i</sup> indicates an internal clade, <sup>t</sup> indicates a tip clade. Rectangles define nesting clades and are connected to clade names at the next level (e.g. 1-1 comprises PE and PF). Clade distance (Dc) and nested clade distance (Dn) in kilometres is presented below clade names. Internal minus tip clade distance (I-T)c and Internal minus tip nested clade distance (I-T)n are indicated within each nesting clade box. Significant and near significant results from permutation tests are indicated by bold outlines around nesting clades. L indicates significantly large distances, s indicates significantly small distances: !p<0.1, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

**Table 4.2:** Interpretation of Nested Clade Analysis (following Templeton 2004).

Species/Group	Clade	Key path	Historical inference
Northern <i>melanotus</i>	1-1	1-19-20-No	Incomplete sampling
<i>P. transvaalensis</i>	1-2	1-2d-3b-5-6-7-8	Past gene flow followed by extinction of intermediate populations
Southern <i>melanotus</i>	1-5	1-19-No	Allopatric fragmentation
northern <i>subviridis/spinosus</i>	1-6	1-2-11b-12-No	Contiguous range expansion
Southern <i>melanotus</i>	2-3	1-19-No	Allopatric fragmentation
southern <i>subviridis</i>	2-9	1-2-two tips	Inconclusive
Southern <i>melanotus</i>	3-3	1-2d-3a,b,c-5-6-only two clades	Range expansion/colonization or restricted dispersal/gene flow
<i>subviridis/microlepidotus</i>	4-6	1-2-11b-12-No	Contiguous range expansion
Southern <i>melanotus/P. transvaalensis</i>	6-2	1-19-20-2-11b-12-No	Contiguous range expansion
southern <i>subviridis/microlepidotus</i>	7-4	1-2-two tips	Inconclusive
<i>subviridis/spinosus/microlepidotus</i>	11-3	1-2a,d-3-4-No	Restricted gene flow with isolation by distance
<i>melanotus/transvaalensis/subviridis</i>	13-2	1-2a-3-4-No	Restricted gene flow with isolation by distance
<i>melanotus/transvaalensis/subviridis/N melanotus</i>	15-1	1-2-11b,c-12-13-14	Past fragmentation and/or long distance colonisation
<i>P. melanotus</i> complex	22-1	1-2-two tips	Inconclusive

#### 4.4 Discussion

The results obtained in this study, using 16S rRNA as a marker, corroborated most of the results obtained in the enzyme electrophoretic analysis (Chapter 3). Firstly, *P. langi* was again found to be basal in the *P. melanotus* species complex. With the addition of *P. microlepidotus* and *P. spinosus* to the ingroup, it in effect means that *P. langi* is the basal species in the genus *Pseudocordylus*. The analyses of Frost *et al.* (2001) and Melville *et al.* (unpublished data) have both indicated that *P. capensis* and *P. nebulosus* do not belong in the *Pseudocordylus* clade. Secondly, the 16S rRNA results confirm that *P. m. melanotus*, as presently construed, is comprised of two clades which are not sister groups. The Nkandla population was, however, found to cluster with the other southern *P. m. melanotus* populations and not with the *P. transvaalensis* populations as was the case in the electrophoretic analysis. However, the most surprising result of the 16S rRNA analysis was the finding that both *P. microlepidotus* and *P. spinosus* are embedded within *P. m. subviridis*.

From the results of both the allozyme and 16S rRNA analyses it is clear that the northern populations of *P. m. melanotus* (Sabie and Lochiel) form a fairly deeply divergent and old clade and may represent a separate species. Although the allozyme analysis (Chapter 3) also suggested that northern *P. m. melanotus* populations formed a separate lineage, there was a fixed allelic difference between the Sabie and Lochiel populations. Nevertheless, the current analysis indicated only 0.24% sequence divergence between the two populations.

The finding that both *P. microlepidotus* and *P. spinosus* are embedded within *P. m. subviridis* makes definitive taxonomic decisions with regards to *P. m. subviridis* impossible at this stage. Both *P. microlepidotus* and *P. spinosus* are morphologically distinct forms and there is no doubt as to the correct specific assignment of specimens used in my analysis. Melville *et al.* (unpublished data) also found *P. microlepidotus* to be embedded within the *P. melanotus* complex and there is therefore no reason to doubt the validity of my finding. It is suggested that *P. m. subviridis*, *P. spinosus* and *P. microlepidotus* should all provisionally be treated as full species.

In the case of *P. spinosus* in particular, there was no resolution at population level between the population of this species and several *P. m. subviridis* populations sampled from the northern Maloti-Drakensberg. In fact, *P. spinosus* shared the same haplotype (PL) as a few specimens of *P. m. subviridis* (Appendix 4.2). One possible explanation for this is that the *P. spinosus* sample represents hybrids between *P. m. subviridis* females and *P. spinosus* males (mtDNA is maternally inherited). However, it is unlikely that all five specimens sampled would have been hybrids, *i.e.* of the “wrong” haplotype. It is more likely that *P. spinosus* evolved from within *P. m. subviridis* and that there was recent, rapid morphological differentiation. The *P. spinosus* lineage may therefore have separated from a *P. m. subviridis* ancestor relatively recently, such that genetic divergence is lacking despite distinct morphological divergence.

The inter-digitation of *P. m. microlepidotus* between populations from the southern part of the geographical range of *P. m. subviridis* suggests that the *P. microlepidotus* species complex originated as a result of a vicariant event/s in this area. In fact, it is clear that the genus *Pseudocordylus* has its roots in the KwaZulu-Natal Drakensberg and from there dispersed to the north (northern *P. m. melanotus*), then south (Southern Lesotho/Hogsback area), and from there to the west to reach the south-western tip of Africa. The close relationship between *P. m. subviridis* and *P. microlepidotus* is corroborated by the findings of Melville *et al.* (unpublished data) as noted below.

Morphological variation is not always correlated with genetic divergence. In the case of some chameleons of the genus *Bradypodion*, morphologically distinct species proved to be genetically very similar (*e.g.* *Bradypodion taeniabronchum* [A. Smith, 1831] and *B. ventrale* [Gray, 1845] – Tolley & Burger 2004; *B. melanocephalum* [Gray, 1865] and *B. thamnobates* Raw, 1976 – Tolley *et al.* 2004). Alternatively, in terms of their mtDNA phylogenies, morphologically similar populations may be diagnosable (*e.g.* populations associated with *B. taeniabronchum* – Tolley & Burger 2004; Tolley *et al.* 2004) or even very distinct (*e.g.* several species in the *Pachydactylus serval* and *P. weberi* Groups – Bauer, Lamb & Branch 2006). Examination of more rapidly evolving genes may help to resolve the taxonomy of the *P. melanotus* species complex or at least provide indications of possible contributing factors involved.

The sequence divergence values obtained for both intra-clade (= inter-population) (0.00-4.06%) and inter-clade (1.20-7.01%) comparisons in the present study were comparatively low. In contrast, Lamb & Bauer (2001) reported 16S rRNA genetic distances of 8.93 to 14.55% between known species of *Rhoptropus* and 3.94% between subspecies of *R. bradfieldi*, although they obtained much greater differentiation using *cytb*; Bauer & Lamb (2002) reported 16S rRNA genetic distances of 4.18 to 16.14% between the five species in the *Pachydactylus capensis* species complex – lowest values (4.18-6.49%) were between members of a temperate lineage comprising the morphologically similar *P. capensis*, *P. vansoni* and *P. affinis* – but once again there were much larger differences with regard to *cytb*; Matthee & Flemming (2002) reported 16S rRNA sequence divergence values of as low as 0.21% for intra-population, and as high as 4.41% for inter-population, comparisons in the *Agama atra* species complex, but found that with *cytb* the differences were much greater between populations (high of 17.8%); Scott, Keogh & Whiting (2004) reported genetic distances of 8.68 to 26.25% for intra-clade, and 19.17 to 31.60% for inter-clade, comparisons in *Platysaurus*; Glor, Kolbe, Powell, Larson & Losos (2003) reported 5 to 18% sequence divergence between 16 allopatric or parapatric groupings of *Anolis*.

Daniels, Mouton & Du Toit (2004) reported 16S rRNA corrected sequence divergence values of 1.69 to 2.85% for intra-clade, and 4.30 to 6.31% for inter-species, comparisons in the *Cordylus cordylus-niger-oelofseni* species complex. However, using ND2, the inter-population (5.82-10.80%) and inter-specific (generally >15%) differences were greater. Despite the low (1.69%) 16S rRNA sequence divergence between *C. oelofseni* populations, the much greater (9-10%) ND2 values led Daniels *et al.* (2004) to suggest that the three populations of this species probably all merit specific recognition.

Sequence divergence values for the 16S rRNA gene appear to be low between taxa in some genera (*e.g.* scincids). For example, Daniels, Heideman, Hendricks & Willson (2002) determined differences of mainly <3% for species and subspecies in the genus *Acontias*, with values of around 2% for intra-specific comparisons; whereas Mausfeld, Vences, Schmits & Veith (2000) determined values of only 1.6 to 6.2% between species of *Mabuya*.

Melville *et al.* (unpublished data) studied the molecular phylogeny of cordylids using the genes ND2 and CO1, and seven tRNA genes. They found that members of the *P. melanotus* (*melanotus*, *subviridis*, *langi*) and *P. microlepidotus* species complexes differed from *Cordylus* by at least 15.7% sequence divergence, and from *Pseudocordylus nebulosus* by at least 15.5%. *Pseudocordylus langi* differed from other members of the *P. melanotus* and *P. microlepidotus* species complexes by at least 12.8%. However, they found that *melanotus* and *subviridis* differed by only 6%, *melanotus* and *microlepidotus* differed by 6.7%, and *subviridis* and *microlepidotus* differed by as little as 4.1%.

The short internal branch lengths for most clades and subclades in the phylogram (Fig. 4.2) suggest recent rapid divergence and radiation of populations. Because of this rapid radiation it is apparent that the 16S rRNA gene is not the optimal gene to use for studying evolutionary relationships in the *P. melanotus* species complex. An analysis of a more rapidly evolving gene such as CO1 may allow better phylogenetic resolution.

In order to obtain further insight into the biogeography of the complex, a Nested Clade Analysis was conducted. The recurrent pattern of small clade distances, some large nested distances for tip clades, with small nested distances for internal clades, suggested an intricate pattern of historical fragmentation with occasional range expansion events that allowed colonization of new areas and a residual pattern of isolation by distance across fragmented populations. The disjunct nature of the three *P. transvaalensis* populations may be explained by past gene flow followed by the extinction of intermediate populations.

Better resolution will be achieved if more populations are included in the analyses. For example, the Eastern population of *P. transvaalensis* should be included so as to establish whether or not this species is monophyletic (the three populations are separable in discriminant analysis – see Chapter 5). Nuclear gene markers could be studied and may provide better insight into evolutionary relationships. The possibility of hybridization - especially between *P. spinosus* and *P. m. subviridis* - should be examined, possibly using allozymes, but preferably using nuclear DNA sequencing or microsatellites. *Pseudocordylus spinosus* samples from areas distant to where *P. m. subviridis* occurs should be included to establish whether or not the Goodoo Pass sample represents an isolated case of hybridization.

In maximum parsimony, phylogenetic resolution and support for relationships is increased when the number of characters used increases (see references in De Queiroz, Lawson & Lemos-Espinal 2002). Therefore, it is hoped that better resolution, especially of shallow nodes (see clades G, F and the group comprising clades C, D and E), will be achieved once the results of the CO1 analysis is complete. However, even though more genes could be sequenced for mtDNA characters, a plateau in resolution and support is eventually expected because, firstly, as the number of characters increases, a point should be reached at which any remaining unresolved clades will be difficult to resolve, and secondly, even if no strongly recalcitrant clades remain, resolution/support should plateau because, as the number of characters increases and clades are resolved, fewer groups remain in the pool of unresolved clades (De Queiroz *et al.* 2002). If additional mitochondrial sequence characters do not resolve ambiguities, sequencing of nuclear introns should be attempted in order to achieve resolution. This may be preferable over the coding regions of nuclear genes, as these generally evolve too slowly to provide resolution.

## CHAPTER 5

### **A morphological analysis of the *Pseudocordylus melanotus* (A. Smith, 1838) species complex (Sauria: Cordylidae)**

#### **5.1 Introduction**

The *Pseudocordylus melanotus* species complex currently consists of five taxa, of which at least three are morphologically poorly defined. The first two taxa were described as *Cordylus (Pseudocordylus) melanotus* and *C. (P.) subviridis* by Andrew Smith in 1838. More than one hundred years later, *Pseudocordylus subviridis transvaalensis* was described by FitzSimons (1943), followed shortly thereafter by *P. langi* Loveridge 1944 and *P. spinosus* FitzSimons 1947. Since then the taxonomic status of the first three taxa has undergone several changes (see Chapter 2). Although FitzSimons (1947) described *P. spinosus*, specimens referable to this species had previously (1943) been treated by him as *P. m. subviridis*. Finally, FitzSimons (1948) considered *Pseudocordylus langi* to be a junior synonym of *P. m. subviridis*.

De Waal (1978) proposed that *P. melanotus* consists of three subspecies, namely *melanotus*, *subviridis* and *transvaalensis*. This was accepted by all subsequent authors (e.g. Branch 1988). However, neither FitzSimons' (1943) Limpopo and Mpumalanga province specimens, nor Broadley's (1964) KwaZulu-Natal records were critically evaluated, with the result that Branch (1988) mapped *P. transvaalensis* as occurring in a large area from Limpopo province southwards into the KwaZulu-Natal midlands. Jacobsen (1989), in an unpublished thesis, later restricted *P. transvaalensis* to three allopatric populations in Limpopo Province and suggested that it be considered a full species. This proposal was put into effect in Branch's (1998) *Field Guide*, but no reasons were given for the action. Both Branch (1985) and Mouton (1997) indicated that the *P. melanotus* species complex was in need of revision.

To a large extent the unresolved taxonomic status of the various forms of the *P. melanotus* species complex is the result of inappropriate methods of evaluation. In the

three most recent revisions, study areas were restricted to political regions (provinces) rather than the natural geographical distribution range of the complex (Broadley 1964; De Waal 1978; Jacobsen 1989). In addition, too much emphasis was placed on particular characters, even though little was known about variation throughout the range. Broadley (1964), for example, separated *P. s. subviridis* and *P. s. transvaalensis* on the basis of differences in spacing between rows of dorsolaterals. This character was found to be fairly variable in several populations (see below). Finally, the way in which these authors summarized variation in scale characters meant that any differences between particular populations were subsumed within the total range of variation. For example, although Jacobsen (1989) noted that the condition of the frontonasal (divided or not) of *P. m. melanotus* varied considerably, and recognized the fact that this form occurred in three allopatric populations in his study area, he did not recognize a geographical pattern to this variation (see below). Jacobsen (1989) also recognized that the three allopatric populations of *P. transvaalensis* were distinguishable on the basis of certain scalation characteristics, but he failed to elaborate.

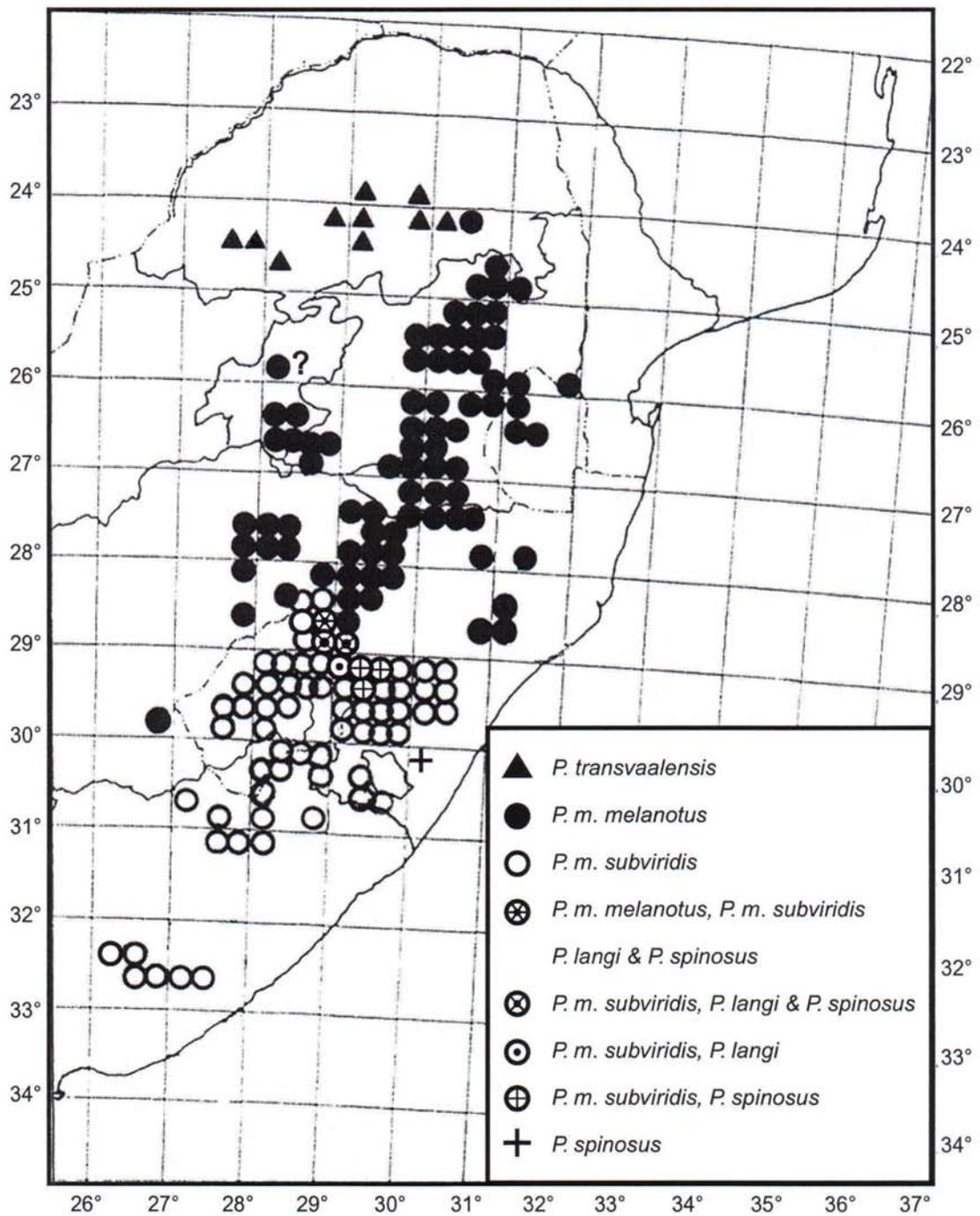
Hillis (1987) argued in favour of the increased combination of molecular and morphological data so as to maximize phylogenetic information. He noted that strong congruence between studies provides good evidence that the underlying historical pattern has been discovered. Therefore, the main aim of this analysis is to establish whether or not there is morphological support for the main genetic assemblages or clades determined by the mtDNA analysis (Chapter 4). A detailed analysis of morphological variation was therefore conducted on a large sample from throughout the range of the *P. melanotus* species complex.

## 5.2 Materials and Methods

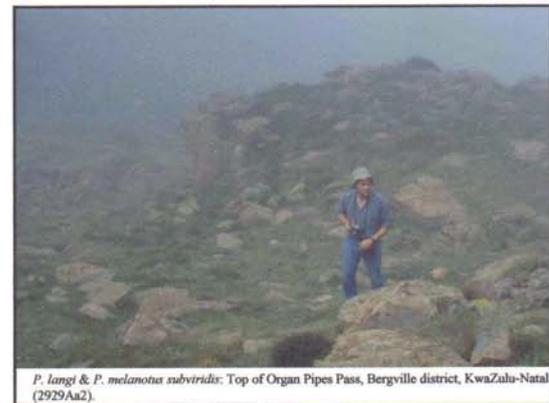
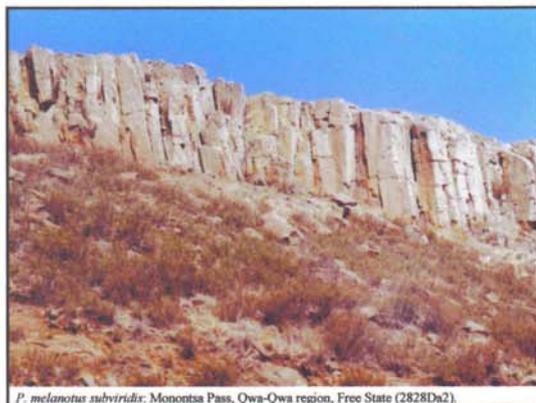
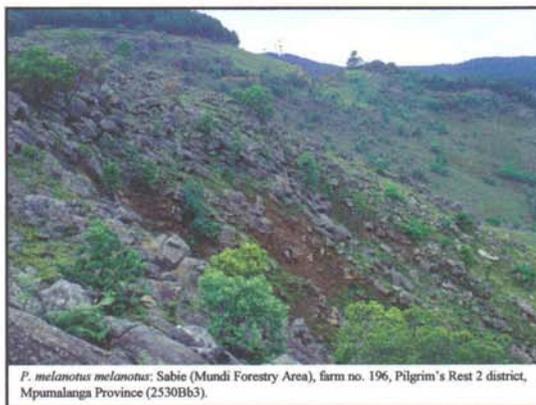
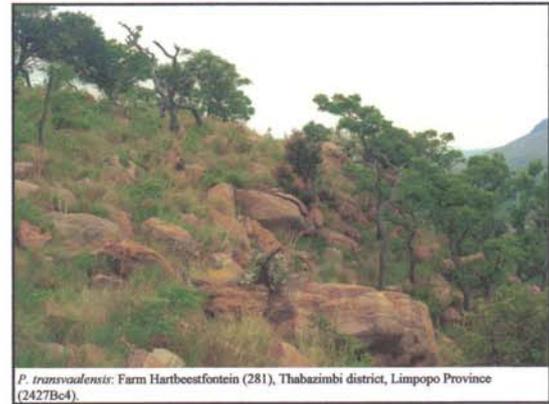
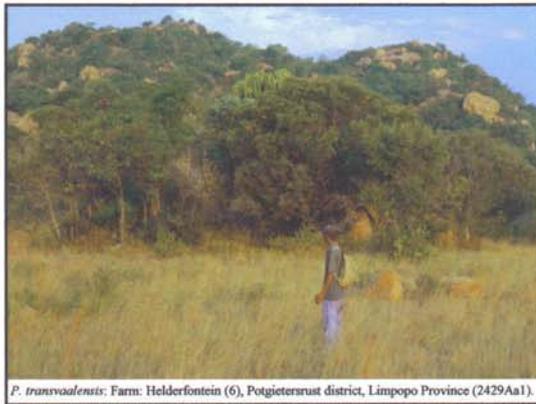
### 5.2.1 Sampling

Populations referable to the *P. melanotus* species complex occur over an extensive area in the eastern part of South Africa - including Swaziland and Lesotho - from about 24° to 33°S latitude and between 26° and 32°E longitude (Fig. 5.1; Appendix 2.1) and are associated with mountainous or rocky terrain (Fig. 5.2). In order to measure

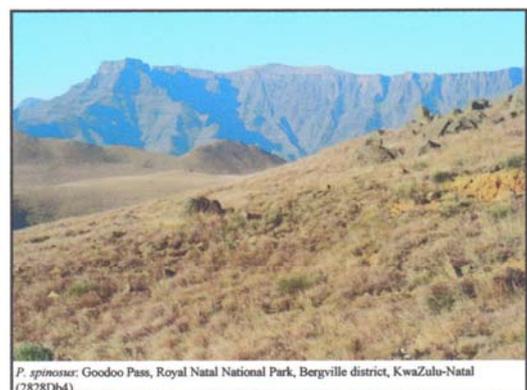
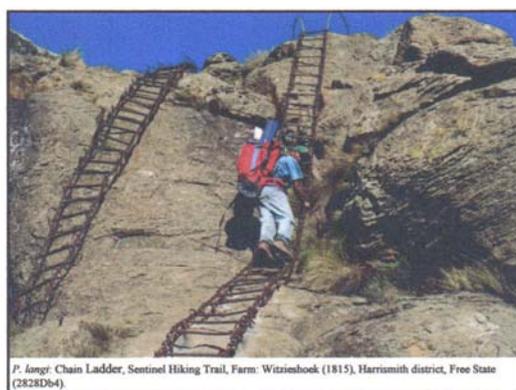
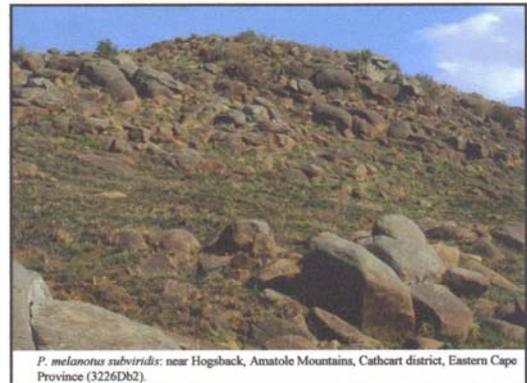
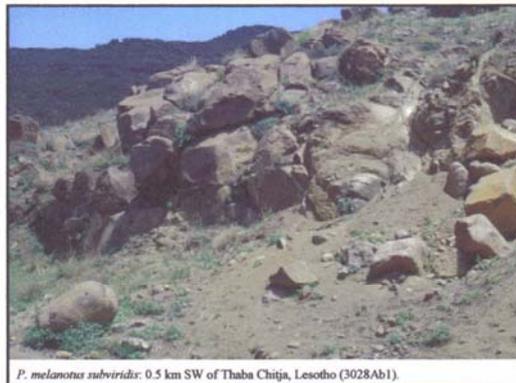
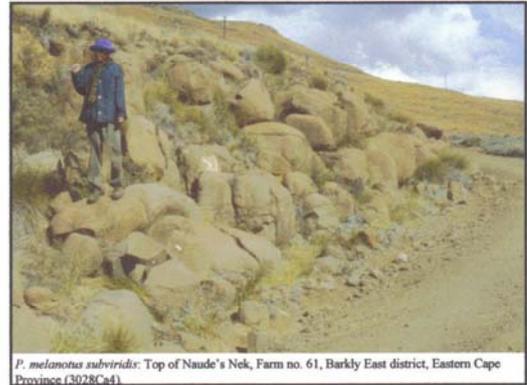
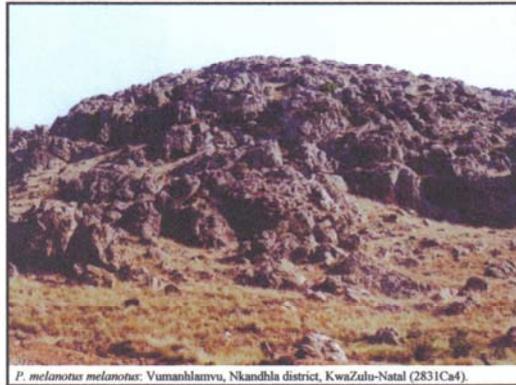
morphological variation in such a widely distributed species complex, specimens from throughout the extensive range were selected for examination. An attempt was also made to include all isolated populations, *e.g.* Suikerbosrand, Nkandhla district and Amatole-Winterberg Mountains (Fig. 5.1).



**Figure 5.1:** Geographical distribution of the *Pseudocordylus melanotus* complex, based on quarter-degree units (see Appendix 2.1 for details).



**Figure 5.2:** Habitats of populations referable to the *Pseudocordylus melanotus* complex.

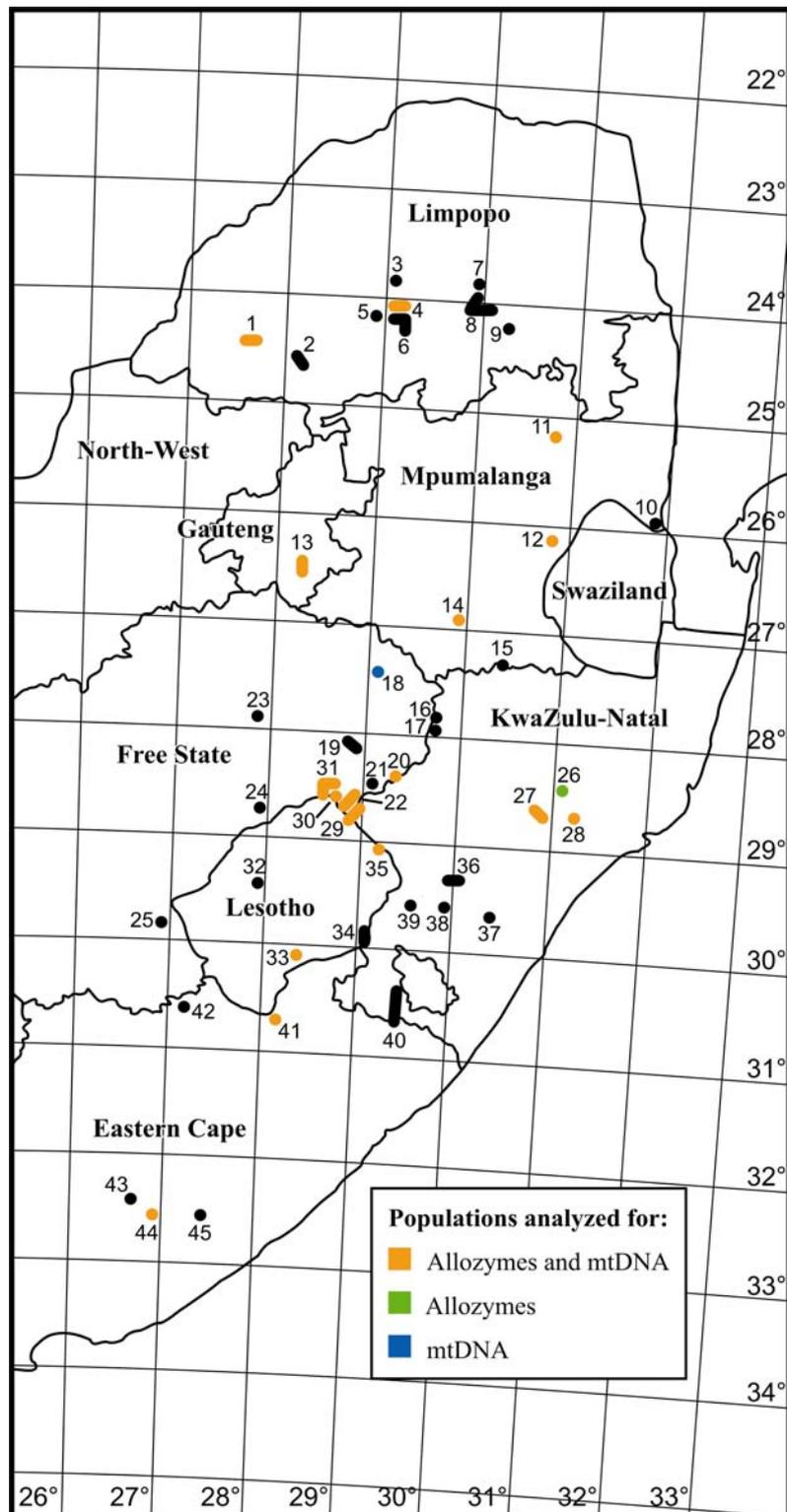


**Figure 5.2** (continued): Habitats of populations referable to the *Pseudocordylus melanotus* complex.

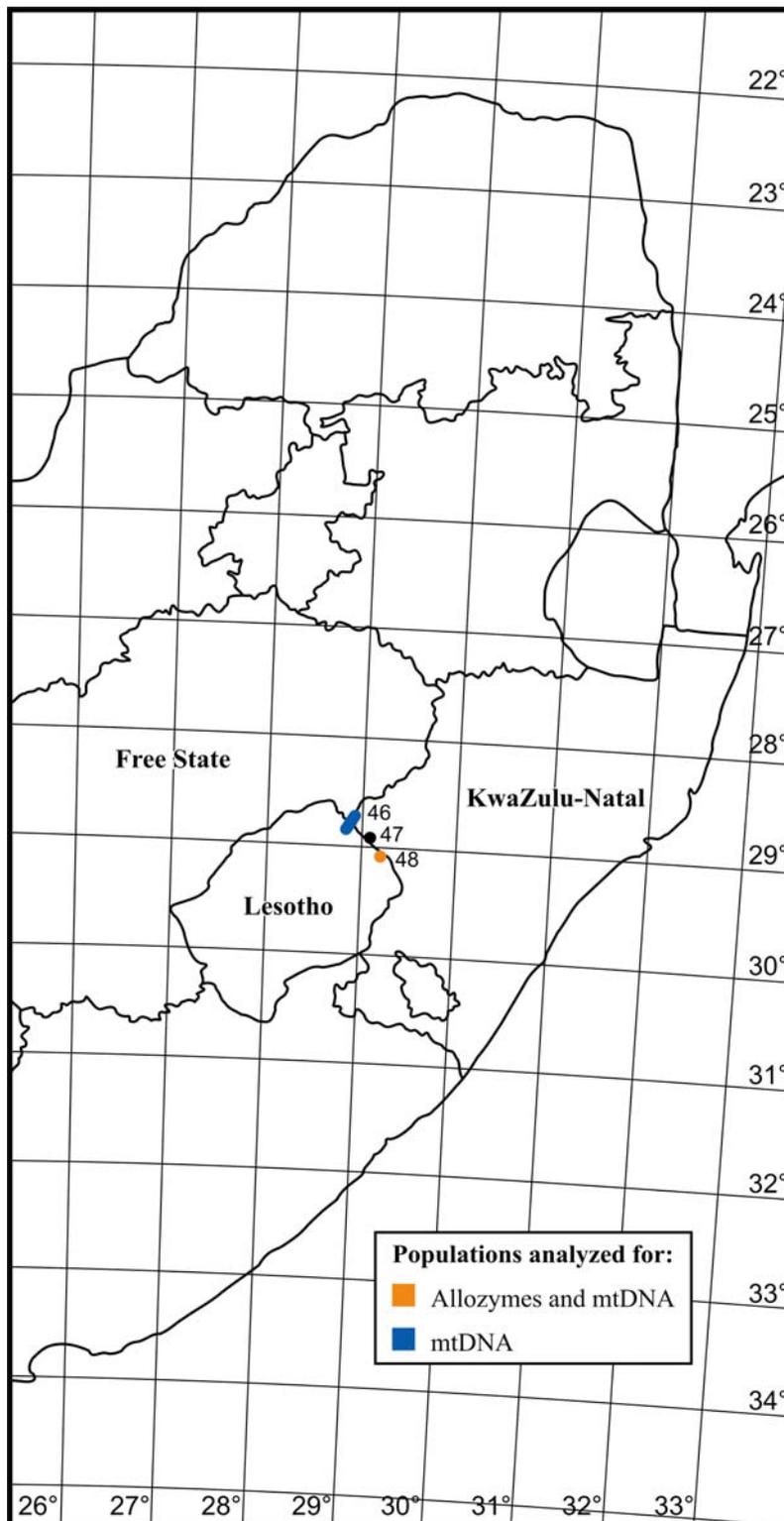
A total of 559 specimens in the *P. melanotus* (51 localities/compound localities) and *P. microlepidotus* (one locality) species complexes were examined in detail, comprising 83 *P. transvaalensis*, 177 *P. m. melanotus* (40 “Northern *melanotus*”, 137 “Southern *melanotus*”), 245 *P. m. subviridis*, 28 *P. langi*, 19 *P. spinosus* and seven *P. microlepidotus fasciatus* (Appendix 5.1). This included 235 specimens from 14 areas collected for use in the allozyme study (Chapter 3; Appendix 3.1) and all 80 specimens used in the mtDNA analysis, including 10 specimens from four localities that did not form part of the allozyme analysis (Chapter 4; Appendix 4.1).

The majority of specimens are housed in the Transvaal Museum, Pretoria (TM) and National Museum, Bloemfontein (NMB), but specimens from various other southern African collections (private collection of John Visser, Jeffrey’s Bay: JV; Natal Museum, Pietermaritzburg: NMSA; Natural History Museum of Zimbabwe, Bulawayo: NMZB; South African Museum, Cape Town: SAM) as well as the Natural History Museum, London (BMNH) were also examined. Specimens listed under “NMB-RY-R” were previously in the private collection of Robert Yeadon (Philippolis) (as “RY”) and have been incorporated into the collection of the National Museum (Bloemfontein).

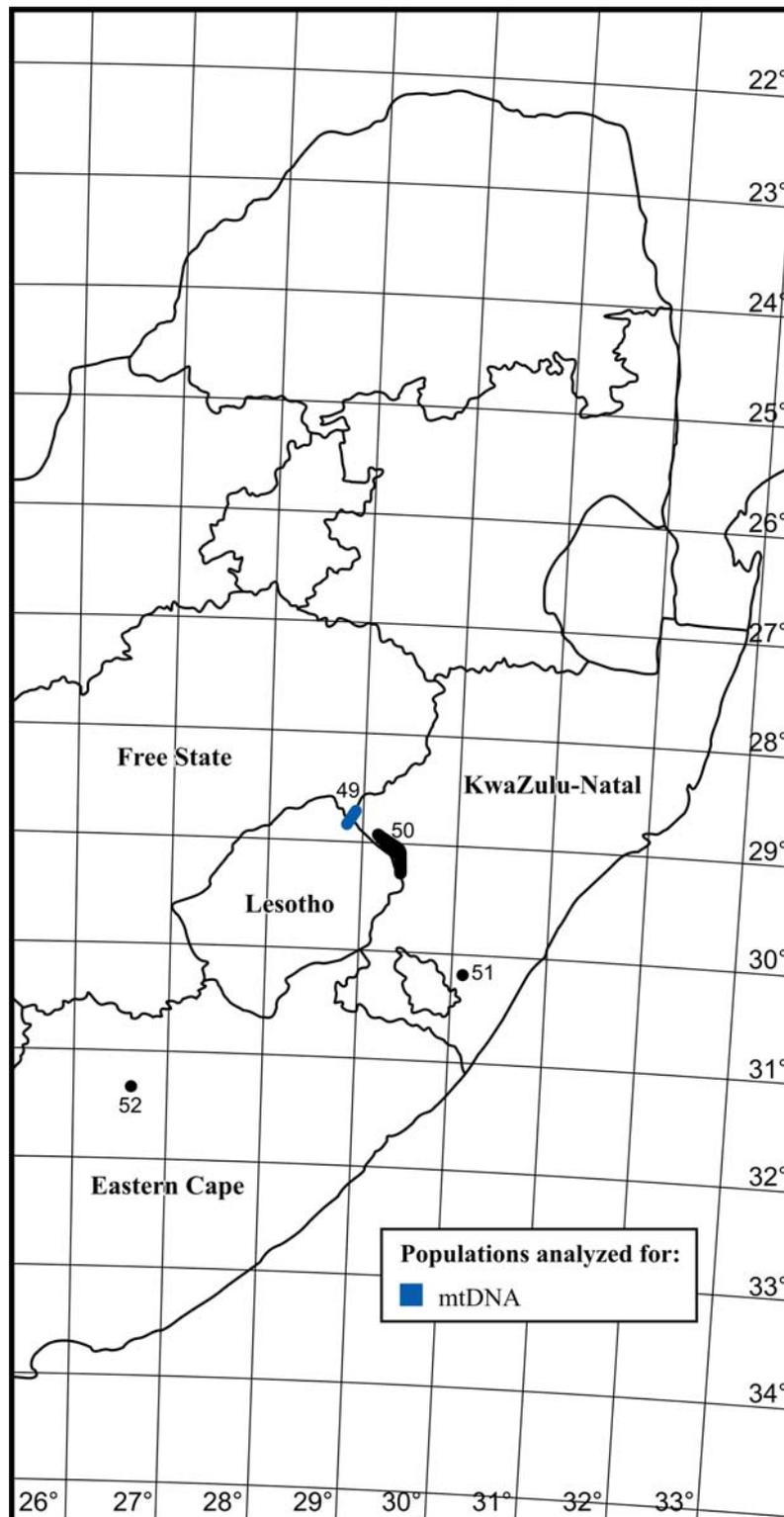
Both *P. m. melanotus* and *P. m. subviridis* have widespread distributions (Fig. 5.1) and therefore localities were selected to represent their total ranges, including isolated populations (Figs 5.3 to 5.5). Large samples from an apparent zone of parapatry (2828DB and vicinity) between the latter two taxa were examined. In a few cases samples from allozyme collecting sites numbered in excess of 20, but in all other cases where more than 20 specimens from the same locality were available, only the 10 largest males and 10 largest females were selected for examination. All available specimens of *P. transvaalensis* (Fig. 5.3) and *P. langi* (Fig. 5.4) in South African collections were examined (Appendix 5.1). Limited material of *P. spinosus* is available, but samples from virtually all known localities were examined (Fig. 5.5; Appendix 5.1). Compound localities were used when sample sizes were small, but only if environmental conditions were considered similar and if gene flow was unlikely to be impeded.



**Figure 5.3:** Geographical distribution of localities and compound localities for the morphological analysis of the *Pseudocordylus melanotus* species complex: *P. transvaalensis*, *P. melanotus melanotus*, *P. melanotus subviridis*. Specimens from the following localities were also used in the genetic analyses (allozymes and mtDNA, marked in orange):- *P. transvaalensis*: 1, 4; *P. m. melanotus* - Northern *melanotus*: 11, 12; *P. m. melanotus* - Southern *melanotus*: 13, 14, 18 (mtDNA only, marked in blue), 20, 22, 26 (allozymes only, marked in green), 27, 28; *P. m. subviridis*: 29, 30, 33, 35, 41, 44. Numbers on the map refer to localities listed in detail in Appendix 5.1.



**Figure 5.4:** Geographical distribution of localities and compound localities for the morphological analysis of the *Pseudocordylus melanotus* species complex: *P. langi*. Specimens from localities 46 (mtDNA only, marked in blue) and 48 (allozymes and mtDNA, marked in orange) were also used in the genetic analyses. Numbers on the map refer to localities listed in detail in Appendix 5.1.



**Figure 5.5:** Geographical distribution of localities and compound localities for the morphological analysis of the *Pseudocordylus melanotus* species complex: *P. spinosus*. Specimens from locality 49 (marked in blue) were also used in the mtDNA analysis. Numbers on the map refer to localities listed in detail in Appendix 5.1.

### 5.2.2 Examination of specimens

Type specimens of *P. melanotus melanotus*, *P. melanotus subviridis*, *P. transvaalensis*, *P. spinosus* and *P. microlepidotus fasciatus* were examined (see Chapter 2). As discussed in chapter 2, digital images of the holotype of *P. langi* were also examined. The latter specimen is morphologically equivalent to the *P. langi* material discussed below. Only the *P. transvaalensis* and *P. spinosus* types examined were included as part of the morphological analysis as there was some uncertainty as to the exact collecting localities of the other specimens.

An exhaustive search for external morphological differences between populations was conducted. A total of 47 characters (eight mensural, 16 qualitative, 23 meristic) were eventually considered informative and objectively scorable (Appendix 5.2) and these were used in the final analyses.

Measurements were performed with digital calipers (0.02 mm). Scales were examined and counted under Carl Zeiss binocular dissecting microscopes at 10 to 40 times magnification. Head and limb measurements were taken on the right side of the body. Transverse rows of dorsal scales were counted in the dorsolateral region on the right side of the body, whereas transverse rows of ventral plates were counted on the left. Scales on both sides of the head were counted and the total used for analysis.

Counts were occasionally complicated due to incompletely divided or damaged scales. Any partly divided scale was counted as two scales. If a scale on one side of the head was damaged, severely fragmented or fused to a different kind of scale (*e.g.* supralabial fused with preocular), the count was made on the other side of the head and the total doubled. Small or extranumerary scales, granules or skin folds present between regular head scales were not counted. For example, a small scale may be present on the right side of the left series of supraoculars, but it would not extend to both sides, *i.e.* not be in broad contact with any other supraocular. When one in a series of scales was divided longitudinally it was counted as a single scale. Further details regarding the manner in which characters were evaluated are provided in Appendix 5.2.

The gender of specimens was determined after dissection and examination of reproductive organs, although a few males were identified by one or two everted hemipenes. If these organs were not found (*e.g.* removed by a previous worker), the specimen was considered “unsexed”. The minimum sizes of specimens examined for reproductive organs were: *P. transvaalensis* and *P. microlepidotus fasciatus*: >70 mm SVL; *P. m. melanotus* and *P. m. subviridis*: >65 mm SVL; *P. langi*: >60 mm SVL; *P. spinosus*: >55 mm SVL.

While examining specimens from the northern parts of the range of *P. m. melanotus* it became evident that the frontonasal was usually undivided, not divided as in other *P. m. melanotus* populations. In order to quantify this difference, a large sample of *P. m. melanotus* ( $N = 272$ , including 61 specimens listed in Appendix 5.1) from Swaziland and the South African provinces of Limpopo, Mpumalanga and Gauteng was examined for this character (Fig. 5.21), as well as the presence or absence of a small scale posterior to the frontonasal (Fig. 5.23), and the numbers of horizontal rows of lateral temporals. Specimens examined additional to those in Appendix 5.1 are listed in Appendix 2.1 under the abbreviation “TM” and marked with an asterisk. Both the allozyme and mtDNA analyses also indicated that the northern-most populations of *P. m. melanotus* (“Northern *melanotus*”) represented a separate lineage. Therefore, “Northern *melanotus*” and “Southern *melanotus*” were analysed separately (see Tables 5.4 to 5.6). Most of the additional specimens had two rows of lateral temporals on either side of the head - the upper row consisting of elongated scales, but occasionally there were two rows on one side of the head and one on the other, or an intermediate condition, or even asymmetrically arranged temporals.

The spacing of longitudinal rows of dorsolaterals in *P. m. subviridis* also proved to be variable and apart from specimens listed in Appendix 5.1, a detailed examination of this character was conducted on 40 additional specimens from the slopes of the Drakensberg in western KwaZulu-Natal. These additional specimens are listed under the abbreviation “NMB-RY-R” and marked with an asterisk in Appendix 2.1. A few additional characters were examined in these specimens to confirm their taxonomic status. The majority of specimens had undivided frontonasals, but in NMB-RY-R 824, 829 and 910 the frontonasal was divided longitudinally, while NMB-RY-R 238 and 241 had partly-divided scales. In NMB-RY-R 824 and 829 there was also a small to moderate sized

scale (respectively) posterior to the frontonasal. Most specimens had a single row of elongated lateral temporals on either side of the head, but a few had two rows - the upper row consisting of elongated scales, or two rows on one side of the head and one on the other, or an intermediate condition. Femoral pore count (both legs) numbered 10 to 18 (seven in one specimen: NMB-RY-R948).

According to the mtDNA analysis (Chapter 4) of 80 specimens in the *P. melanotus* species complex, the latter consists of the following main clades/groupings: *P. langi*, *P. m. melanotus* (= “Northern *melanotus*”), southern *P. m. melanotus* (= “Southern *melanotus*”), *P. transvaalensis* and *P. m. subviridis*. The allozyme analysis also provided support for most of these assemblages (Chapter 3). According to the mtDNA analysis *P. spinosus* is imbedded within a *P. m. subviridis* clade, but as it is considerably different morphologically and apparently also differs in terms of its habitat, it is treated separately. *Pseudocordylus microlepidotus* is imbedded within a Hogsback-S Lesotho-Naude’s Nek *P. m. subviridis* clade, but it too is treated separately as it differs morphologically (*e.g.* adult *P. microlepidotus* often have generation glands on the back; Tables 5.1 to 5.6). All populations of *P. m. subviridis* – in clades C, D and E - are morphologically very similar or indistinguishable. The only population that is (largely) distinguishable, using discriminant analysis, from other consubspecifics, is the Hogsback (Amatole-Winterberg) population, which represents a subclade of clade E (Chapter 4). *Pseudocordylus m. subviridis* has therefore been evaluated mainly as a unit for the purposes of the morphological analysis. The morphological analysis therefore attempts to find concordance with the groupings determined by the mtDNA analysis as discussed above.

The additional 479 specimens examined were assigned to the various groupings on the basis of their morphological similarity to specimens used in the mtDNA analysis. With reference to Tables 5.1 to 5.3, populations at localities 1-8 were assigned to *P. transvaalensis*, 9-12 to Northern *melanotus*, 13-28 to Southern *melanotus*, 29-45 to *P. melanotus subviridis*, 46-48 to *P. langi*, 49-51 to *P. spinosus*, and population 52 to *P. microlepidotus fasciatus*.

### 5.2.3 Statistical analyses

All statistical procedures were conducted using the Statistica version 6 computer package. Variables were tested for normality using the Shapiro-Wilk's *W* test. To determine whether or not there was a significant difference in the numbers of femoral pores between males and females of each grouping (taxon or subdivision thereof), One-way ANOVA was used for normally distributed data, whereas the Mann-Whitney U Test was used if either male or female pores per grouping were non-parametrically distributed. Probability values ( $p$ ) < 0.05 were considered significant.

A combination of quantitative and qualitative scale data and morphometric data was used to identify species. Such a character-based approach involves looking for diagnostic character states representing apparently fixed (or near-fixed) differences (*e.g.* 10 versus 12 infralabials) between populations and/or non-overlapping (or near-non-overlapping) differences (*e.g.* 10-12 versus 14-16 rows of ventrals). If diagnostic traits are in fact genetically based and truly fixed, it is unlikely that gene flow occurs between species (Wiens & Penkrot 2002).

Meristic, qualitative and mensural data were then combined in multivariate analyses. Two types of ordination comparisons were conducted to determine whether or not samples could be separated in multivariate space. Both Principal Components Analysis (PCA) and Canonical Discriminant Analysis (CDA) were conducted using Statistica version 6. Because of a high incidence of regenerated or missing tails, tail length was excluded from the analyses. Other characters excluded were either invariable or displayed negligible variation (*e.g.* presence/absence of glands anterior to vent). Characters that were fixed or nearly fixed for particular taxa or groupings (*i.e.* spinosity of lateral scales; femoral pores pore- or pit-like; markings on throat) were excluded from the PCA and CDA as their inclusion would have swamped the quantitative analyses. The number of differentiated femoral scales (overlying generation glands) was also excluded as it was sometimes difficult to count these, both males and females of *P. spinosus* have them (although sample size was small), and there is evidence [see section 5.4.1.4] that numbers are dependent on environmental conditions. Separate analyses were conducted for the whole *P. melanotus* species complex (including seven specimens of *P. microlepidotus fasciatus*), *P. melanotus* (comprising Northern *melanotus*, Southern

*melanotus* and *P. m. subviridis*), *P. m. subviridis* (Maloti-Drakensberg and Amatole populations) with Southern *melanotus*, and *P. transvaalensis* (Western, Central and Eastern populations). For all analyses the same 38 characters were used (seven morphometric, 20 meristic and 11 qualitative), except for *P. transvaalensis*, in which case only 35 of these were used as the other three (all qualitative characters) were invariable between populations or in one case (texture of posterior infralabials) exhibited variance below the minimum tolerance permitted by the program. In all cases the same variables were used in both PCA and CDA. For missing data, the pairwise option was used for PCA and casewise for CDA.

### 5.3 Results

#### 5.3.1 Character analysis

Some characters were invariable across all populations – e.g. the large (“median”) subocular situated below the eye was in contact with the lip on both sides of the head in all specimens except on the right side of SAM 11314 (*P. m. subviridis*). The median subocular was also divided vertically on its lower half on both sides of the head in two specimens - NMB R8182 (*P. m. melanotus*) and NMB R4609 (*P. m. subviridis*) - and fully divided vertically on the right side of the head in NMB R6830 (*P. m. subviridis*). Some other characters exhibited only infrequent variation – e.g. ventral plates were smooth in all specimens except for one in which they were weakly keeled. These characters are not discussed further. The majority of characters evaluated displayed at least some intra-locality variation, with several cases of regional variation (Tables 5.1 to 5.6).

**Table 5.1:** Frequency of occurrence (%) for qualitative characters at 52 localities in the *Pseudocordylus melanotus* and *P. microlepidotus* complexes. Superscripts indicate that sample size differs from that given in the second column – e.g. a superscript value of 1 means that the sample size for that character is  $N - 1$ . For femoral pores in females sample sizes are indicated in parentheses. Interspaces between longitudinal rows of dorsolaterals: equal to larger + >0.5 but not equal +  $\delta$  0.5, A = granular scales only and in contact, B = enlarged scales in contact. Gular pattern: A = throat pale with a parallel pair of dark longitudinal median stripes with arrow-like anterior ends, B = throat black, C = throat pale with a single dark median longitudinal stripe D = like A but without arrow-like ends; <sup>a</sup> = black in 4% of sample, <sup>b</sup> = black in 14%, <sup>c</sup> = black in 36%. Population groups: T = *transvaalensis*, NM = Northern *melanotus*, Gauteng *melanotus*, SM = Southern *melanotus*, nM = Nkandla *melanotus*, DS = Drakensberg *subviridis*, AS = Amatole *subviridis*, L = *langi*, Sp = *spinosus*, MF = *microlepidotus fasciatus*.

Locality	N	Population group	Femoral pores in females large with secretions	FN wider than long (* as wide as long)	Scale behind FN	FN separates supranasal	FN divided + partly divided	FN contacts lorals	Anterior frontal present	Anterior parietal divided + partly divided	Median dorsals >0.5 size of dorsolaterals	Lateral dorsals $\geq$ 0.75 size of dorsolaterals	Dorsolaterals larger than median dorsals	Interspaces between longitudinal rows of dorsolaterals	Gular pattern	Posterior infralabials smooth	Lateralis spinose
1	19	T	0 (8)	95+5 <sup>1</sup>	50 <sup>1</sup>	89	89+5	100	42	47+37	0	0	100	0+0+100	B	0	0
2	4	T	0 (3)	100	0	100 <sup>1</sup>	0+50	100	25	0+75	0	25	100	0+0+100	B	0 <sup>1</sup>	0
3	4	T	0 (2)	100	75	75	100+0	100	0	0+25	0	0	100	0+0+100	B	0	0
4	17	T	0 (9)	94+6	41	71	18+53	100	82	6+18	0	0	100	0+12+88	B	0	0
5	4	T	0 (1)	50	0	100	0+50	100	0	0+50	0	0	100	0+0+100	B <sup>1</sup>	50	0
6	6	T	0 (4)	100	17	17	50+17	100	83	0+83	0	0	100	0+0+100	B	0	0
7	22	T	0 (10)	95+5	62 <sup>1</sup>	18	45+36	100 <sup>2</sup>	27	0+14 <sup>1</sup>	0	36	100	0+41+59	B	0	0
8	7	T	0 (4)	100	67 <sup>1</sup>	14	100+0	100	29	0+0	0	14	100	0+14+86	B	14	0
9	5	NM	0 (1)	100	0 <sup>2</sup>	20	40+20	75 <sup>1</sup>	0	0+0	0	60	100	0+40+60	A <sup>1</sup>	0 <sup>1</sup>	0
10	1	NM	-	100	0	100	0+0	-	-	-	0	100	100	-	B	0	0
11	23	NM	0 (11)	61+26	55 <sup>1</sup>	30	26+17	100	0	0+0	4	0	100	0+74+26	A+B <sup>a</sup>	4	0
12	11	NM	0 (6)	100	18	0	18+9	100	0	0+0	0	9	100	0+27+73	A+B <sup>c</sup>	0	0
13	15	GM	0 (7)	100	47	40	100+0	100 <sup>1</sup>	0	0+0	0	33	100	0+0+100	A	0	0
14	9	SM	0 (5)	100	78	38 <sup>1</sup>	89+11	100	33	0+11	11	11	100	0+0+100	A	0 <sup>1</sup>	0
15	3	SM	-	100	100 <sup>2</sup>	33	100+0	100 <sup>1</sup>	-	0+0 <sup>2</sup>	0	67	100	-	A <sup>1</sup>	0	0
16	8	SM	0 (5)	100	38	0	88+13	100	0	0+0	25	25	100	0+25+75	A	0	0
17	3	SM	0 (2)	100	33	0	33+67	100	0 <sup>1</sup>	0+0 <sup>1</sup>	0	67	100	0+0+100 <sup>1</sup>	A <sup>1</sup>	0	0
18	2	SM	0 (1)	100	0	0	100+0	100	0	0+0	0	50	100	0+0+100	A	0	0
19	8	SM	0 (3)	88	38	13	75+25	100	0	0+0	0	63	100	0+50+50	A	0 <sup>1</sup>	0
20	23	SM	0 (16)	100	18 <sup>1</sup>	9	95+0 <sup>1</sup>	100 <sup>2</sup>	0	0+0	0 <sup>1</sup>	18 <sup>1</sup>	100 <sup>1</sup>	0+26+74	A	0 <sup>1</sup>	0
21	20	SM	0 (9)	100	68 <sup>1</sup>	0	95+5	100 <sup>1</sup>	0 <sup>1</sup>	5+0 <sup>1</sup>	0	30	100	0+20+80	A	0 <sup>2</sup>	0
22	3	SM	-	100	67	0	100+0	100	0	0+0	0	100	100	0+100+0	A	0	0
23	3	SM	0 (1)	100	0	0	100+0	100	0	0+0	0	100	100	0+33+67	A <sup>1</sup>	0 <sup>1</sup>	0
24	7	SM	0 (4)	100	29	0	100+0	100	0	0+0	0	29	100	0+14+86	A+B <sup>b</sup>	0	0
25	2	SM	0 (2)	100	0	0	0+100	100	0	0+0	0	50	100	0+0+100	A	0	0
26	1	nM	0 (1)	100	0	0	100+0	100	0	0+0	0	100	100	0+0+100	A	0	0
27	19	nM	0 (6)	100	50 <sup>1</sup>	26	68+26	100	0 <sup>8</sup>	0+0 <sup>9</sup>	0	74	100	0+9+91 <sup>8</sup>	A <sup>2</sup>	0 <sup>1</sup>	0
28	11	nM	0 (7)	100	9	0	36+45	100	0	0+0	0	64	100	0+0+100	A <sup>1</sup>	0 <sup>1</sup>	0
22	6	DS	50(2)	83	33	0	50+0	100	0	0+0	17	33	100	0+33+67	A	0	0
29	44	DS	88 (17)	98 <sup>1</sup>	0 <sup>1</sup>	9 <sup>1</sup>	0+5 <sup>1</sup>	100	0	0+0 <sup>1</sup>	89	89	100	27+55+18	A	0	0
30	40	DS	71 (17)	98	8	3	25+13	95 <sup>1</sup>	0	0+0	60	75	100	38+43+20	A <sup>2</sup>	3 <sup>1</sup>	0
31	8	DS	100 (4)	100	0 <sup>1</sup>	0	0+0	100	0	0+0	25	100	100	88+13+0	A <sup>1</sup>	13	0
32	20	DS	73 (11)	100	5	5	0+5	94 <sup>1</sup>	0	0+5	100	90	100	55+45+0	A	0	0
33	10	DS	100 (4)	100	0	10	0+0	100 <sup>1</sup>	0	0+0	100	60	100	0+100+0	A	0	0
34	16	DS	43 (7)	100	0	0	0+0	88	0	0+0 <sup>1</sup>	100	100	100	75+25+0	A	0	0
35	18	DS	33 (9)	100	0	6	0+0	100	0	0+0	100	89	100	50+50+0	A	0 <sup>1</sup>	0
36	12	DS	100 (2)	100	27 <sup>1</sup>	8	0+25	100 <sup>1</sup>	0 <sup>1</sup>	0+0 <sup>1</sup>	0	75	100	0+0+100 <sup>1</sup>	A	0 <sup>1</sup>	0
37	1	DS	-	100	0	100	0+0	100	0	0+0	0	0	100	0+0+100	A	0	0
38	4	DS	100 (1)	100	0 <sup>2</sup>	0	0+0	50	0 <sup>2</sup>	0+0 <sup>2</sup>	0	50	100	0+0+100 <sup>2</sup>	A	0	0
39	6	DS	100 (1)	100	0	0	0+0	20 <sup>1</sup>	0	0+0	0	83	100	0+67+33	A <sup>2</sup>	0	0
40	6	DS	-	100	0	0	0+17	67	0	0+0	50	100	100	17+50+33	A	0	0
41	23	DS	100 (13)	96	9	4	0+0	91 <sup>1</sup>	0	0+0	91	57	100	22+78+0	A <sup>3</sup>	0	0
42	3	DS	100 (1)	100	-	33	33+0	100	-	-	67	100	100	33+33+33	A	0	0
43	1	AS	-	100	-	0	0+0	100	-	-	0	0	100	-	A	0	0
44	25	AS	55 (11)	92	0 <sup>2</sup>	0	0+0	96 <sup>1</sup>	0 <sup>2</sup>	0+0 <sup>2</sup>	12	4	100	0+26+74 <sup>2</sup>	A <sup>1</sup>	0 <sup>2</sup>	0
45	2	AS	-	100	-	0	0+0	100	-	-	50	50	100	-	A	0	0
46	8	L	100 (2)	100	0	0	0+25	100	0	0+0	100	88	0	A	C	88	0
47	1	L	-	100	0	0	0+100	100	0	0+0	100	100	0	A	C	100	0
48	19	L	100 (6)	100	7 <sup>1</sup>	0	16+5	100 <sup>1</sup>	0 <sup>1</sup>	0+0 <sup>1</sup>	100	89	0	A	C	100 <sup>1</sup>	0
49	10	Sp	100 (1)	0	10	0	0+0	0	0	0+0	0	100	100	0+0+10+B	D	0	100
50	7	Sp	100 (2)	0* <sup>1</sup>	14*	0*	0+17* <sup>1</sup>	0* <sup>1</sup>	0 <sup>1</sup>	0+0	0	86	100	0+0+57+B	D	0	86
51	2	Sp	-	0+50	0	0	0+0	0	0	0+0	0	50	100	B	D	0	100
52	7	MF	67 (3)	57+43	0	0	0+0	100	0	0+0	0	0	100	0+0+100	D	0	0

\* Frontonasal absent in one specimen (14%).

**Table 5.2:** Meristic data for head scalation characters at 52 localities in the *Pseudocordylus melanotus* and *P. microlepidotus* complexes. Superscripts indicate that sample size differs from that given in the second column – e.g. a superscript value of 1 means that the sample size for that character is  $N - 1$ . Population groups: T = *transvaalensis*, NM = Northern *melanotus*, Gauteng *melanotus*, SM = Southern *melanotus*, nM = Nkandla *melanotus*, DS = Drakensberg *subviridis*, AS = Amatole *subviridis*, L = *langi*, Sp = *spinotus*, MF = *microlepidotus fasciatus*.

Locality	Population group	N	Upper temporals	Horizontal lateral temporals	Supraoculars	Supraciliaries	Suboculars anterior to median	Suboculars posterior to median	Supralabials	Infralabials	Sublabials	Gulars anterior to first sublabials	Gulars across throat	Small scales behind interparietal	Occipitals
1	T	19	5-8	6-8	8	10-11	2-4	3-5	8-10	12-17	10-14	2-4	29-40	2-12	7-10 <sup>4</sup>
2	T	4	6	5-6	8	10-11	2	2-4	8-9	12	10	2	33-36	4-7	7-9 <sup>1</sup>
3	T	4	6	4-6	8	10	2-3	4-5	7-9	12	10-11	2	29-34	5-8	7-8 <sup>1</sup>
4	T	17	6	4-6	8	10-11	2	3-6	8-9	12-14	10-13	2-4	27-36	5-10	6-9 <sup>2</sup>
5	T	4	6-8	5-7	8-9	10	2	3-4	8-9	12-14	10-12	2-3	31-41	2-5	9-10 <sup>2</sup>
6	T	6	6	4-6	8	9-10	2-4	4-5	8-9	12	10-12	2-3	25-34	4-7	6-9
7	T	22	5-7	4-7	8-10	10-12	2-4 <sup>1</sup>	3-6 <sup>1</sup>	8-10	12-14	10-13	2-3	27-39 <sup>1</sup>	2-6 <sup>1</sup>	6-10 <sup>1</sup>
8	T	7	6-7	4-6	8	10	2-3	3-4	8-10	12-13	10-13	2-3	28-41	3-5	7-9 <sup>1</sup>
9	NM	5	6	3-5	8	10-12	2	2-3	8-9	12-13	10	2	24-29	0	9-11
10	NM	1	6	-	8	10	2	2	8	12	10	3	26	0	9
11	NM	23	6-8	4-6	8-10	10-12	2-3	2-4	8-10	12-13	10	2-3	23-32 <sup>1</sup>	0-4	6-10
12	NM	11	6	4	8	10-11	2-5	2-4	8-11	12	10	2-4	23-28 <sup>1</sup>	0	8-12
13	GM	15	6-7	3-5	8	9-11	2-3 <sup>1</sup>	2 <sup>1</sup>	7-10	11-12	10	2-4	21-28	0-1	6-12
14	SM	9	6	4	8	10-11	2-3	2-3	8-10	12-13	10-12	2-5	24-27	0-2	6-8
15	SM	3	6	2-4 <sup>1</sup>	8	10	2	2	8	12	10	2	25-27	0-2	8-9
16	SM	8	6	3-4	8	10	2-3	2-3	8	12	10	2-3	24-28	0	8-12
17	SM	3	6	4-5	8-9	10	2	2-4	8	12	10	2-4	22-27	0	7-11
18	SM	2	6	4	8	10-11	2	2	8	12	10	2	22-28	0	12
19	SM	8	6	4	8	10	2-3	2-3	8-9	12-13	10	2	25-31	0-2	7-11
20	SM	23	6	3-4	8-9	8-11	2	2-4	8-10	10-12	10	2-4 <sup>1</sup>	22-33	0-2	7-11 <sup>2</sup>
21	SM	20	5-8	3-5	8-9	10-11	2-5	2-3	8	11-13	10	2-4	20-29 <sup>2</sup>	0-2	7-13 <sup>3</sup>
22	SM	3	6	4-6	8	10	2	2-3	8	12	10	2-3	22-25	0	8-11
23	SM	3	6	4	8	10	2	2	8-9	12	10	2	25-26	0-1	6-8
24	SM	7	6	4	8	10-11	2-3	2-3	8	10-12	9-10	2-3 <sup>1</sup>	23-27 <sup>1</sup>	0	7-8 <sup>1</sup>
25	SM	2	6	4	8	10	2	2	8	12	10	2	28-32	0	6-8
26	nM	1	6	4	8	10	2	2	9	11	10	4	30	1	12
27	nM	19	6	4 <sup>2</sup>	8 <sup>1</sup>	9-10	2	2-4	8-10	10-14	9-10	2-3	24-30 <sup>1</sup>	0-1	7-12 <sup>1</sup>
28	nM	11	6	4-5	8	10	2-4	2-3	8-9	12	10	2-4	22-32	0-1	7-11 <sup>1</sup>
29	DS	6	6	4	8	10	2-3	2	7-9	12	10	2-4	21-27	0-3	7-10
29	DS	44	6	2-4	8-10	9-12	2-3	2-4	7-10	11-13	10	2-4	23-33 <sup>1</sup>	0-3	6-12
30	DS	40	6-8	2-4	8-9	9-12	2-3	2-4	6-10	11-13	10-11	2-4	22-34	0-2	5-11 <sup>1</sup>
31	DS	8	6	2-4	8	10	2	2-4	8-9	12	10	2-4	25-31 <sup>1</sup>	0-1	7-9
32	DS	20	6	2-4	8	10-11	2-4	2-3	8-11	11-14	10	2-4	24-32	0-2	5-10
33	DS	10	6-8	2-4	8	10	2-3	2-3	8-10	12-13	10	2	25-35	0	6-9
34	DS	16	6-7	2-4	8	10-11	2-3	2-3	8-11	10-13	10-11	2-3	24-33	0-2	6-11
35	DS	18	6-7	2-4	8	10-12	2-4	2-4	7-9	11-13	10	2-4	25-33	0-3	7-13
36	DS	12	5-6 <sup>1</sup>	2-4 <sup>1</sup>	8	10-11	2	2-4	8	12	10	2	25-32 <sup>1</sup>	0-2	10-14 <sup>1</sup>
37	DS	1	6	4	8	10	2	4	8	12	10	2	27	0	9
38	DS	4	6	2-4 <sup>2</sup>	8	10	2	2-4	8-9	12	10	2	25-31	0	9-11 <sup>1</sup>
39	DS	6	6	2-4	8	10-11	2-3	2	8	12	10-11	2-3	22-30	0	6-8
40	DS	6	6	2-4	8	10	2-3	2-3	8-10	10-12	10	2-3	25-31	0	7-11
41	DS	23	6 <sup>1</sup>	2-4	8	10-12	2-4	2	7-9	10-12	10	2-4	23-32	0-1	6-10
42	DS	3	6	2-4	8	8-10	2	2	8	12	10	2	23-27	0	7-8
43	AS	1	6	4	8	10	2	2	8	12	10	2	26	0	8
44	AS	25	6-7	2-4 <sup>1</sup>	8	10-12	2-3	2	6-9	10-13	10 <sup>1</sup>	2-5 <sup>1</sup>	23-34 <sup>3</sup>	0	6-11
45	AS	2	6-7	4	8	10-12	2-5	2	9-10	13	10	3-4	31-33	0	9-11
46	L	8	6	2-4	8	9-11	2	2	8-9	10-11	10-11	2-4	26-43	0-3	0
47	L	1	6	2	8	11	2	3	8	10	10	2	33	0	0
48	L	19	4-7	2-4 <sup>3</sup>	8	9-12	2-3 <sup>1</sup>	2-6 <sup>1</sup>	8-10	10-11	9-11	2-4	25-45	0-3	0
49	Sp	10	6	2-4	8	10-11	2-3	2-4	8-9	10-14	10-11	2	21-26	0	0
50	Sp	7	5-6	2-4	8	10	2	2-4	6-8	12-13	10-11	2	20-25	0	0
51	Sp	2	6	2	8	10	2	2-3	6-9	12	10	2	21-23	0	0
52	MF	7	6	4-6	8	10	2	3-4	8-11	12-13	10	2	22-33	0-2	7-10

**Table 5.3:** Meristic data for scalation characters at 52 localities in the *Pseudocordylus melanotus* and *P. microlepidotus* complexes. Superscripts indicate that sample size differs from that given in the second column – e.g. a superscript value of 1 means that the sample size for that character is  $N - 1$ . For femoral pore counts, male and female sample sizes are indicated in parentheses. Superscripts used for counts of differentiated femoral scales apply to sample sizes for males as indicated under femoral pore counts. Population groups: T = *transvaalensis*, NM = Northern *melanotus*, Gauteng *melanotus*, SM = Southern *melanotus*, nM = Nkandla *melanotus*, DS = Drakensberg *subviridis*, AS = Amatole *subviridis*, L = *langi*, Sp = *spinus*, MF = *microlepidotus fasciatus*.

Locality	Population group	N	Transverse rows of dorsals	Longitudinal rows of dorsals	Transverse rows of ventrals	Longitudinal rows of ventrals	Lamellae under fourth finger	Lamellae under fourth toe	Femoral pores: Males	Femoral pores: Females	Differentiated femoral scales in males	Pre-cloacal glands
1	T	19	40-51	40-50	28-33	12-14	13-16 <sup>1</sup>	18-22	12-17 (10)	13-15 (8)	17-28	0-1
2	T	4	41-44	39-45	28-31	12	13-15	18-19	-	12-14 (3)	-	0
3	T	4	41-49	44-49	28-32	12-14	16	20-21	13-15 (2)	10-16 (2)	14-15	0
4	T	17	39-48	42-53	28-31	12-14	13-16	17-21	10-15 (8)	11-14 (9)	8-24	0-6
5	T	4	43-49	43-55	28-30	12	14-18	20-23	13-15 (2)	15 (1)	8-14	0
6	T	6	42-48	40-48	29-31	12-14	14-17	18-21	13-15 (2)	0-15 (4)	17-27	0-1
7	T	22	38-46	41-49	28-31	12-14	14-17	18-22	13-15 (8)	13-16 (10)	7-24	0
8	T	7	41-45	40-45	28-32	12-14	15-16	19-22	12-17 (3)	13-16 (3)	14-28	0-2
9	NM	5	45-54	44-46	28-31	12	14-18	20-22	15 (1)	12 (1)	11	0
10	NM	1	48	39	29	12	18	22	-	-	14	0
11	NM	23	48-57	40-52	28-31	12-14	15-19	19-25 <sup>1</sup>	11-15 (10)	12-18 (10)	18-40	0-1
12	NM	11	43-50	37-50	28-30	12	14-17	18-21	15-16 (2)	12-15 (6)	22-24	0
13	GM	15	40-47	34-42	27-32	12	15-18	18-23	14-20 (6)	14-16 (7)	18-27 <sup>1</sup>	0
14	SM	9	41-47	41-47	27-30	12	14-17	18-20	15-18 (4)	15-17 (5)	0-12	0
15	SM	3	43-50	36-43	26-29	12	15-17	20-22	14-18 (2)	-	8-17	0
16	SM	8	43-50	36-42	28-33 <sup>1</sup>	12	15-18	18-22	14 (2)	14-17 (5)	0	0
17	SM	3	44-48	36	29-32	12	15-19	19-22	15 (1)	14-15 (2)	14	0
18	SM	2	48-49	44-46	30-31	12	16	20-21	15 (1)	13 (1)	18	0
19	SM	8	45-50	36-45	29-31	12	14-16	18-21	15-19 (3)	15-17 (4)	6-16	0
20	SM	23	37-47	36-46	26-32	11-14	14-18	18-23	17-19 (3)	14-20 (14)	11-21	0
21	SM	20	41-51	33-41	28-32	12-14	13-18	16-21	14-16 (5)	12-21 (9)	16-32	0
22	SM	3	45-52	37-38	30-32	12	14-19	19-22	-	-	-	0
23	SM	3	45-46	38-40	28-29	12	16-17	18-21	14 (1)	15 (1)	0	0
24	SM	7	40-50	38-43	26-29	12	14-17	18-20	15-17 (2)	12-16 (4)	24	0
25	SM	2	51-56	43-45	28-31	12	17-18	20-23	-	10-13 (2)	0	0
26	nM	1	40	39	28	12	14	18	-	12 (1)	0	-
27	nM	19	40-50	34-43	27-31	12-14	14-18	17-22	12-17 (7)	14-17 (6)	10-27	0
28	nM	11	40-53	36-44	28-31	12-14	13-17	18-21	14-15 (2)	13-18 (7)	17-19	0
22	DS	6	43-53	34-41	30-32	12-14	14-16	17-21	16-17 (2)	15-16 (2)	13	0
29	DS	44	41-57	27-43	26-30	12-14	15-20	18-25	16-25 (21)	14-20 (17)	0-63	0
30	DS	40	41-59	29-53	26-33	12-14	14-19	17-23	12-17 (17)	13-19 (17)	0-46	0
31	DS	8	44-55	32-44	27-30	12-14	15-18	18-22	14-15 (2)	16-18 (4)	47-63	0 <sup>1</sup>
32	DS	20	42-56	30-46	25-31	12-14	14-19	18-23	13-17 (3)	13-19 (11)	0-58	0
33	DS	10	43-54	33-41	28-31	12-14	15-17	18-21 <sup>1</sup>	13-16 (6)	10-14 (4)	11-30	0
34	DS	16	42-59	28-38	26-31	12-14	14-18	18-23	13-19 (7)	13-18 (7)	0-75	0-2
35	DS	18	41-56	30-45	26-30	12-14	15-19	18-24	14-19 (7)	13-21 (9)	0-58	0
36	DS	12	43-50	35-47	27-32	12-14	14-16	18-21	10-14 (9)	11-13 (2)	15-45	0
37	DS	1	46	41	27	12	17	19	13 (1)	-	36	0
38	DS	4	42-51	36-46	27-28	12	14-16	19-21	14 (1)	14 (1)	20	0
39	DS	6	42-51	30-41	25-29	12	14-17	18-22	14-18 (2)	16 (1)	28-59	0
40	DS	6	42-55	32-38	28-31 <sup>1</sup>	12	15-18	17-22	13-15 (2)	-	13 <sup>1</sup>	0
41	DS	23	43-52	32-44	27-31	12-14	14-18	18-22	12-17 (7)	11-17 (13)	0-24	0-1
42	DS	3	46-50	32-42	28-30	12	15-17	19-20	12-14 (2)	19 (1)	18-20	0
43	AS	1	44	45	27	12	16	18	12 (1)	-	17	0
44	AS	25	40-52 <sup>1</sup>	36-46	26-31	12-14	14-17	17-21	10-15 (8)	10-13 (12)	10-16	0
45	AS	2	47-49	43-44	31-32	12	18	20-21	-	-	-	0
46	L	8	0	7-9	29-31	10-12	17-21	20-25	27-31 (3)	26 (2)	0-21	0
47	L	1	0	9	31	12	18	22	29 (1)	-	0	0
48	L	19	0	6-8	29-32	10-12	16-20	21-26	25-34 (9)	26-30 (8)	0-50 <sup>1</sup>	0
49	Sp	10	35-40	32-37	26-28	10	13-15	16-18	6-9 (8)	8 (1)	26-40	0
50	Sp	7	37-43	31-37	27-30	10	14-15	17-20	8 (3)	8 (2)	31-44	0
51	Sp	2	35-39	35-36	26-27	10	14-16	15-19	7 (2)	-	30-35	0
52	MF	7	45-49	42-49	29-31	14-16	16-17	18-23	10-13 (3)	10-14 (3)	14-23	0

**Table 5.4:** Frequency of occurrence (%) for qualitative characters in populations of the *Pseudocoryphus melanotus* and *P. microlepidotus* complexes. Sample sizes are indicated in parentheses. Interspaces between longitudinal rows of dorsolaterals: equal to larger +  $\geq 0.5$  but not equal +  $\leq 0.5$ . A = granular scales only and in contact, B = enlarged scales in contact. Gular pattern: A = throat pale with a parallel pair of dark longitudinal median stripes with arrow-like anterior ends, B = throat black, C = throat pale with a single dark median longitudinal stripe, D = like A but without arrow-like ends.

	Female Femoral pores	Large sections	FN wider than long ( $\tau$ as wide as long)	Scale behind FN	Supranasal FN	FN separates	FN divided + partly divided	FN contacts	Anterior frontal present	Anterior divided + partially divided	Median dorsals of >0.5 size	Lateral dorsolaterals	Lateral dorsolaterals $\geq 0.75$ size	Dorso- lateral larger than median dorsals	Interspaces between longitudinal rows of dorso- lateral dorsals	Gular pattern	Posterior smooth infralabials	Lateralis
<i>P. transvaalensis</i>	0 (41)	94+4 (83)	48 (80)	55 (82)	53+28 (83)	100 (81)	43 (83)	12+43 (82)	0 (83)	100 (83)	0+14+86 (83)	4 (82)	0 (83)	0 (83)	B (82)	4 (82)	0 (83)	
Western	0 (11)	96+4 (23)	41 (22)	91 (22)	74+13 (23)	100 (23)	39 (23)	39+43 (23)	0 (23)	100 (23)	0+0+100 (23)	0 (22)	0 (23)	B (23)	0 (22)	0 (23)	0 (23)	
Central	0 (16)	90+3 (31)	35 (31)	65 (31)	32+39 (31)	100 (31)	61 (31)	3+71 (31)	0 (31)	100 (31)	0+6+94 (31)	6 (31)	0 (31)	B (30)	6 (31)	0 (31)	0 (31)	
Eastern	0 (14)	97+3 (29)	67 (27)	17 (29)	59+28 (29)	100 (27)	28 (29)	0+11 (28)	0 (29)	100 (29)	0+34+66 (29)	3 (29)	0 (29)	B (29)	3 (29)	0 (29)	0 (29)	
<i>P. m. melanotus</i>	0 (87)	94+3 (177)	40 (165)	15 (176)	70+14 (176)	99 (170)	2 (163)	1+1 (163)	2 (176)	100 (177)	0+27+73 (164)	1 (168)	0 (177)	A(96)+B(4) (171)	1 (168)	0 (177)	0 (177)	
Northern	0 (18)	78+15 (40)	38 (37)	23 (40)	25+15 (40)	97 (38)	0 (39)	0+0 (39)	3 (40)	100 (40)	0+56+44 (39)	3 (39)	0 (40)	A(85)+B(15) (40)	3 (39)	0 (40)	0 (40)	
Southern	0 (69)	99 (137)	40 (128)	13 (136)	84+14 (136)	100 (132)	2 (124)	1+1 (124)	2 (136)	100 (136)	0+18+82 (125)	0 (129)	0 (137)	A(99)+B(1) (131)	0 (129)	0 (137)	0 (137)	
Suikerbosrand	0 (7)	100 (15)	47 (15)	40 (15)	100+0 (15)	100 (14)	0 (15)	0+0 (15)	0 (15)	100 (15)	0+0+100 (15)	0 (15)	0 (15)	A (15)	0 (15)	0 (15)	0 (15)	
Nkandhla	0 (14)	100 (31)	31 (26)	16 (31)	58+32 (31)	100 (31)	0 (23)	0+0 (22)	0 (31)	100 (31)	0+4+96 (23)	0 (29)	0 (31)	A (28)	0 (29)	0 (31)	0 (31)	
Other S mel	0 (48)	99 (91)	41 (87)	8 (90)	90+10 (90)	100 (87)	3 (86)	1+1 (87)	3 (90)	100 (90)	0+24+76 (87)	0 (85)	0 (91)	A(99)+B(1) (88)	0 (85)	0 (91)	0 (91)	
<i>P. m. subviridis</i>	74 (100)	98 (244)	5 (232)	5 (244)	6+5 (244)	94 (238)	0 (236)	0+0.4 (232)	65 (245)	100 (245)	31+46+24 (237)	1 (240)	0 (245)	A (233)	1 (240)	0 (245)	0 (245)	
Drakensberg	76 (89)	98 (216)	5 (209)	6 (216)	6+6 (216)	93 (211)	0 (211)	0+0.5 (209)	72 (217)	100 (217)	34+48+18 (214)	1 (214)	0 (217)	A (209)	1 (214)	0 (217)	0 (217)	
Amatole	55 (11)	93 (28)	0 (26)	0 (28)	0+0 (28)	96 (27)	0 (26)	0+0 (26)	14 (28)	100 (28)	0+26+74 (23)	0 (26)	0 (28)	A (24)	0 (26)	0 (28)	0 (28)	
<i>P. lungi</i>	100 (8)	100 (28)	4 (23)	0 (28)	11+14 (28)	100 (27)	0 (23)	0+0 (23)	100 (28)	0 (28)	A (28)	96 (27)	0 (28)	C (28)	96 (27)	0 (28)	0 (28)	
<i>P. spinosus</i>	100 (3)	0+6* (18)	11* (19)	0* (19)	0+6* (18)	0* (18)	0 (18)	0+0 (19)	0 (19)	100 (19)	0+0+26+B(74) (19)	0 (19)	0 (19)	D (19)	0 (19)	0 (19)	95 (19)	
<i>P. m. fasciatus</i>	67 (3)	57+43 (7)	0 (7)	0 (7)	0+0 (7)	100 (7)	0 (7)	0+0 (7)	0 (7)	100 (7)	0+0+100 (7)	0 (7)	0 (7)	D (7)	0 (7)	0 (7)	0 (7)	

\* Frontonasal absent in one specimen (14%).

**Table 5.5:** Meristic data for head scalation in populations of the *Pseudocorymbus melanotus* and *P. microlepidotus* complexes. The range (minimum to maximum) for each character is followed by the sample size (in parentheses) and mean  $\pm$  one standard deviation.

	Upper temporals	Horizontal lateral temporals	Supraoculars	Supraciliaries	Suboculars anterior to median	Suboculars posterior to median	Supralabials	Infralabials	Sublabials	Gulars anterior to 1st sublabials	Gulars across throat	Small scales behind interparietal	Occipitals
<i>P. transvaalensis</i>	5-8 (83) 6.1 $\pm$ 0.45	4-8 (83) 5.7 $\pm$ 0.82	8-10 (83) 8.1 $\pm$ 0.29	9-12 (83) 10.1 $\pm$ 0.40	2-4 (82) 2.2 $\pm$ 0.57	2-6 (82) 4.0 $\pm$ 0.66	7-10 (83) 8.5 $\pm$ 0.65	12-17 (83) 12.4 $\pm$ 0.83	10-14 (83) 11.2 $\pm$ 1.00	2-4 (83) 2.1 $\pm$ 0.41	25-41 (82) 32.4 $\pm$ 3.24	2-12 (82) 5.7 $\pm$ 2.04	6-10 (71) 7.9 $\pm$ 1.04
Western	5-8 (23) 6.0 $\pm$ 0.52	5-8 (23) 6.1 $\pm$ 0.63	8 (23) 8.0 $\pm$ 0.00	10-11 (23) 10.2 $\pm$ 0.42	2-4 (23) 2.3 $\pm$ 0.62	2-5 (23) 3.9 $\pm$ 0.60	8-10 (23) 8.6 $\pm$ 0.66	12-17 (23) 12.3 $\pm$ 1.06	10-14 (23) 11.0 $\pm$ 1.15	2-4 (23) 2.1 $\pm$ 0.42	29-40 (23) 34.0 $\pm$ 2.64	2-12 (23) 6.8 $\pm$ 2.52	7-10 (18) 8.6 $\pm$ 0.98
Central	6-8 (31) 6.1 $\pm$ 0.36	4-7 (31) 5.4 $\pm$ 0.95	8-9 (31) 8.1 $\pm$ 0.25	9-11 (31) 10.1 $\pm$ 0.36	2-4 (31) 2.1 $\pm$ 0.40	3-6 (31) 4.2 $\pm$ 0.72	7-9 (31) 8.4 $\pm$ 0.55	12-14 (31) 12.3 $\pm$ 0.59	10-13 (31) 10.9 $\pm$ 0.92	2-4 (31) 2.2 $\pm$ 0.50	25-41 (31) 31.5 $\pm$ 3.03	2-10 (31) 6.1 $\pm$ 1.65	6-10 (26) 7.8 $\pm$ 1.05
Eastern	5-7 (29) 6.2 $\pm$ 0.47	4-7 (29) 5.8 $\pm$ 0.62	8-10 (29) 8.1 $\pm$ 0.41	10-12 (29) 10.1 $\pm$ 0.41	2-4 (28) 2.4 $\pm$ 0.68	3-6 (28) 3.9 $\pm$ 0.59	8-10 (29) 8.5 $\pm$ 0.74	12-14 (29) 12.7 $\pm$ 0.80	10-13 (29) 11.7 $\pm$ 0.76	2-3 (29) 2.1 $\pm$ 0.26	27-41 (28) 32.1 $\pm$ 3.55	2-6 (28) 4.3 $\pm$ 0.98	6-10 (27) 7.6 $\pm$ 0.89
<i>P. m. melanotus</i>	5-8 (177) 6.0 $\pm$ 0.28	2-6 (173) 4.1 $\pm$ 0.46	8-10 (176) 8.1 $\pm$ 0.31	8-12 (177) 10.1 $\pm$ 0.37	2-5 (176) 2.1 $\pm$ 0.46	2-4 (176) 2.3 $\pm$ 0.52	7-11 (177) 8.3 $\pm$ 0.60	10-14 (177) 12.0 $\pm$ 0.45	9-12 (177) 10.0 $\pm$ 0.19	2-5 (175) 2.3 $\pm$ 0.62	20-33 (171) 25.8 $\pm$ 2.37	0-4 (177) 0.2 $\pm$ 0.59	6-13 (169) 8.9 $\pm$ 1.59
Northern	6-8 (40) 6.1 $\pm$ 0.32	3-6 (39) 4.2 $\pm$ 0.63	8-10 (40) 8.3 $\pm$ 0.54	10-12 (40) 10.1 $\pm$ 0.46	2-5 (40) 2.2 $\pm$ 0.61	2-4 (40) 2.5 $\pm$ 0.64	8-11 (40) 8.6 $\pm$ 0.81	12-13 (40) 12.1 $\pm$ 0.22	10 (40) 10.0 $\pm$ 0.00	2-4 (40) 2.2 $\pm$ 0.43	23-32 (38) 26.4 $\pm$ 2.27	0-4 (40) 0.23 $\pm$ 0.70	6-12 (40) 8.6 $\pm$ 1.56
Southern	5-8 (137) 6.0 $\pm$ 0.27	2-6 (134) 4.0 $\pm$ 0.38	8-9 (136) 8.0 $\pm$ 0.17	8-11 (137) 10.0 $\pm$ 0.33	2-5 (136) 2.1 $\pm$ 0.40	2-4 (136) 2.2 $\pm$ 0.45	7-10 (137) 8.2 $\pm$ 0.49	10-14 (137) 12.0 $\pm$ 0.50	9-12 (137) 10.0 $\pm$ 0.21	2-5 (135) 2.4 $\pm$ 0.66	20-33 (133) 25.6 $\pm$ 2.38	0-2 (137) 0.2 $\pm$ 0.55	6-13 (129) 9.0 $\pm$ 1.59
Suikerbosrand	6-7 (15) 6.1 $\pm$ 0.26	3-5 (15) 4.0 $\pm$ 0.38	8 (15) 8.0 $\pm$ 0.00	9-11 (15) 10.0 $\pm$ 0.38	2-3 (14) 2.1 $\pm$ 0.27	2 (14) 2.0 $\pm$ 0.00	7-10 (15) 8.1 $\pm$ 0.59	11-12 (15) 11.9 $\pm$ 0.35	10 (15) 10.0 $\pm$ 0.00	2-4 (15) 2.3 $\pm$ 0.59	21-28 (15) 24.7 $\pm$ 1.68	0-1 (15) 0.1 $\pm$ 0.26	6-12 (15) 9.1 $\pm$ 1.69
Nkandhla	6 (31) 6.0 $\pm$ 0.00	4-5 (29) 4.0 $\pm$ 0.19	8 (30) 8.0 $\pm$ 0.00	9-10 (31) 10.0 $\pm$ 0.18	2-4 (31) 2.1 $\pm$ 0.36	2-4 (31) 2.2 $\pm$ 0.45	8-10 (31) 8.5 $\pm$ 0.68	10-14 (31) 11.9 $\pm$ 0.65	9-10 (31) 10.0 $\pm$ 0.18	2-4 (31) 2.6 $\pm$ 0.67	22-32 (30) 26.7 $\pm$ 2.33	0-1 (31) 0.2 $\pm$ 0.40	7-12 (29) 9.6 $\pm$ 1.24
Other S mel	5-8 (91) 6.0 $\pm$ 0.31	2-6 (90) 4.0 $\pm$ 0.42	8-9 (91) 8.0 $\pm$ 0.21	8-11 (91) 10.0 $\pm$ 0.36	2-5 (91) 2.1 $\pm$ 0.43	2-4 (91) 2.2 $\pm$ 0.48	8-10 (91) 8.1 $\pm$ 0.35	10-13 (91) 12.0 $\pm$ 0.46	9-12 (91) 10.0 $\pm$ 0.24	2-5 (89) 2.3 $\pm$ 0.66	20-33 (88) 25.3 $\pm$ 2.38	0-2 (91) 0.3 $\pm$ 0.63	6-13 (85) 8.7 $\pm$ 1.64
<i>P. m. subviridis</i>	5-8 (243) 6.1 $\pm$ 0.28	2-4 (241) 3.0 $\pm$ 0.94	8-10 (245) 8.0 $\pm$ 0.14	8-12 (245) 10.1 $\pm$ 0.45	2-5 (245) 2.1 $\pm$ 0.37	2-4 (245) 2.2 $\pm$ 0.55	6-11 (245) 8.2 $\pm$ 0.61	10-14 (245) 12.0 $\pm$ 0.41	10-11 (244) 10.0 $\pm$ 0.11	2-5 (244) 2.4 $\pm$ 0.70	21-35 (239) 27.7 $\pm$ 2.60	0-3 (245) 0.2 $\pm$ 0.49	5-14 (242) 8.2 $\pm$ 1.68
Drakensberg	5-8 (215) 6.0 $\pm$ 0.28	2-4 (214) 2.9 $\pm$ 0.95	8-10 (217) 8.0 $\pm$ 0.15	8-12 (217) 10.1 $\pm$ 0.43	2-4 (217) 2.1 $\pm$ 0.33	2-4 (217) 2.3 $\pm$ 0.58	6-11 (217) 8.2 $\pm$ 0.60	10-14 (217) 12.0 $\pm$ 0.39	10-11 (217) 10.0 $\pm$ 0.12	2-4 (217) 2.3 $\pm$ 0.64	21-35 (214) 27.5 $\pm$ 2.59	0-3 (217) 0.2 $\pm$ 0.52	5-14 (214) 8.2 $\pm$ 1.70
Amatole	6-7 (28) 6.1 $\pm$ 0.26	2-4 (27) 3.5 $\pm$ 0.75	8 (28) 8.0 $\pm$ 0.00	10-12 (28) 10.2 $\pm$ 0.57	2-5 (28) 2.2 $\pm$ 0.61	2 (28) 2.0 $\pm$ 0.00	6-10 (28) 8.0 $\pm$ 0.64	10-13 (28) 12.1 $\pm$ 0.54	10 (27) 10.0 $\pm$ 0.00	2-5 (27) 2.7 $\pm$ 1.02	23-34 (25) 28.8 $\pm$ 2.48	0 (28) 0.0 $\pm$ 0.00	6-11 (28) 8.0 $\pm$ 1.55
<i>P. langi</i>	4-7 (28) 5.8 $\pm$ 0.77	2-4 (25) 2.3 $\pm$ 0.63	8 (28) 8.0 $\pm$ 0.00	9-12 (28) 10.3 $\pm$ 0.67	2-3 (27) 2.1 $\pm$ 0.32	2-6 (27) 2.4 $\pm$ 0.88	8-10 (28) 8.1 $\pm$ 0.45	10-11 (28) 10.1 $\pm$ 0.32	9-11 (28) 10.1 $\pm$ 0.38	2-4 (28) 2.6 $\pm$ 0.87	23-45 (28) 30.4 $\pm$ 4.99	0-3 (28) 0.3 $\pm$ 0.81	0 (28) 0.0 $\pm$ 0.00
<i>P. spinosus</i>	5-6 (19) 6.0 $\pm$ 0.23	2-4 (19) 2.6 $\pm$ 0.84	8 (19) 8.0 $\pm$ 0.00	10-11 (19) 10.1 $\pm$ 0.23	2-3 (19) 2.1 $\pm$ 0.32	2-4 (19) 2.8 $\pm$ 0.79	6-9 (19) 8.0 $\pm$ 0.88	10-14 (19) 12.2 $\pm$ 0.77	10-11 (19) 10.2 $\pm$ 0.42	2 (19) 2.0 $\pm$ 0.00	20-26 (19) 23.2 $\pm$ 1.78	0 (19) 0.0 $\pm$ 0.00	0 (19) 0.0 $\pm$ 0.00
<i>P. m. fuscicatus</i>	6 (7) 6.0 $\pm$ 0.00	4-6 (7) 5.1 $\pm$ 0.69	8 (7) 8.0 $\pm$ 0.00	10 (7) 10.0 $\pm$ 0.00	2 (7) 2.0 $\pm$ 0.00	3-4 (7) 3.6 $\pm$ 0.54	8-11 (7) 9.6 $\pm$ 0.98	12-13 (7) 12.1 $\pm$ 0.38	10 (7) 10.0 $\pm$ 0.00	2 (7) 2.0 $\pm$ 0.00	22-33 (7) 27.9 $\pm$ 3.44	0-2 (7) 0.4 $\pm$ 0.79	7-10 (7) 8.9 $\pm$ 1.22

**Table 5.6:** Meristic data for scalation characters in populations of the *Pseudocordylus melanotus* and *P. microlepidotus* complexes. The range (minimum to maximum) for each character is followed by the sample size (in parentheses) and mean  $\pm$  one standard deviation.

	Transverse rows of dorsals	Longitudinal rows of dorsals	Transverse rows of Ventrals	Longitudinal Ventrals	Lamellae under fourth finger	Lamellae under fourth toe	Differentiated scutes in males	Female pores	Male pores	Female pores	Female pores	Female pores	Female pores	Pre-cloacal Glands
<i>P. transvaalensis</i>	38-51 (83) 43.6 $\pm$ 2.57	39-55 (83) 44.7 $\pm$ 3.18	28-33 (83) 29.9 $\pm$ 1.28	12-14 (83) 12.4 $\pm$ 0.83	13-18 (82) 15.0 $\pm$ 0.95	17-23 (83) 19.8 $\pm$ 1.08	7-28 (35) 18.0 $\pm$ 5.73	10-17 (35) 13.9 $\pm$ 1.43	12-17 (10) 13.9 $\pm$ 1.45	12-15 (11) 13.8 $\pm$ 0.87	0-16 (40) 13.5 $\pm$ 2.59	0-17 (75) 13.7 $\pm$ 2.13	0-6 (83) 0.1 $\pm$ 0.71	
Western	40-51 (23) 44.3 $\pm$ 2.93	39-50 (23) 43.3 $\pm$ 2.77	28-33 (23) 30.1 $\pm$ 1.53	12-14 (23) 12.9 $\pm$ 1.00	13-16 (22) 14.5 $\pm$ 0.91	18-22 (23) 19.3 $\pm$ 0.88	17-28 (10) 21.8 $\pm$ 4.24	12-17 (10) 13.9 $\pm$ 1.45	12-15 (11) 13.8 $\pm$ 0.87	12-17 (21) 13.9 $\pm$ 1.15	0-1 (23) 0.0 $\pm$ 0.21	12-17 (21) 13.9 $\pm$ 1.15	0-1 (23) 0.0 $\pm$ 0.21	
Central	39-49 (31) 44.2 $\pm$ 2.51	40-55 (31) 46.3 $\pm$ 3.58	28-32 (31) 29.6 $\pm$ 1.15	12-14 (31) 12.3 $\pm$ 0.68	13-18 (31) 15.2 $\pm$ 1.07	17-23 (31) 19.7 $\pm$ 1.18	8-27 (14) 16.3 $\pm$ 5.31	10-15 (14) 13.5 $\pm$ 1.45	10-15 (14) 13.5 $\pm$ 1.45	0-16 (16) 12.4 $\pm$ 3.71	0-16 (30) 12.9 $\pm$ 2.89	0-16 (30) 12.9 $\pm$ 2.89	0-6 (31) 0.2 $\pm$ 1.09	
Eastern	38-46 (29) 42.2 $\pm$ 1.72	40-49 (29) 44.2 $\pm$ 2.24	28-32 (29) 30.0 $\pm$ 1.18	12-14 (29) 12.3 $\pm$ 0.70	14-17 (29) 15.3 $\pm$ 0.67	18-22 (29) 20.2 $\pm$ 0.98	7-28 (11) 16.6 $\pm$ 6.17	12-17 (11) 14.4 $\pm$ 1.36	12-17 (11) 14.4 $\pm$ 1.36	13-16 (13) 14.5 $\pm$ 1.20	12-17 (24) 14.4 $\pm$ 1.25	12-17 (24) 14.4 $\pm$ 1.25	0-2 (29) 0.1 $\pm$ 0.37	
<i>P. m. melanotus</i>	37-57 (177) 45.8 $\pm$ 3.70	33-52 (177) 40.4 $\pm$ 3.67	26-33 (176) 29.5 $\pm$ 1.25	11-14 (177) 12.1 $\pm$ 0.49	13-19 (177) 16.1 $\pm$ 1.38	16-25 (176) 19.9 $\pm$ 1.66	0-40 (51) 18.0 $\pm$ 9.53	11-20 (52) 15.3 $\pm$ 1.89	11-20 (52) 15.3 $\pm$ 1.89	10-21 (85) 15.1 $\pm$ 2.07	10-21 (137) 15.1 $\pm$ 2.00	10-21 (137) 15.1 $\pm$ 2.00	0-1 (176) 0.0 $\pm$ 0.08	
Northern	43-57 (40) 49.5 $\pm$ 3.23	37-52 (40) 44.0 $\pm$ 3.46	28-31 (40) 29.3 $\pm$ 0.82	12-14 (40) 12.1 $\pm$ 0.32	14-19 (40) 17.0 $\pm$ 1.55	18-25 (39) 21.4 $\pm$ 1.76	11-40 (13) 25.7 $\pm$ 8.06	11-16 (13) 13.6 $\pm$ 1.45	11-16 (13) 13.6 $\pm$ 1.45	12-18 (17) 13.7 $\pm$ 2.05	11-18 (30) 13.7 $\pm$ 1.79	11-18 (30) 13.7 $\pm$ 1.79	0-1 (40) 0.0 $\pm$ 0.16	
Southern	37-56 (137) 44.8 $\pm$ 3.11	33-47 (137) 39.3 $\pm$ 3.02	26-33 (136) 29.5 $\pm$ 1.34	11-14 (137) 12.1 $\pm$ 0.53	13-19 (137) 15.8 $\pm$ 1.21	16-23 (137) 19.5 $\pm$ 1.38	0-32 (38) 15.3 $\pm$ 8.58	12-20 (39) 15.8 $\pm$ 1.70	12-20 (39) 15.8 $\pm$ 1.70	10-21 (68) 15.4 $\pm$ 1.95	10-21 (107) 15.5 $\pm$ 1.86	10-21 (107) 15.5 $\pm$ 1.86	0 (136) 0.0 $\pm$ 0.00	
Suikerbosrand	40-47 (15) 42.2 $\pm$ 2.04	34-42 (15) 37.7 $\pm$ 2.43	27-32 (15) 29.5 $\pm$ 1.25	12 (15) 12.0 $\pm$ 0.00	15-18 (15) 16.6 $\pm$ 0.83	18-23 (15) 20.2 $\pm$ 1.47	18-27 (5) 22.0 $\pm$ 3.39	14-20 (6) 16.5 $\pm$ 2.17	14-20 (6) 16.5 $\pm$ 2.17	14-16 (7) 15.1 $\pm$ 0.90	14-20 (13) 15.8 $\pm$ 1.69	14-20 (13) 15.8 $\pm$ 1.69	0 (15) 0.0 $\pm$ 0.00	
Nkandhla	40-53 (31) 44.5 $\pm$ 2.76	34-44 (31) 38.7 $\pm$ 2.78	27-31 (31) 29.3 $\pm$ 1.08	12-14 (31) 12.5 $\pm$ 0.85	13-18 (31) 15.5 $\pm$ 1.21	17-22 (31) 19.4 $\pm$ 1.41	10-27 (9) 16.3 $\pm$ 5.27	12-17 (9) 15.0 $\pm$ 1.50	12-17 (9) 15.0 $\pm$ 1.50	12-18 (14) 14.7 $\pm$ 1.64	10-21 (47) 15.6 $\pm$ 2.11	10-21 (47) 15.6 $\pm$ 2.11	0 (91) 0.0 $\pm$ 0.00	
Other S mel	37-56 (91) 45.3 $\pm$ 3.16	33-47 (91) 39.8 $\pm$ 3.09	26-33 (90) 29.6 $\pm$ 1.44	11-14 (91) 12.1 $\pm$ 0.38	13-19 (91) 15.8 $\pm$ 1.22	16-23 (91) 19.4 $\pm$ 1.34	0-32 (24) 13.6 $\pm$ 9.66	14-19 (24) 15.9 $\pm$ 1.61	14-19 (24) 15.9 $\pm$ 1.61	10-21 (47) 15.6 $\pm$ 2.11	10-21 (71) 15.7 $\pm$ 1.95	10-21 (71) 15.7 $\pm$ 1.95	0 (91) 0.0 $\pm$ 0.00	
<i>P. m. subviridis</i>	40-59 (244) 47.9 $\pm$ 4.08	27-53 (245) 36.6 $\pm$ 4.39	25-33 (244) 28.6 $\pm$ 1.40	12-14 (245) 12.4 $\pm$ 0.81	14-20 (245) 16.4 $\pm$ 1.29	17-25 (244) 20.2 $\pm$ 1.66	0-75 (97) 28.0 $\pm$ 18.49	10-25 (98) 15.6 $\pm$ 2.87	10-25 (98) 15.6 $\pm$ 2.87	10-21 (101) 15.0 $\pm$ 2.32	10-25 (244) 15.3 $\pm$ 2.62	10-25 (244) 15.3 $\pm$ 2.62	0-2 (244) 0.0 $\pm$ 0.14	
Drakensberg	41-59 (217) 48.3 $\pm$ 4.05	27-53 (217) 36.0 $\pm$ 4.27	25-33 (216) 28.6 $\pm$ 1.38	12-14 (217) 12.4 $\pm$ 0.81	14-20 (217) 16.5 $\pm$ 1.27	17-25 (216) 20.3 $\pm$ 1.68	0-75 (88) 29.5 $\pm$ 18.76	10-25 (89) 15.9 $\pm$ 2.75	10-25 (89) 15.9 $\pm$ 2.75	10-21 (89) 15.5 $\pm$ 2.01	10-25 (178) 15.7 $\pm$ 2.41	10-25 (178) 15.7 $\pm$ 2.41	0-2 (216) 0.0 $\pm$ 0.15	
Amatole	40-52 (27) 45.1 $\pm$ 3.18	36-46 (28) 41.0 $\pm$ 2.47	26-32 (28) 28.5 $\pm$ 1.50	12-14 (28) 12.4 $\pm$ 0.84	14-18 (28) 15.6 $\pm$ 1.20	17-21 (28) 19.3 $\pm$ 1.21	10-17 (9) 13.3 $\pm$ 2.69	10-15 (9) 12.2 $\pm$ 1.72	10-15 (9) 12.2 $\pm$ 1.72	10-13 (12) 11.4 $\pm$ 1.00	10-15 (21) 11.8 $\pm$ 1.37	10-15 (21) 11.8 $\pm$ 1.37	0 (28) 0.0 $\pm$ 0.00	
<i>P. langi</i>	0 (28) 0.0 $\pm$ 0.00	6-9 (27) 7.7 $\pm$ 0.88	29-32 (28) 30.6 $\pm$ 1.03	10-12 (28) 11.3 $\pm$ 0.98	16-21 (28) 18.0 $\pm$ 1.23	20-26 (28) 22.6 $\pm$ 1.34	0-50 (10) 16.3 $\pm$ 18.80	25-34 (11) 28.9 $\pm$ 2.59	25-34 (11) 28.9 $\pm$ 2.59	26-30 (10) 27.9 $\pm$ 1.73	25-34 (21) 28.4 $\pm$ 2.23	25-34 (21) 28.4 $\pm$ 2.23	0 (28) 0.0 $\pm$ 0.00	
<i>P. spinosus</i>	35-43 (19) 38.5 $\pm$ 2.27	31-37 (19) 34.6 $\pm$ 1.50	26-30 (19) 27.4 $\pm$ 0.96	10 (19) 10.0 $\pm$ 0.00	13-16 (19) 14.4 $\pm$ 0.77	15-20 (19) 17.7 $\pm$ 1.05	26-44 (13) 34.8 $\pm$ 5.51	6-9 (13) 7.6 $\pm$ 0.77	6-9 (13) 7.6 $\pm$ 0.77	8 (3) 8.0 $\pm$ 0.00	6-9 (16) 7.7 $\pm$ 0.70	6-9 (16) 7.7 $\pm$ 0.70	0 (19) 0.0 $\pm$ 0.00	
<i>P. m. fasciatus</i>	45-49 (7) 47.1 $\pm$ 1.77	42-49 (7) 46.1 $\pm$ 2.61	29-31 (7) 30.1 $\pm$ 0.90	14-16 (7) 14.6 $\pm$ 0.98	16-17 (7) 16.7 $\pm$ 0.49	18-23 (7) 21.4 $\pm$ 1.62	14-23 (3) 18.7 $\pm$ 4.51	10-13 (3) 11.7 $\pm$ 1.53	10-13 (3) 11.7 $\pm$ 1.53	10-14 (3) 11.7 $\pm$ 2.08	10-14 (6) 11.7 $\pm$ 1.63	10-14 (6) 11.7 $\pm$ 1.63	0 (7) 0.0 $\pm$ 0.00	

Characters for which distinct patterns of geographic variation were observed are discussed below:

#### 5.3.1.1 Colour pattern (Fig. 5.6)

Dorsal colour pattern, especially in adults, proved to be a fairly reliable character for distinguishing between at least some taxa in the *P. melanotus* species complex. For example, *P. transvaalensis* differed from all other groups in having dark crossbands (sometimes in a zig-zag pattern) over a pale yellow to orange back (Figs 5.6 and 5.8). The flanks were usually a vivid orange colour, especially in males. *Pseudocordylus m. melanotus* and *P. m. subviridis* were similar, but as a group they were usually easily distinguished from all others in the complex. The colour patterns of both *P. langi* and *P. spinosus* were also distinctive (Fig. 5.6). See below for a detailed discussion. *Pseudocordylus microlepidotus fasciatus* had a variable colour pattern (Branch 1998; pers. obs.), but it never had the appearance of any of the *P. melanotus* species complex groupings.

Distinct sexual dichromatism occurred in all groups referable to both *P. m. melanotus* and *P. m. subviridis*. Mature males generally had a dark median band on the back, with yellow to orange flanks, whereas females and juveniles had a grey back with darker markings. In both sexes there were often scattered pale spots on the back. In some females these spots were arranged in the form of transverse bars across the back (e.g. NMB R8225 from near Hogsback). The pattern over the middle of the back varied in males from different localities, from black with little or no other markings to a pattern similar to that of females (e.g. S Lesotho *P. m. subviridis* males). The extent of the bright colouration on the flanks also varied considerably in males, and females occasionally also had at least some colour on the flanks. However, occasional females had exactly the same colour pattern as typical, mature males in their population – e.g. at Suikerbosrand Nature Reserve in Gauteng (Fig. 5.7, but note the narrow head typical of females). Mouton & Van Wyk (1993) studied sexual dichromatism and dimorphism in *P. m. subviridis* from the highlands of Lesotho. They determined that although most females were dull coloured (olive to olive-brown or olive-yellow), 5% had pale yellow flanks and 3% had bright orange or lemon flanks. Juveniles and subadult males were similar to

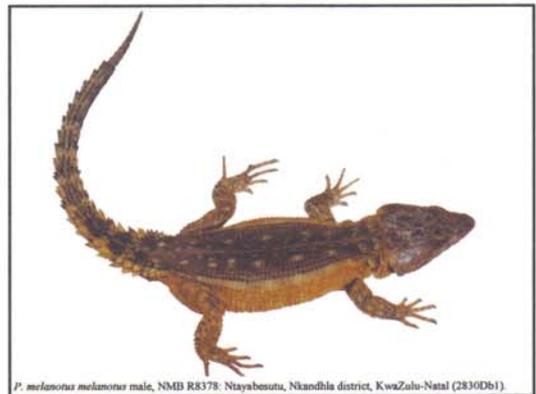
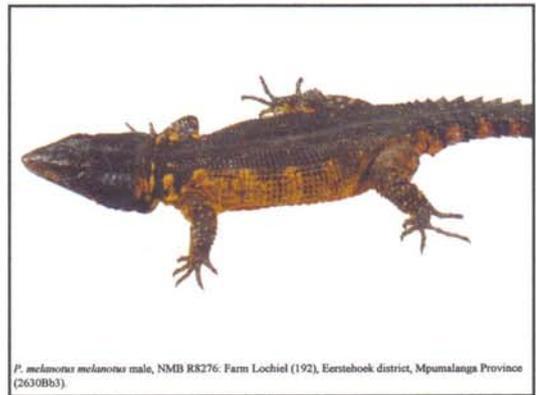
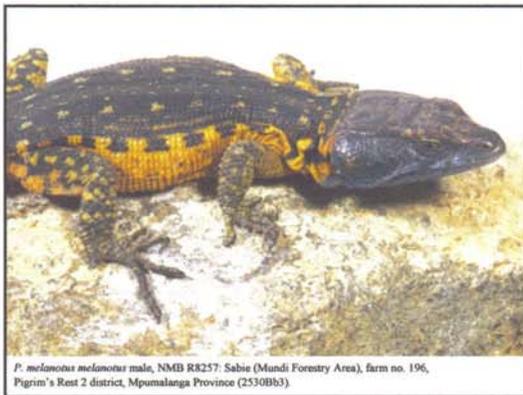
females. Most mature males (80 mm SVL and larger) had brightly coloured flanks (turquoise, lemon or orange).

Based on live specimens from localities 1 (NMB R8430-44; Appendix 5.1; Fig. 5.6), 4 (NMB R8195-208; Appendix 5.1; Fig. 5.8) and 7 (NMB R8546-51, Appendix 5.1), from the Western, Central and Eastern regions respectively, sexual dichromatism was evident but weakly developed in *P. transvaalensis*. Males had bright, orange or yellow bodies with dark crossbands over the back that did not extend onto the flanks. The bands were often unevenly arranged, resulting in a zigzag pattern mid-dorsally. Females were similar but had a dull yellow to olive body colour. However, two live males from locality 7 (NMB R8041-2; Appendix 5.1) in the Eastern region had greyish backs with dark crossbands and pale orange flanks. Juveniles had distinctly banded backs. *Pseudocordylus transvaalensis* often had black heads, a condition occasionally also occurring in male *P. m. melanotus* (e.g. NMB R8257, Sabie). In *P. transvaalensis* the throat was also black, a condition occurring in only a few *P. m. melanotus* (e.g. NMB R8257). The chest – and sometimes also the rest of the venter – was grey in several specimens of *P. transvaalensis*. The only other member of the complex that regularly had a grey venter (but not a completely black throat) was *P. langi*.

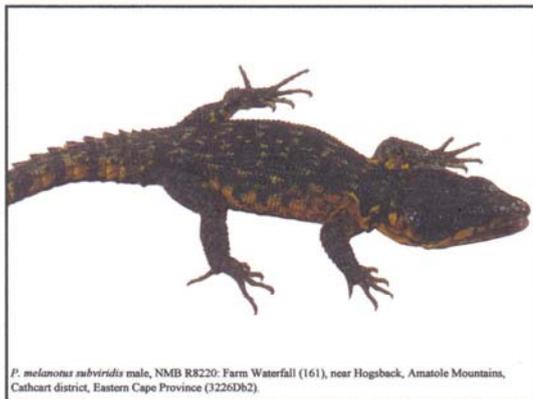
There was no apparent difference in colour pattern between males and females in both *P. spinosus* and *P. langi*. However, *P. spinosus* is poorly known and seldom collected, and a more detailed study of living specimens needs to be conducted with regard to possible differences in colour pattern. Figure 5.6 indicates that *P. spinosus* has a dark brown back with distinct cream to golden yellow spots and orange flanks. However, based on five specimens (four males, one juvenile) collected at Goodoo Pass (Appendix 5.1), the spots may be pale to cream yellow, while the flanks may be dull orange to yellow or lack bright colouration.

Close examination of *P. langi* showed that it had a distinct dorsal pattern. The overall colour was grey, with dark longitudinal streaks over the middle and dorsolateral parts of the back, between which were distinct pale, cream or light greenish spots (Fig. 5.6). Broadley (1964) noted that specimens from Organ Pipes Pass had a series of 1-6 bright sky-blue spots on either side of the body. In the new Organ Pipes Pass material, a distinct series of at least 3-4 pale blue spots were present on either side of the body, although

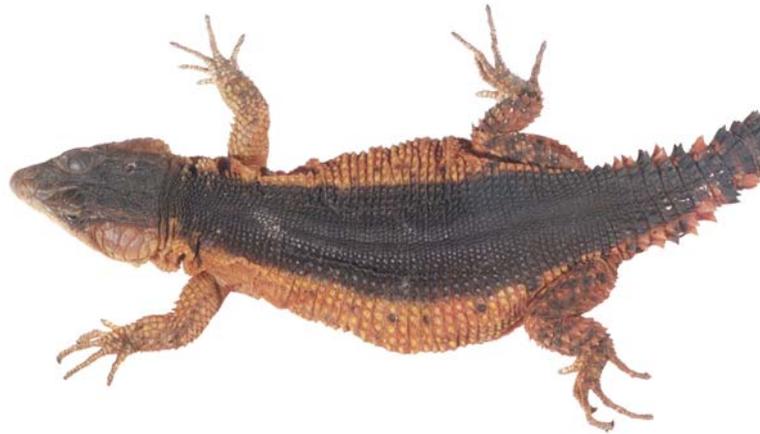
there were sometimes additional small spots. Some specimens from the Chain Ladder near Mont-aux-Sources had two rows of blue spots, the lowermost row consisting of much smaller spots.



**Figure 5.6:** Representatives of the *Pseudocordylus melanotus* and *P. microlepidotus* complexes.



**Figure 5.6** (continued): Representatives of the *Pseudocordylus melanotus* and *P. microlepidotus* complexes.



**Figure 5.7:** Female *Pseudocordylus melanotus melanotus* (NMB R8417) from Suikerbosrand Nature Reserve with colour pattern typical of mature males from this locality (but note the narrow head typical of females).



**Figure 5.8:** *Pseudocordylus transvaalensis* male (top, NMB R8195) and female (below, NMB R8196) from the farm Helderfontein, Potgietersrust district, Limpopo Province. Females have duller colours.

### 5.3.1.2 Morphometrics

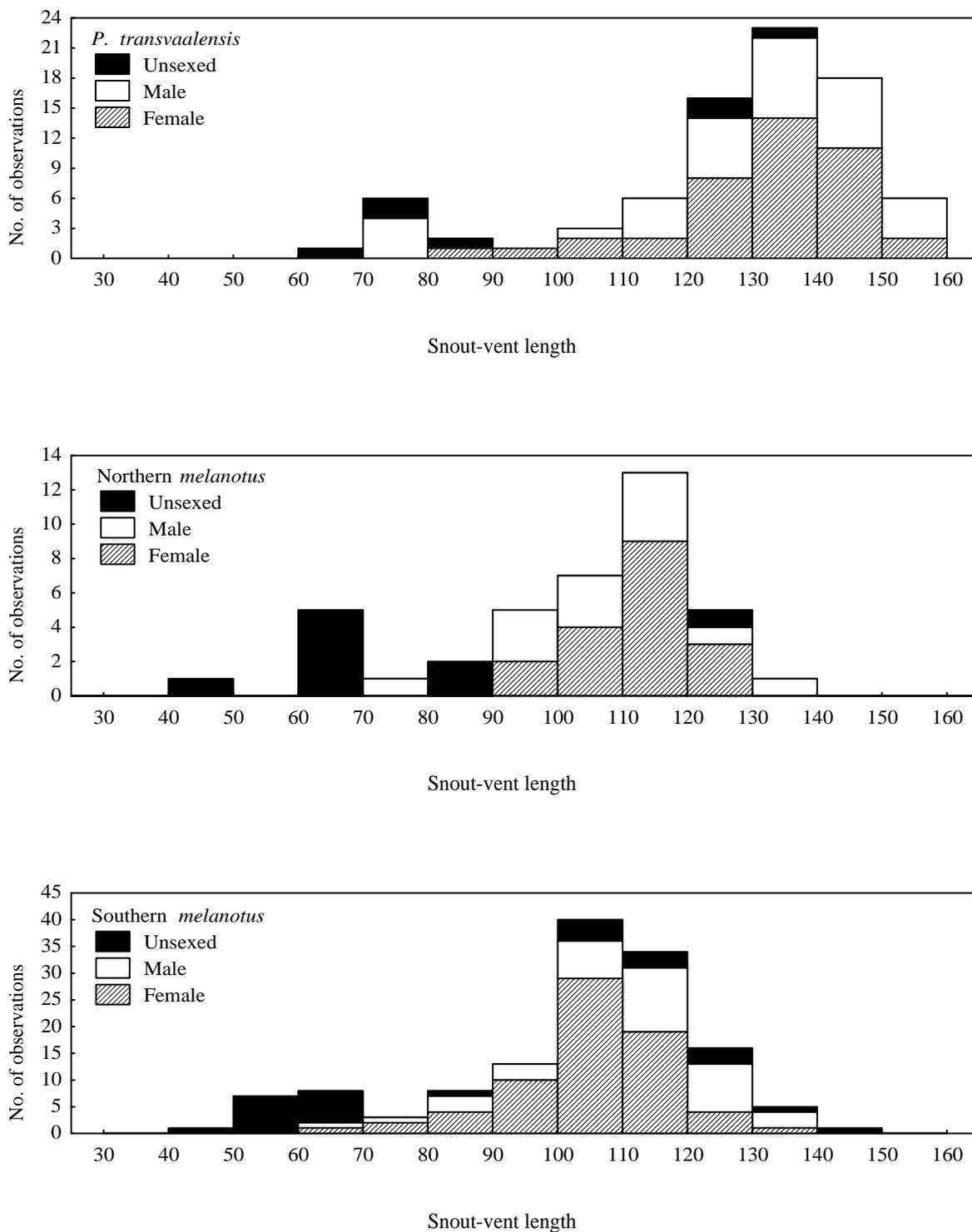
#### 1. Snout-vent length (Fig. 5.9):

The largest males and females in the various groupings were as follows:- *P. transvaalensis*: male 157 mm SVL : female 157 mm SVL; Northern *melanotus*: 136 : 121; Southern *melanotus*: 135 : 132; *P. m. subviridis*: 140 : 111; *P. langi*: 103 : 85; *P. spinosus*: 93 : 87. Generally *P. transvaalensis* achieved by far the greatest SVL, followed by the two *P. melanotus* groups, *P. m. subviridis*, *P. langi* and finally *P. spinosus*.

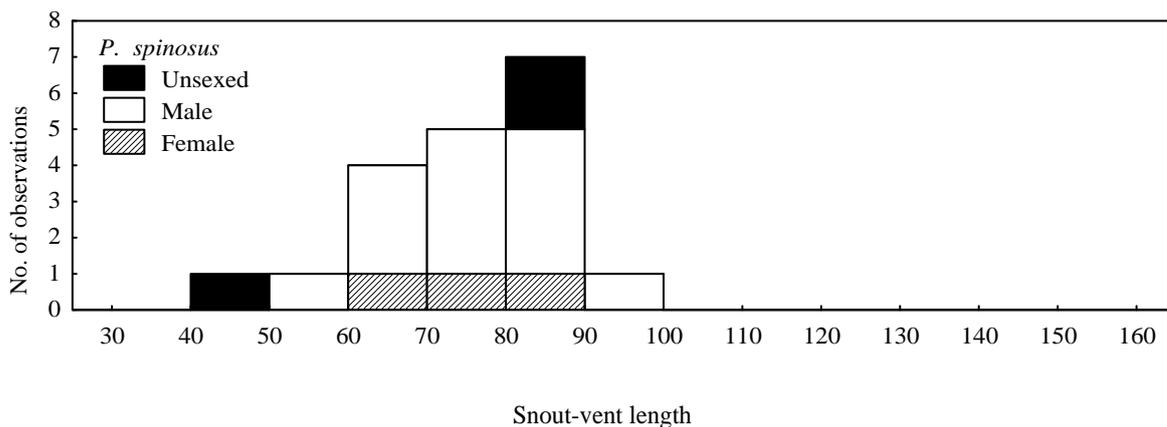
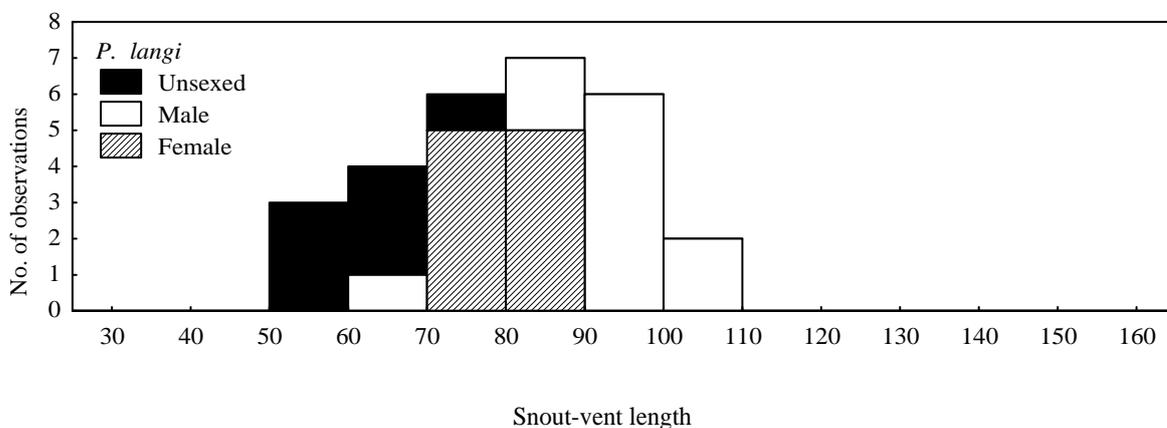
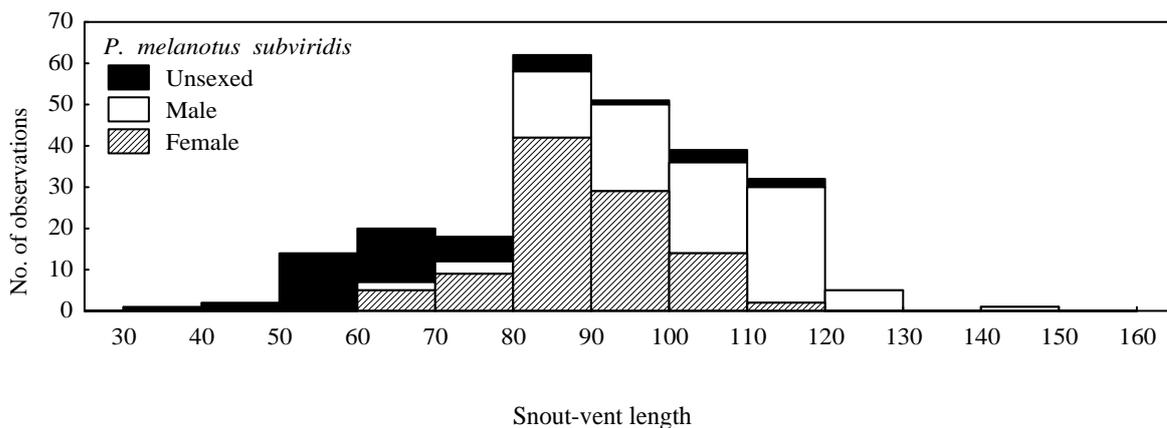
Regarding sexual dimorphism in body size, in Northern *melanotus*, *subviridis*, *langi*, *spinosus* and *microlepidotus fasciatus*, males comprised the largest size classes, while in the case of *transvaalensis* and Southern *melanotus* both males and females were represented in the largest size classes. Mouton & Van Wyk (1993) determined that male *subviridis* in the Lesotho highlands achieved a much larger SVL, and generally had longer and wider heads, than females (see below).

#### 2. Head dimensions (Figs 5.10-5.16):

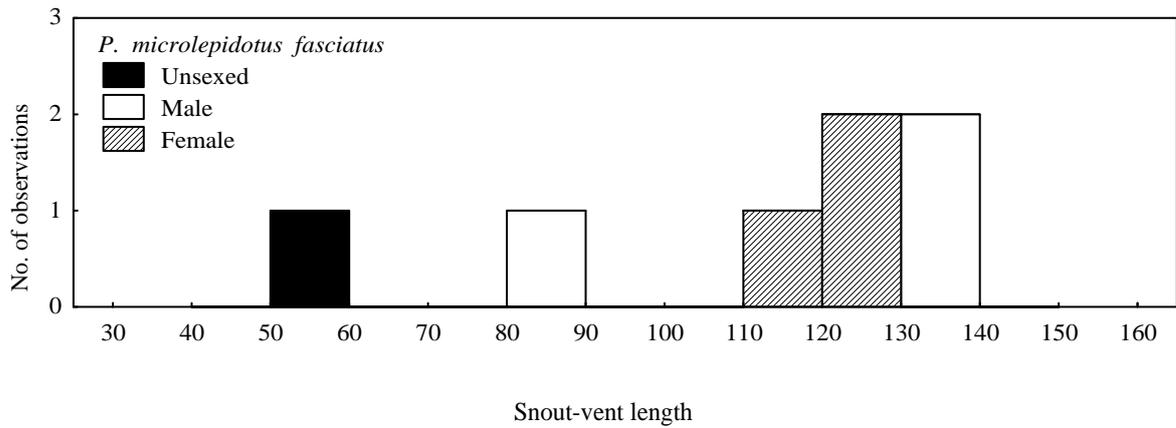
Adult males in the *transvaalensis*, Northern *melanotus*, Southern *melanotus* and *subviridis* groups tended to have longer, wider and deeper heads than females (Figs 5.10-5.13). In *transvaalensis* this distinction between the sexes occurred at a SVL of about 131 mm for both head length and width, and 149 mm for head depth; in Northern *melanotus* it occurred at a SVL of about 107 mm for all head dimensions; in Southern *melanotus* it occurred at a SVL of 109 mm for length, 110 mm for width and 99 mm for depth; and in *subviridis* it occurred at a SVL of about 98 mm for length, 96 mm for width and 105 mm for depth. The sample sizes for *spinosus* and *microlepidotus fasciatus* were small, but in the case of *spinosus* males also had larger heads, the distinction occurring at a SVL of about 79 mm for head length and width. Nothing meaningful can be said with regard to *langi* as most males examined were larger than females.



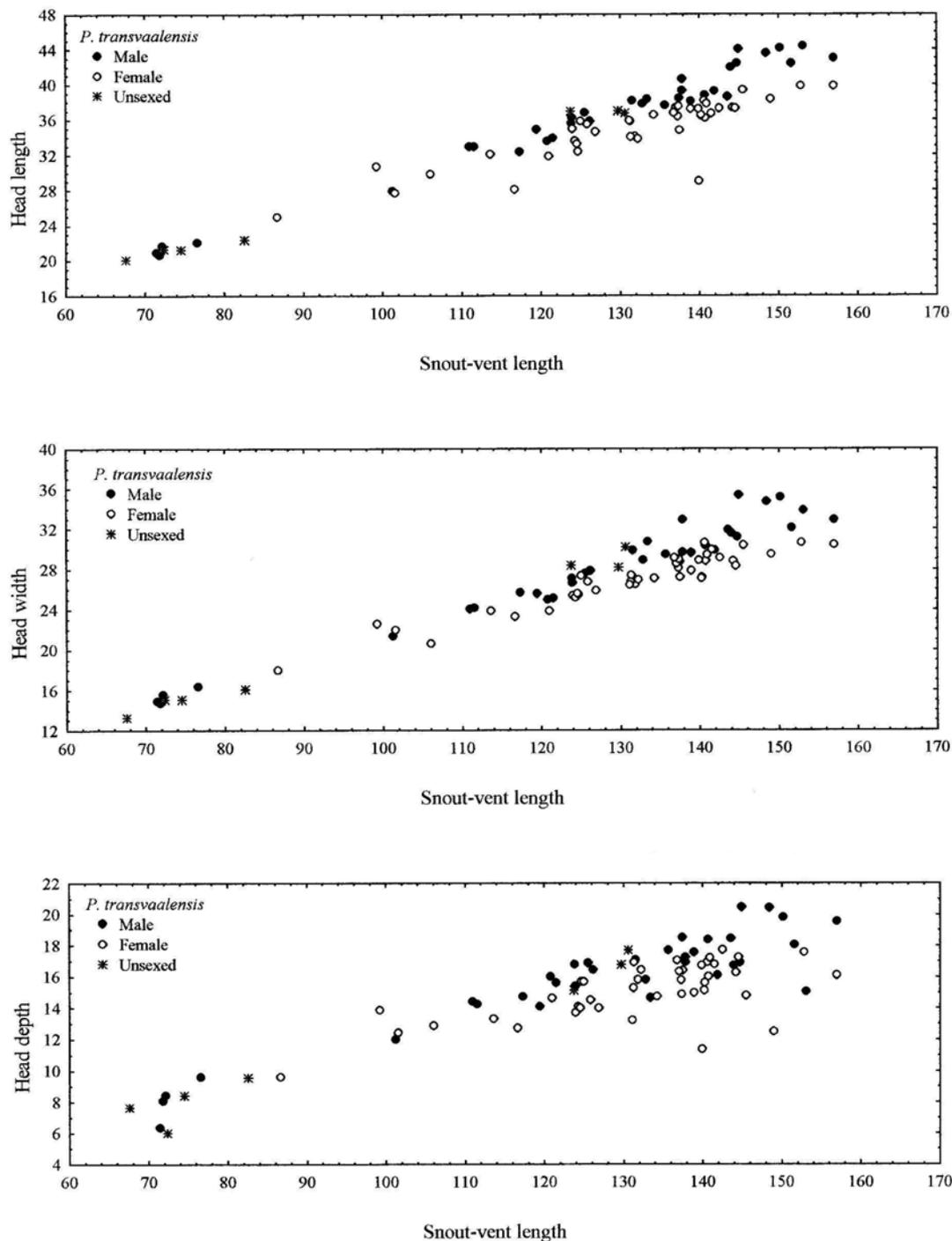
**Figure 5.9:** Histograms showing size (snout-vent length) distribution of *Pseudocordylus* specimens examined by sex and grouping.



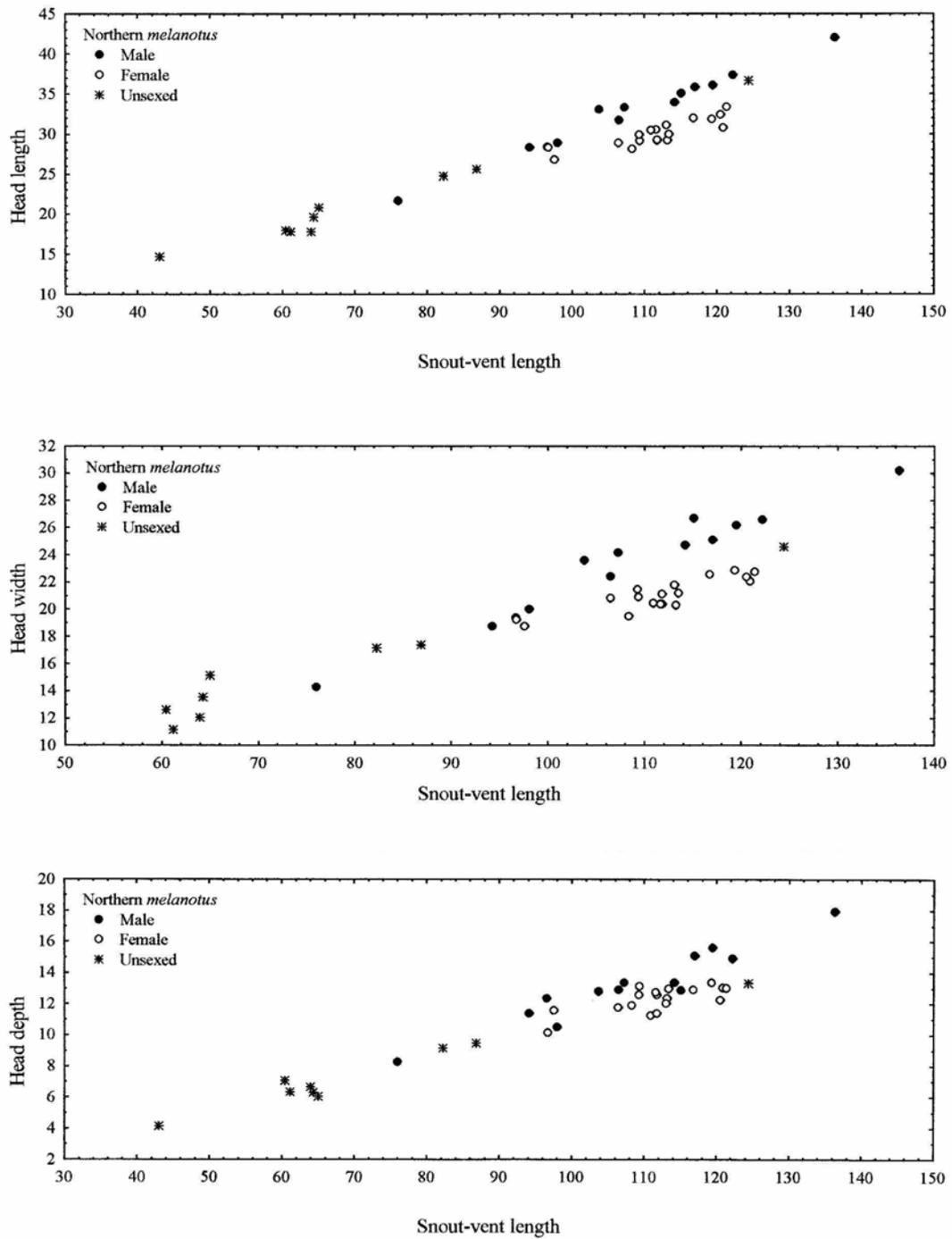
**Figure 5.9** (continued): Histograms showing size (snout-vent length) distribution of *Pseudocordylus* specimens examined by sex and grouping.



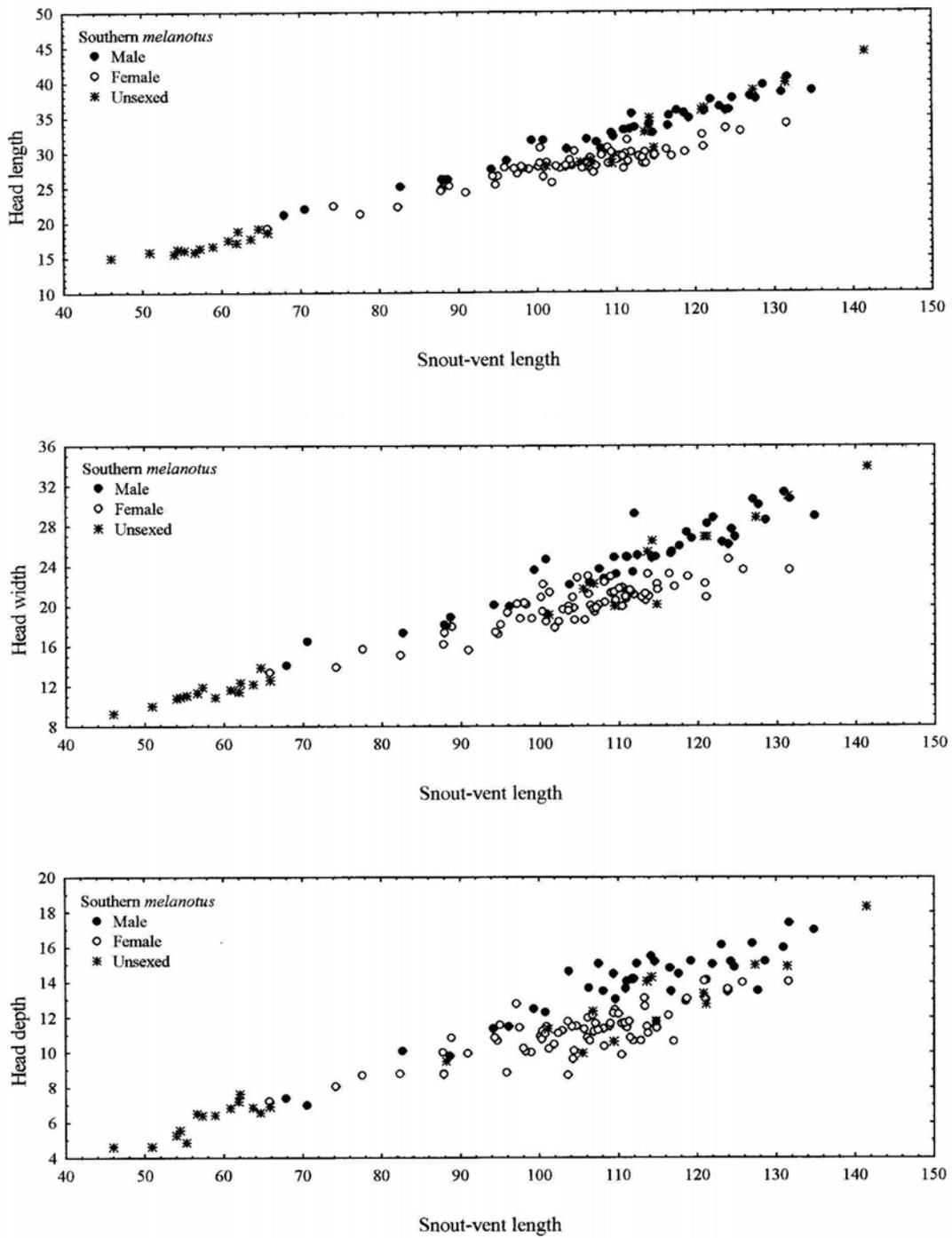
**Figure 5.9** (continued): Histograms showing size (snout-vent length) distribution of *Pseudocordylus* specimens examined by sex and grouping.



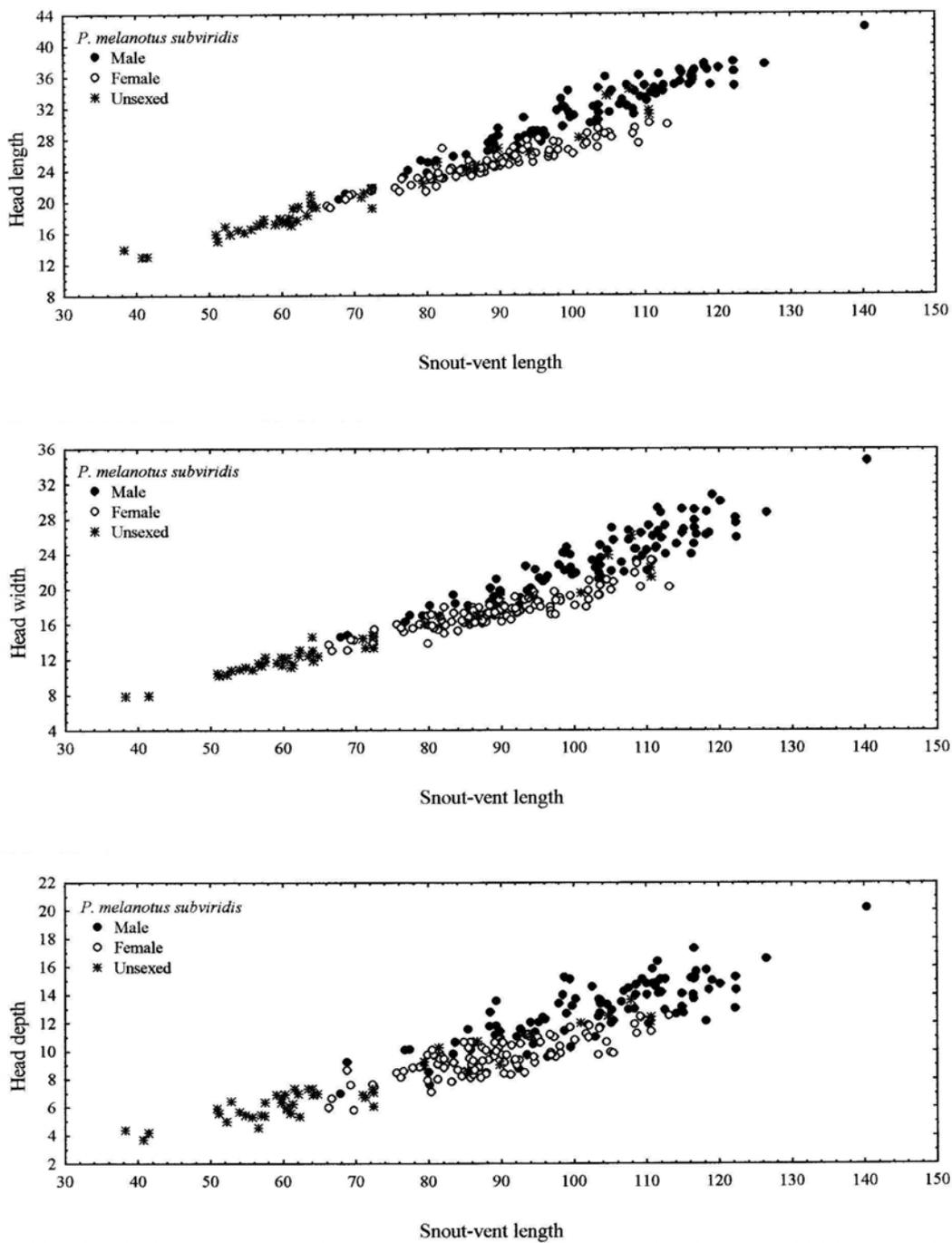
**Figure 5.10:** Head dimensions (length, width, depth) in males, females and unsexed specimens of *Pseudocordylus transvaalensis*.



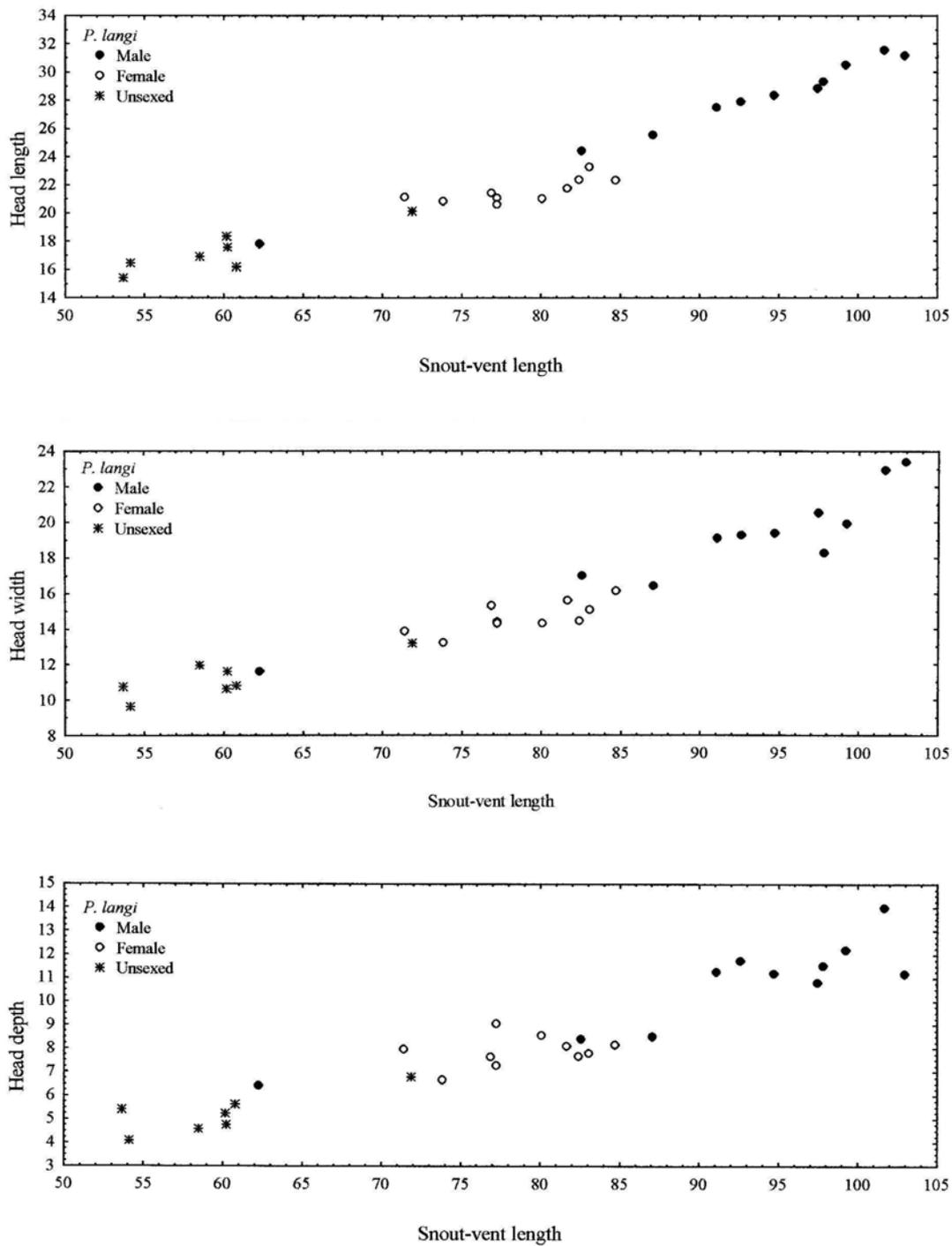
**Figure 5.11:** Head dimensions (length, width, depth) in males, females and unsexed specimens from the northern population of *Pseudocordylus melanotus melanotus* (= “Northern *melanotus*”).



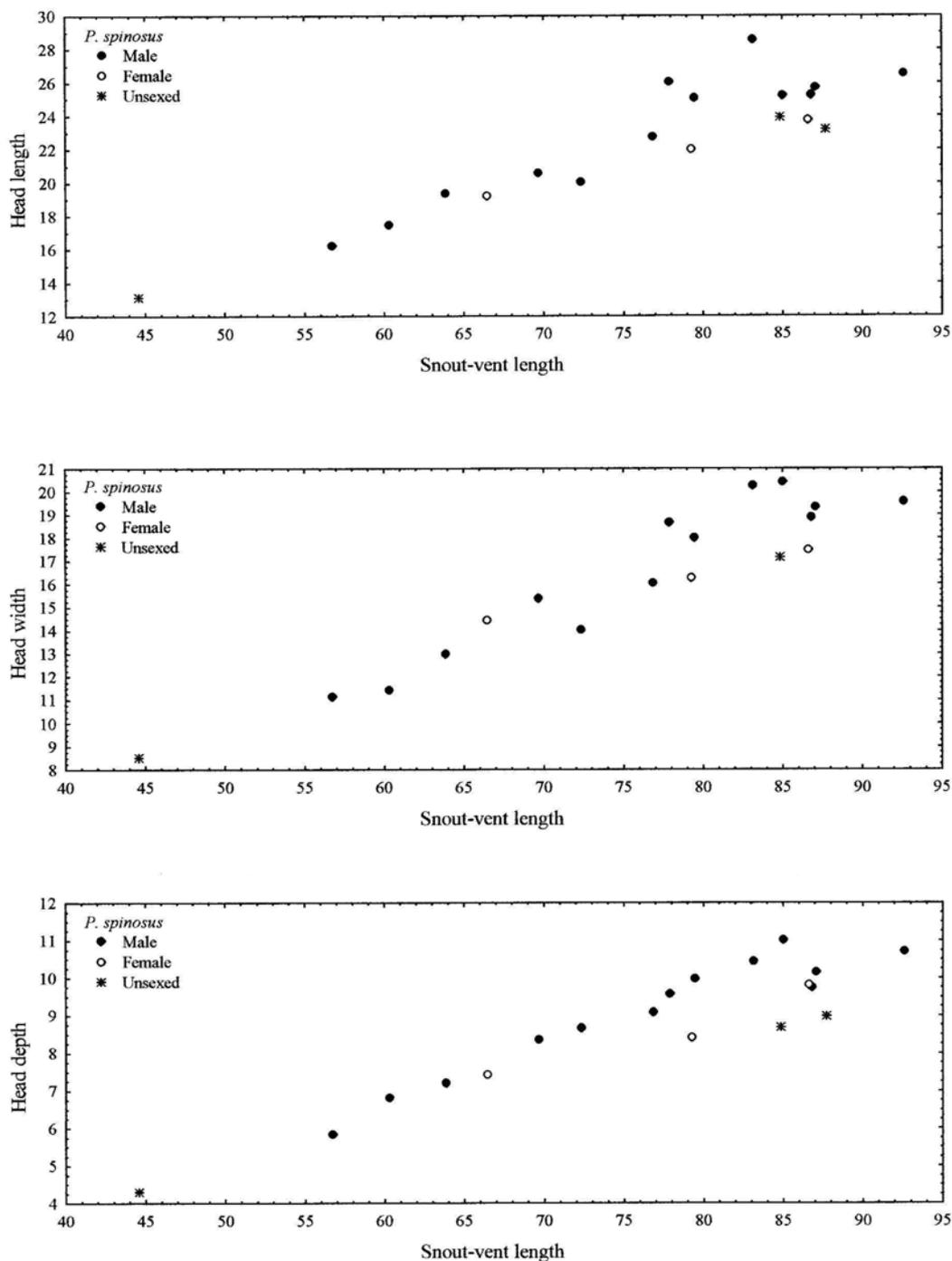
**Figure 5.12:** Head dimensions (length, width, depth) in males, females and unsexed specimens from the southern population of *Pseudocordylus melanotus melanotus* (= “Southern *melanotus*”).



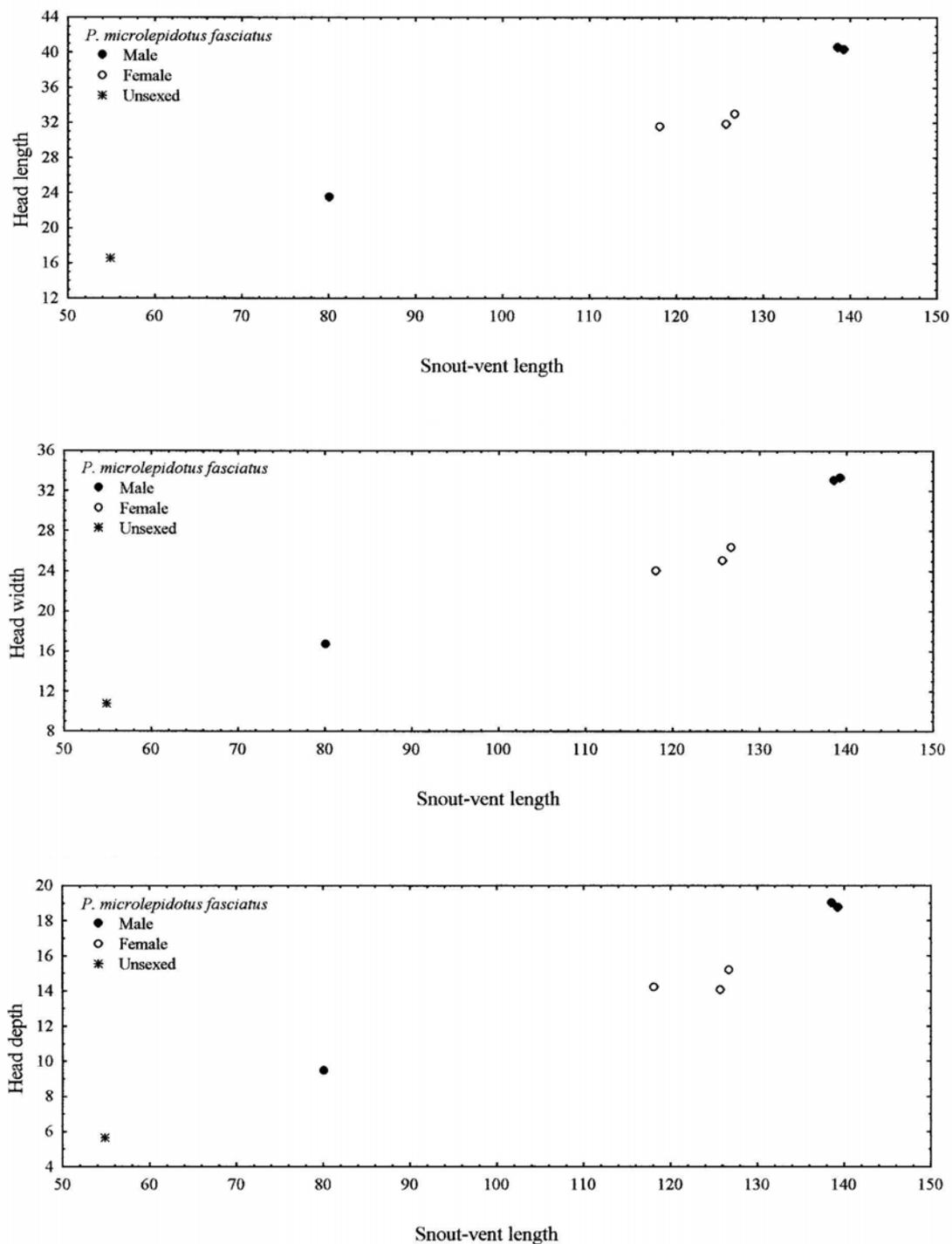
**Figure 5.13:** Head dimensions (length, width, depth) in males, females and unsexed specimens of *Pseudocordylus melanotus subviridis*.



**Figure 5.14:** Head dimensions (length, width, depth) in males, females and unsexed specimens of *Pseudocordylus langi*.



**Figure 5.15:** Head dimensions (length, width, depth) in males, females and unsexed specimens of *Pseudocordylus spinosus*.



**Figure 5.16:** Head dimensions (length, width, depth) in males, females and unsexed specimens of *Pseudocordylus microlepidotus fasciatus*.

### 5.3.1.3 Qualitative characters

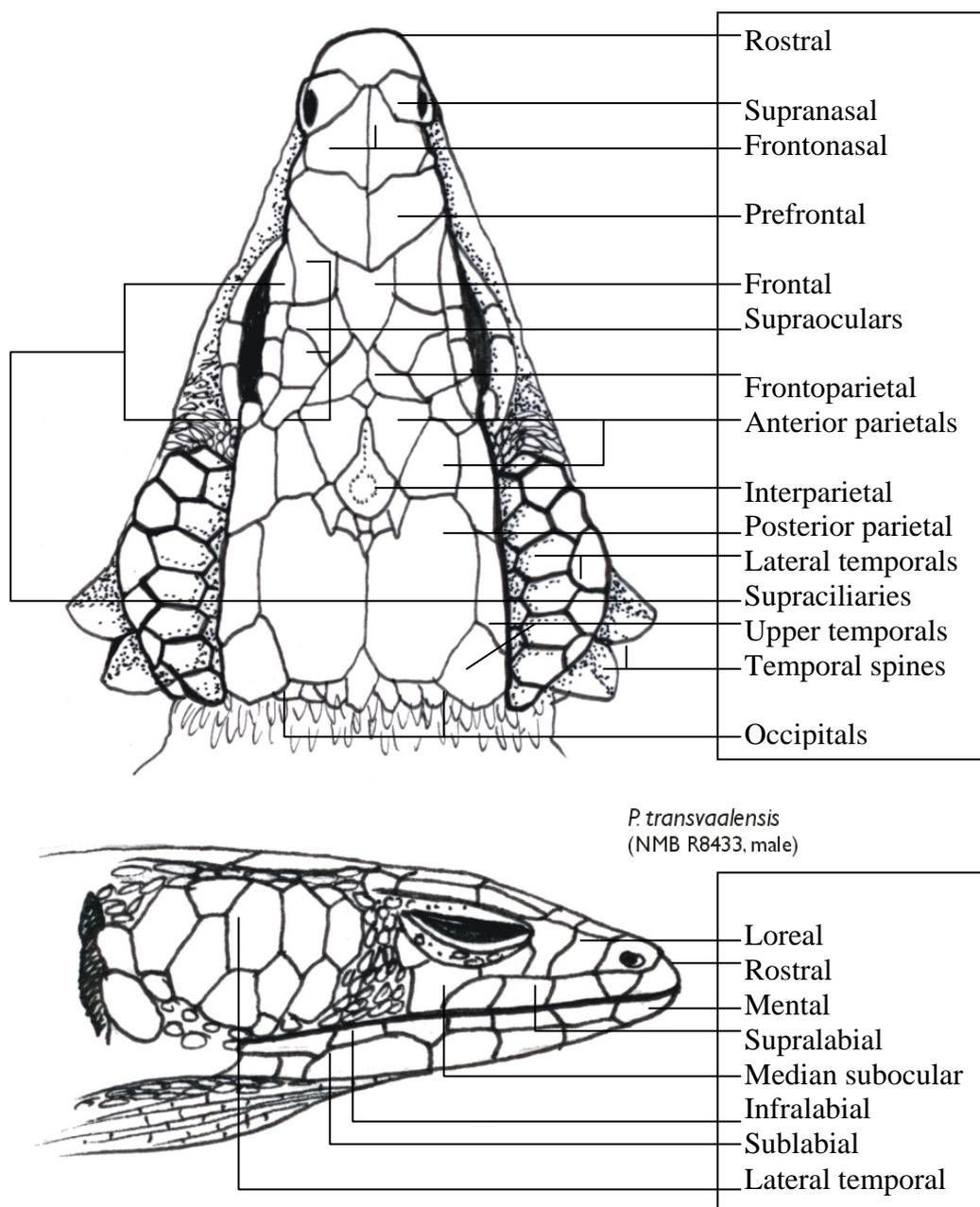
The names and positions of taxonomically important head shields in the *Pseudocordylus melanotus* species complex are shown in Figure 5.17.

#### 1. **Shape of the frontonasal** (width vs length) (Table 5.1; Figs 5.18-5.20):

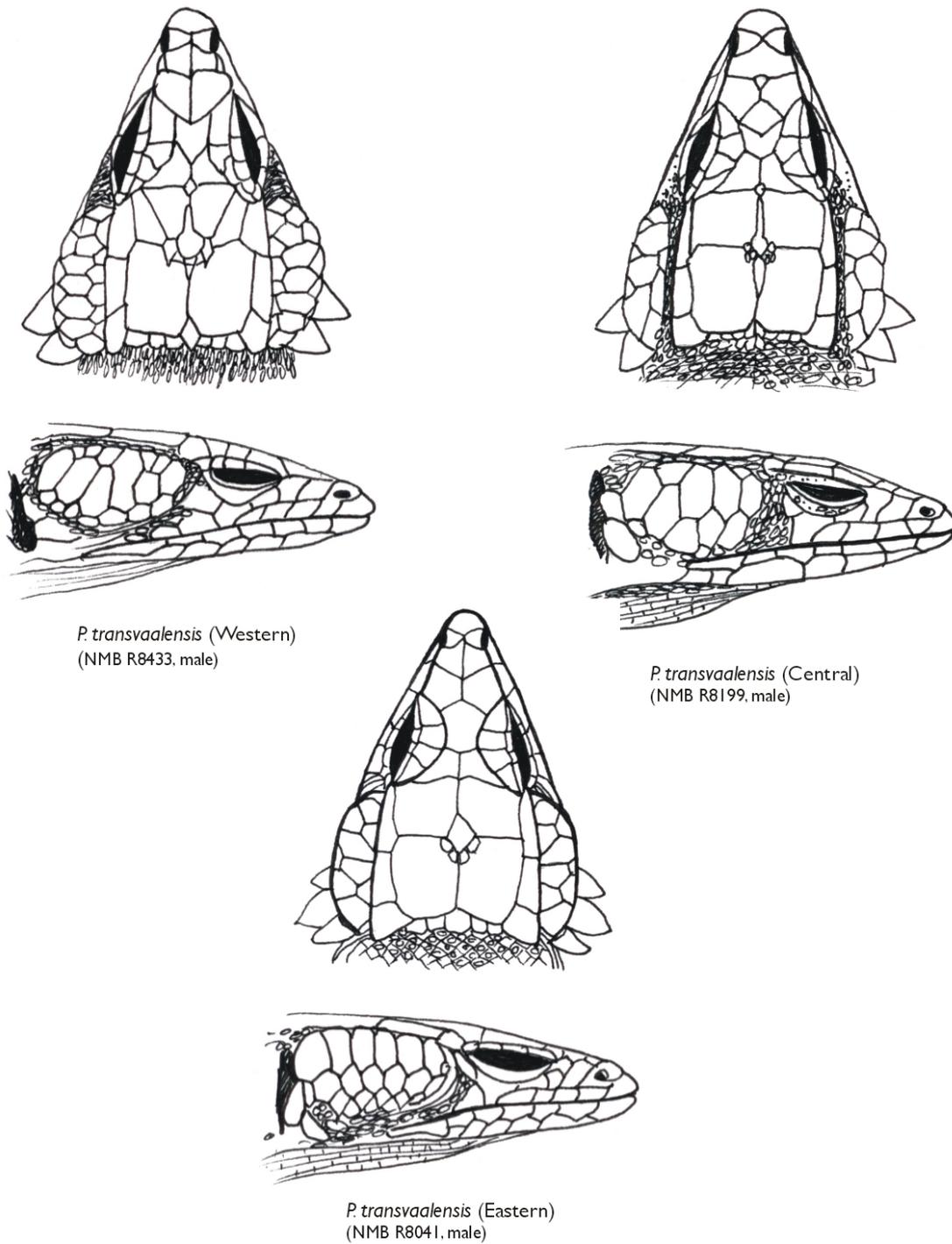
In most populations the frontonasal was wider than long, although it was occasionally as wide as long. However, *P. spinosus* differed in this regard in having a frontonasal that was almost always longer than it was wide (width equal to length in TM 55302; frontonasal absent in TM 50085), whereas it was either wider than long (57%) or equal (43%) in *P. microlepidotus fasciatus*.

#### 2. **Frontonasal divided or not** (Table 5.1; Figs 5.18-5.20):

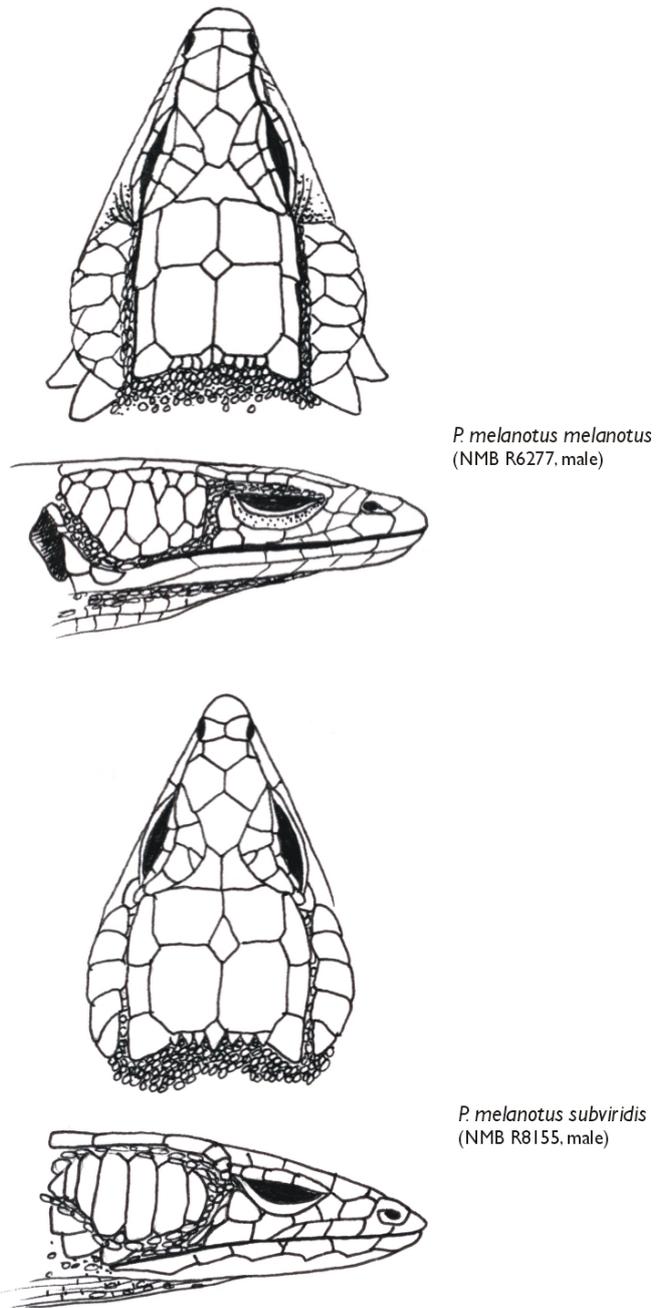
The frontonasal was usually undivided in all populations of Drakensberg *P. m. subviridis* and *P. spinosus* (absent in TM 50085), and always undivided in Amatole populations of *P. m. subviridis*. It was also usually undivided in two out of three *P. langi* populations, although it was partly divided in the single specimen referable to population 47. In most populations of Southern *melanotus* the frontonasal was usually divided, although in population 17, two of the three specimens had only partly divided frontonasals. Partly divided scales occurred frequently in Southern *melanotus*, but were particularly common in *P. m. melanotus* from Nkandhla district. Northern *melanotus* differed from Southern *melanotus* in that the frontonasal was frequently undivided. This was confirmed after examining a large sample ( $N = 272$ ) of *P. m. melanotus* collected north of the Vaal River (Fig. 5.21; see Appendix 2.1 for material examined, but excluding TM 74200, 74202: fragmented frontonasals, and TM 24116, 24106: locality not traced on maps). This may be a case of character displacement as parapatric *P. m. melanotus* and *P. m. subviridis* were usually distinguished by a divided versus undivided frontonasal respectively. In all *P. transvaalensis* populations the frontonasal was usually divided or partly divided, but while specimens from the Western region almost always had divided frontonasals, those from the Eastern region often had either divided or undivided frontonasals, and in the Central region there was an almost equal occurrence of divided, partly divided and undivided scales (Fig. 5.22).



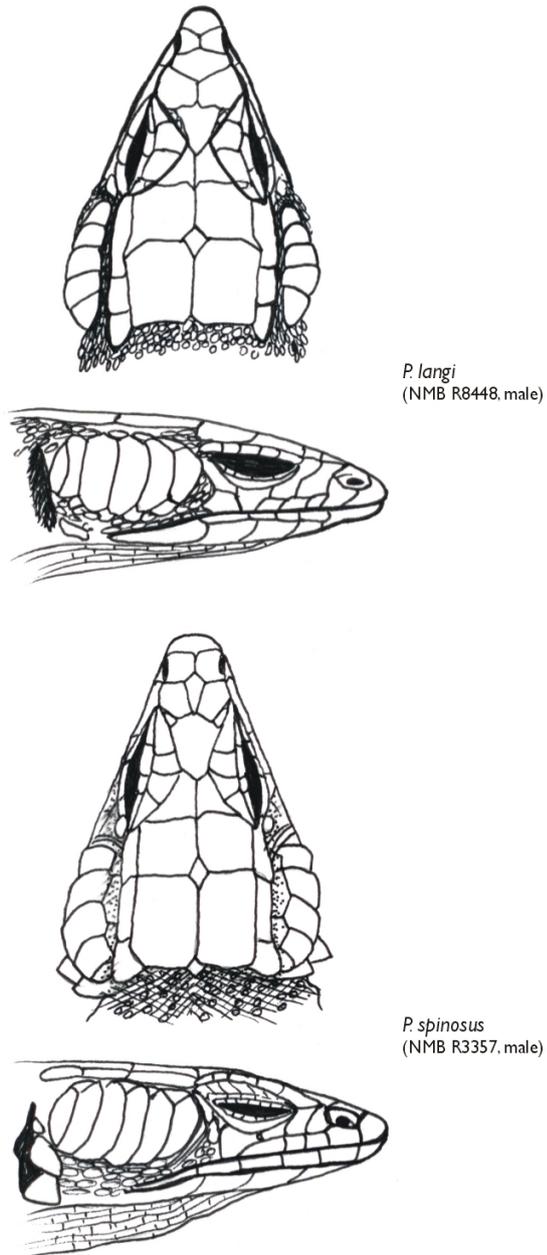
**Figure 5.17:** Scapulation of the dorsal and lateral aspects of the head of a representative of the *Pseudocordylus melanotus* species complex (*P. transvaalensis*, NMB R8433, male).



**Figure 5.18:** Scallation of the dorsal and lateral aspects of the head of *Pseudocordylus transvaalensis* from three populations (Western, Central, Eastern).

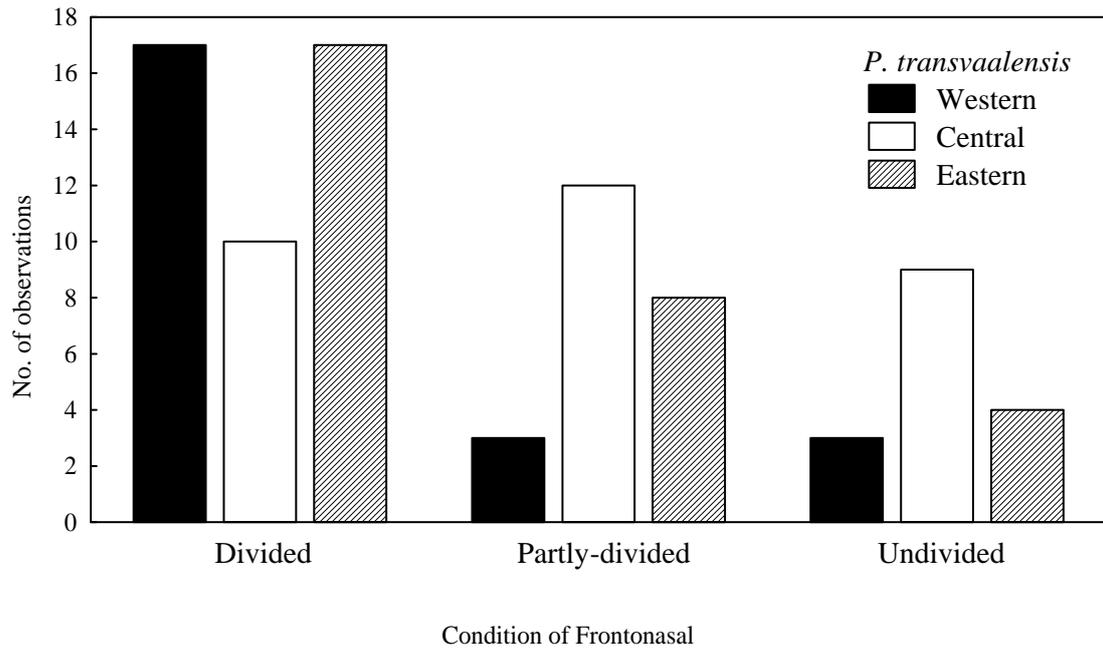


**Figure 5.19:** Scalation of the dorsal and lateral aspects of the head of *Pseudocordylus melanotus melanotus* and *P. melanotus subviridis*.



**Figure 5.20:** Sculation of the dorsal and lateral aspects of the head of *Pseudocordylus langi* and *P. spinosus*.





**Figure 5.22:** Condition of the frontonasal in three allopatric populations of *Pseudocordylus transvaalensis*.

**3. Scale behind frontonasal** (Table 5.1; Figs 5.18-5.20):

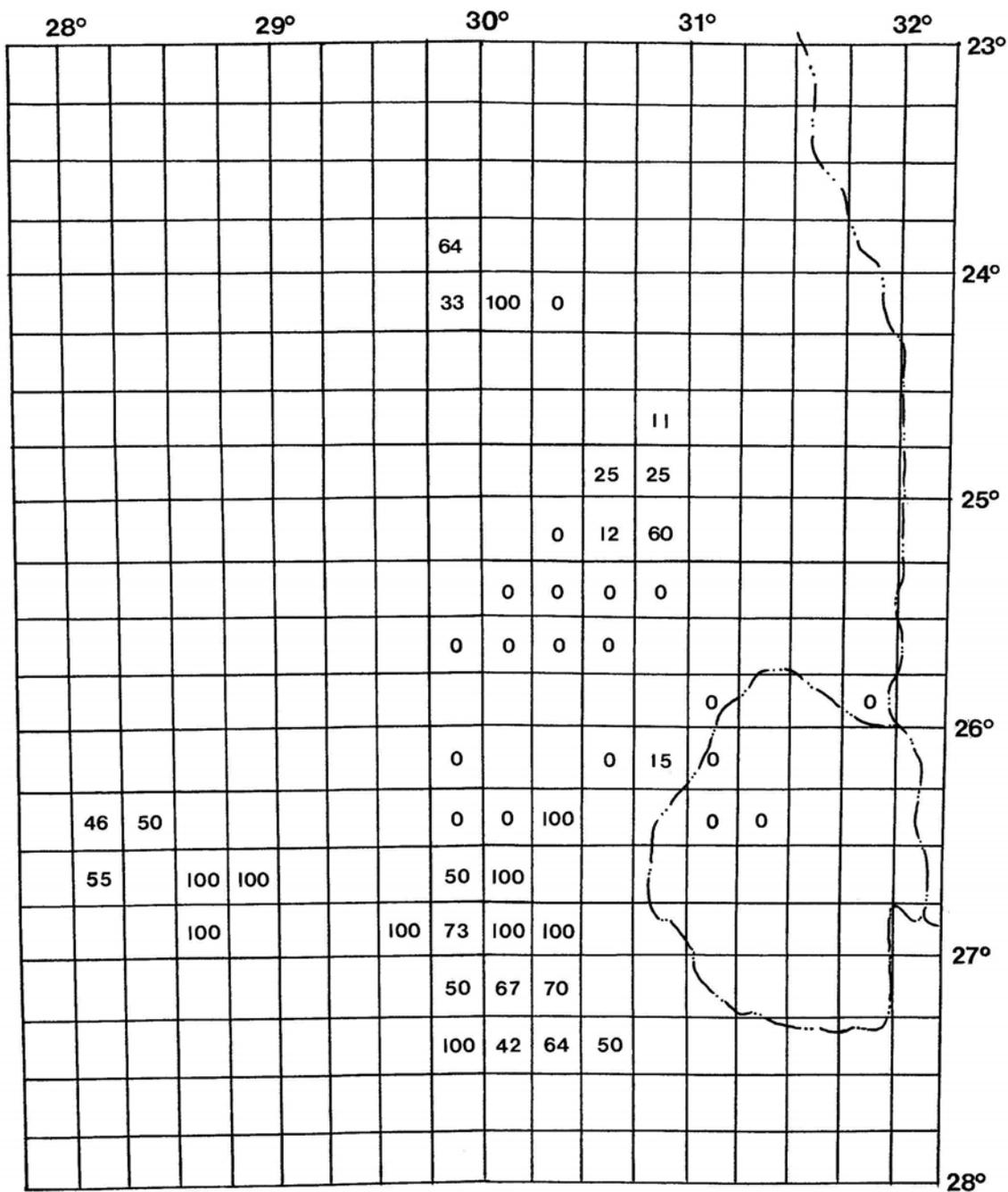
A small scale posterior to the frontonasal was fairly common in most populations of *transvaalensis* and *melanotus*, but infrequent in all *subviridis*, *langi* and *spinosus*, and absent in *microlepidotus fasciatus*. A detailed evaluation of this character in a large sample of *melanotus* from north of the Vaal River (Gauteng, Mpumalanga and Limpopo provinces) showed that while a scale behind the frontonasal was common in southern areas (Gauteng, southern Mpumalanga), it occurred infrequently in the north, with the exception of the Sabie area (60% presence) (Fig. 5.23).

**4. Frontonasal separates supranasals** (Table 5.1; Figs 5.18-5.20):

In most populations the supranasals were usually in contact. However, in the majority of specimens from populations 1-5 (*transvaalensis*) the frontonasal was in contact with the frontal, separating the supranasals. This was also the condition in high percentages of samples of Northern *melanotus* (populations 11, 13-15). The supranasals were usually separated in Western and Central *transvaalensis*, but usually in contact in Eastern *transvaalensis* (Fig. 5.24).

**5. Proximity of the frontonasal to the loreals** (Table 5.1; Figs 5.18-5.20):

The lateral extensions of the frontonasal were almost always in contact with the loreals on either side of the head in all populations except *spinosus* (populations 49-51; frontonasal absent in TM 50085) and population 39 of *subviridis* (separated in four out of five specimens). In the latter populations the frontonasal was excluded from the loreal on either side of the head by a supranasal and prefrontal. This also applied to a few specimens in other populations. The frequency of exclusion was highest in the south-eastern part of the range of *subviridis*.



**Figure 5.23:** Frequencies at which a small to moderate sized scale is present posterior to the frontonasal in *Pseudocordylus melanotus melanotus* from Limpopo, Mpumalanga and Gauteng provinces, and Swaziland; and the Eastern population of *P. transvaalensis* in Limpopo Province (2329DD, 2429BB, 2430AA). Details of localities and specimens examined are provided in Appendix 2.1.

**6. Anterior frontal present or absent** (Table 5.1; Figs 5.18-5.20):

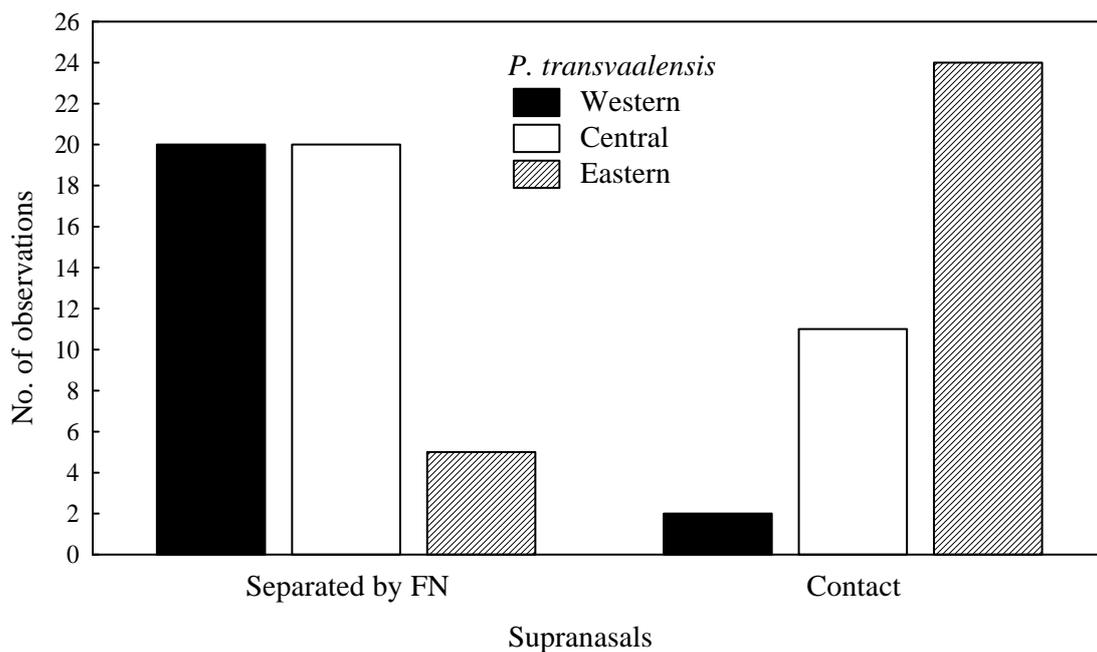
In most populations of *transvaalensis* a medium to large scale may be present anterior to the frontal (“anterior frontal”). Such a scale was absent in the *microlepidotus fasciatus* sample and all other populations in the *P. melanotus* species complex with the exception of population 14 (33% frequency) referable to Southern *melanotus*.

**7. Anterior parietals entire or divided** (Table 5.1; Figs 5.18-5.20):

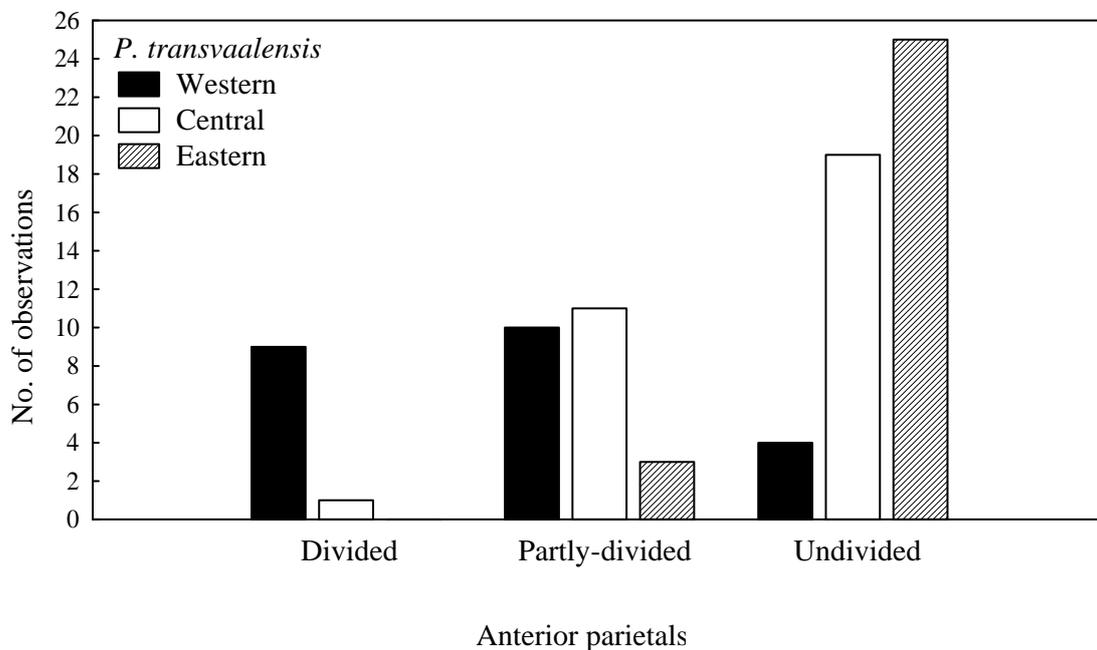
The anterior parietals were often divided or partly divided – in the way indicated in Fig. 5.17 - in populations 1-6 of *transvaalensis*. Western *transvaalensis* (populations 1-2) usually had the parietals either divided or at least partly divided; Central *transvaalensis* (populations 3-6) often had undivided anterior parietals, although a large proportion of the sample had partly-divided scales; whereas Eastern *transvaalensis* (populations 7-8) almost always had undivided parietals (Fig. 5.25). The other populations in the complex almost always had the anterior (and posterior) parietals undivided. Only a few exceptions occurred: one out of 20 specimens from population 21 had divided anterior parietals, while one specimen each from populations 14 ( $N = 9$ ) and 32 ( $N = 20$ ) had partly divided scales. The anterior parietals were always undivided in the *microlepidotus fasciatus* sample.

**8. Texture of posterior infralabial** (Table 5.1):

The posterior infralabial on either side of the head was smooth in all *langi* except one of the eight specimens from population 46 that had ridged (weekly keeled) scales. All *microlepidotus fasciatus* examined, and almost all other specimens in the *P. melanotus* species complex, had either distinctly keeled or ridged posterior infralabials. The exceptions were: one specimen from population 8 ( $N = 7$ ), one from population 11 ( $N = 23$ ), one from population 30 ( $N = 40$ ), and one from population 31 ( $N = 8$ ), all of which had smooth scales.



**Figure 5.24:** Proximity of the supranasals in three allopatric populations of *Pseudocordylus transvaalensis*.



**Figure 5.25:** Condition of the anterior parietals in three allopatric populations of *Pseudocordylus transvaalensis*.

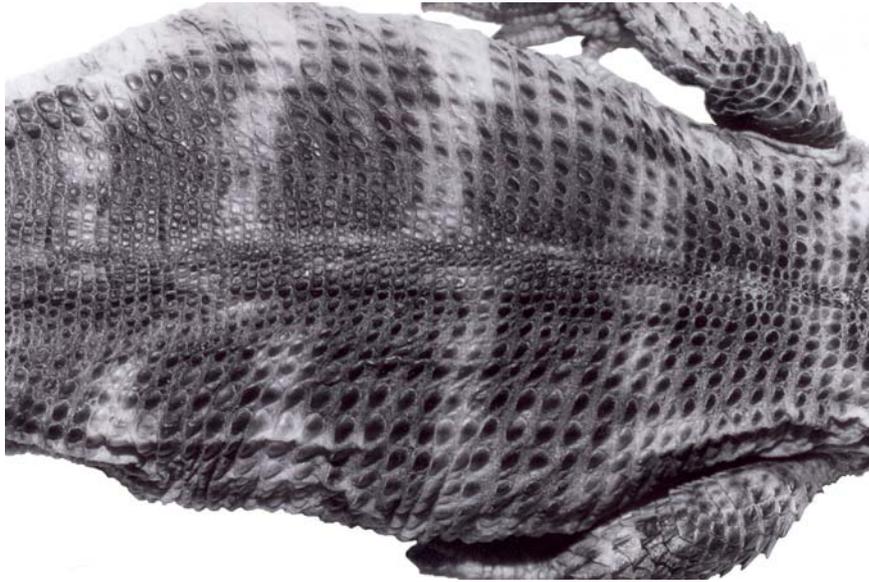
**9. Size of median dorsals in relation to dorsolaterals** (Table 5.1; Fig. 5.26):

There was a fixed difference between *langi* and all other populations and groupings with regard to the relative size of the median dorsal scales. The median scales were larger than the (granular) dorsolaterals in *langi*, whereas the dorsolaterals were always larger in other populations.

**10. Size of granular interspaces between longitudinal rows of dorsolaterals** (Table 5.1; Fig. 5.26):

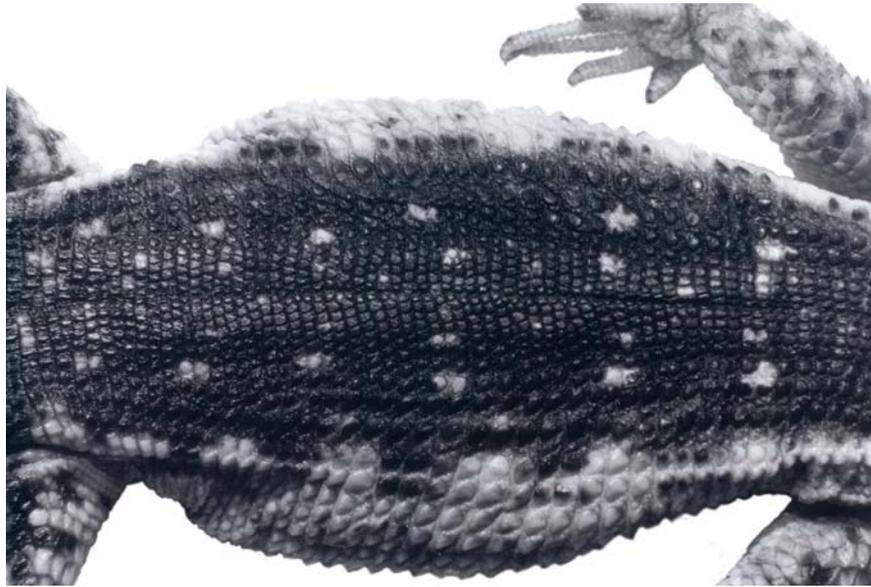
In *langi* the dorsolaterals were granular and in contact, whereas in *spinosus* they were enlarged and keeled, and either in contact or very closely spaced (spaces less than one-quarter the size of average adjacent dorsolaterals). Specimens of *microlepidotus fasciatus* all had closely spaced rows of dorsolaterals (spaces < 0.5 size of adjacent dorsolaterals). All populations of *transvaalensis* had mostly closely spaced dorsolaterals (spaces  $\leq$  0.5 size of adjacent dorsolaterals) and this was also the case with most populations of *melanotus*, the exceptions being population 11 that had 74%, and population 22 that had 56%, with spaces between dorsolateral scale rows greater than half the size of adjacent scales. In Drakensberg *subviridis* the majority of specimens in most populations had widely separated dorsolaterals - spaces equal to or larger than adjacent scales or at least larger than half the size of adjacent scales. However, there were a few exceptions in the south-eastern part of the range (populations 36-38; Fig. 5.27). While populations of *subviridis* in high altitude areas tended to have widely-spaced rows, populations at lower elevations, at least in this area, almost always had the longitudinal rows of dorsals arranged in typically *melanotus*-like fashion. This may be likened to character displacement as the two taxa do not come into contact in this region as in Qwa-Qwa. Amatole *subviridis* (population 44) also had mostly closely-set dorsolaterals (spaces  $\leq$  0.5 size of adjacent dorsolaterals).

**A**

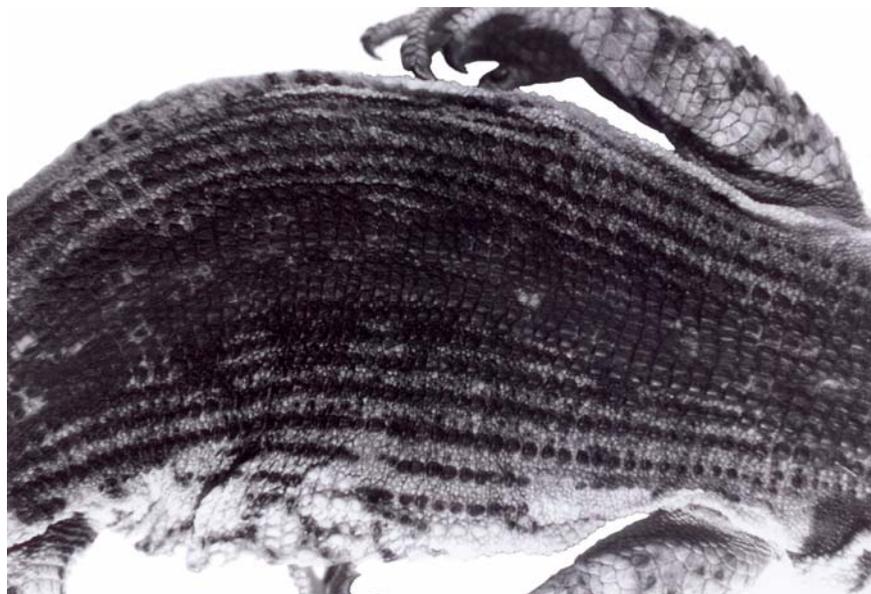


**Figure 5.26:** Arrangement of dorsal scales in the *Pseudocordylus melanotus* species complex. **A:** *Pseudocordylus transvaalensis* (NMB R8442, female: Farm Hartbeestfontein, Thabazimbi district, Limpopo Province).

**B**

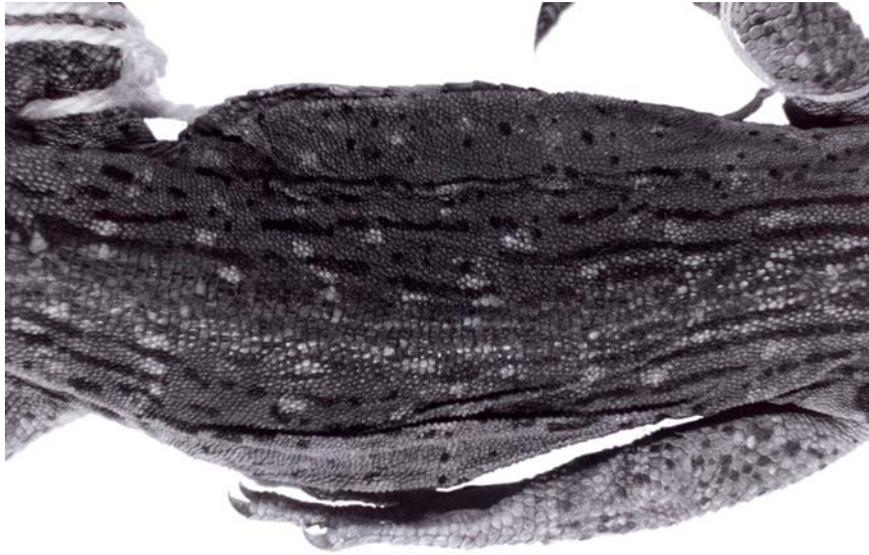


**C**

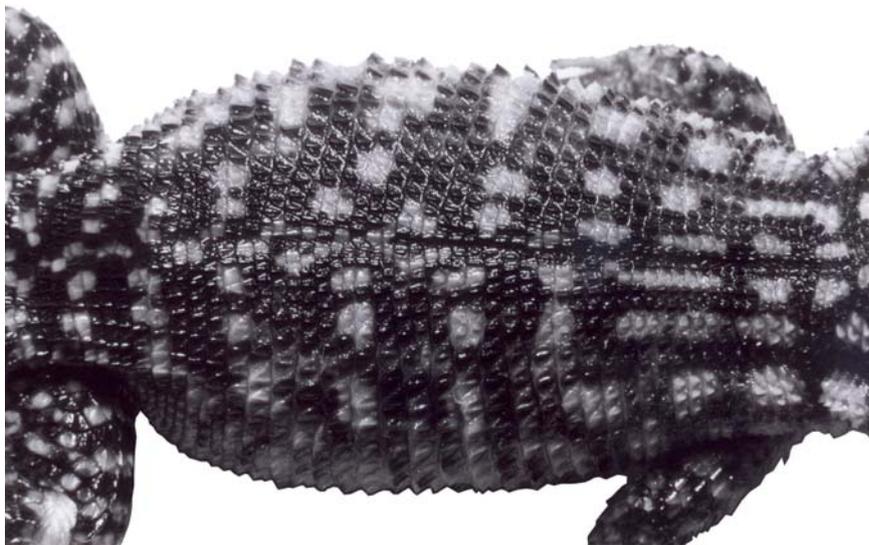


**Figure 5.26** (continued): Arrangement of dorsal scales in the *Pseudocordylus melanotus* species complex. B: *Pseudocordylus melanotus melanotus* (NMB R8184, male: Farm Uyshoek, Harrismith district, Free State), C: *Pseudocordylus melanotus subviridis* (NMB R8154, male: Organ Pipes Pass, KwaZulu-Natal).

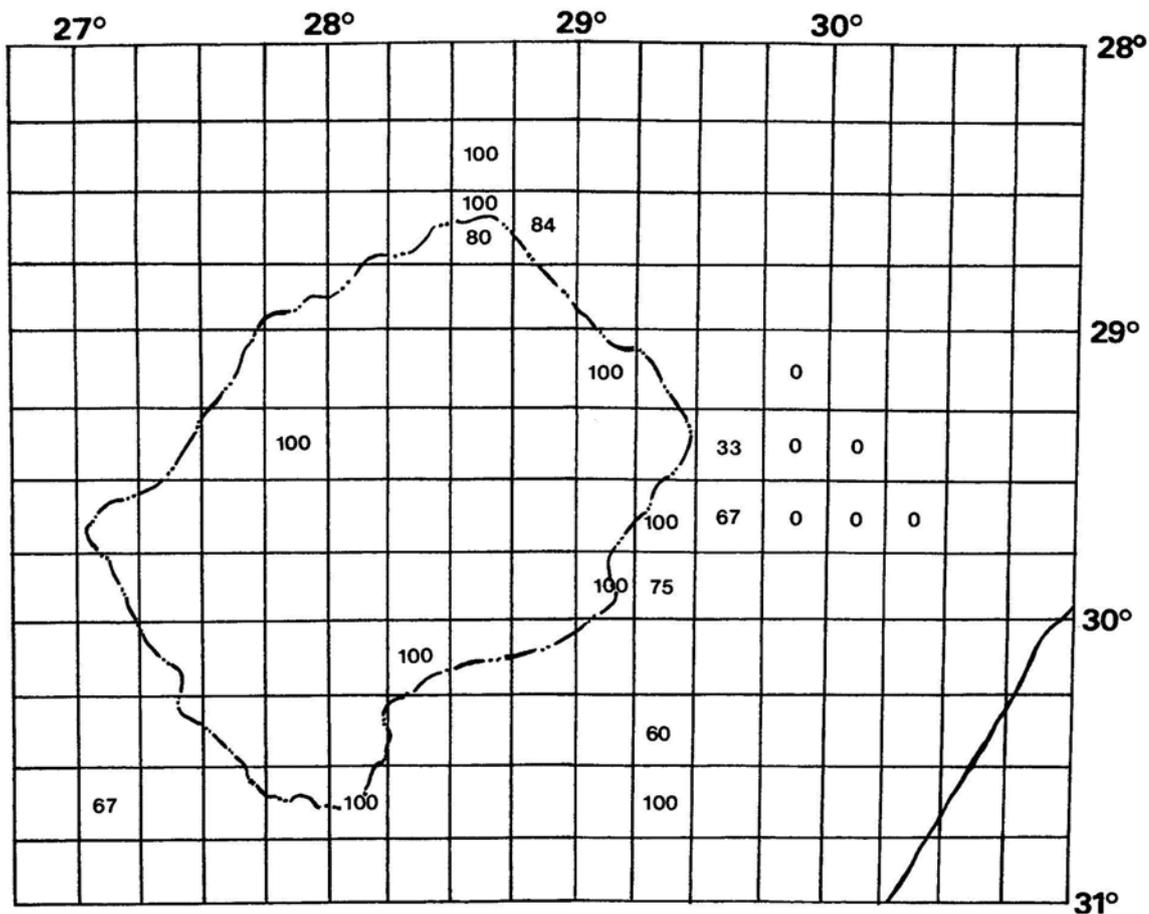
**D**



**E**



**Figure 5.26** (continued): Arrangement of dorsal scales in the *Pseudocordylus melanotus* species complex. D: *Pseudocordylus langi* (NMB R8448, male: Organ Pipes Pass, KwaZulu-Natal), E: *Pseudocordylus spinosus* (NMB R3357, male: “Sentinel”, Free State).



**Figure 5.27:** Frequencies at which the longitudinal rows of dorsolaterals are widely spaced (spaces >0.5 size of adjacent dorsolaterals) in *Pseudocordulus melanotus subviridis* from the Drakensberg and Lesotho. The frequency for the Monontsa Pass population (80%) is shown separately from others in unit 2828DA. Details of localities and specimens examined are provided in Appendix 2.1.

**11. Spinosity of lateral dorsal scales** (Table 5.1):

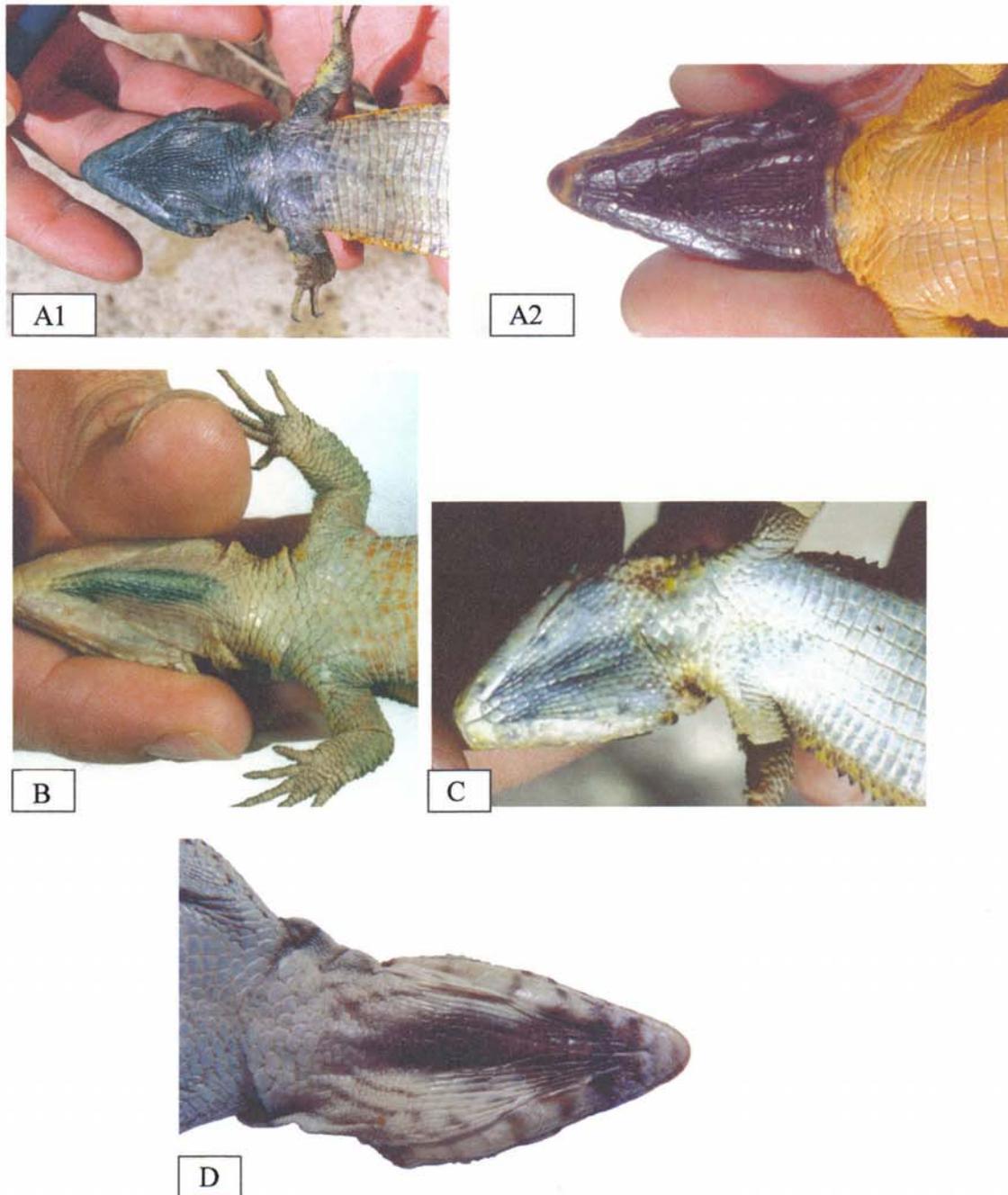
In *spinosus* the lateral dorsal scales were usually distinctly spinose. The only exception was NMB-RY-R125 - the second smallest specimen examined (SVL = 57 mm) - which had what appeared to be non-spinose laterals. Although this character may be affected by age, the smallest specimen of *spinosus* (NMB R8571, SVL = 44.6 mm) had spinose laterals. All other specimens in the complex, as well as the sample of *microlepidotus fasciatus*, had non-spinose laterals. There was considerable variation in the texture of the lateral scales, especially in Drakensberg *subviridis* (e.g. distinctly keeled, weakly keeled, smooth). In the case of *langi* all but the median (paravertebral) dorsals were granular, whereas in *transvaalensis* and some populations of *melanotus*, the laterals were usually largely or completely smooth.

**12. Femoral pores in females** (Table 5.1):

Femoral pores in all females from populations 1-28 (*transvaalensis* and *melanotus*) were small, shallow, pit-like and lacked secretions. Moderate to large, distinct, deep pores with yellow-brown secretory plugs were the norm for females in populations of *subviridis* and occurred invariably in *langi* and *spinosus*. Two of the three female *microlepidotus fasciatus* also had similar pores. In a few populations of *subviridis* there were high percentages of pit-like pores: 57% in population 34, 67% in population 35 (both Drakensberg *subviridis*) and 45% in population 44 (Amatole *subviridis*).

**13. Colour pattern on the throat** (Table 5.1; Fig. 5.28):

In *transvaalensis* the throat was always black or dark grey. This colouration often extended onto the lower labials as well as the chest and sometimes also further down on the belly. However, black throats did also occur occasionally in Northern *melanotus* as well as population 24 of Southern *melanotus*, but in such cases the chest and belly were not black or grey. In *langi* the pale throat was marked by a dark median stripe that expanded into a ball-like shape anteriorly. In all other populations the gular pattern normally consisted of a pair of dark median longitudinal stripes on a pale background. In *melanotus* and *subviridis* these stripes were thick-set and the anterior end of each was shaped like a half arrow-head, whereas in *spinosus* and *microlepidotus fasciatus* the stripes were thin and lacked arrow-head-like ends. For all groups there were often also dark spots or streaks on the throat, but these did not change the overall appearance as described above.



**Figure 5.28:** The four classes of gular colour pattern in the *Pseudocordylus melanotus* complex:- A: black - A1 (*P. transvaalensis*, NMB R8550, male, Monte Christo), A2 (*P. melanotus melanotus*, NMB R8276, male, Lochiel); B: paired stripes with arrow-head-like anterior ends (*P. melanotus melanotus*, NMB R8382, male, Ntayabesutu); C: narrow, paired stripes lacking arrow-head-like anterior ends (*P. spinosus*, NMB R8569, male, Goodoo Pass); D: arrow-shaped marking (*P. langi*, NMB R8448, male, Organ Pipes Pass).

#### 5.3.1.4 Meristic characters

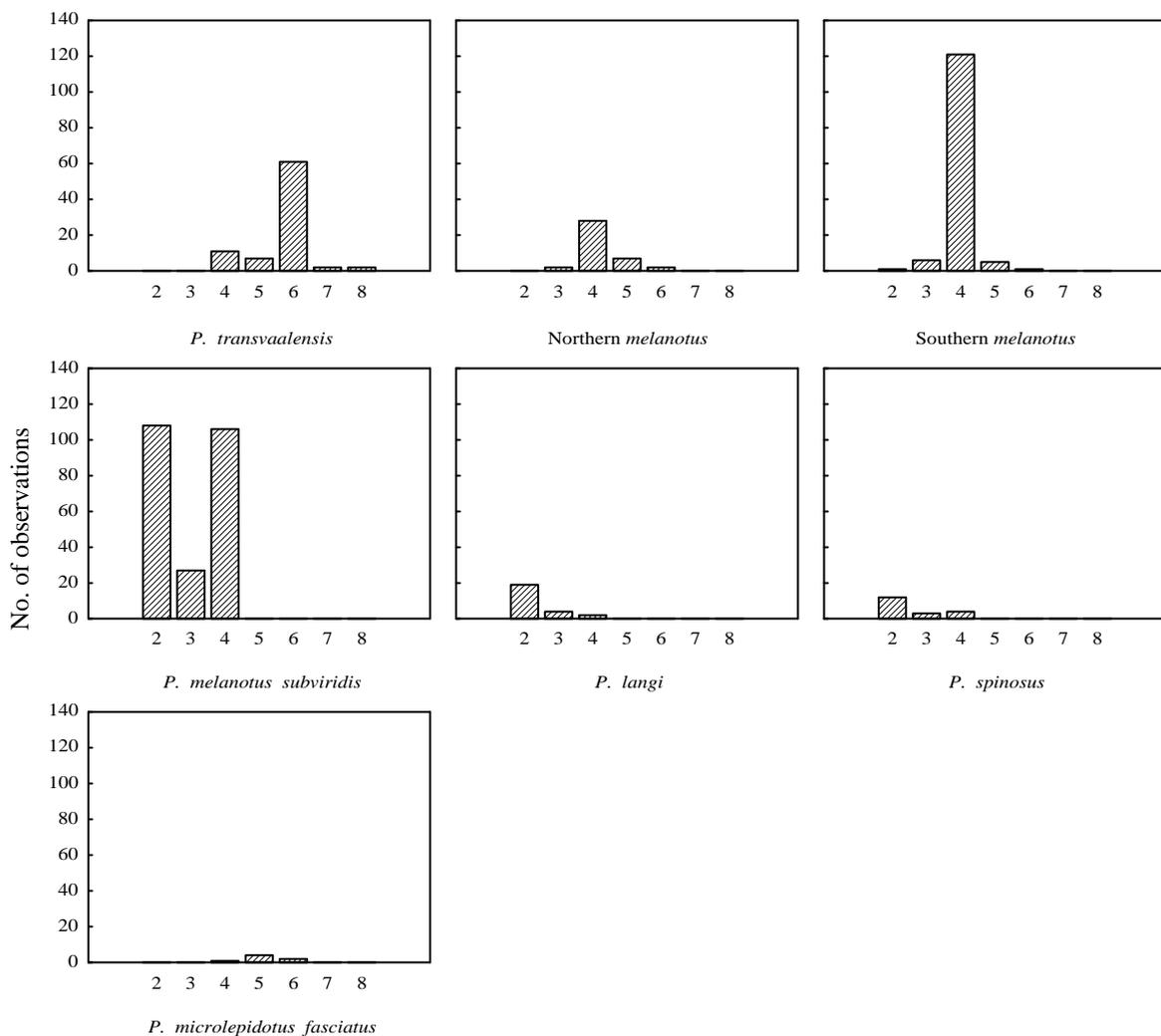
##### 1. **Horizontal rows of lateral temporals** (Table 5.2, Figs 5.18-5.20, 5.29-5.30):

While most populations had 2-4 rows of lateral temporals, counts as high as 6-8 occurred in all *P. transvaalensis* populations (1-8). The mode was 6 for all three populations of *P. transvaalensis*. In *P. microlepidotus fasciatus* there were 4-6 rows, which was also the case for most Northern *melanotus*. However, in both *P. langi* and *P. spinosus* there were usually only two rows (one on either side of the head). In Drakensberg *P. m. subviridis* the mode was also two, but an almost equally large proportion of specimens had four rows (usually two on each side). Amatole *P. m. subviridis* usually had four rows, as was usually the case in all four *P. melanotus* groupings.

When a single row was present the scales were greatly elongated; when two rows were present, the scales of the upper row were elongated, while those of the lower row were square to hexagonal or only slightly elongated; and when three or four rows were present, the scales of the upper row were slightly elongated and those of each lower row were mostly progressively less elongate until the lowest row which had square to round scales (Fig. 5.30).

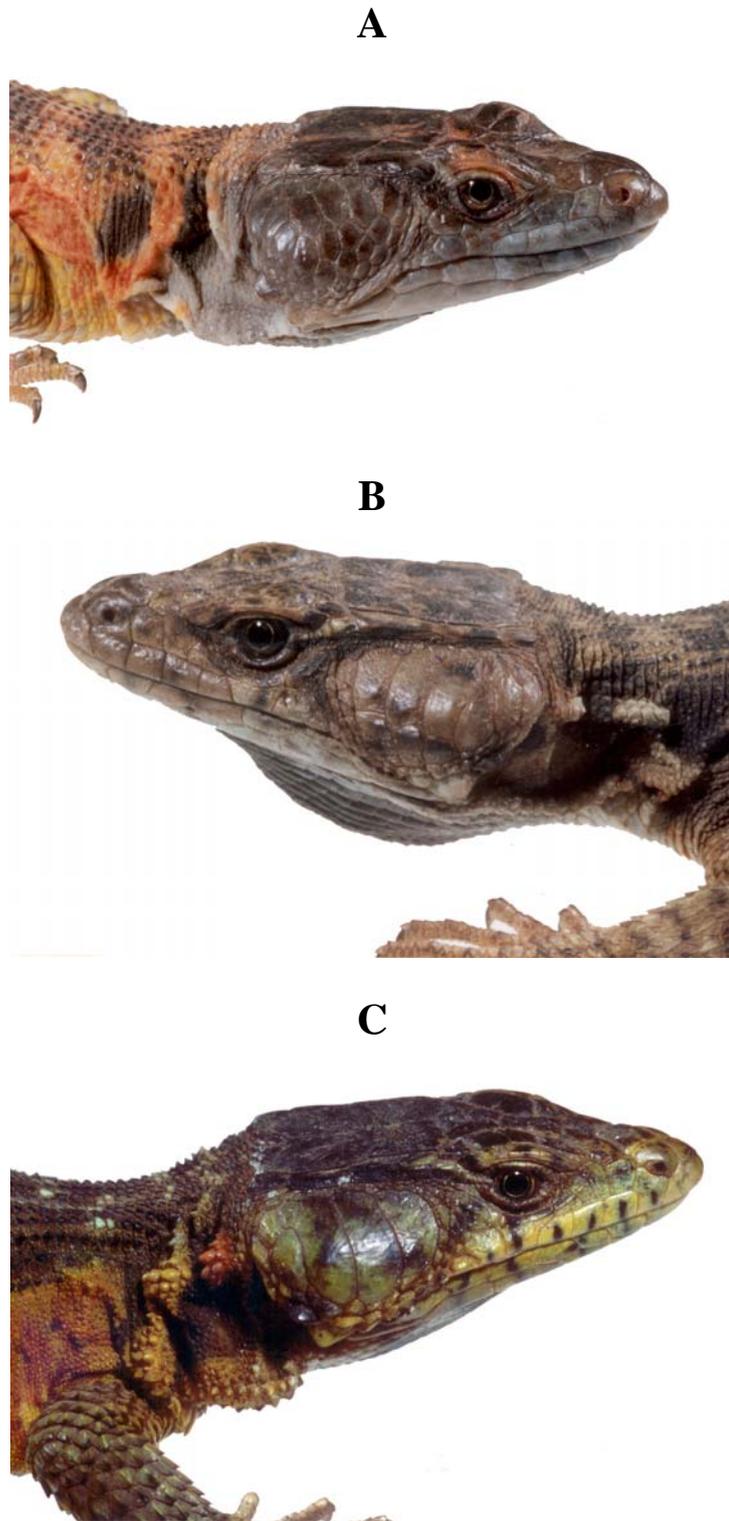
##### 2. **Suboculars posterior to the median subocular** (Table 5.2; Fig. 5.31):

In most populations and groupings in the *P. melanotus* species complex there were usually two suboculars posterior to the median (one on either side of the head), but in *transvaalensis* the mode was four (range 2-6), usually two scales on either side of the head. However, while all *langi* populations usually had two (one on either side) and only occasionally four (two on either side) suboculars posterior to the median, one specimen (NHMZ 2419) had six (four on the left side, two on the right). In *microlepidotus fasciatus* there were 3-4 suboculars posterior to the median.

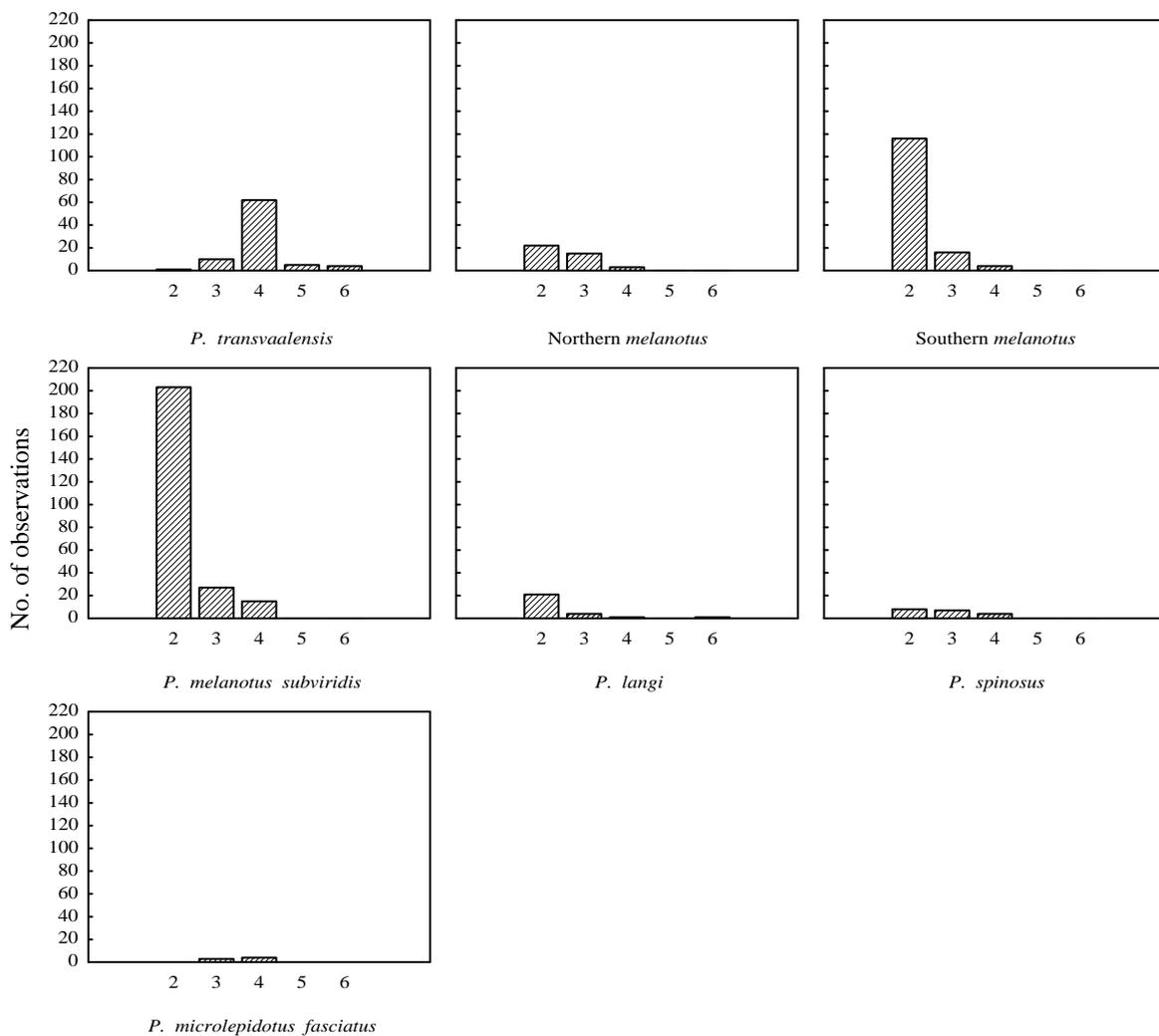


Horizontal rows lateral temporals

**Figure 5.29:** Number of horizontal rows of lateral temporals in the *Pseudocordylus melanotus* species complex.



**Figure 5.30:** Lateral aspects of the head illustrating three classes of lateral temporal scale arrangement in the *Pseudocordylus melanotus* species complex: A: three horizontal rows (*P. transvaalensis*, NMB R8434, male, Hartbeestfontein); B: two rows (*P. melanotus subviridis*, NMB R8363, male, Qoqolosing); C: one row (*P. melanotus subviridis*, NMB R8309, male, Naude's Nek).



Suboculars posterior to median

**Figure 5.31:** Number of suboculars posterior to the median subocular in the *Pseudocordylus melanotus* species complex.

3. **Infralabials** (Table 5.2; Fig. 5.32):

*Pseudocordylus langi* differed from the others in that it usually had only 10 infralabials (five on either side of the head), rather than 12 (six on either side). Only three specimens of *langi* differed, having 11 infralabials (five on one side of the head and six on the other). Only one specimen of *spinosus* had 10 infralabials (five per side) and another had 14 (six on left side, eight on right), the rest having 12 or 13. Infralabial counts were often higher than 12 (13-14, 17) in *P. transvaalensis*.

4. **Sublabials** (Table 5.2):

Specimens from most populations had 10 sublabials, although this varied from 9 to 11 (12 in one specimen from population 14). However, in *transvaalensis* (populations 1-8) it varied from 10 to 14 (mean per population varied from 10.0 to 11.2, but was higher in populations 7 [11.6] and 8 [11.9]). The Western and Central populations of *transvaalensis* had modes of 10, with high proportions of specimens with 11 and 12 sublabials, but the Eastern population (populations 7-8) usually had 12 sublabials, although 11 was also common (Fig. 5.33).

5. **Gular scales between posterior sublabials** (Table 5.2):

There was considerable variation in this count, even within populations (*e.g.* populations 1, 30, 46, 48). However, *spinosus* tended to have relatively low counts (20-26) and was almost always distinguishable from at least *transvaalensis* (25-41) and *langi* (25-45).

6. **Small scales posterior to the interparietal** (Table 5.2; Figs 5.18-5.20):

In populations 1-8 (*transvaalensis*) there were 2-12 small scales posterior to the interparietal (Fig. 5.34). Specimens from other populations usually lacked scales in this region, but occasionally up to four such scales were present in populations of *melanotus* and *subviridis*. The mean did not exceed one in any of these populations (9-52) and was 0.4 or less in all groupings except *transvaalensis*. These scales were always absent in Amatole populations of *subviridis* as well as all *spinosus* and were present in only one specimen of *microlepidotus fasciatus* ( $N = 7$ ) and four *langi* ( $N = 28$ ).

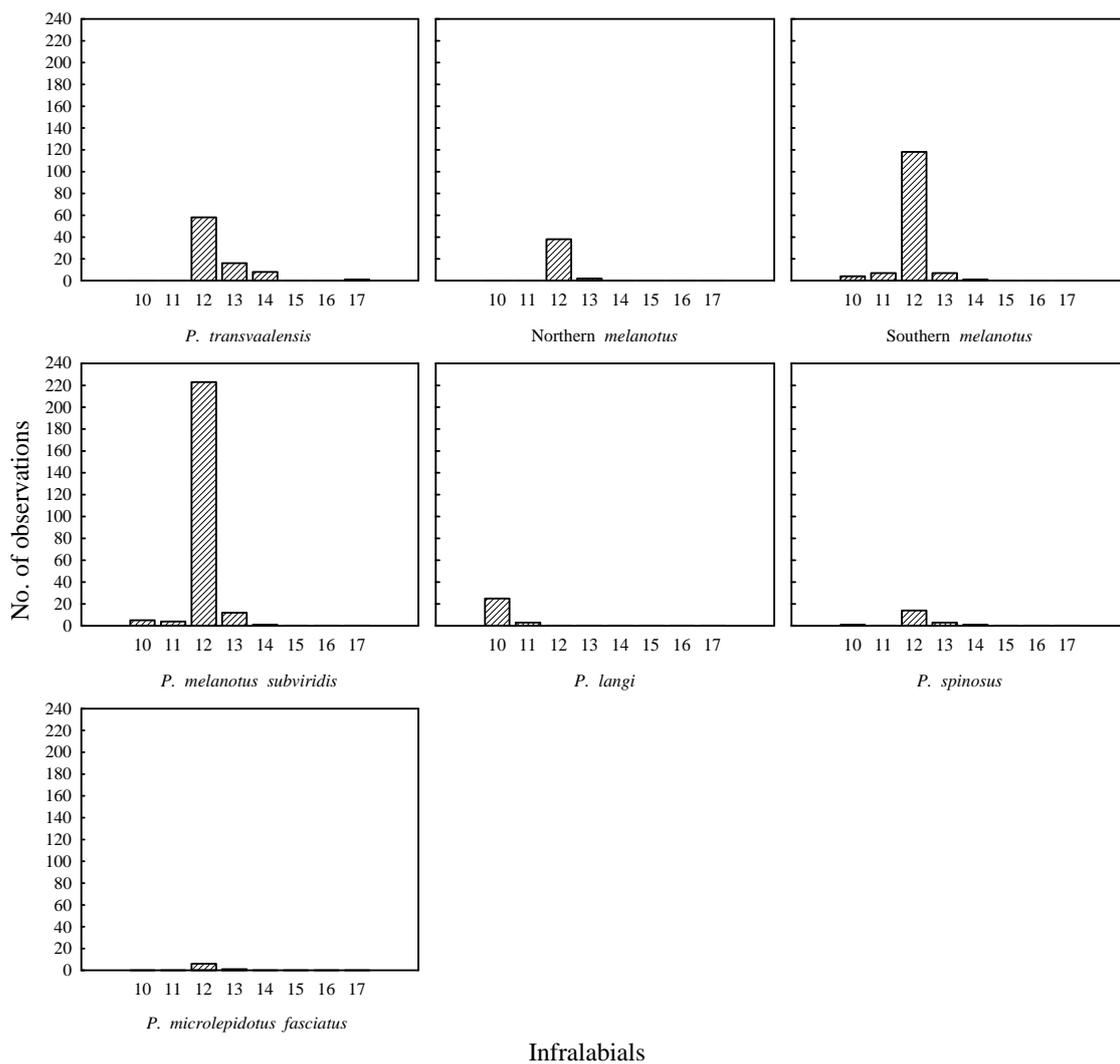
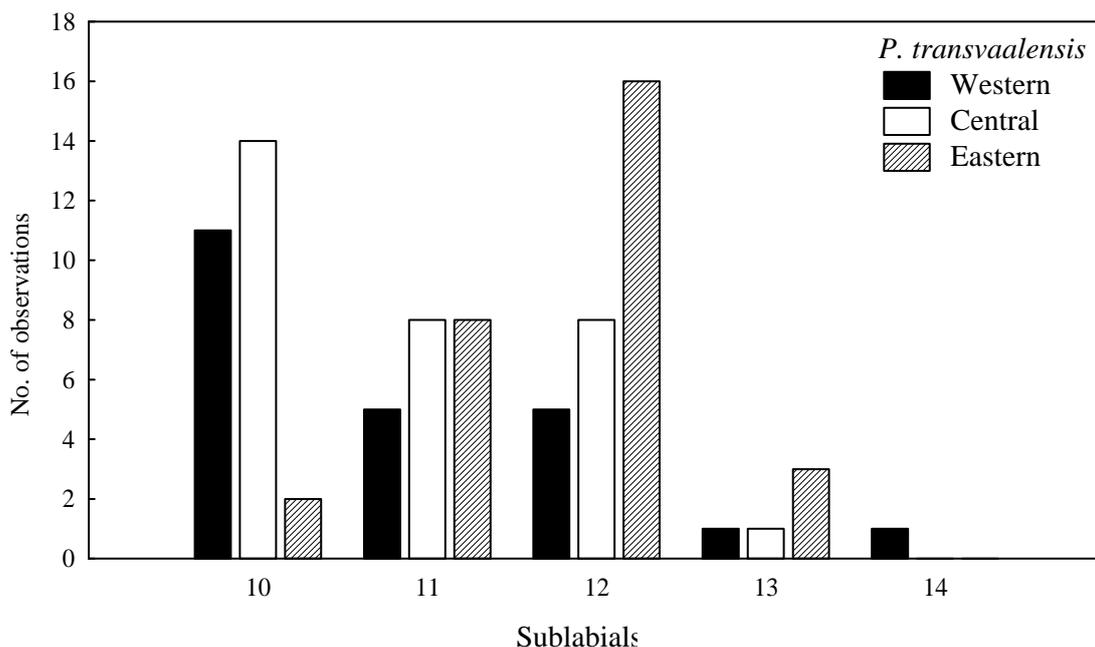
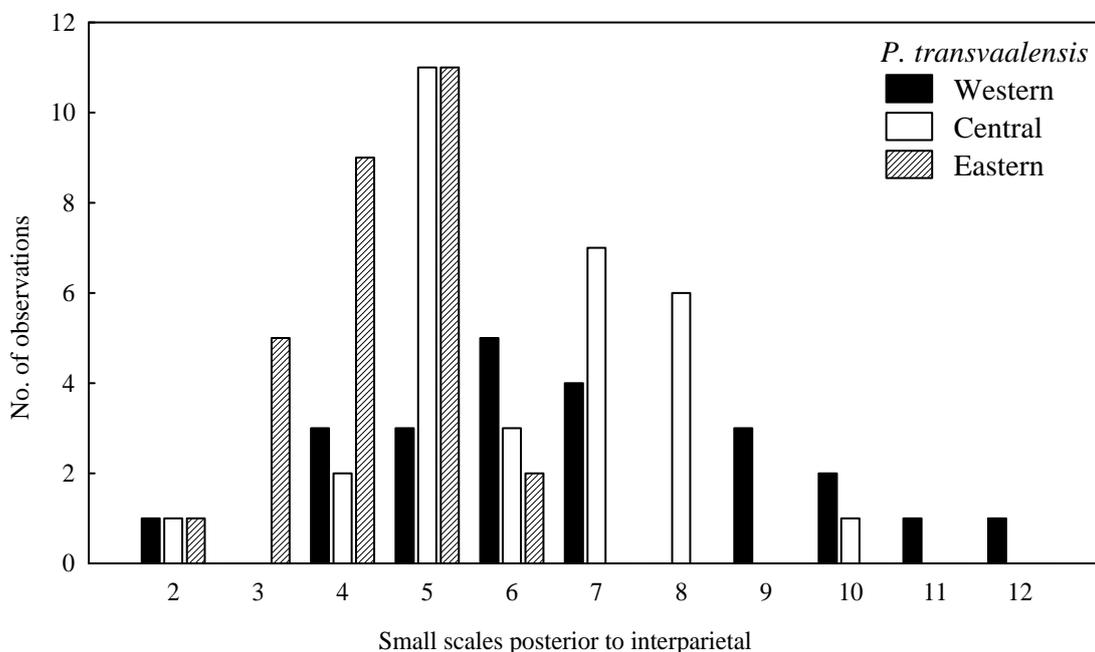


Figure 5.32: Number of infralabials in the *Pseudocordylus melanotus* species complex.



**Figure 5.33:** Number of sublabials in three allopatric populations of *Pseudocordylus transvaalensis*.



**Figure 5.34:** Number of small scales posterior to the interparietal in three allopatric populations of *Pseudocordylus transvaalensis*.

**7. Occipitals** (Table 5.2):

The numbers of occipitals varied considerably in most groups, but these scales were absent in *langi* and *spinosus*. In *langi* there were granular scales on the occiput. Although *spinosus* had slightly enlarged, keeled scales behind the parietals, they were about the same size or smaller than the adjacent dorsals and were therefore not considered as occipitals.

**8. Transverse rows of dorsal scales** (Table 5.3):

There was considerable variation in this character. Apart from *langi* that was scored as zero for this character as it had granular dorsals over most of the back except the paravertebral rows, *spinosus* had the lowest counts (35-43).

**9. Longitudinal rows of dorsal scales** (Table 5.3):

There was also considerable variation in this character. However, populations (46-48) of *langi* had only 6-9 rows, as only the (non-granular) paravertebral dorsals were counted, whereas *spinosus* had the next lowest counts (31-37).

**10. Longitudinal rows of ventral scales** (Table 5.3):

There were invariably 10 rows of ventrals in *spinosus*, 10-12 rows (mean 11.3) in *langi*, 12-14 rows in most other groups (11 in one *melanotus* from population 20: NMB R8189), but 14-16 (mean 14.6) in *microlepidotus fasciatus*.

**11. Lamellae under the fourth finger** (Table 5.3):

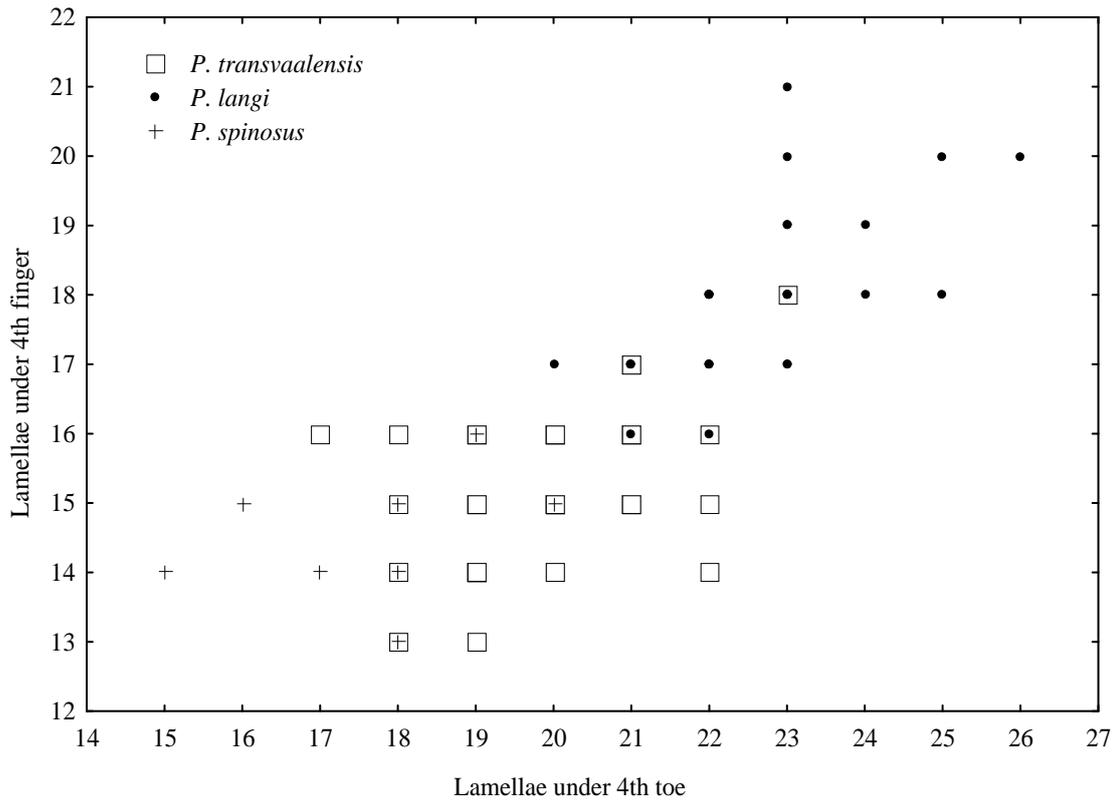
With regard to this character, most *transvaalensis* (13-18 lamellae) and *spinosus* (13-16) had lower counts than *langi* (16-21) (Fig. 5.35).

**12. Lamellae under the fourth toe** (Table 5.3):

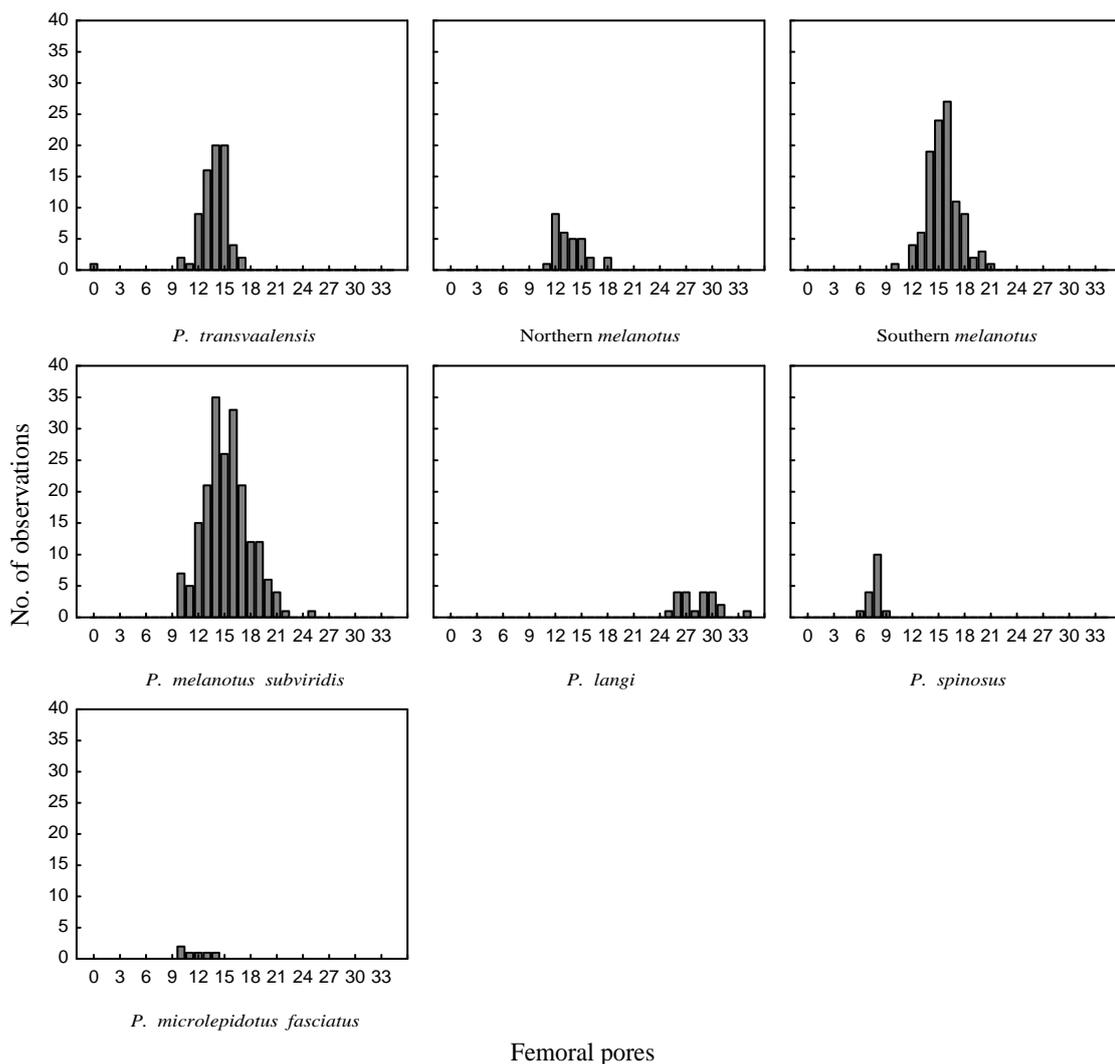
*Pseudocordylus spinosus* (15-20) and *P. langi* (20-26) were usually separable on the basis of this character. These two taxa were separated in morphological space when the number of lamellae under the fourth finger and fourth toe were plotted together (Fig. 5.35).

### 13. Femoral pores (Table 5.3; Fig. 5.36):

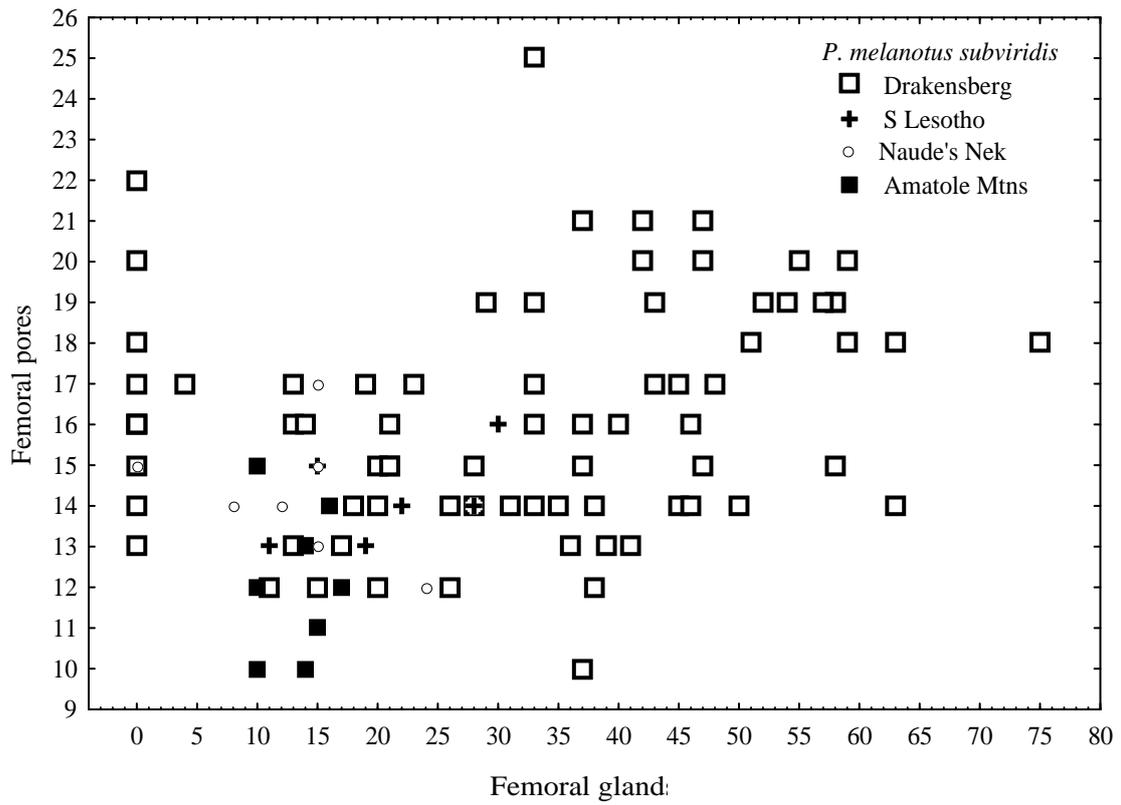
There were no significant differences between males and females of any of the seven groupings with regard to numbers of femoral pores (ANOVA:- *transvaalensis*:  $F_{73} = 0.693$ ,  $p = 0.408$ ; Southern *melanotus*:  $F_{105} = 1.215$ ,  $p = 0.273$ ; *spinosus*:  $F_{14} = 0.713$ ,  $p = 0.413$ ; Mann-Whitney U Test:- Northern *melanotus*:  $Z = 0.356$ ,  $p = 0.722$ , 13 males and 17 females; *subviridis*:  $Z = 0.970$ ,  $p = 0.332$ , 98 males and 101 females; *langi*:  $Z = 0.880$ ,  $p = 0.379$ , 11 males and 10 females; *microlepidotus fasciatus*:  $Z = 0.000$ ,  $p = 1.000$ , three males and three females). Femoral pore counts for males and females were therefore combined (also for multivariate analyses, section 5.3.2). *Pseudocordylus langi* had the highest numbers of femoral pores (25-34) and *spinosus* the lowest (6-9). Each of these populations differed from all other populations with regard to this character, except that one male Drakensberg *subviridis* (population 29) also had 25 pores. However, the next highest pore count was 21, for both Southern *melanotus* and Drakensberg *subviridis*. Northern *melanotus* had a slightly lower mean pore count compared to Southern *melanotus* (means 13.7 versus 15.5 respectively); and Amatole *subviridis* had a much lower mean than Maloti-Drakensberg *subviridis* (means 11.8 versus 15.7 respectively) (Table 5.6). The Amatole *subviridis* population had low numbers of both femoral pores and differentiated femoral scales when compared to Maloti-Drakensberg *subviridis* (Fig. 5.37). One female *transvaalensis* (TM 33278, 102 mm SVL) lacked femoral pores.



**Figure 5.35:** Relationship between number of lamellae under the fourth toe and number of lamellae under the fourth finger in *Pseudocordylus transvaalensis*, *P. langi* and *P. spinosus*.



**Figure 5.36:** Number of femoral pores (males and females) in the *Pseudocordylus melanotus* species complex.



**Figure 5.37:** Relationship between numbers of differentiated femoral scales (femoral glands) and femoral pores in male *Pseudocordylus melanotus subviridis* from four areas, namely the Drakensberg, southern Lesotho, Naude’s Nek and the Amatole Mountains.

**14. Glandular femoral scales (generation glands) (Table 5.3):**

Glandular femoral scales occurred in males from all populations, although they were occasionally absent. Although there was much variation in the number of these scales in the various populations, there were a few distinct differences. *Pseudocordylus transvaalensis* (7-28) and *spinosus* (26-44) had largely exclusive ranges and the maximum number (75) in *subviridis* exceeded that of all other groups. Several populations of *subviridis* had counts in excess of 40. Only half of the 10 male *langi* had glandular femoral scales (numbering 21, 24, 32, 36 and 50) and the next highest count apart from *subviridis* was 40 for a Northern *melanotus* male from population 11. Glandular femoral scales were usually absent in females, but *P. spinosus* differed from the rest in this regard as all three females examined had glandular scales (10 and 12 in population 50; 22 in population 49). However, they were also present in some females from the southern part of the distribution range of Drakensberg *subviridis*: 38% of females ( $N = 13$ ) from locality 41 had glandular scales (2-19), and the single female from locality 42 had six such scales. Also, one of the three female *microlepidotus fasciatus* had (13) glandular femoral scales.

As noted by Mouton *et al.* (2003) the numbers of generation glands varied geographically and appeared to be influenced by environmental factors. Du Toit *et al.* (2004) determined that females of the *Cordylus-cordylus-niger-oelofseni* species complex from western coastal localities in the Cape Fold Mountains usually lacked generation glands, whereas females from inland lowland areas usually possessed these glands. They concluded that this was due to differing climatic factors such as fog and cloud cover in the west that might cause females to invest less in such secondary sexual characteristics.

### 5.3.2 Multivariate analyses

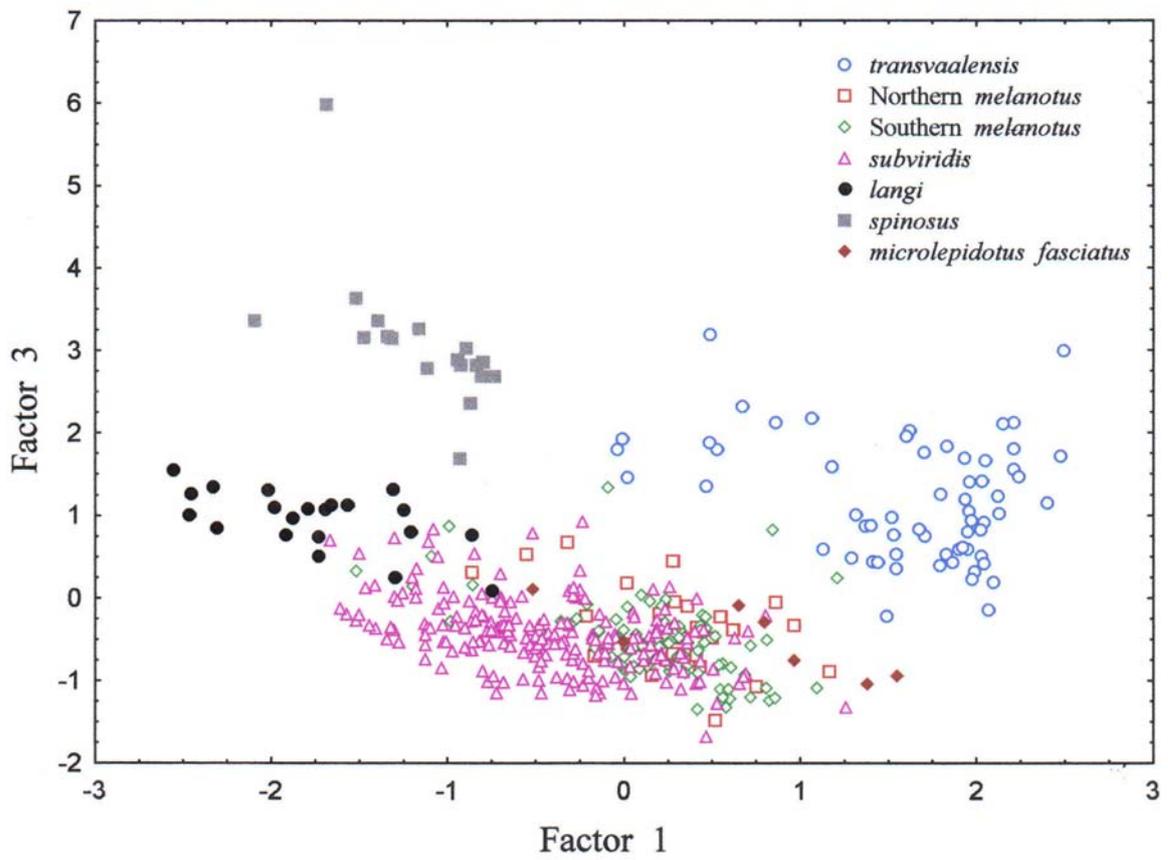
The majority of variables used in the multivariate analyses were normally distributed per grouping. For *P. transvaalensis* and Southern *melanotus* all variables were normally distributed. In the case of the large *P. m. subviridis* sample only head depth (HD) was not normally distributed ( $p = 0.243$ ). All variables were normally distributed in the Drakensberg *P. m. subviridis* sub-sample, but for the small Amatole *P. m. subviridis* sub-sample forelimb length (FL) was not normally distributed ( $p = 0.061$ ). In Northern *melanotus* the following variables were not normally distributed: head length (HL), head width (HW), gulars between posterior sublabials (G), occipitals (Occ), transverse dorsals (TD) and longitudinal dorsals (LD) ( $p = 0.051-0.518$ ). For other groupings, represented by small samples, several variables were not normally distributed:- *P. langi*: HL, HW, HD, FL, hindlimb length (H), length of 4<sup>th</sup> toe (4T), snout-vent length (SVL), lamellae under 4<sup>th</sup> toe, femoral pores (FP) ( $p = 0.063-0.555$ ); *P. spinosus*: HL, HW, HD, SVL, G, TD, LD ( $p = 0.096-0.499$ ); *P. microlepidotus fasciatus*: HL, HW, HD, FL, HL, 4T, SVL, horizontal rows of lateral temporals, supralabials, G, Occ, LD, transverse ventrals, FP ( $p = 0.078-0.975$ ). Bivariate scatterplots showing the relationship between the various measurement variables and SVL indicated that the data was linear and homoscedastic.

#### 5.3.2.1 *Pseudocordylus melanotus* species complex

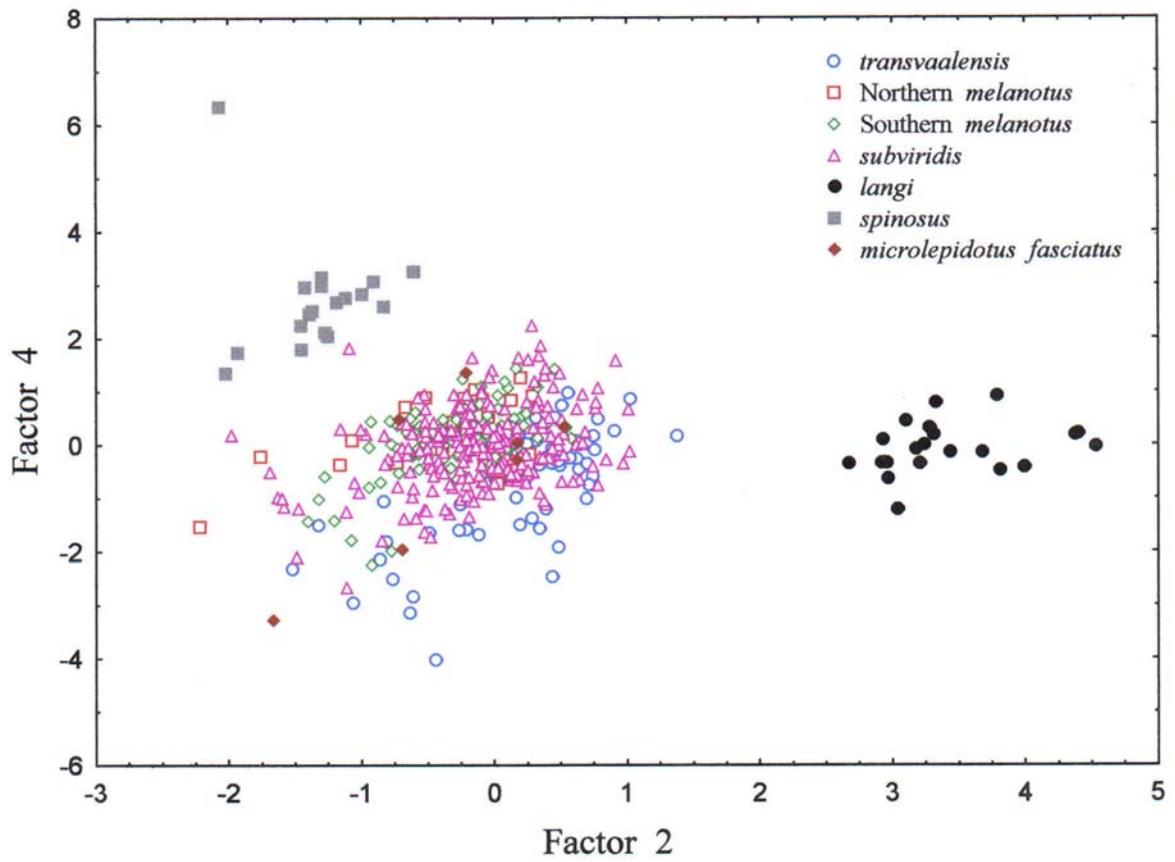
##### 5.3.2.1.1 Principal Components Analysis of the *Pseudocordylus melanotus* species complex

Principal Components Analysis partitions total variation among specimens without reference to pre-defined groups. For the analysis of the *Pseudocordylus melanotus* species complex, a total of 38 characters were used (Table 5.7). Of the 559 processed cases (specimens), 507 were accepted as valid, and the principal components scores of 431 specimens were plotted (62 *P. transvaalensis*, 29 Northern *melanotus*, 90 Southern *melanotus*, 204 *P. m. subviridis*, 21 *P. langi*, 18 *P. spinosus*, seven *P. microlepidotus fasciatus*). Four distinct clusters (natural groupings) were discerned, namely *P. transvaalensis*, *P. langi*, *P. spinosus* and a cluster consisting of all other groups. *Pseudocordylus spinosus* is separated from *P. transvaalensis* along principal component (PC) 1, and from all other taxa along PC3 (Fig. 5.38); whereas *P. langi* is separated from all other taxa along PC2 (Fig. 5.39). The highest factor loadings for PC1 were all for

morphometric characters: head length (0.960), head width (0.939), hindlimb (0.939), forelimb (0.933), SVL (0.930), fourth toe (0.927) and head depth (0.925) (Table 5.7). This indicates that body proportions differed substantially between *P. transvaalensis* and the two smaller species, namely *P. spinosus* and *P. langi*. For PC2 the highest loadings were: transverse rows of dorsals (0.911), longitudinal rows of dorsals (0.808), occipitals (0.744), texture of posterior infralabial (0.709), femoral pores (0.686), size of interspaces between longitudinal rows of dorsolaterals (0.671) and infralabials (0.652) (Table 5.7). These are characters that largely distinguish *P. langi* from the other taxa. For PC3 the highest loadings were: small scales posterior to interparietal (0.818), suboculars posterior to median (0.772), sublabials (0.708), anterior frontal present/absent (0.657), gulars between posterior sublabials (0.640), anterior parietals undivided/partly divided/divided (0.592) and horizontal rows of lateral temporals (0.591) (Table 5.7). The PCA explained 56.3% of variance (26.0% in PC1, 11.5% in PC2, 7.6% in PC3) (Table 5.7), indicating that much residual variation is not explained on these axes.



**Figure 5.38:** Principal Components Analysis of the *Pseudocordylus melanotus* complex: Plots of principal components 1 and 3 are shown.



**Figure 5.39:** Principal Components Analysis of the *Pseudocordylus melanotus* complex: Plots of principal components 2 and 4 are shown.

**Table 5.7:** Factor loadings (Varimax normalized) for the Principal Components Analysis of the *Pseudocordylus melanotus* complex.

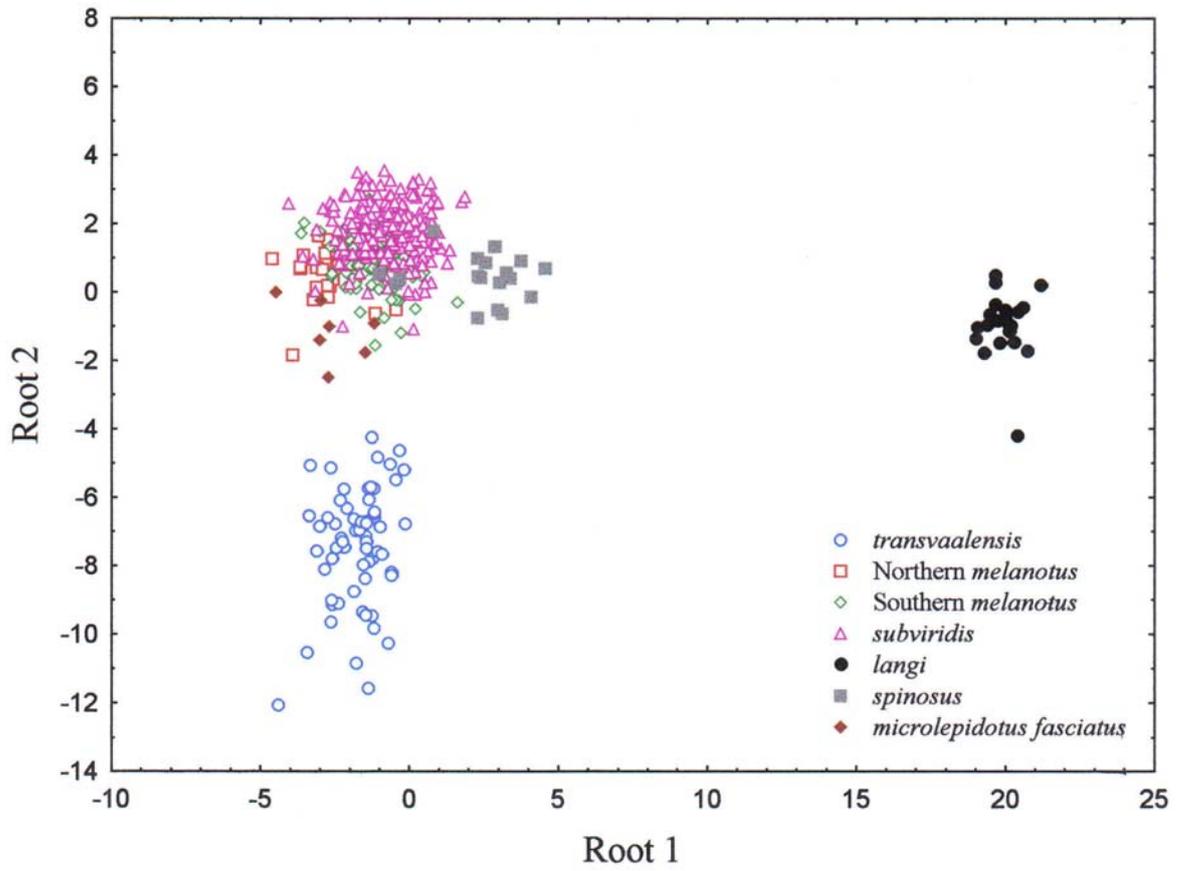
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Head length	0.960	0.138	0.137	-0.003	-0.109
Head width	0.939	0.138	0.176	-0.044	-0.127
Head depth	0.925	0.163	0.210	-0.004	-0.083
Forelimb	0.933	0.051	0.237	0.085	-0.126
Hindlimb	0.939	0.062	0.232	0.072	-0.118
Fourth toe	0.927	-0.009	0.171	0.122	-0.063
Snout-vent length	0.930	0.138	0.197	-0.006	-0.186
Upper temporals	0.039	0.219	0.103	0.043	0.134
Horizontal rows of lateral temporals	0.264	0.244	0.591	0.035	-0.440
Supraoculars	0.054	0.036	0.043	-0.037	-0.020
Supraciliaries	-0.042	-0.101	0.162	0.082	0.131
Suboculars anterior to median	0.017	0.007	0.155	0.049	-0.048
Suboculars posterior to median	0.146	0.045	0.772	-0.146	-0.065
Supralabials	0.013	0.144	0.277	0.108	0.055
Infralabials	0.108	0.652	0.249	-0.155	-0.051
Sublabials	0.154	0.038	0.708	-0.103	-0.081
Gulars in contact with anterior sublabials	-0.043	-0.085	-0.055	0.191	-0.008
Gulars between posterior sublabials	0.235	-0.046	0.640	0.260	0.241
Occipitals	0.106	0.744	-0.084	0.385	-0.246
Transverse rows of dorsals	0.037	0.911	-0.109	-0.015	0.019
Longitudinal rows of dorsals	0.227	0.808	0.220	-0.123	-0.269
Transverse rows of ventrals	-0.190	0.244	-0.188	-0.279	0.366
Longitudinal rows of ventrals	0.099	0.432	0.121	0.487	0.045
Lamellae under 4th finger	-0.034	-0.235	-0.142	0.519	0.385
Lamellae under 4th toe	-0.046	-0.258	0.104	0.527	0.278
Femoral pores	-0.028	0.686	0.061	-0.515	-0.139
Small scales posterior to interparietal	0.269	0.066	0.818	-0.059	-0.146
Frontonasal width in relation to length	0.078	0.088	0.012	0.790	-0.149
Small scale present/absent behind frontonasal	-0.152	-0.021	-0.206	-0.015	0.507
Frontonasal separates supranasals or not	0.295	0.054	0.438	-0.075	-0.111
Frontonasal undivided/partly divided/divided	-0.019	0.006	0.070	-0.214	0.646
Frontonasal in contact with loreals or not	0.135	0.061	0.086	0.773	-0.206
Anterior frontal present/absent	0.159	0.049	0.657	-0.087	-0.131
Anterior parietals undivided/partly divided/divided	0.140	0.069	0.592	-0.046	-0.087
Median dorsals as a proportion of dorsolaterals	-0.205	-0.213	-0.154	0.320	0.690
Lateral dorsals as a proportion of dorsolaterals	-0.227	<b>-0.156</b>	-0.257	0.022	0.512
Size of interspaces between long. rows dorsolaterals	0.170	0.671	0.110	0.023	-0.531
Texture of posterior infralabial	0.095	0.709	0.020	-0.219	-0.033
Eigenvalue	9.872	4.378	2.894	2.456	1.784
Percentage contribution towards total variance	26.0	11.5	7.6	6.5	4.7
Cumulative Eigenvalue	9.872	14.249	17.143	19.600	21.383
Cumulative proportion	0.260	0.375	0.451	0.516	0.563

### 5.3.2.1.2 Canonical Discriminant Analysis of the *Pseudocordylus melanotus* species complex

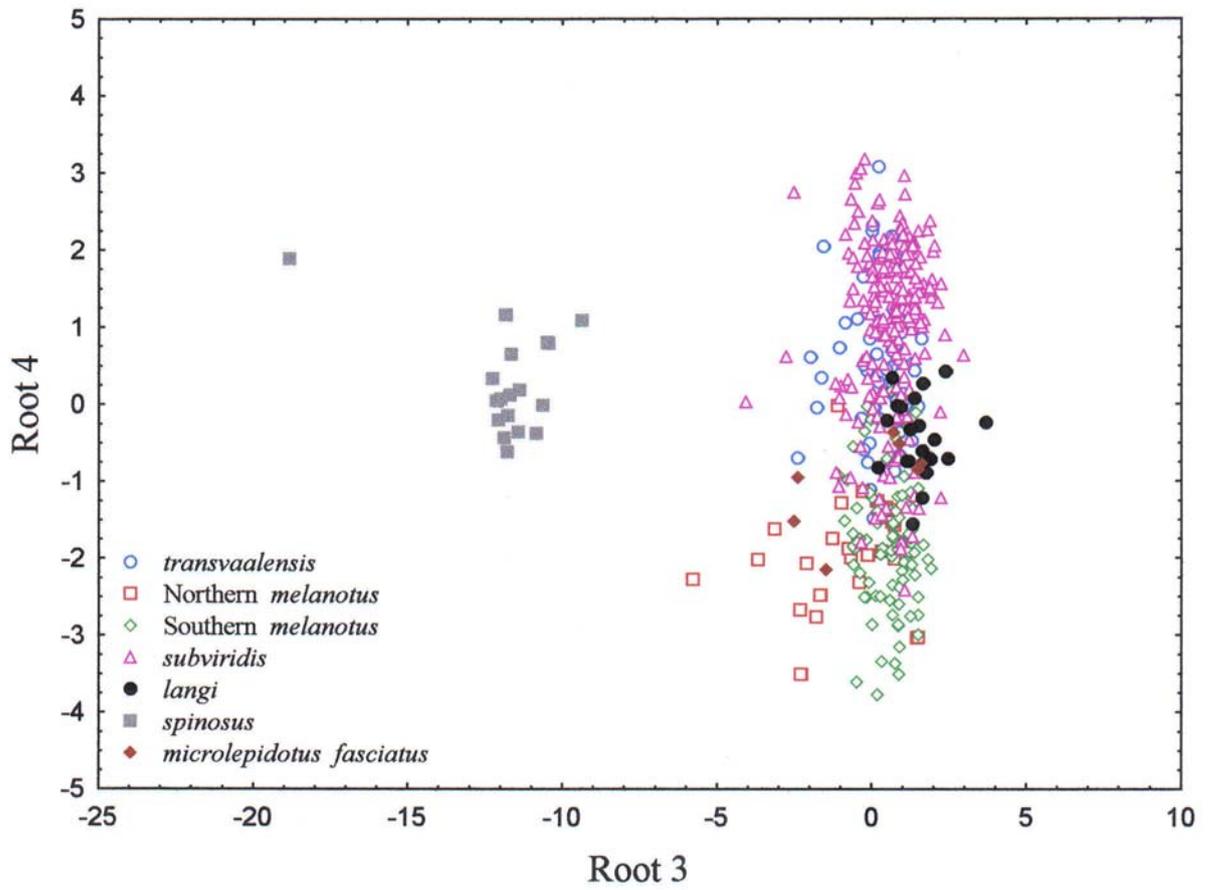
Discriminant function analyses are based on a posteriori classification of individuals into groups using the distinguishing characters determined by the analysis. The same 38 characters used for the PCA were used in this analysis (see Table 5.9). Of the 559 specimens, 431 were accepted as valid (62 *P. transvaalensis*, 29 Northern *melanotus*, 90 Southern *melanotus*, 204 *P. m. subviridis*, 21 *P. langi*, 18 *P. spinosus*, seven *P. microlepidotus fasciatus*). Table 5.8 indicates that *P. transvaalensis*, *P. langi*, *P. spinosus* and *P. microlepidotus fasciatus* are 100% distinguishable in morphometric space. The majority of *P. m. subviridis* (89.7%) and Southern *melanotus* (88.9%) were also correctly identified, but this applied to only 72.4% of Northern *melanotus*. As in PCA, the CDA discerned four distinct clusters, namely *P. transvaalensis*, *P. langi*, *P. spinosus* and a cluster consisting of all other groups (Figs 5.40 and 5.41). The distribution of samples along the first two canonical axes accounted for 78.1% of variation (Table 5.9). *Pseudocordylus langi* is distinguished from all other taxa along axis 1 (Fig. 5.40), which loads most heavily for transverse rows of dorsals (0.907), followed distantly by head width (-0.498) and size of interspaces between longitudinal rows of dorsolaterals (0.418) (Table 5.9). *Pseudocordylus transvaalensis* is separated from all others along axis 2 (Fig. 5.40), which loads most heavily for hindlimb length (-0.715), small scales posterior to interparietal (0.579), head length (0.546) and sublabials (0.403); while *spinosus* is distinguished from all others along axis 3 (Fig. 5.41), which loads most heavily for forelimb (0.761), frontonasal width in relation to length (0.726), head depth (0.567), head length (0.521) and head width (0.494) (Table 5.9). Axis 3 accounted for 15.7% of variation.

**Table 5.8:** Observed (rows) and predicted (columns) classifications of specimens in the *Pseudocordylus melanotus* complex according to the Canonical Discriminant Analysis.

	Percent correct	<i>trans</i> p=.144	<i>N mel</i> p=.067	<i>S mel</i> p=.209	<i>sub</i> p=.473	<i>langi</i> p=.049	<i>spinosus</i> p=.042	<i>m. fasc.</i> p=.016
<i>transvaalensis</i>	100.0	62	0	0	0	0	0	0
Northern <i>melanotus</i>	72.4	0	21	7	1	0	0	0
Southern <i>melanotus</i>	88.9	0	1	80	9	0	0	0
<i>subviridis</i>	89.7	0	1	20	183	0	0	0
<i>langi</i>	100.0	0	0	0	0	21	0	0
<i>spinosus</i>	100.0	0	0	0	0	0	18	0
<i>microlepidotus fasciatus</i>	100.0	0	0	0	0	0	0	7
Total	91.0	62	23	107	193	21	18	7



**Figure 5.40:** Canonical Discriminant Analysis of the *Pseudocordylus melanotus* complex: Plots of the first two canonical axes are shown.



**Figure 5.41:** Canonical Discriminant Analysis of the *Pseudocordylus melanotus* complex: Plots of the third and fourth canonical axes are shown.

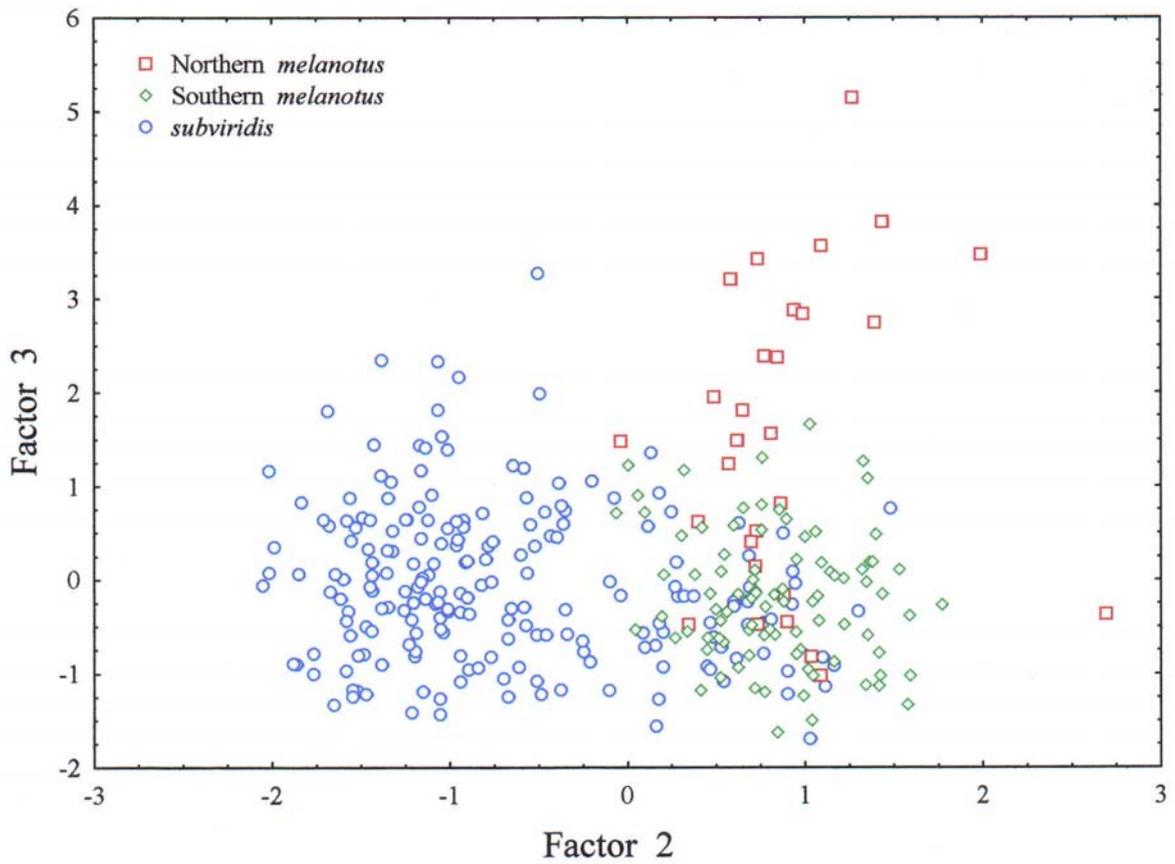
**Table 5.9:** Standardized coefficients in the Canonical Discriminant Analysis of the *Pseudocordylus melanotus* complex.

Variable	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6
Head length	0.374	0.546	-0.521	0.085	0.041	2.226
Head width	-0.498	-0.113	0.494	1.045	0.113	-1.876
Head depth	0.130	0.325	-0.567	-0.192	0.016	-0.272
Forelimb	0.053	-0.324	0.761	0.238	0.085	-0.715
Hindlimb	0.104	-0.715	-0.100	-0.065	0.272	0.166
Fourth toe	0.119	0.212	0.244	-0.351	-0.301	0.736
Snout-vent length	-0.395	-0.066	-0.267	-0.777	-0.173	-0.335
Upper temporals	0.028	0.010	-0.010	-0.034	-0.054	0.030
Horizontal rows of lateral temporals	-0.075	-0.370	-0.017	-0.219	0.162	-0.010
Supraoculars	-0.068	-0.017	0.180	-0.039	0.095	0.367
Supraciliaries	0.059	0.023	-0.010	0.032	-0.033	-0.112
Suboculars anterior to median	-0.075	0.031	0.030	-0.000	-0.098	0.242
Suboculars posterior to median	-0.082	-0.332	-0.106	0.124	0.193	-0.177
Supralabials	0.081	-0.068	0.052	-0.156	0.349	-0.137
Infralabials	-0.148	0.106	-0.081	0.098	-0.111	-0.024
Sublabials	-0.039	-0.403	-0.085	0.192	-0.048	0.022
Gulars in contact with anterior sublabials	0.118	0.212	0.059	0.082	-0.162	-0.014
Gulars between posterior sublabials	0.184	-0.128	0.172	0.153	-0.116	0.051
Occipitals	-0.228	0.057	0.367	-0.042	0.041	-0.046
Transverse rows dorsals	-0.907	0.240	-0.029	0.190	-0.115	0.181
Longitudinal rows dorsals	-0.169	-0.117	-0.103	-0.324	0.179	0.064
Transverse rows ventrals	0.324	-0.062	0.116	-0.200	0.038	0.044
Longitudinal rows ventrals	-0.015	0.005	0.242	0.158	0.449	-0.398
Lamellae under 4th finger	-0.010	0.140	0.029	-0.125	0.104	0.052
Lamellae under 4th toe	0.063	-0.134	-0.010	0.097	0.454	0.142
Femoral pores	0.236	0.135	0.225	-0.194	-0.270	-0.078
Small scales posterior to interparietal	-0.053	-0.579	-0.026	0.250	-0.161	0.131
Frontonasal width in relation to length	-0.035	-0.012	-0.726	-0.086	0.209	-0.192
Small scale present/absent behind frontonasal	0.017	0.063	0.019	-0.228	-0.153	0.086
Frontonasal separates supranasals or not	0.052	0.137	-0.162	-0.038	0.065	-0.211
Frontonasal undivided/partly divided/divided	0.070	0.063	0.162	0.253	0.314	0.199
Frontonasal in contact with loreals or not	0.119	-0.036	-0.145	0.061	-0.179	0.019
Anterior frontal present/absent	-0.128	0.066	-0.072	0.058	0.119	0.006
Anterior parietals undivided/partly divided/divided	0.014	0.273	0.036	-0.059	0.048	-0.073
Median dorsals as a proportion of dorsolaterals	0.026	0.028	0.231	0.420	0.116	-0.076
Lateral dorsals as a proportion of dorsolaterals	0.041	0.047	-0.101	0.031	-0.339	-0.249
Size of interspaces between long. rows dorsolaterals	0.418	-0.107	-0.314	0.005	0.229	0.390
Texture of posterior infralabial	0.223	0.018	0.192	-0.051	0.103	-0.030
Eigenvalue	21.567	9.927	6.334	1.437	0.671	0.402
Percentage contribution	53.5	24.6	15.7	3.5	1.7	1.0
Cumulative proportion	0.535	0.781	0.938	0.973	0.990	1.000

5.3.2.2 ***Pseudocordylus melanotus* (comprising Northern *melanotus*, Southern *melanotus* and *P. m. subviridis*)**

5.3.2.2.1 **Principal Components Analysis of *Pseudocordylus melanotus***

For the analysis of *Pseudocordylus melanotus* (including Northern *melanotus*, Southern *melanotus* and *P. m. subviridis*) the same 38 characters as mentioned above were used (Table 5.10). Of the 422 processed cases (specimens), 384 were accepted as valid, and the principal components scores of 323 specimens were plotted (29 Northern *melanotus*, 90 Southern *melanotus*, 204 *P. m. subviridis*). The PCA did not distinguish clearly between groups. Although the latter groups did form clusters, there was considerable overlap between them (Fig. 5.42). The highest factor loadings for PC1 were once again all for morphometric characters: head length (0.973), hindlimb (0.966), forelimb (0.965), head depth (0.956), head width (0.952), SVL (0.946) and fourth toe (0.925) (Table 5.10). For PC2 the highest loadings were: median dorsals as a proportion of dorsolaterals (0.835), size of interspaces between longitudinal rows of dorsolaterals (0.793), horizontal rows of lateral temporals (0.663) and lateral dorsals as a proportion of dorsolaterals (0.575); whereas as for PC3 the highest loadings were: lamellae under fourth toe (0.779), lamellae under fourth finger (0.718) and frontonasal width in relation to length (0.502) (Table 5.10). The PCA explained 43.1% of variance (19.0% in PC1, 9.1% in PC2, 5.7% in PC3) (Table 5.10), indicating that much residual variation is not explained on these axes.



**Figure 5.42:** Principal Components Analysis of *Pseudocordylus melanotus* (Northern *melanotus*, Southern *melanotus*, *subviridis*): Plots of principal components 2 and 3 are shown.

**Table 5.10:** Factor loadings (Varimax normalized) for the Principal Components Analysis of *Pseudocordylus melanotus* (Northern *melanotus*, Southern *melanotus*, *subviridis*).

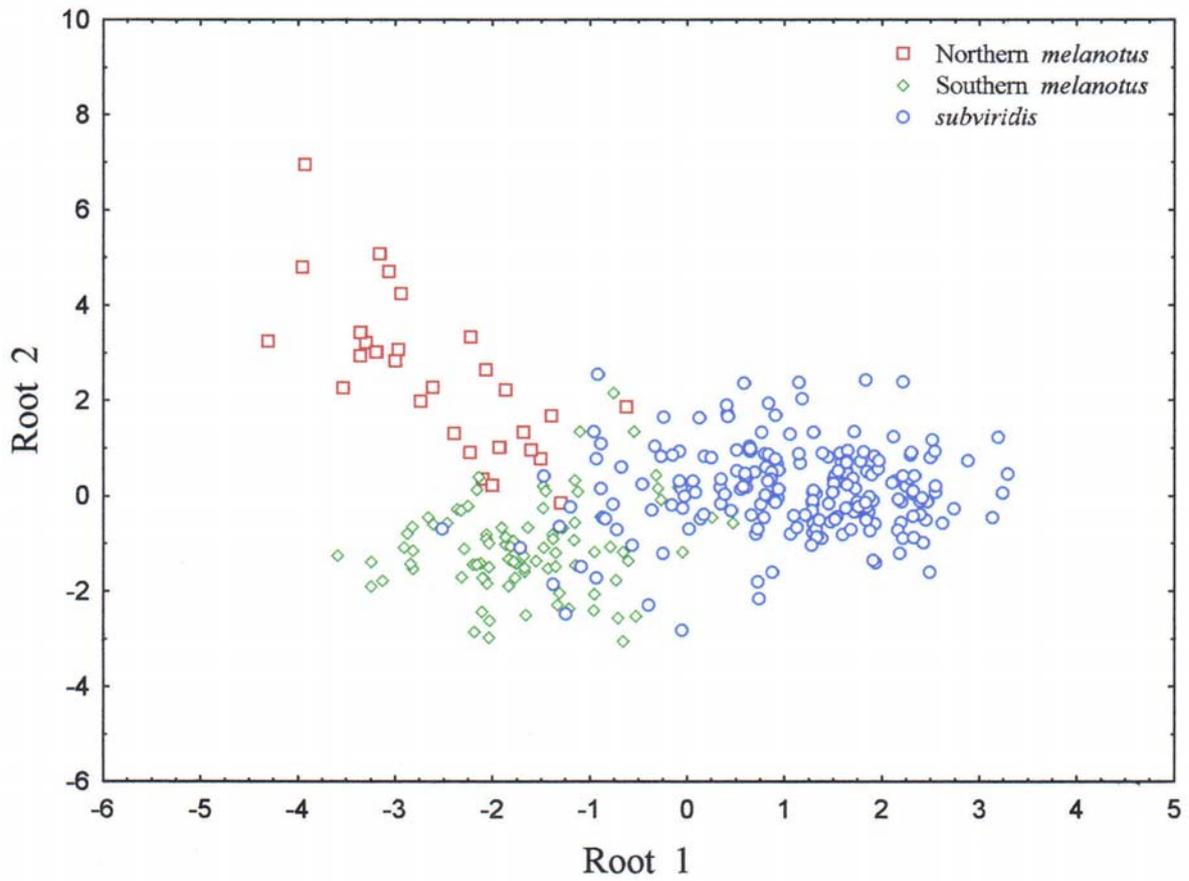
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Head length	0.973	0.116	-0.024	-0.013	0.035
Head width	0.952	0.118	-0.046	-0.012	0.011
Head depth	0.956	0.076	0.021	-0.031	0.040
Forelimb	0.965	0.114	-0.012	-0.014	0.008
Hindlimb	0.966	0.125	0.012	-0.025	0.001
Fourth toe	0.925	0.092	0.162	-0.032	-0.033
Snout-vent length	0.946	0.214	-0.032	-0.033	0.026
Upper temporals	0.006	-0.027	-0.001	0.019	0.207
Horizontal rows of lateral temporals	0.111	0.663	0.254	0.098	-0.002
Supraoculars	0.006	0.184	0.467	-0.151	0.064
Supraciliaries	-0.020	-0.098	0.077	0.268	0.168
Suboculars anterior to median	-0.011	0.084	0.095	0.003	0.182
Suboculars posterior to median	-0.011	0.039	0.326	0.108	0.033
Supralabials	0.010	0.030	0.179	0.146	0.288
Infralabials	0.096	-0.043	0.058	0.271	0.274
Sublabials	-0.008	-0.030	-0.003	0.508	0.059
Gulars in contact with anterior sublabials	-0.045	0.078	-0.033	0.333	0.123
Gulars between posterior sublabials	-0.175	0.447	-0.100	-0.074	-0.430
Occipitals	0.046	0.277	-0.082	-0.068	0.006
Transverse rows of dorsals	-0.004	0.453	-0.299	-0.089	-0.428
Longitudinal rows of dorsals	0.179	0.490	0.133	0.162	0.519
Transverse rows of ventrals	0.173	0.314	0.063	0.025	0.090
Longitudinal rows of ventrals	0.031	-0.261	-0.139	0.020	0.396
Lamellae under 4th finger	0.003	-0.328	0.718	0.010	-0.143
Lamellae under 4th toe	-0.042	-0.208	0.779	0.021	-0.114
Femoral pores	-0.119	0.304	-0.188	-0.117	0.574
Small scales posterior to interparietal	0.032	0.003	0.081	0.403	-0.095
Frontonasal width in relation to length	0.057	0.139	0.502	-0.147	0.255
Small scales present/absent behind frontonasal	0.166	0.479	0.113	0.296	-0.077
Frontonasal separates supranasals or not	0.215	0.098	0.245	0.149	-0.064
Frontonasal undivided/partly divided/divided	0.118	0.462	-0.111	0.180	-0.359
Frontonasal in contact with loreals or not	-0.110	-0.043	-0.136	-0.058	0.254
Anterior frontal present/absent	0.027	0.098	-0.057	0.783	-0.064
Anterior parietals undivided/partly divided/divided	-0.075	0.028	-0.055	0.490	-0.075
Median dorsals as a proportion of dorsolaterals	0.143	0.835	-0.022	-0.014	0.001
Lateral dorsals as a proportion of dorsolaterals	0.134	0.575	0.082	0.005	0.197
Size of interspaces between long. rows dorsolaterals	0.050	0.793	-0.063	-0.043	-0.093
Texture of posterior infralabial	0.081	0.043	-0.017	-0.110	0.146
Eigenvalue	7.222	3.454	2.178	1.774	1.751
Percentage contribution towards total variance	19.0	9.1	5.7	4.7	4.6
Cumulative Eigenvalue	7.222	10.675	12.854	14.627	16.379
Cumulative proportion	0.190	0.281	0.338	0.385	0.431

5.3.2.2.2 **Canonical Discriminant Analysis of *Pseudocordylus melanotus***

The same 38 characters used above were used in this analysis (see Table 5.12). Of the 422 specimens, 323 were accepted as valid (29 Northern *melanotus*, 90 Southern *melanotus*, 204 *P. m. subviridis*). Table 5.11 indicates that the three groups are largely distinguishable in morphometric space. The majority of *P. m. subviridis* (91.2%) and Southern *melanotus* (88.9%) were correctly identified, but this applied to only 72.4% of Northern *melanotus*. The three groups formed fairly distinct clusters (Fig. 5.43). Although Southern *melanotus* and *P. m. subviridis* overlapped considerably, Northern *melanotus* was largely separated from the others in morphometric space. Southern *melanotus* and *P. m. subviridis* exhibit partial separation along axis 1, which loads most heavily for SVL (-0.831), head width (0.811), longitudinal rows of dorsals (-0.378), median dorsals as a proportion of dorsolaterals (0.374) and horizontal rows of lateral temporals (-0.359) (Table 5.12). Northern *melanotus* shows the greatest separation from the other two groupings along axis 2, which loads most heavily for head length (1.723), head width (0.974), forelimb length (-0.652), size of interspaces between longitudinal rows of dorsolaterals (0.432), femoral pores (-0.423), SVL (-0.391) and frontonasal undivided/partly divided/divided (0.364) (Table 5.12).

**Table 5.11:** Observed (rows) and predicted (columns) classifications of specimens of *Pseudocordylus melanotus* (Northern *melanotus*, Southern *melanotus*, *subviridis*) according to the Canonical Discriminant Analysis.

	Percent correct	N <i>mel</i> p = 0.090	S <i>mel</i> p = 0.279	<i>sub</i> p = 0.632
Northern <i>melanotus</i>	72.4	21	7	1
Southern <i>melanotus</i>	88.9	1	80	9
<i>subviridis</i>	91.2	1	17	186
Total	88.9	23	104	196



**Figure 5.43:** Canonical Discriminant Analysis of *Pseudocordylus melanotus* (Northern *melanotus*, Southern *melanotus*, *subviridis*).

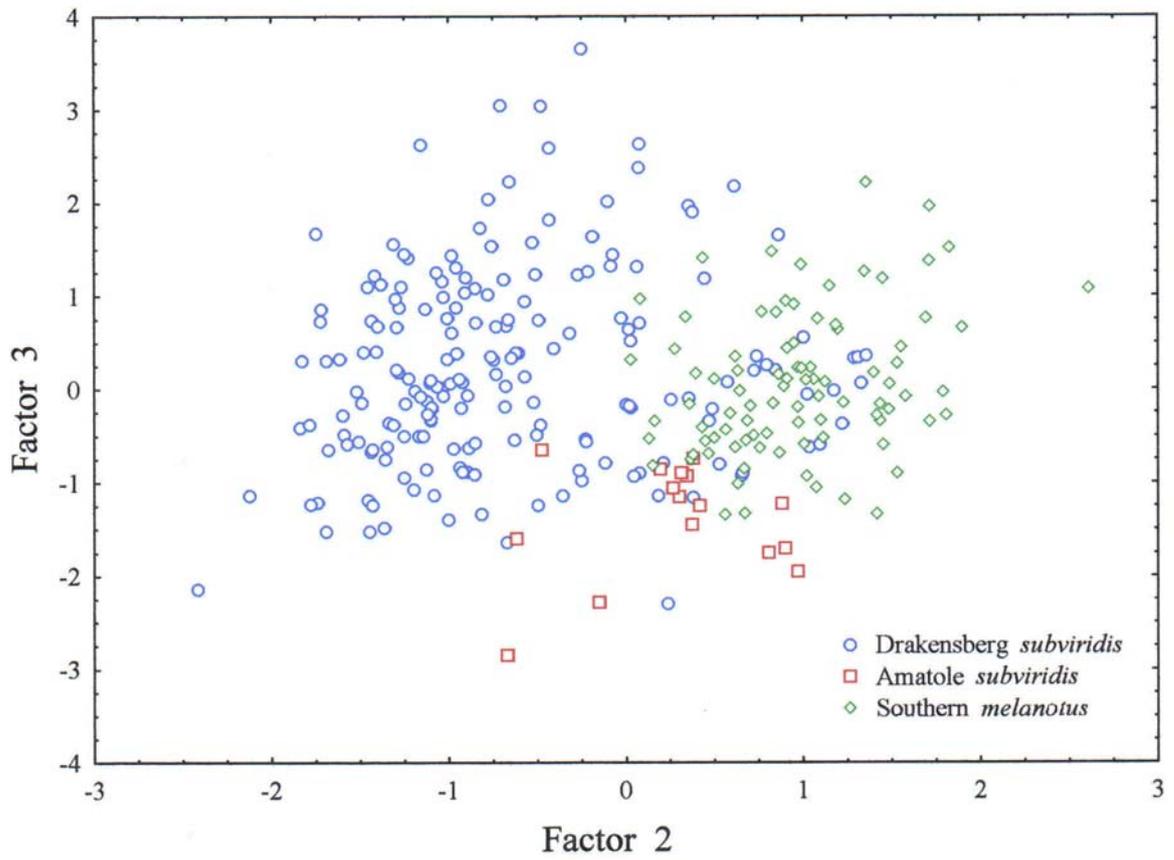
**Table 5.12:** Standardized coefficients in the Canonical Discriminant Analysis of *Pseudocordylus melanotus* (Northern *melanotus*, Southern *melanotus*, *subviridis*).

<b>Variable</b>	<b>Root 1</b>	<b>Root 2</b>
Head length	0.271	1.723
Head width	0.811	-0.974
Head depth	0.028	-0.022
Forelimb	0.223	-0.652
Hindlimb	-0.312	0.159
Fourth toe	-0.245	0.150
Snout-vent length	-0.831	-0.391
Upper temporals	0.016	0.071
Horizontal rows of lateral temporals	-0.359	0.078
Supraoculars	-0.054	0.278
Supraciliaries	0.054	-0.094
Suboculars anterior to median	-0.059	0.005
Suboculars posterior to median	-0.022	0.122
Supralabials	-0.134	0.085
Infralabials	-0.005	-0.083
Sublabials	0.144	-0.004
Gulars in contact with anterior sublabials	0.202	-0.132
Gulars between posterior sublabials	0.208	-0.214
Occipitals	-0.035	-0.019
Transverse rows of dorsals	-0.156	0.257
Longitudinal rows of dorsals	-0.378	0.207
Transverse rows of ventrals	-0.063	-0.107
Longitudinal rows of ventrals	0.199	-0.046
Lamellae under 4th finger	-0.070	0.033
Lamellae under 4th toe	0.051	0.329
Femoral pores	-0.015	-0.423
Small scales posterior to interparietal	0.052	0.036
Frontonasal width in relation to length	-0.152	0.228
Small scale present/absent behind frontonasal	-0.129	-0.020
Frontonasal separates supranasals or not	0.064	-0.035
Frontonasal undivided/partly divided/divided	0.280	0.364
Frontonasal in contact with loreals or not	0.098	0.047
Anterior frontal present/absent	0.112	0.128
Anterior parietals undivided/partly divided/divided	0.019	0.021
Median dorsals as a proportion of dorsolaterals	0.374	0.048
Lateral dorsals as a proportion of dorsolaterals	0.054	-0.355
Size of interspaces between long. rows dorsolaterals	0.028	0.432
Texture of posterior infralabial	0.084	-0.082
Eigenvalue	2.093	0.907
Percentage contribution	69.8	30.2
Cumulative proportion	0.698	1.000

5.3.2.3 ***Pseudocordylus melanotus subviridis* (comprising Maloti-Drakensberg and Amatole populations) and Southern *melanotus***

5.3.2.3.1 **Principal Components Analysis of *Pseudocordylus melanotus subviridis* and Southern *melanotus***

For the analysis of *Pseudocordylus melanotus subviridis* and Southern *melanotus* the same 38 characters mentioned above were used (Table 5.13). Of the 382 processed cases (specimens), 346 were accepted as valid, and the principal components scores of 294 specimens were plotted (188 Drakensberg *P. m. subviridis*, 16 Amatole *P. m. subviridis*, 90 Southern *melanotus*). The analysis did not distinguish clearly between groups. Although the latter groups did form vague clusters, there was considerable overlap between them (Fig. 5.44). The highest factor loadings for PC1 were once again all for morphometric characters: head length (0.971), hindlimb (0.968), forelimb (0.959), head depth (0.952), head width (0.952), SVL (0.940) and fourth toe (0.932) (Table 5.13). Drakensberg *P. m. subviridis* and Southern *melanotus* were partially separated along PC2, which loaded most heavily for: median dorsals as a proportion of dorsolaterals (0.791), size of interspaces between longitudinal rows of dorsolaterals (0.761), horizontal rows of lateral temporals (0.721), frontonasal undivided/partly divided/divided (0.562), small scale present/absent behind frontonasal (0.528) and transverse rows of dorsals (0.493) (Table 5.13). Amatole *P. m. subviridis* was partly separated from the others along PC3, which loaded most heavily for lamellae under 4<sup>th</sup> toe (0.762), lamellae under 4<sup>th</sup> finger (0.738) and femoral pores (0.598) (Table 5.13). The PCA explained 43.2% of variance (19.2% in PC1, 9.4% in PC2, 5.2% in PC3) (Table 5.13), indicating that much residual variation is not explained on these axes.



**Figure 5.44:** Principal Components Analysis of *Pseudocordylus melanotus subviridis* (Drakensberg and Amatole populations) and *Southern melanotus*: Plots of principal components 2 and 3 are shown.

**Table 5.13:** Factor loadings (Varimax normalized) for the Principal Components Analysis of *Pseudocordylus melanotus subviridis* (Drakensberg and Amatole populations) and Southern *melanotus*.

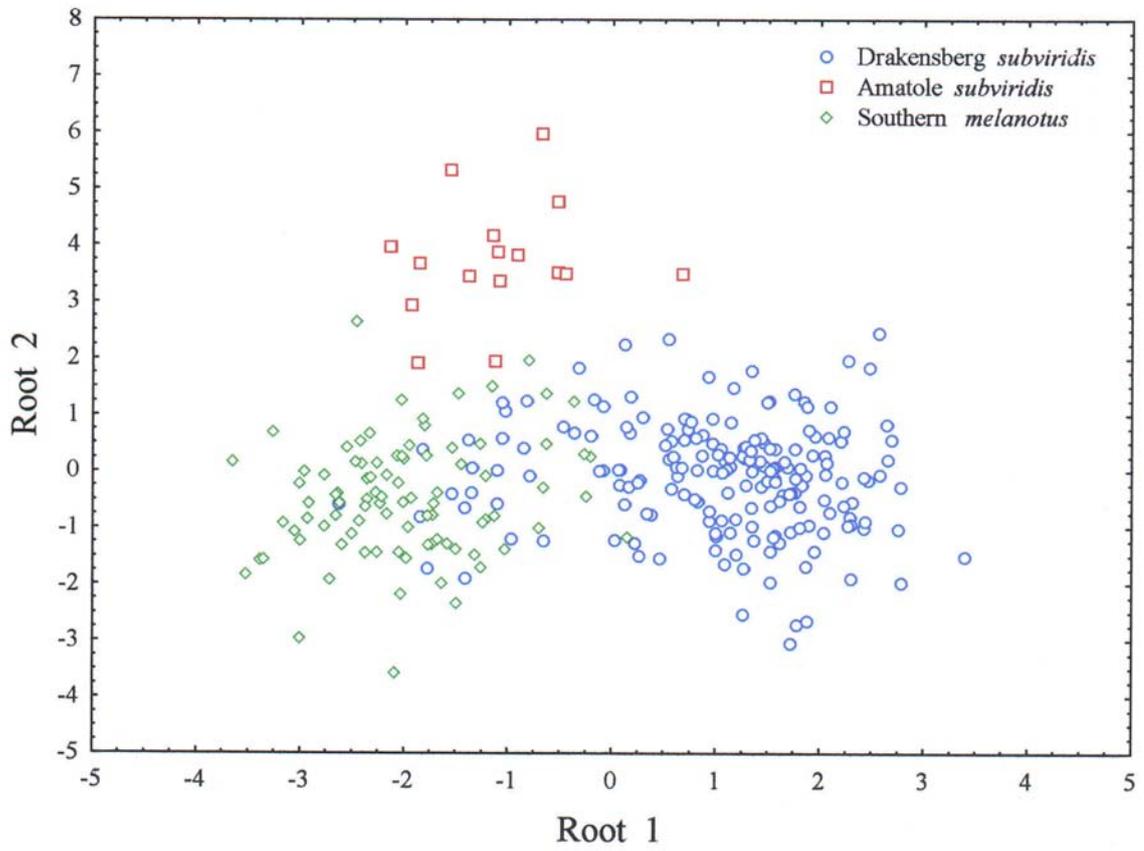
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Head length	0.971	0.119	-0.059	0.007	0.038
Head width	0.952	0.119	-0.071	-0.000	0.014
Head depth	0.952	0.094	0.001	-0.025	0.073
Forelimb	0.959	0.130	-0.039	0.005	0.028
Hindlimb	0.968	0.132	-0.015	-0.001	0.018
Fourth toe	0.932	0.098	0.115	0.004	0.003
Snout-vent length	0.940	0.227	-0.077	-0.027	0.031
Upper temporals	-0.031	0.013	-0.033	-0.091	0.291
Horizontal rows of lateral temporals	0.077	0.721	0.142	0.035	0.075
Supraoculars	0.060	-0.089	-0.066	0.057	0.220
Supraciliaries	-0.010	-0.085	0.053	0.222	0.244
Suboculars anterior to median	-0.016	0.119	0.178	-0.078	0.273
Suboculars posterior to median	-0.014	0.049	0.293	0.056	0.148
Supralabials	0.026	0.012	0.142	0.067	0.336
Infralabials	0.096	0.039	0.074	0.161	0.400
Sublabials	-0.015	-0.042	-0.020	0.543	0.063
Gulars in contact with anterior sublabials	-0.088	0.204	0.067	0.241	0.332
Gulars between posterior sublabials	0.153	-0.419	-0.049	0.010	0.492
Occipitals	0.037	0.383	0.107	-0.216	0.159
Transverse rows of dorsals	0.021	0.493	-0.078	-0.024	-0.478
Longitudinal rows of dorsals	0.173	0.443	-0.324	0.134	0.424
Transverse rows of ventrals	0.154	0.372	0.016	-0.070	0.185
Longitudinal rows of ventrals	0.007	-0.184	-0.155	-0.084	0.456
Lamellae under 4th finger	-0.024	-0.266	0.738	-0.027	0.099
Lamellae under 4th toe	-0.074	-0.178	0.762	-0.021	0.091
Femoral pores	0.096	-0.130	0.598	0.081	-0.230
Small scales posterior to interparietal	0.022	0.065	0.139	0.362	0.085
Frontonasal width in relation to length	-0.060	0.125	0.183	-0.082	0.122
Small scale present/absent behind frontonasal	0.177	0.528	0.057	0.227	-0.017
Frontonasal separates supranasals or not	0.248	-0.049	-0.014	0.378	-0.300
Frontonasal undivided/partly divided/divided	0.120	0.562	0.094	0.166	-0.231
Frontonasal in contact with loreals or not	0.078	0.129	0.283	-0.004	-0.043
Anterior frontal present/absent	0.013	0.092	-0.060	0.839	-0.013
Anterior parietal undivided/partly divided/divided	-0.097	0.049	-0.012	0.492	0.009
Median dorsals as a proportion of dorsolaterals	0.146	0.791	-0.200	-0.009	-0.131
Lateral dorsals as a proportion of dorsolaterals	0.136	0.470	-0.284	0.058	-0.009
Size of interspaces between long. rows dorsolaterals	0.077	0.761	-0.138	-0.011	-0.199
Texture of posterior infralabials	-0.059	0.009	0.090	0.039	-0.026
Eigenvalue	7.290	3.590	1.980	1.904	1.640
Percentage contribution towards total variance	19.2	9.4	5.2	5.0	4.3
Cumulative Eigenvalue	7.290	10.880	12.860	14.764	16.404
Cumulative proportion	0.192	0.286	0.338	0.389	0.432

5.3.2.3.2 **Canonical Discriminant Analysis of *Pseudocordylus melanotus subviridis* and Southern *melanotus***

The same 38 characters used above were used in this analysis (see Table 5.15). Of the 385 specimens, 294 were accepted as valid (188 Drakensberg *P. m. subviridis*, 16 Amatole *P. m. subviridis*, 90 Southern *melanotus*). Table 5.14 indicates that the three groups are largely distinguishable in morphometric space. The majority of Drakensberg *subviridis* (89.9%) and Southern *melanotus* (88.9%) were correctly identified, as were 87.5% of Amatole *P. m. subviridis*. The three groups formed fairly distinct clusters (Fig. 45). Although Drakensberg *P. m. subviridis* and Southern *melanotus* overlapped considerably, Amatole *P. m. subviridis* was largely separated from the others in morphometric space. Drakensberg *P. m. subviridis* and Southern *melanotus* exhibit partial separation along axis 1, which loads most heavily for head width (1.272), SVL (-0.776) and median dorsals as a proportion of dorsolaterals (0.430); whereas Amatole *P. m. subviridis* is largely separated from the others along axis 2, which loads most heavily for head length (3.416), head width (-2.274), hindlimb (-1.090), forelimb (0.580) and femoral pores (-0.513) (Table 5.15).

**Table 5.14:** Observed (rows) and predicted (columns) classifications of *Pseudocordylus melanotus subviridis* (Drakensberg and Amatole populations) and Southern *melanotus* according to the Canonical Discriminant Analysis.

	Percent correct	Drak. sub p = 0.639	Amat. sub p = 0.054	S mel p = 0.306
Drakensberg <i>subviridis</i>	89.9	169	0	19
Amatole <i>subviridis</i>	87.5	0	14	2
Southern <i>melanotus</i>	88.9	9	1	80
Total	89.5	178	15	101



**Figure 5.45:** Canonical Discriminant Analysis of *Pseudocordylus melanotus subviridis* (Drakensberg and Amatole populations) and Southern *melanotus*.

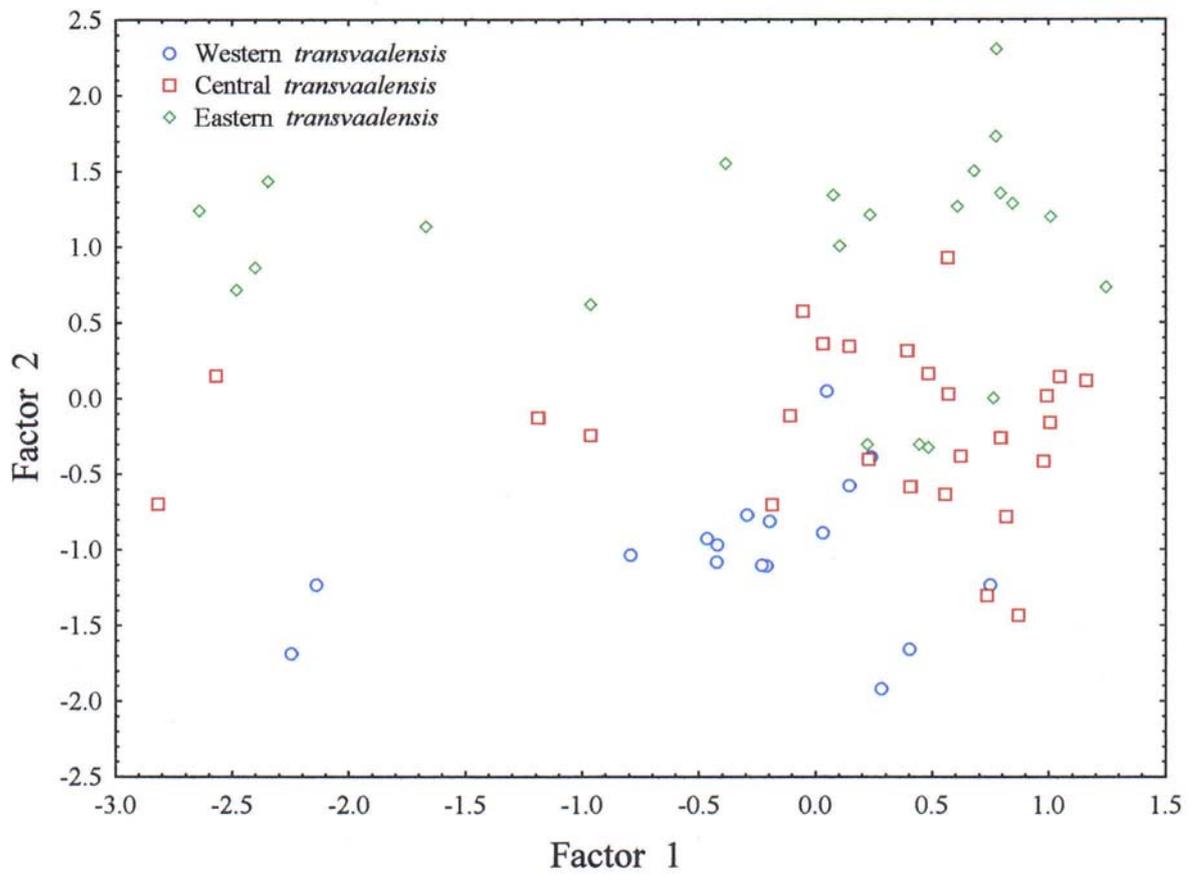
**Table 5.15:** Standardized coefficients in the Canonical Discriminant Analysis of *Pseudocordylus melanotus subviridis* (Drakensberg and Amatole populations) and Southern *melanotus*.

<b>Variable</b>	<b>Root 1</b>	<b>Root 2</b>
Head length	-0.375	3.416
Head width	1.272	-2.274
Head depth	0.142	-0.190
Forelimb	-0.267	0.580
Hindlimb	0.177	-1.090
Fourth toe	-0.127	0.005
Snout-vent length	-0.776	-0.668
Upper temporals	0.016	0.033
Horizontal rows of lateral temporals	-0.293	-0.127
Supraoculars	0.048	-0.003
Supraciliaries	0.011	-0.014
Suboculars anterior to median	-0.062	0.039
Suboculars posterior to median	0.032	-0.026
Supralabials	-0.012	-0.233
Infralabials	-0.051	0.088
Sublabials	0.149	-0.013
Gulars in contact with anterior sublabials	0.072	0.192
Gulars between posterior sublabials	0.008	0.239
Occipitals	0.079	-0.281
Transverse rows of dorsals	0.012	-0.184
Longitudinal rows of dorsals	-0.282	0.063
Transverse rows of ventrals	-0.025	-0.207
Longitudinal rows of ventrals	0.124	0.121
Lamellae under 4th finger	-0.027	-0.064
Lamellae under 4th toe	0.177	0.045
Femoral pores	-0.005	-0.513
Small scales posterior to interparietal	0.116	-0.177
Frontonasal width in relation to length	-0.052	0.059
Small scales present/absent behind frontonasal	-0.103	-0.202
Frontonasal separates supranasals or not	0.036	0.096
Frontonasal undivided/partly divided/divided	0.314	0.158
Frontonasal in contact with loreals or not	0.143	-0.009
Anterior frontal present/absent	0.096	0.118
Anterior parietal undivided/partly divided/divided	0.011	0.061
Median dorsals as a proportion of dorsolaterals	0.430	-0.197
Lateral dorsals as a proportion of dorsolaterals	0.061	-0.357
Size of interspaces between long. rows dorsolaterals	0.222	-0.061
Texture of posterior infralabials	0.046	-0.012
Eigenvalue	1.987	0.842
Percentage contribution	70.2	29.8
Cumulative proportion	0.702	1.000

5.3.2.4 ***Pseudocordylus transvaalensis* (comprising Western, Central and Eastern populations)**

5.3.2.4.1 **Principal Components Analysis of *Pseudocordylus transvaalensis***

For the analysis of *Pseudocordylus transvaalensis* a total of 35 characters were used (see Table 5.16). Of the 83 processed cases (specimens), 70 were accepted as valid, and the principal components scores of 65 specimens were plotted (17 Western, 26 Central, 22 Eastern). The PCA of *P. transvaalensis* (including the three allopatric population groups, namely Western, Central and Eastern) did not distinguish clearly between groups. There were no distinct clusters of plots (Fig. 5.46). Although the Eastern and Western population groups were largely separated on PC2, both overlapped with the Central group. The highest factor loadings for PC1 were once again all for morphometric characters: head width (0.980), head length (0.972), SVL (0.964), hindlimb (0.964), forelimb (0.946), fourth toe (0.920) and head depth (0.916) (Table 5.16). For PC2 the highest loadings were: frontonasal separates supranasals or not (0.622), occipitals (0.548), lateral dorsals as a proportion of dorsolaterals (0.531), anterior parietals undivided/partly divided/divided (0.510) and small scales posterior to interparietal (0.507) (Table 5.16). The PCA explained 47.9% of variance (19.9% in PC1, 8.4% in PC2) (Table 5.16), indicating that much residual variation is not explained on these axes.



**Figure 5.46:** Principal Components Analysis of *Pseudocordylus transvaalensis* (Western, Central and Eastern populations): Plots of the first two principal components are shown.

**Table 5.16:** Factor loadings (Varimax normalized) for the Principal Components Analysis of *Pseudocordylus transvaalensis* (Western, Central and Eastern populations).

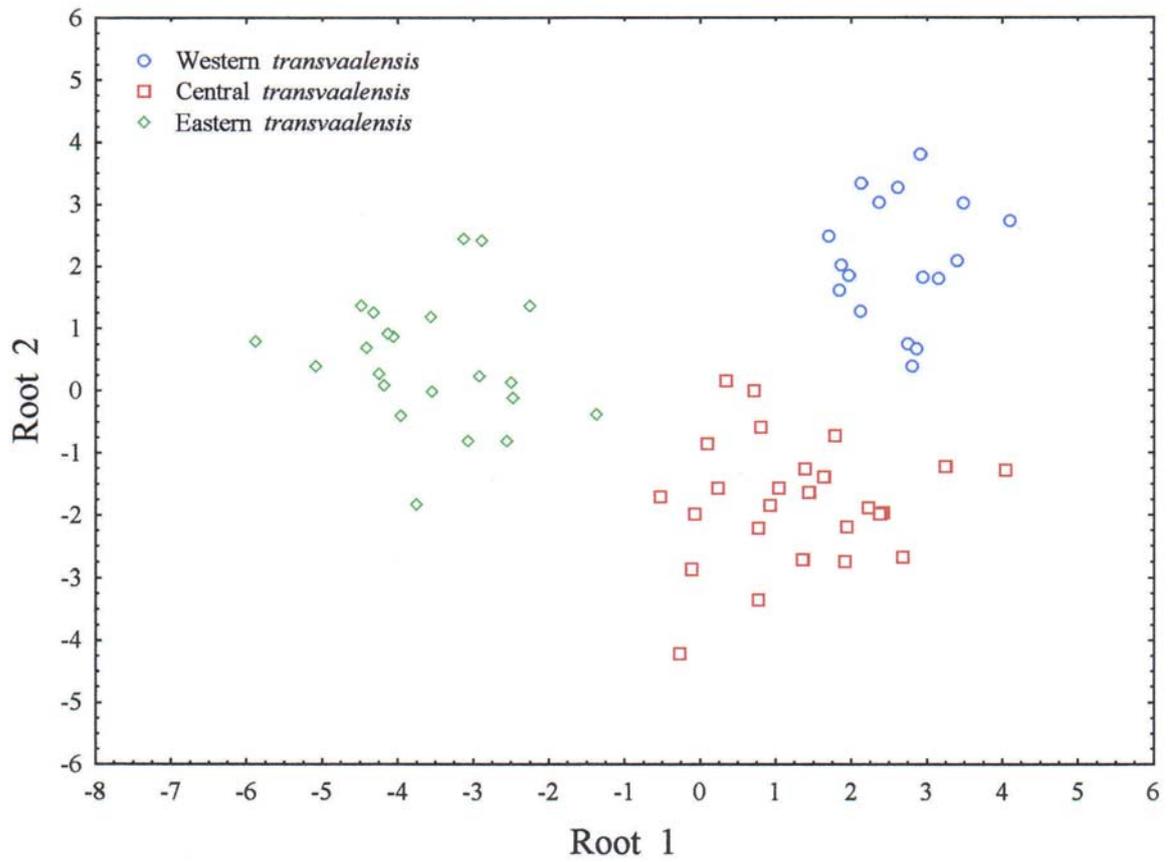
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Head length	0.972	-0.002	-0.018	-0.043	0.098
Head width	0.980	-0.014	-0.020	-0.007	0.057
Head depth	0.916	-0.135	-0.015	0.033	0.056
Forelimb	0.946	0.065	0.011	-0.055	0.040
Hindlimb	0.964	0.015	0.013	-0.048	0.060
Fourth toe	0.920	0.112	0.056	-0.066	-0.014
Snout-vent length	0.964	0.009	-0.017	-0.071	0.051
Upper temporals	-0.067	0.091	0.662	-0.046	0.154
Horizontal rows of lateral temporals	-0.180	0.052	0.116	0.328	-0.015
Supraoculars	-0.029	0.078	0.541	-0.015	0.178
Supraciliaries	0.104	0.189	-0.020	-0.118	0.278
Suboculars anterior to median	0.052	0.163	-0.256	0.552	0.179
Suboculars posterior to median	0.205	-0.023	0.001	0.041	0.678
Supralabials	-0.075	-0.003	0.015	0.435	0.218
Infralabials	0.020	0.161	0.060	0.460	-0.358
Sublabials	-0.030	0.346	0.077	0.478	-0.095
Gulars in contact with anterior sublabials	0.191	-0.174	0.509	-0.058	-0.095
Gulars between posterior sublabials	-0.205	-0.319	0.217	0.451	-0.079
Occipitals	0.162	0.548	-0.027	-0.058	-0.130
Transverse rows of dorsals	-0.005	0.402	-0.348	-0.324	0.254
Longitudinal rows of dorsals	0.215	-0.076	0.551	0.094	-0.345
Transverse rows of ventrals	0.167	-0.172	0.125	0.481	-0.087
Longitudinal rows of ventrals	0.097	-0.413	-0.272	0.433	0.127
Lamellae under 4th finger	0.163	0.368	0.483	0.269	0.026
Lamellae under 4th toe	-0.134	0.345	0.429	0.206	0.004
Femoral pores	0.307	-0.057	0.155	0.326	0.544
Small scales posterior to interparietal	-0.073	0.507	0.165	-0.056	0.243
Frontonasal width in relation to length	-0.257	-0.143	0.543	-0.094	0.190
Small scale present/absent behind frontonasal	0.015	0.255	-0.191	0.422	-0.267
Frontonasal separates supranasals or not	-0.170	0.622	-0.047	0.180	-0.002
Frontonasal undivided/partly divided/divided	-0.152	-0.187	-0.232	0.429	0.137
Anterior frontal present/absent	-0.080	0.070	0.142	0.035	0.636
Anterior parietals undivided/partly divided/divided	-0.025	0.510	-0.042	0.018	-0.142
Lateral dorsals as a proportion of dorsolaterals	-0.055	0.531	0.034	0.024	0.143
Size of interspaces between long. rows dorsolaterals	0.144	0.487	-0.001	-0.001	0.087
Eigenvalue	6.971	2.950	2.562	2.347	1.941
Percentage contribution towards total variance	19.9	8.4	7.3	6.7	5.5
Cumulative Eigenvalue	6.971	9.920	12.482	14.830	16.770
Cumulative proportion	0.199	0.283	0.357	0.424	0.479

5.3.2.4.2 **Canonical Discriminant Analysis of *Pseudocordylus transvaalensis***

The same 35 characters mentioned above were used in this analysis (Table 5.18). Out of a total of 83 cases (specimens), 65 were accepted as valid (17 Western, 26 Central, 22 Eastern). All three populations - namely Western, Central and Eastern - were classified correctly 100% of the time (Table 5.17). This is clearly evident in Fig. 5.47 showing three distinct and separate clusters of points. The Eastern population was separated from the others along axis 1, which loaded most heavily for: head length (-3.046), SVL (1.312), hindlimb length (1.196), forelimb length (0.946), anterior parietal undivided/partly divided/divided (0.793), small scales posterior to interparietal (0.744), infralabials (-0.721) and frontonasal separates supranasal or not (-0.673) (Table 5.18). Western and Central populations are largely separated along axis 2, which loads most heavily for: SVL (1.923), head width (-1.799), head depth (1.060), forelimb length (-0.955), hindlimb length (-0.897), head length (0.726) and horizontal rows of lateral temporals (0.686) (Table 5.18).

**Table 5.17:** Observed (rows) and predicted (columns) classifications of *Pseudocordylus transvaalensis* (Western, Central and Eastern populations) according to the Canonical Discriminant Analysis.

	Percent correct	W trans p = 0.262	C trans p = 0.400	E trans p = 0.338
Western <i>transvaalensis</i>	100	17	0	0
Central <i>transvaalensis</i>	100	0	26	0
Eastern <i>transvaalensis</i>	100	0	0	22
Total	100	17	26	22



**Figure 5.47:** Canonical Discriminant Analysis of *Pseudocordylus transvaalensis* (Western, Central and Eastern populations).

**Table 5.18:** Standardized coefficients in the Canonical Discriminant Analysis of *Pseudocordylus transvaalensis* (Western, Central and Eastern populations).

<b>Variable</b>	<b>Root 1</b>	<b>Root 2</b>
Head length	-3.046	0.726
Head width	0.571	-1.799
Head depth	-0.624	1.060
Forelimb	0.945	-0.955
Hindlimb	1.196	-0.897
Fourth toe	0.050	-0.008
Snout-vent length	1.312	1.923
Upper temporals	-0.026	0.480
Horizontal rows of lateral temporals	0.305	0.686
Supraoculars	-0.234	-0.184
Supraciliaries	-0.353	0.025
Suboculars anterior to median	-0.225	0.171
Suboculars posterior to median	0.490	-0.303
Supralabial	0.257	-0.251
Infralabial	-0.721	0.016
Sublabials	-0.144	0.273
Gulars in contact with anterior sublabials	-0.111	-0.175
Gulars between posterior sublabials	0.596	0.219
Occipitals	0.134	0.069
Transverse rows of dorsals	0.615	0.127
Longitudinal rows of dorsals	-0.011	-0.561
Transverse rows of ventrals	-0.571	0.279
Longitudinal rows of ventrals	0.186	0.089
Lamellae under 4th finger	-0.025	-0.548
Lamellae under 4th toe	-0.302	-0.141
Femoral pores	-0.228	0.085
Small scales posterior to interparietal	0.744	0.082
Frontonasal width in relation to length	0.244	0.104
Small scale present/absent behind frontonasal	0.037	0.412
Frontonasal separates supranasal or not	-0.673	0.012
Frontonasal undivided/partly divided/divided	-0.027	-0.308
Anterior frontal present/absent	-0.174	0.299
Anterior parietals undivided/partly divided/divided	-0.792	-0.248
Lateral dorsals as a proportion of dorsolaterals	-0.580	0.486
Size of interspace between long. rows dorsolaterals	-0.111	-0.076
Eigenvalue	7.156	2.633
Percentage contribution	73.1	26.9
Cumulative proportion	0.731	1.000

#### 5.4 Discussion

The morphological analysis indicated support for most of the genetic assemblages determined by the mtDNA analysis. For example, the northern-most member of the complex, namely *P. transvaalensis*, has a unique colour pattern (Figs 5.6 and 5.8) and is the largest species. Jacobsen (1990) pointed out that colour pattern was the most consistent character separating this taxon and *P. m. melanotus*. *Pseudocordylus transvaalensis* also differs from virtually all others in the complex in having a black throat and a series of small scales immediately posterior to the interparietal (a character first noted by De Waal 1978), as well as high frequencies of divided or partly divided anterior parietals and presence of an anterior frontal; usually four (two on either side of the head) instead of two suboculars posterior to the median subocular; and usually three (others have 1-2) horizontal rows of lateral temporals. Both PCA and CDA indicated that *P. transvaalensis* is 100% distinguishable from other taxa in the complex.

Jacobsen (1989) analyzed the three allopatric populations of *P. transvaalensis* and noted that they could be distinguished on the basis of certain scalation characteristics. As indicated in the character analysis above, distinct frequency differences do exist for the three populations with regard to at least four head shield characters, namely condition of frontonasal (divided or not), position of the frontonasal (separates supranasals or not), condition of the anterior parietals (divided or not) and number of sublabials. A CDA of the three populations showed that they were 100% distinguishable in morphological space.

De Waal (1978) distinguished the two subspecies of *P. melanotus* as follows: frontonasal usually divided in *melanotus*, entire in *subviridis*; femoral pores are shallow pits in female *melanotus*, but distinct pores in female *subviridis*; differentiated femoral scales 1-17 in *melanotus*, usually 19-34 in *subviridis*; dorsolateral scales closely spaced or in contact in *melanotus*, well separated in *subviridis*; lateral temporals usually in two rows - the upper row consisting of elongated scales - in *melanotus*, usually in a single row of much elongated scales in *subviridis*. The present study found that the frontonasal was usually divided in *melanotus* populations and undivided in *subviridis* populations. However, northern-most *melanotus* populations had mostly undivided frontonasals. Although most *melanotus* had two rows of lateral temporals on either side of the head, the number varied

from one to two in *subviridis*. Nevertheless, when two rows were present in *subviridis* the scales of the upper row were usually much more elongated than in *melanotus*. Most *melanotus* females had pit-like femoral pores and most *subviridis* females had larger pores with secretary plugs. However, there was some intralocality variation in this character amongst *subviridis* (Table 5). There was also considerable variation in the number of differentiated femoral scales in males, although *subviridis* tended to have higher counts. In *subviridis* the longitudinal rows of dorsolaterals were usually well separated, whereas they were in close proximity in *melanotus*. Although Broadley (1964) used this character to separate populations of *melanotus* and *subviridis* in KwaZulu-Natal, there was considerable variation. Morphologically, Southern *melanotus* and *P. m. subviridis* were the least distinct of the taxa/groupings studied. The Hogsback (Amatole-Winterberg) subclade of *P. m. subviridis* is diagnosable using discriminant analysis, but specimens from the other populations (clades C, D, E) cannot be distinguished from one another morphologically (e.g. Table 5.1 to 5.3).

Branch (1988a, 1998: 207) appears to follow De Waal (1978) with regard to the characterization of the two subspecies of *P. melanotus*, but incorrectly states that in *P. m. subviridis* the “lateral scales” (probably in reference to the dorsolaterals) are “larger than the spaces between them” (this refers to the typical *melanotus* condition). Branch (1998) appears to follow Jacobsen’s (1989) concept of *P. transvaalensis*, but is wrong in stating that in *P. transvaalensis* the lateral (probably meaning dorsolateral) scales are smaller than the spaces between them (they are bigger – see also Jacobsen 1989), and that female *P. transvaalensis* have well developed femoral pores (the latter are shallow pits - paratypes and others examined, similar to female *P. m. melanotus*). In addition, Branch (1998) did not plot Jacobsen’s (1989) isolated Gauteng sub-population of *P. m. melanotus*, or Broadley’s (1964) somewhat isolated Qudeni Forest record (2830DB) for this subspecies.

The northern-most population of *P. m. melanotus* (Northern *melanotus*) differs from other consubspecifics in having high frequencies of undivided frontonasals and low occurrences of a small scale posterior to the frontonasal. The mtDNA phylogeny indicated that Northern *melanotus* is deeply divergent and genetically very distinct from all other taxa in the *P. melanotus* species complex. It also indicated distinct genetic structuring between the Sabie and Lochiel populations, but not significant genetic

divergence. With regard to morphology, the Sabie population tends to have greater numbers of transverse and longitudinal rows of dorsals, and greater numbers of lamellae under both the fourth finger and fourth toe (Table 5.3). The CDA of morphological characters – using Northern and Southern *melanotus* and *P. m. subviridis* - indicated that Northern *melanotus* was largely distinguishable and may be considered a diagnosable group.

The isolated *P. m. melanotus* populations at Suikerbosrand and Nkandhla district did not exhibit meaningful morphological or genetic differentiation from other *P. m. melanotus*.

Monontsha Pass and the nearby locality Thibella (see Appendix 5.1) were the only sites where morphologically intermediate specimens were collected. However, while most specimens from the locality Qoqolosing were morphologically *melanotus*-like, all but one (= *melanotus*) of the specimens sequenced was included in the *subviridis* group. The single specimen from Thibella was largely *subviridis*-like morphologically and was referable to this group in the mtDNA analysis.

The mtDNA analysis indicated that the Amatole, S Lesotho and Naude's Nek populations, together with *P. microlepidotus*, formed a clade within the *P. m. subviridis* group. Although none of these populations can be distinguished from one another or from other *P. m. subviridis* on the basis of one or more individual characters, a CDA of morphological characters – using the Drakensberg and Amatole populations of *P. m. subviridis* and Southern *melanotus* – showed that the Amatole population was largely distinguishable on the basis of a unique combination of character states and can be regarded as a diagnosable unit. This population may have been separated from the main population in relatively recent times and although it has developed some morphological differences it has not yet reached a significant level of genetic divergence.

*Pseudocordylus langi* differs from all others in the complex with regard to the granular nature of its dorsal scales and its distinct colour pattern (e.g. blue spots on the flanks). With very few exceptions it also differs in having a smooth posterior infralabial, high numbers of femoral pores (25-34) and low numbers of infralabials (usually five on either side). Both the PCA and CDA showed that *P. langi* is 100% distinguishable in morphological space. Although *P. langi* has been confused with *P. m. subviridis* in the

past (*e.g.* Loveridge 1944, FitzSimons 1948), Broadley (1964) showed that the two taxa differed morphologically. The findings of the present study confirm the latter author's conclusions in this regard.

FitzSimons described *Pseudocordylus spinosus* in 1947. In his earlier revision he had included some specimens of *P. spinosus* under the name *P. subviridis subviridis* (FitzSimons 1943). However, *P. spinosus* differs from all others in the complex in that the lateral scales are spinose (except for one juvenile), the frontonasal is almost always distinctly longer than wide and in contact with the loreals, "occipitals" are keeled but smaller than the scales behind them, dorsals are in contact or very narrowly separated, femoral pore counts are very low (6-9), and females have differentiated glandular femoral scales (generation glands). Both the PCA and CDA showed that *P. spinosus* is 100% distinguishable in morphological space.

In conclusion, the analysis of morphological variation in the *P. melanotus* species complex indicated support for the following genetic assemblages identified in the mtDNA analysis: *P. transvaalensis*, Northern *melanotus*, Southern *melanotus* and *P. langi*. *Pseudocordylus m. subviridis* is part of an assemblage comprised of three clades, one including *P. spinosus* and another including *P. microlepidotus*. The latter three species are morphologically distinguishable from all others in the complex, although *P. m. subviridis* is occasionally difficult or even impossible to separate from Southern *melanotus*. However, apart from the Hogsback (Amatole-Winterberg) subclade of *P. m. subviridis*, which is diagnosable using discriminant analysis (Chapter 5), specimens of *P. m. subviridis* from the other populations (clades C, D, E) are morphologically indistinguishable from one another (*e.g.* Table 5.1 to 5.3).

## CHAPTER 6

### Conclusions

#### 6.1 Type specimens and type localities

Name-bearing types provide a standard against which specimens can be compared to determine whether or not they are conspecific (or consubspecific). In the present study, lectotypes and paralectotypes were designated for *Cordylus microlepidotus* (= *Pseudocordylus microlepidotus microlepidotus*), *Cordylus (Pseudocordylus) melanotus* (= *P. melanotus melanotus*) and *Cordylus (Pseudocordylus) subviridis* (= *P. melanotus subviridis*), whereas lectotypes and alloparalectotypes were designated for both *Cordylus (Pseudocordylus) fasciatus* (= *P. microlepidotus fasciatus*) and *Pseudocordylus subviridis transvaalensis* (= *P. transvaalensis*). Bradley's (1964) relegation of the male "cotype" of *C. (P.) melanotus* to the synonymy of *C. (P.) fasciatus* was shown to be untenable. The type specimens of *melanotus*, *subviridis* and *fasciatus* have all long been considered lost, but their current designation provides future researchers with a starting point for comparisons.

Appropriate restriction of type localities helps define the spatial distribution of taxa. The type locality of *Pseudocordylus microlepidotus namaquensis* was restricted to the vicinity of the Roggeveldberg, Komsberg and Nuweveldberg mountains of the Great Escarpment in the Northern and Western Cape Provinces; whereas the type locality of *C. (P.) subviridis* was restricted to the upper reaches of Menyameng Pass in the Maloti Mountains of Lesotho, based on entries in Andrew Smith's diary and journal. The morphology of specimens designated as types of *melanotus* and *subviridis*, and restriction of the type locality of *subviridis* to a site in the western Maloti Mountains, confirms De Waal's (1978) interpretation and definition of the two taxa, which were difficult to distinguish based on Smith's (1838, 1843) descriptions.

## 6.2 Taxonomic status

Sequence data from the 16S rRNA mtDNA gene indicated seven clades within the *P. melanotus* species complex, comprising *P. langi* (most basal), Northern *melanotus*, Southern *melanotus*, *P. transvaalensis* and three clades containing all *P. melanotus subviridis*. One of these includes *P. spinosus*, whereas another includes *P. microlepidotus*. Despite support for the clades (MP 77-100%, BI 1.0, ML 69-100% except clades D, G), relationships between *P. transvaalensis*, Southern *melanotus* and the *P. m. subviridis-spinosus-microlepidotus* assemblage were not resolved. Nevertheless, the MP topology indicated that Southern *melanotus* was more closely related to *P. transvaalensis* than to Northern *melanotus*. Although the topology indicated that both the *P. langi* and Northern *melanotus* clades were deeply divergent, the short internal branch lengths for the other groups suggest a recent, rapid divergence and radiation of populations.

According to the mtDNA analysis, *Pseudocordylus transvaalensis* forms a distinct clade more closely related to Southern *melanotus* than Northern *melanotus*. This is at least partly supported by allozyme data. *Pseudocordylus transvaalensis* is distinguished morphologically from all others in the complex in that it is larger, has a distinct colour pattern on the back and throat, and a series of small scales posterior to the interparietal. There are also other less definitive features (*e.g.* usually two suboculars behind the median subocular on either side of the head). Both Principal Components Analysis (PCA) and CDA, using morphometric and scale characters, indicated that this species is 100% distinguishable from other taxa in morphological space. It can be concluded that, on the basis of genetic and morphological data, *P. transvaalensis* is a distinct and valid species. This species consists of three allopatric populations in Limpopo Province. Jacobsen (1989) reported morphological differences between these populations, but failed to provide supporting data. In the present study it was determined that there were noteworthy frequency differences between the three populations with regard to certain head shield characters. A separate CDA of the three *P. transvaalensis* populations using the same morphometric and scale characters mentioned above showed that they were 100% distinguishable in morphological space. However, neither the allozyme nor mtDNA analyses indicated significant genetic divergence between the sampled central and eastern populations, suggesting that they have only recently been separated.

Nevertheless, Wiley (1981) noted that electrophoretic similarity might be decoupled from morphological divergence, while Wiens & Penkrot (2002: 73) stated that “species may be distinct and even morphologically diagnosable from one another but still have non exclusive gene genealogies.” While it is clear from both the genetic and morphological evidence that *P. transvaalensis* must be considered a species separate from *P. melanotus*, the observed morphological differences suggest that some other form of molecular analysis (*e.g.* microsatellites) of all three regional populations may be required to evaluate the possibility that this species is polyphyletic.

Specimens from the vicinity of GaSelati River in the eastern escarpment, previously identified as *P. transvaalensis*, were determined to be *P. m. melanotus*, indicating that these two species may be parapatric. As noted below, an attempt should be made to collect *P. m. melanotus* (or *P. transvaalensis*) from this area to establish whether the two taxa are in fact parapatric, or possibly even sympatric. If the *P. m. melanotus* population is confirmed and the Olifants River gorge is indeed a major geographic barrier that caused the separation of *P. transvaalensis* from other populations of *Pseudocordylus*, then, considering its isolation, this population may represent a genetically differentiated relict.

There are also ecological differences between *P. transvaalensis* and *P. melanotus*: the former is usually solitary, found alone in crevices and alone on rocky outcrops, whereas 2-3 *P. m. melanotus* (and *P. spinosus*) and up to a dozen *P. m. subviridis* occasionally occupy the same crevice and they are often found in groups on outcrops (Jacobsen 1990, Branch 1998; pers. obs.).

Both the northern (Northern *melanotus*) and southern (Southern *melanotus*) populations of *P. m. melanotus* are distinct from *P. m. subviridis* according to the mtDNA analysis. There was some supporting evidence from the allozyme analysis in that populations of *P. m. melanotus* were separated from *P. m. subviridis* by a fixed allelic difference at locus *AAT-2*. However, the sequence data indicated that Northern *melanotus* is deeply divergent from all other populations of *P. melanotus* (Southern *melanotus* and *subviridis*). There is also allozyme evidence for this, as both the Sabie and Lochiel populations differ from others analyzed at locus *GPI*. However, there is a fixed allelic difference between the populations from Sabie (Mpumalanga Escarpment proper) and Lochiel (Barberton Mountainlands). The latter situation may, however, be indicative of recent fragmentation

and inbreeding rather than a long period of isolation. According to the mtDNA analysis there is only 0.24% sequence divergence between the Sabie and Lochiel populations. While the whole Northern *melanotus* population appears to be allopatric to the rest of *P. m. melanotus*, there are no clear indications that the Sabie and Lochiel populations are isolated from one another. Morphologically, Northern *melanotus* is characterized by having a high frequency of undivided frontonasals (usually divided in Southern *melanotus*) and there is seldom a small scale posterior to the frontonasal (often present in Southern *melanotus*). A Canonical Discriminant Analysis (CDA) of the three populations of *P. melanotus* (Northern *melanotus*, Southern *melanotus* and *P. m. subviridis*) indicated that Northern *melanotus* is largely distinguishable in morphological space. Several allozyme and mtDNA studies have resulted in the detection of morphologically cryptic - or nearly indistinguishable - species (see Hillis 1987). The genetic and morphological data indicated that Northern *melanotus* represents a distinct lineage and should be described as a new species. Southern *melanotus* should be recognized as a monotypic species, *i.e.* *P. melanotus*. It is genetically, and to a large extent morphologically, distinct from *P. m. subviridis* (see below). Smith's (1838, 1843) descriptions of *P. melanotus* were based on specimens from the eastern Free State (Ficksburg administrative district according to De Waal 1978), *i.e.* within the range of "Southern *melanotus*". Therefore it is "Northern *melanotus*" that requires a new name.

Both the allozyme and mtDNA analyses suggested high levels of gene flow between populations of Southern *melanotus* from Harrismith, Vrede and Amersfoort, despite the fact that rocky outcrops in these areas are often separated by intervening grassland. Neither the Suikerbosrand nor Nkandla populations, despite their apparent isolation, differed significantly genetically or morphologically from other Southern *melanotus*. However, with regard to allozymes, the Nkandla population was indistinguishable from *P. transvaalensis*. It appears to have become fixed for the same allele as *P. transvaalensis* (rather than all other *P. m. melanotus*) at genetic locus *GLDH* by chance rather than due to recent migration. The fixed difference relative to other *P. m. melanotus* suggests that fragmentation and inbreeding, or recent isolation, has occurred.

As noted above, the allozyme analysis determined that there was also a fixed allelic difference between all *P. m. melanotus* and *P. m. subviridis* populations at locus *AAT-2*. The mtDNA analysis showed that the three clades containing *P. m. subviridis* (with *P.*

*spinosus* and *P. microlepidotus*) formed a group most closely related to Southern *melanotus* and *P. transvaalensis*. In clade C, representing populations from the north of *P. m. subviridis*, inter-digitation of samples suggests high levels of gene flow between the populations from Monontsha Pass, Organ Pipes Pass, Witzieshoek and Qoqolosing. However, the three populations in clade D are also represented in clade C. Clade D is the sister group to clade E, which contains all southern populations of *P. m. subviridis*, including the isolated Amatole-Winterberg population. The relationships of clades C to E therefore require further investigation. Morphologically, *P. m. subviridis* differs from the two populations of *P. m. melanotus* as follows: frontonasal usually undivided versus usually divided respectively (but usually undivided in Northern *melanotus*), lateral temporals usually in a single row of greatly elongated scales versus usually two rows (upper elongated, lower not), longitudinal rows of dorsolaterals widely spaced versus closely spaced. A CDA using morphometric and scale characters indicated that *P. m. subviridis* is largely distinguishable from other populations in the complex. However, even a separate CDA of the three populations of *P. melanotus* (Northern *melanotus*, Southern *melanotus* and *subviridis*) indicated considerable overlap in morphological space. Although it is apparent that the *P. m. subviridis* populations together form a paraphyletic assemblage, with *P. spinosus* and *P. microlepidotus* embedded within, *P. m. subviridis*, *P. spinosus* and *P. microlepidotus* (which are all morphologically distinguishable) should all, as an interim measure, be considered full species pending a detailed analysis of the *subviridis-spinosus-microlepidotus* complex.

Both the allozyme and mtDNA analyses indicated that the population from Monontsha Pass - in an apparent contact zone between *P. m. melanotus* and *P. m. subviridis* - is in fact referable to *P. m. subviridis*. De Waal (1978) had assigned specimens from this locality to both taxa as well as the category "intermediates". It was in fact this apparent hybridization and resultant morphological intermediacy that had prompted De Waal (1978) to treat the two taxa as subspecies of *P. melanotus*. However, there were no heterozygous individuals in the sample used in the allozyme analysis. There was some conflict between the allozyme and mtDNA analyses with regard to populations from the Qwa-Qwa region. All specimens from the localities Qoqolosing (usually morphologically *melanotus*-like) and Thibella (morphologically intermediate but closer to *subviridis*) grouped with *P. m. melanotus* in the allozyme study, but with *P. m. subviridis* (except for one specimen from Qoqolosing [= *melanotus*]) in the sequencing analysis.

With regard to the allozyme results, this may merely imply random fixation of alleles. The mtDNA results indicate that this region represents a zone of parapatry. Intensive collecting and further analysis of lizards from this area would elucidate relationships.

The taxonomic status of the allopatric Amatole-Great Winterberg population of *P. m. subviridis*, and possibly other populations (see below), requires further investigation. The allozyme analysis indicated that there was a fixed allelic difference between the Hogsback (Amatole) and S Lesotho versus other *P. m. subviridis* populations at locus *GLDH*. S Lesotho *P. m. subviridis* was genetically inseparable from the Amatole group, rather than the Drakensberg group, as might have been suspected considering their geographical proximity. Also, the mtDNA analysis indicated that the latter two populations, the population from Naude's Nek and *P. microlepidotus* formed a clade (E). A CDA of *P. m. subviridis* (Maloti-Drakensberg and Amatole populations) and Southern *melanotus* indicated that the Amatole population is largely differentiated morphologically.

Historical confusion over the status of *P. langi* was the result of poor judgment on the part of both Loveridge and FitzSimons. Loveridge (1944) described the species on the basis of a single specimen, but then assigned paratype status to specimens he had not personally examined. FitzSimons (1948), who had examined these specimens for Loveridge, later re-examined them and decided that the latter had described nothing but a sparsely scaled *P. m. subviridis* as a new species. However, FitzSimons did not examine the holotype of *P. langi* and thus relegated a perfectly good species – as revealed by Broadley in 1964 - to the synonymy of *P. m. subviridis*. Both the allozyme (*e.g.* fixed allelic difference with sympatric *P. m. subviridis*) and mtDNA analyses demonstrated that *P. langi* is genetically distinct from others in the complex. A detailed morphological examination showed that *P. langi* is distinguishable by its dorsal scalation: granular scales with a paravertebral row of 6 to 9 enlarged, flat scales; five smooth infralabials on either side of the head, and as many as 12 to 17 femoral pores on each thigh. In both PCA and CDA *P. langi* was distinct from all other taxa/groups.

Morphologically, *P. spinosus* is easily distinguished from all other taxa in the *P. melanotus* species complex by: spinose lateral scales, low numbers of femoral pores (6-9 on both thighs), frontonasal longer than it is wide and excluded from the loreals. In both

PCA and CDA it is clearly separated from other taxa. This species was not included in the allozyme analysis as its status was not considered problematic, but in the mtDNA phylogram it was, rather surprisingly considering its morphological distinctness, imbedded within *P. m. subviridis*. The specimens used in the mtDNA analysis, although morphologically typically *spinosus*-like, shared the same 16S rRNA haplotype as several specimens referable, morphologically and genetically, to *P. m. subviridis* (see Chapter 4). As mtDNA is a reflection of maternal inheritance, one explanation is that the sampled population consists of hybrids between male *P. spinosus* and female *P. m. subviridis*, but it is more likely that this topology indicates that *P. spinosus* arose from a *P. m. subviridis* ancestor (see section 4.4). In order to clarify the status of *P. spinosus* it will be necessary to collect samples from further away and from areas where “pure” *P. spinosus* is likely to occur - *i.e.* where *P. m. subviridis* does not occur - such as at lower elevations in the Drakensberg of western KwaZulu-Natal (see below).

According to Figure 4.2, *P. microlepidotus* also arose from a *P. m. subviridis* ancestor. The close relationship between these two taxa was supported by J. Melville’s (unpublished data) finding that these two species were the sister group to *P. m. melanotus*.

This study demonstrates a few instances of mismatches between morphology and genetics in the various taxa in the *P. melanotus* species complex. In the case of *P. spinosus* there is high morphological resolution but no genetic resolution. The opposite situation occurs with regard to Northern *melanotus*, although there are minor morphological differences. With regard to *P. langi* there are high levels of morphological and genetic resolution, with high levels of concordance. However, in the case of *P. transvaalensis* there is high morphological resolution but only moderate genetic resolution. Some populations of *P. m. subviridis* are morphologically similar to Southern *melanotus*, while most others are at least moderately dissimilar, but there is moderate genetic resolution from other taxa.

### 6.3 Biogeography

Representatives of the Cordyloidea (*i.e.* *Paramacellodus*, *Saurillus*, *Pseudosaurillus*), also known as cordyloids, were present in England in the Upper (= Late) Jurassic (Estes

1983; Rocek 1984). Other fossils found in Europe, namely *Pseudolacerta lamandini* Filhol, 1888 and related forms (early to middle Eocene, c. 50 million years ago), and *Palaeocordylus bohemicus* Rocek, 1984 (Lower Miocene, c. 20 mya) are referable to the family Cordylidae, which led Rocek (1984) to suggest that cordylids inhabited Europe during the Jurassic-Miocene period. Estes (1983) suggested that the Cordylidae moved southwards from Europe and were restricted to sub-Saharan Africa and Madagascar after the Sahara Desert was formed. Cordylids may thus have evolved following the restriction of widespread primitive cordylids to areas in Africa as a result of separation by Cretaceous epicontinental seas. Alternatively, the cordylids may have had an African centre of origin and dispersed northwards into Europe, possibly during peak Eocene tropicality when other lizard groups also extended their ranges (Estes 1983). It should be noted that much of Europe was submerged during the Cretaceous. Lang's (1991) morphology-based phylogeny supported an African origin for the Cordyliformes (Cordylidae and Gerrhosauridae). He was of the opinion that both *Pseudolacerta* and *Palaeocordylus* represented either a single, or two separate, northward dispersals during peak Eocene tropicality.

The present day geographical distribution of the various taxa in the *P. melanotus* species complex was discussed in detail in section 2.8 of Chapter 2 and is illustrated in Fig. 2.1 (localities listed in Appendix 2.1). *Pseudocordylus transvaalensis* occurs in three allopatric populations (1700-2000 m) in Limpopo Province. *Pseudocordylus m. melanotus* has an extensive distribution in Mpumalanga Province, N Swaziland, NW KwaZulu-Natal and NE Free State (1400-2300 m), with an isolated population at Suikerbosrand and adjacent areas (1500-1860 m) in Gauteng, and in the Nkandhla district of central KwaZulu-Natal (1100-1500 m). *Pseudocordylus m. subviridis* occurs in two allopatric populations, one in the Maloti-Drakensberg and associated areas (1400-3200 m) and another in the Amatole Mountains and vicinity (1400-1600+ m). The two subspecies of *P. melanotus* are parapatric in the Qwa-Qwa region. *Pseudocordylus langi* is restricted to the edge and summit of the Drakensberg (2805-3048 m) in the area from Mont-aux-Sources to Organ Pipes Pass, where it is sympatric (and microsympatric) with *P. m. subviridis*. *Pseudocordylus spinosus* occurs on the lower to middle slopes (900-2517 m) of the Drakensberg in KwaZulu-Natal and the Free State where it is sometimes sympatric (apparently not microsympatric) with *P. m. subviridis*. *Pseudocordylus m. microlepidotus* is widespread in the Cape Fold Mountains (20-1920 m a.s.l.), *P. m.*

*fasciatus* occurs in the inland mountains of the Eastern Cape Province (440-1900 m), whereas *P. m. namaquensis* is restricted to the Nuweveldberg and Komsberg ranges (around 1600 m). Microgeographic occurrence depends on suitable narrow, deep rock crevices.

Based on Wiens & Penkrot's (2002) criteria (see section 1.6), *P. langi* must have been separated from other members of the *P. melanotus* (and *P. microlepidotus*) species complex for a relatively long period of time (it has an exclusive haplotype phylogeny and is morphologically distinct). With reference to the same criteria, a similar situation applies with regard to Southern *melanotus* (occasionally indistinguishable from *P. m. subviridis*) and *P. transvaalensis*. *Pseudocordylus spinosus*, on the other hand, is morphologically distinct but has a non-exclusive haplotype phylogeny, being nested within a clade (C) containing *P. m. subviridis*. Despite its morphological distinctness, the former species may therefore have been reproductively isolated only recently. A similar situation occurs with regard to *P. microlepidotus*, which is morphologically less distinct (from *melanotus*, *subviridis* and *transvaalensis*). According to Wiens & Penkrot's (2002) criteria (but see below) Northern *melanotus* may have been separated for only an intermediate period, because although it has an exclusive haplotype phylogeny, it is not always distinguishable morphologically from Southern *melanotus*. Although *P. m. subviridis* populations in clades C, D and E form a genetically and partly morphologically (sometimes indistinguishable from Southern *melanotus*) discernable group, relationships between clades are less clear (see above). While the three clades are genetically distinct, they are not morphologically distinct, and may also therefore have been separated for only intermediate periods of time.

According to Bauer (1999), the majority of mountain chains in southern Africa were produced during two periods of uplift in the Oligocene-Miocene and Pliocene-Pleistocene. Africa underwent planation during the Tertiary sub-era, resulting in a relatively even land surface. However, during the Pliocene, down-flexing of continental margins occurred with a concomitant rise of the escarpment to, in most places, greater elevations than those of today; and the Pleistocene in southern Africa was marked by the upheaval of the sub-continent to its present plateau form (Partridge & Maud 2000).

During the Cenozoic Era the earth experienced a progressive decline in temperature, starting in the Paleocene and continuing until the end of the Miocene, after which time a series of oscillations occurred (Brain 1985). At the end of the Miocene, about 6.5 to 5 million years ago, an Antarctic ice sheet grew rapidly, to a size and extent much greater than at present. This drop in temperature is known as the Terminal Miocene Event and resulted in a rapid sea level drop of 100 m worldwide. During the subsequent Pliocene, temperatures increased by a few degrees Celsius, but then dropped again about 2.6 to 2.5 million years ago, at which time a major ice cap formed in the northern hemisphere. In the following Pleistocene, temperatures oscillated between glacials and interglacials at least 17 times (Brain 1985). Several sites with reported glacial and/or periglacial landforms or processes are located at high altitudes in the Maloti-Drakensberg, Amatole Mountains and Cape Fold Mountains (Boelhouwers & Meiklejohn 2002). According to Deacon & Lancaster (1988: 95), the Basutolian Ecotone (*i.e.* Maloti-Drakensberg and adjacent plateau areas) “is unique in possessing periglacial features that are direct evidence of colder temperatures in the Late Pleistocene.” However, although there is evidence of glaciation in southern Africa during earlier periods in the earth’s history, as evidenced in Dwyka Group sediments, Deacon & Lancaster (1988) noted that this area was never glaciated during the Quaternary, while Boelhouwers & Meiklejohn (2002) consider the literature in support of Quaternary glaciation in the subcontinent to be contentious. Support for such glaciation in Lesotho and on the southern African escarpment is based on the idea that Antarctic polar fronts were situated further north and thereby increased the amount of winter snow. Nevertheless, southern Africa was 5-9°C cooler during the Quaternary than at present with marked changes in rainfall patterns (Deacon & Lancaster 1988). According to Boelhouwers & Meiklejohn (2002) conditions in the subcontinent during the Last Glacial Maximum were probably colder (by 5-10°C) and drier (precipitation about 70% of current values in high elevation areas) than at present, with at least deep seasonal ground freezing.

The Quaternary sub-era was characterized by relatively long (100 000 years), cold and mostly dry glacial periods alternating with relatively short (10 000 years), warm and mostly wet interglacials. During glacials the polar ice sheets expanded and sea levels dropped; whereas during interglacials there were smaller ice fields and higher sea levels. In the southern hemisphere the ice sheets advanced northwards from the south pole, resulting in colder conditions in southern Africa. During the Plio-Pleistocene, according

to Hewitt (2000: 907), species “went extinct over large parts of their range, some dispersed to new locations, some survived in refugia and then expanded again, and this must have occurred repeatedly.” Hewitt also noted that different parts of a species’ range might have been colonized at different rates because of physical barriers or previous inhabitants, leading to distinct genetic structures. Also, the varied topography of mountain chains may have subdivided species into populations that evolved independently with only occasional gene flow. Distinct genetic differences between individuals of *P. m. subviridis* from certain localities in the northern Drakensberg may be the result of long-isolated parts of the population moving to an area where prior inhabitants were present. Alternatively, the movements of lizards with distinct genotypes, from two different areas, may have converged.

According to Brain (1985: 49), African habitats “have been repeatedly affected by low temperature episodes during the last few million years”, with minimum winter temperatures “depressed by between 5°C and 10°C on each occasion.” Brain (1985: 51) also added that the “alternating cycles of higher and lower temperature, which have repeatedly affected southern Africa, have caused expansions and contractions of temperature zones. In turn, ranges of plants and animals sensitive to temperature thresholds will have expanded and contracted, such expansions and contractions being similar to the rising and sinking of a water level around intricate topography - the moving front will have created many islands, serving to break up a once continuous range into disjunct patches. It is possible that such patches will have served as centres of allopatric speciation.”

Jacobsen (1989) suggested that the distribution ranges of Cape Temperate reptiles and amphibians were already established prior to the Pleistocene, at a time when the Limpopo depression, with its mainly arid climate, was not a barrier to movement. The Limpopo depression was formed about 7-15 million years ago during the Miocene epoch, at the time of the marginal down-flexing of coastal land surfaces. According to Jacobsen (1989: 1473), the occurrence of species such as *Bitis atropos* in the highlands of eastern Zimbabwe, separated from other conspecific montane populations along the Great Escarpment by the Limpopo gap (Broadley 1983; Branch 1998), is indicative of a previous widespread distribution (of a temperate fauna) during cooler Pliocene [and Miocene] climates. He added (p. 1473) that “climatic events during the Pleistocene were

not of significant magnitude to permit migration routes across the Limpopo gap and it is considered that these faunas are relics of a former wider distribution.” Six more snake species occur in association with the Cape Fold Mountains, Maloti-Drakensberg and Mpumalanga escarpment, and are also found in the highlands of eastern Zimbabwe, namely *Amphlorhinus multimaculatus*, *Psammophis crucifer*, *Hemachatus haemachatus*, *Duberria lutrix* (Zimbabwean population is a separate subspecies), *Causus rhombeatus* and *Lycodonomorphus rufulus*; while an additional two snakes (*Typhlops bibronii* and *Leptotyphlops conjunctus*) and one lizard (*Acontias plumbeus*) have similar ranges, but do not occur in the Cape Fold Mountains (Broadley 1983; Branch 1998). Frogs with similar distribution ranges associated with the South African and Zimbabwean escarpments include *Vandijkophrynus*, *Strongylopus fasciatus*, and the paired grouping of *S. grayii* (south) and *S. rhodesianus* (north), until recently considered subspecies (Poynton 1964; Channing 2001; Minter, Burger, Harrison, Braack, Bishop & Kloepfer 2004). The Vlei Rat, *Otomys irroratis* (Brants, 1827), also has a similar distribution (De Graaff 1981; Skinner & Chimimba 2005). There is thus considerable evidence that the establishment of the hot and dry Limpopo gap caused the separation of widely distributed, mostly temperate terrestrial vertebrates. Many of the above species are not restricted to mountainous areas and appear to have expanded their ranges in association with the expansion of grassland (see Branch 1990).

Although there are no known populations of *Pseudocordylus* north of the Limpopo valley, *Cordylus mossambicus* FitzSimons 1958, occurs in the Gorongosa Mountain in Mozambique, extending to the lower slopes of the Chimanimani Mountains in the highlands of eastern Zimbabwe. It is morphologically similar to the larger *Pseudocordylus* (i.e. *P. microlepidotus*, *P. transvaalensis*, *P. m. melanotus*) – e.g. the enlarged dorsal scales are embedded in granular skin, and the back is blackish with bright orange flanks in breeding males (similar to *melanotus* and *subviridis*) and dark brown with transverse rows of small white or yellow spots in females and juveniles (again similar to *melanotus* and *subviridis*) (FitzSimons 1958; Broadley 1966; Branch 1998). *Cordylus regius* Broadley, 1962, is found in granite outcrops in the Umtali district of eastern Zimbabwe. It is a similar species with bright yellow or orange flanks in males and yellowish-brown flanks in females and juveniles, although it lacks granules on the back (Broadley 1962, 1966; Branch 1998). *Cordylus mossambicus* and *C. regius* are the only two species of *Cordylus* in which males have distinct, bright orange or yellow

flanks, a characteristic of several taxa in the genus *Pseudocordylus* (Chapter 5; Branch 1998). The relationships of these two species to other cordylids has not yet been the subject of a molecular analysis, but the possibility of the two species being related to, if not conspecific with, *Pseudocordylus* cannot be discounted. If one or both species is closely related to *Pseudocordylus* it is possible that a once continuous population stretching from the highlands of Zimbabwe into the Great Escarpment in the eastern and southern parts of southern Africa was separated by the formation of the dry Limpopo valley about 7-15 million years ago. Because the Limpopo depression was formed during the Miocene, *Pseudocordylus* populations may have been present throughout their current range during the Pliocene.

Several additional reptile species, species complexes and genera occur in association with the escarpment, but do not occur north of the Limpopo. Although they may have occurred in the highlands of Zimbabwe and Mocambique and became extinct there, they may in fact never have occurred that far north. Apart from the *P. melanotus* and *P. microlepidotus* complexes (Fig. 2.1), the following taxa are involved: *Tropidosaura* and *Bradypodion* (also widespread in the Karoo and coastal areas), *Trachylepis homalocephala*, *Pedioplanis burchelli*, *Nucras lalandii* and *Tetradactylus seps*, and the snake *Lamprophis guttatus* (Branch 1998). The cordylid *Chamaesaura aenea* is restricted to the montane grasslands of the Drakensberg and associated areas, whereas the gecko genus *Afroedura* is associated with rocky habitat along the western and eastern escarpment of southern Africa (Branch 1998). Frogs associated with the southern and eastern escarpment are *Heleophryne*, *Vandijkophrynus garipeensis*, *Semnodactylus wealii* and *Cacosternum nanum parvum*; while three species are restricted to the Maloti-Drakensberg, namely *Amietia vertebralis*, *A. dracomontana* and *Strongylopus hymenopus* (Poynton 1964; Channing 2001; Minter *et al.* 2004). A few mammals are also distributed on, and in the vicinity of, the escarpment, namely Grey Rhebok *Palea capreolus* (Forster, 1790), Hewitt's Red Rock Rabbit *Pronolagus saundersiae* (Hewitt, 1927) and Sclater's Golden Mole *Chlorotalpa sclateri* (Broom, 1907); while Slogett's Vlei Rat *Otomys sloggetti* Thomas, 1902, is found only in the Maloti-Drakensberg and adjacent eastern escarpment with peripheral isolates to the south-west (De Graaff 1981; Friedmann & Daly 2004; Skinner & Chimimba 2005).

Bauer (1999: 59) noted that: “If earth history explains distribution (and thus endemism and regional diversity), its explanatory power should be general, and many taxa should exhibit congruent patterns of lineage splitting attributable to common events.” Branch (1990) in fact noted that there were similarities between the herpetofauna of the Winterberg (including the Amatole Mountains) and Drakensberg. For example, like the Amatole Mountain (and Winterberg) population of *P. m. subviridis*, the gecko *Afroedura amatolica* (Hewitt, 1925) and the frogs *Vandijkophrynus amatolicus* (Hewitt, 1925) (a component of what was formerly known as the [widespread] *Bufo angusticeps* group) and *Anhydrophryne rattrayi* are restricted to this range. A recent study by Cunningham & Vences (2006) indicated that populations of *Vandijkophrynus* in the Amatole Mountains, Maloti-Drakensberg, Mpumalanga escarpment and Inyanga highlands of Zimbabwe are all Pleistocene relicts. The adder *Bitis atropos* has isolated populations in all of these areas (and the Cape Fold Mountains) but is absent from the Amatole Mountains. Also, the Olifants River gorge has separated populations of *P. transvaalensis* from others in the complex. According to Jacobsen (1989: 632): “Its isolation from the greater body of *Pseudocordylus* species by the dry and hot Olifants river gap and the fact that each isolated population of this form has characteristics of its own, indicate a time of very long separation dating prior to the development of the gorge through the escarpment.” The Olifants River gorge has also separated populations of reptiles like *Bitis atropos*, *Bradypodion*, *Lygodactylus* and *Afroedura* (Jacobsen 1989), and the frog *Heleophryne natalensis* (Cunningham & Bloomer 2004); and the mole *Neamblysomus gunningi* (Broom, 1908) is restricted to the Woodbush area (De Graaff 1981; Friedmann & Daly 2004; Skinner & Chimimba 2005). However, I have not been able to identify any terrestrial vertebrates (species, species complexes, genera) with populations isolated at Suikerbosrand, Nkandhla district or any of the areas where the three populations of *P. transvaalensis* occur. If vicariance were the only factor explaining the current geographical distribution of populations in the *P. melanotus* complex then several animal species and species complexes would have similar distribution patterns. However, although several species are restricted to the vicinity of the Great Escarpment in eastern southern Africa (see above), no known terrestrial vertebrate taxa or species complexes share a similar range to that of the *P. melanotus* complex as a whole (e.g. Northern *melanotus*; allopatric populations at Suikerbosrand and Nkandhla district).

According to Broadley (1964) the north-eastern part of the Maloti-Drakensberg was the evolutionary center for *Pseudocordylus* because it is only here that three taxa (*P. m. subviridis*, *P. langi*, *P. spinosus*) occur together. In fact, *P. m. melanotus* also occurs in the adjacent Qwa-Qwa region. It can be noted here that the Maloti-Drakensberg may also be the evolutionary center for the frog genus *Strongylopus* as three species occur in this region (*S. fasciatus*, *S. grayii*, *S. hymenopus*). Broadley noted that the larva ramparts of the escarpment provide ideal rocky habitat for *Pseudocordylus* and climatic oscillations could be avoided by simply moving up or down the steep slopes. He was of the opinion that populations isolated by these movements diverged and gave rise to *P. langi* (summit) and *P. spinosus* (lower slopes). It is apparent that Broadley was considering the Pleistocene, which means that - if his theory is correct - the latter two species evolved sometime during the last 1.8 million years (based on [www.fossilmuseum.net](http://www.fossilmuseum.net)).

Broadley (1964) believed that the ancestor of *Pseudocordylus* was large, similar to *P. microlepidotus* and occurred in an extensive area from the Cape Fold Mountains in the Western Cape to the eastern escarpment in Mpumalanga. During dry periods [possibly inter-glacials] it would have been restricted to high elevation summits [which would have experienced higher levels of precipitation than the lowlands] and gene flow between populations would be interrupted, resulting in the isolated groups diverging. Broadley (p. 109) added that: "The central (Basutoland) group always had the largest area of suitable temperate habitats and therefore the largest populations and the most rapid rate of evolution. These Basutoland lizards gradually became smaller in size (a common evolutionary trend) and the small temporals fused to form elongate shields." He then noted (p. 109): "During pluvial periods there was enough intermittent gene flow between the central and northern populations to prevent them diverging beyond the subspecific level, but by the time the central and southern populations came into contact again they had diverged to a point where they were reproductively isolated, giving rise to two sympatric species [*P. melanotus subviridis* and *P. microlepidotus fasciatus*] in the north-eastern [Eastern] Cape Province." Broadley's interpretation appears to be based mainly on the potential influence of pluvial and non-pluvial periods during the Pleistocene (e.g. Brain & Meester 1964).

Broadley (1964) concluded that *Pseudocordylus* evolved by centrifugal speciation (see Brown 1957). He noted that taxa at the periphery of the range of the genus, namely *P.*

*microlepidotus* and *P. transvaalensis*, resemble one another (and the ancestral form) in that both are large and possess numerous temporals. Broadley also added that *P. m. subviridis*, *P. langi* and *P. spinosus*, all occurring at the evolutionary center of the genus, are the most advanced morphologically. This was with reference to the fusion of small lateral temporals into vertically elongated shields. Centrifugal speciation or evolution is akin to “evolutionary biogeography” and suggests that higher taxa have a geographical center of origin where new species are produced. Newly evolved species are considered better adapted than older species and displace them towards the periphery of the range. Advanced or derived species (*i.e. subviridis*, *langi* and *spinosus*) are thus found in or near the center of origin, with the most primitive species (*i.e. microlepidotus* and *transvaalensis*) at the periphery of the range. According to Wiley (1981: 286) this notion was derived from the idea that “there was increasing perfection within a lineage as evolution proceeded”, but he added that: “progressive evolution has not been taken seriously for years.”

Proponents of “phylogenetic biogeography” believe the opposite, *i.e.* phylogenetically most primitive members of a taxon occur near the center of origin of the group, with more advanced members at the periphery. Evolution proceeds by allopatric speciation of peripheral isolates (Wiley 1981). However, a more modern and generally acceptable approach is that of “vicariance biogeography” which stresses the importance of seeking common patterns of distribution among different animal groups and then postulating explanations for vicariant events that split ancestral biota (Wiley 1981). According to this approach, allopatric speciation may occur by means of fragmentation of an ancestral species as a result of vicariance, or by dispersal and eventual peripheral isolation of populations. Unlike the other approaches to biogeography discussed above, the concept of a center of origin does not play a central role in vicariance biogeography. Congruence between phylogenetic and geographic patterns suggests that monophyletic clades share a common history in space and time. This approach, which is less rigid than the others mentioned above, and which considers both vicariance and dispersal, will be followed when attempting to explain the evolution of the *P. melanotus* species complex (see below).

On the basis of the topology of the mtDNA phylogram (Fig. 4.2) and knowledge of climatic oscillations during the Cenozoic, the series of vicariant events that gave rise to

*Pseudocordylus* (excluding *P. capensis* and *P. nebulosus*) can be reconstructed. The genus probably originated in the general area where the most basal species in the complex (*P. langi*) occurs today, namely the Maloti-Drakensberg. The altitudinal gradient associated with these mountains renders them a perfect refugium for cool-adapted species. By moving up and down such mountains species can effectively circumvent the effects of climatic oscillations and survive unchanged over time (e.g. Mouton & Oelofsen 1988; Costandius, Mouton & Boucher 2006). The *P. langi*-like ancestor, during a period of cool, moist conditions similar to those experienced at the high altitudes where *P. langi* occurs today, may have had an extensive range along the eastern escarpment, with the Maloti-Drakensberg forming its southern limit. During a subsequent rise in global temperatures, range contraction and fragmentation took place, leaving an isolated population in the south and another in the north. The southern population (*P. langi*) survived unchanged in its Maloti-Drakensberg refugium, but the northern population was forced to adapt to warmer conditions. This northern population was the ancestor to all other *Pseudocordylus* (see Fig. 4.2). After adapting to the warmer conditions, the northern form was able to expand its range again, but during a subsequent cooler period range contraction occurred, resulting in an isolated north-eastern population in the Sabie-Lochiel area in Mpumalanga (Northern *melanotus*) and a western population (ancestral to all *Pseudocordylus* excluding *P. langi* and Northern *melanotus*). Relationships in this latter clade are not sufficiently resolved to allow further reconstruction of the biogeographic history of the *Pseudocordylus* clade. It is, however, clear that a *P. m. subviridis*-like form eventually became isolated in the south where it came into contact with *P. langi*. It eventually extended its range SW to the inland mountains of the Eastern Cape and Cape Fold Mountains to give rise to the *P. microlepidotus* species complex.

The spiny morphology of *P. spinosus* is a clear indication that this species had a lowland origin. According to Mouton & Flemming (2001) the predators of cordylids can be divided into two classes: avian predators and non-avian ones. Avian predators are highly visually orientated, mainly diurnal and unable to extract prey from crevices. Besides crypsis, a speedy retreat into a shelter is the most effective way for cordylid lizards to avoid capture by these predators. Non-avian predators use vision and smell to locate lizard prey, many are able to extract lizards from their shelters, and many are nocturnal and can prey on the lizards when the latter are inactive. All cordylid species will be preyed upon while inactive in their shelters. Speed will be ineffective in such situations

as the lizards will either be too cold to flee, or will have no place to run to. Body armour will, however, provide protection against many of these predators. According to Mouton & Flemming (2001) and Mouton, Flemming, Effenberger & Visagie (2005), the visibility of species during periods of activity will determine the relative importance of birds of prey in shaping anti-predator devices and hence their morphology. Heavy armour is ineffective against birds of prey and a speedy retreat to a shelter is probably the best means of escape. Speed and heavy armour are, however, conflicting traits (Losos, Mouton, Bickel, Cornelius & Ruddock 2002). Thus, the greater the visibility of a species, the greater the need for speed and the less developed the armour will be. Cordylids are all diurnal heliothermic baskers. Those species in cold environments (*e.g. P. langi*) will bask for longer periods than those in warm environments, increasing their visibility to avian predators and the selective influence of avian predation on morphology. Species occurring in warm environments will bask less often and will therefore be less exposed to avian predators. Terrestrial predation will therefore have an over-riding effect on morphology, resulting in well-developed armour (*cf. granular P. langi*). The origin of *P. spinosus* may therefore be ascribed to a *P. m. subviridis*-like ancestral population that became isolated in the lowlands (lower slopes of the Drakensberg) where terrestrial predation pressure resulted in a quick shift in morphology from fairly smooth body scales to a more spiny morphology.

According to the Nested Clade Analysis, long-distance colonization also played a role following the first fragmentation event splitting the original *Pseudocordylus* population. There followed a period of restricted gene flow (isolation by distance) and continuous range expansion in the case of southern populations of *P. m. subviridis* (S Lesotho, Naude's Nek and Hogsback). The ancestor of *P. melanotus/P. transvaalensis* also experienced a period of continuous range expansion. Southern *melanotus* is associated with restricted dispersal or range expansion, whereas allopatric fragmentation apparently played a role in the case of derived populations, such as the one at Suikerbosrand. *Pseudocordylus transvaalensis* is associated with past gene flow followed by the extinction of intermediate populations resulting in its three allopatric populations. It therefore seems likely that both vicariance as well as the processes mentioned above, such as dispersal, played a role in the evolution of taxa in the *P. melanotus* species complex.

Fragmentation of lizard populations may be the result of physical factors. In the case of some species, sand flows caused fragmentation of rocky habitat isolating rupicolous

species. Alternatively, exposure of rocky substrates may restrict psammophilous forms. It has also been suggested that populations of *Pachydactylus* became entrapped on sand islands surrounded by extensive savannah (Bauer & Lamb 2002). According to Bauer, Lamb & Branch (2006), *Pachydactylus* geckos, cordylids and scorpions show high substrate specificity and are therefore likely, historically, to have been subjected to vicariance. This often results in elevated rates of localized speciation and therefore increased diversity and endemism. The formation of islands following a rise in sea level caused isolation of *Cordylus* gene pools and lead to allopatric speciation (*e.g.* Mouton, 1985). Formation of rivers may also represent a vicariant event (*Platysaurus*: Scott *et al.* 2004; *Pachydactylus*: Bauer 1999; Bauer & Lamb 2002), as may the formation of mountains and the development of intervening flatlands (Matthee & Flemming 2002). The distribution of animals may also be influenced by habitat choice, dispersal capabilities (*e.g.* more limited in saxicolous species as compared to most birds) and behavioural attributes (*e.g.* territoriality, social structure).

However, climate change is probably the main cause of fragmentation and range expansion in many other animals. For example, Tolley *et al.* (2006: 790) noted that: “climatic fluctuations have periodically created islands of differing vegetation types, some of which may have persisted as isolated patches for periods of time. It is likely that for chameleons in the CFR (= Cape Fold Region), several periods of repeated allopatry and subsequent contact have occurred as a result of these vegetation fluctuations and changes. Periods of contact between lineages could have led to repeated periods of hybridization or introgression, followed by episodes of diversification in allopatry.” Climatic oscillations, namely temperature fluctuations and associated wet-dry cycles during the Pleistocene, were also offered as an explanation for the fragmentation of *Agama atra* populations and their resultant isolation in mountain refugia (Matthee & Flemming 2002). According to Mouton & Van Wyk (1994), rupicolous cordylids are especially likely to give rise to geographical isolates.

Severe climatic oscillations (high-low temperatures and wet-dry cycles) causing expansion and contraction of suitable rocky habitat (resulting in the formation of “island” populations) is most likely to be the main factor explaining the historical fragmentation and range expansion of populations in the *P. melanotus* species complex. Considering the fact that several populations of *Pseudocordylus* share mtDNA haplotypes (*i.e.* *P. m.*

*subviridis*, *P. spinosus*), the isolating agent disrupting gene flow was probably recent, at least in some cases. It is suggested that during glacials in the Pleistocene, especially in pluvial climates, conditions may have been suitable to allow populations of *Pseudocordylus* in the cooler, wetter highlands to disperse down the slopes and occupy the previously hotter and drier surrounding lowlands. If temperatures at the top of mountains were too cold and conditions too severe, populations may even have vacated these high elevations. However, with the return of warmer, drier conditions, populations of crag lizards - which had probably become cold-adapted (considering their current distribution) - may have returned to the cooler and wetter upper slopes, leaving behind remnant populations in pockets of suitable habitat, possibly including warm-adapted populations. Vicariance leads to the establishment of disjunct populations, which results in genetic isolation and differentiation, and possibly allopatric speciation. Over time, populations on the various peaks and highlands may have been linked and even interbred, before being isolated. These isolated populations remained in their rocky habitat and in some cases evolved into different species. The process probably repeated itself several times with climatic oscillations. This cycle of range expansion and contraction may account for the isolated "island" populations at Suikerbosrand, Nkandhla district and Amatole-Winterberg, all found in relatively cool and wet, high elevation rocky habitat.

Changes in vegetation due to climatic changes may also have influenced what was once suitable habitat. Pure grasslands replaced other forms of vegetation during the last glacial maximum (Brain 1985). For example, transformation of bushveld into open grassland during cold conditions may have exposed rocky outcrops, rendering them suitable for occupation by *Pseudocordylus*. A similar scenario was suggested by Boycott (1992) to explain the range expansion of temperate lizards, such as *Cordylus vittifer*, in Swaziland during a drier and cooler phase of the mid-Holocene. During warmer and wetter times more luxuriant plant growth may have choked crevices and covered rocky outcrops, shading them from the sun and making them unsuitable habitat for cordylids. There may also have been areas where series of rocky outcrops created corridors along which lizards moved from one area of prime habitat to another.

The two main populations of *P. m. subviridis*, namely Maloti-Drakensberg and Amatole-Winterberg, may have been separated during an interglacial, but during a subsequent glacial, when temperate conditions were more widespread, they were not successful in

occupying (or re-occupying) the lower-lying areas between these ranges. This may have been due, in part, to competition with the larger *P. microlepidotus fasciatus*, which still occurs in the Eastern Cape today. In the case of *P. m. melanotus*, the two isolated populations were also apparently not able to link up with the main population. It should be noted that *P. transvaalensis*, Southern *melanotus* and *P. microlepidotus* apparently all occur in at least three allopatric populations. Once continuous populations of these taxa/groups may have been divided following severe climatic changes such as those occurring during glacial periods. It is suggested that populations of *P. m. melanotus* and *P. m. subviridis* were initially separated, probably due to range contraction following unfavourable climatic conditions, but eventually range expansion of one or both taxa resulted in their parapatric distributions in the Qwa-Qwa region.

Weathering of rock by wind, sand and chemical action and the resultant reduction in the number of available shelters may also have caused fragmentation of populations. In some areas competition with similar sized lizards (*e.g.* *Cordylus warreni* complex in Limpopo and Mpumalanga provinces; *C. vittifer* in Mpumalanga, Free State and KwaZulu-Natal; *P. microlepidotus fasciatus* in the Eastern Cape) that use the same habitat may have caused local extinctions. Also, because specialized saxicolous lizards are considered to be poor dispersers, individuals may not have crossed open areas to colonize nearby rocky outcrops. However, the possibility that ancestral *Pseudocordylus* was only partly rupicolous and thus capable of some degree of terrestriality, cannot be discounted. Nevertheless, as was suggested in a recent study on the rupicolous lizard *Agama atra* (Matthee & Flemming 2002), it seems that to a great extent the phylogeographic structure of the *P. melanotus* species complex can be attributed to the distribution of mountains and rocky outcrops.

The quartzitic rocky outcrops of Suikerbosrand in Gauteng are home to a population of *P. m. melanotus* isolated from other *Pseudocordylus* by extensive grasslands and the Vaal River (Figs 2.1 and 5.1). This area may have been a refugium during the last glacial maximum. Old museum records (Irene; Pretoria District; Appendix 2.1) suggest that this taxon also occurred on the quartzitic rocks of the Witwatersrand and vicinity. A recent study determined that the critical minimum temperature (loss of righting as end point) for *P. m. melanotus* in Suikerbosrand Nature Reserve is as high as 10°C, while lower lethal temperature (death as end point) is -4°C (McConnachie, Alexander & Whiting 2004).

Lizards survived by supercooling, but in dry conditions they perished when freezing occurred at  $-5^{\circ}\text{C}$ . Freezing probably occurs at higher temperatures in wet conditions. Temperatures in retreat crevices are often near to the lower lethal temperature and frequently below the critical minimum at night (McConnachie *et al.* 2004). These authors speculated that even a slight cooling of the climate ( $>2^{\circ}\text{C}$ ), or a switch to winter rainfall, could cause the extinction of this population. They also noted that rocky crevices are essential for preventing predation of unconscious lizards. Considering the fact that during the last glacial maximum temperatures were  $5\text{-}10^{\circ}\text{C}$  lower than today (Boelhouwers & Meikeljohn 2002), the Suikerbosrand study demonstrates how easily populations may be exterminated in periods of extreme cold (*e.g.* glacials). Temperature tolerances of *Pseudocordylus* in the Maloti-Drakensberg, for example, are expected to be higher considering the colder conditions there (*e.g.* regular snowfalls on the higher peaks). Projected global temperature increases over the next 50-100 years pose a serious threat to the survival of the cold-adapted, montane restricted, Cape form *Pseudocordylus nebulosus* (Costandius *et al.* 2006). Even a slight increase in mean temperature may result in lowland animals moving into its range and competing for limited resources. Rising global temperatures may also negatively affect other populations of *Pseudocordylus*.

#### 6.4 Conservation status

Two species of *Pseudocordylus* are currently regarded as being of conservation concern. Both *P. langi* and *P. spinosus* are listed as “Restricted” in the current South African Red Data Book – Reptiles and Amphibians. This category is applied to taxa endemic to South Africa (including Lesotho and Swaziland) that have very localized geographical ranges. It is felt that these taxa could easily be threatened and their status should thus be carefully monitored. As South Africa is considered their sole guardian, elimination locally would mean extinction. All cordylids are listed in Appendix 2 of the Convention on the International Trade in Endangered Species of Fauna and Flora (CITES).

In 1988 *P. langi* was in fact known from an even smaller area than that shown on Branch’s (1988b) map, because at least two of the quarter-degree units plotted were erroneous (see Chapter 2). This taxon is currently known from only four units in the

Mont-aux-Sources – Cathedral Peak area, but is likely to occur more widely along the homogeneous summit and escarpment edge of the Drakensberg. It is not restricted to South Africa itself as there is a record from Cleft Peak (Appendix 2.1) in Lesotho and the two countries share the same summit habitat. The species is almost certain to occur at other sites in KwaZulu-Natal and Lesotho. It appears to be fairly abundant in at least some of the sites (*e.g.* 2828DB) where it has been found during the last nine years (*pers. obs.*; M. Cunningham, *pers. comm.*). The Organ Pipes Pass population (Fig. 5.2) is protected within the Cathedral Peak State Forest (and probably elsewhere) in the uKhahlamba-Drakensberg Park, a World Heritage Site. The inhospitable habitat of *P. langi* (Fig. 5.2) should also provide it with a large degree of protection from both habitat destruction and harvesting by collectors. Nevertheless, until it has been shown to have a more extensive range, its conservation status should be retained. The species is protected by provincial ordinances in KwaZulu-Natal (see Branch 1988b). As it has now been confirmed as occurring in the Free State (Appendix 2.1), the latter province should also legislate for its protection. According to Goedbloed & Cunningham (2006), *P. langi* is restricted to basalt cliffs above 2700 m along the northern edge of the Drakenberg in a narrow strip of land about 100 km long, from Mechachane in the Free State to Giant's Castle in KwaZulu-Natal. Within this range it occurs in dispersed colonies on north-facing slopes associated with seepages. These authors found little evidence of expansion or past vicariance and suggested that the species occurs in an area with relatively invariant climatic factors, with cohesion being maintained by gene flow among nearby colonies.

*Pseudocordylus spinosus* is still known from essentially the same area mapped by Branch (1988c). It occurs mainly on the lower slopes of the Drakensberg in KwaZulu-Natal and the Free State, but one record is from an area in excess of 2500 m (Appendix 2.1). Bourquin (2004) recently plotted a record that appears to bridge the gap between the main Drakensberg population and the isolated population in the Ixopo district of southern KwaZulu-Natal. Both of the latter records require confirmation, but conservation authorities should, in the meantime, provide protection for these populations as they may be genetically differentiated. The latter thinking applies to any small, isolated population (see next paragraph). A detailed survey of the foothills and middle slopes of the Drakensberg in southern KwaZulu-Natal is required to establish the true range of this species. At a site in Goodoo Pass in Royal Natal National Park (Fig. 5.2; E6-7 in

Appendix 2.1) the species was found to be locally common in rocky outcrops separated by grassland (pers. obs., 7 June 2005). Crevices were near or even at ground level and not very deep in comparison with those usually used by *P. m. subviridis*. Much of the range of *P. spinosus* falls within the uKhahlamba-Drakensberg Park. However, until a survey has been conducted it is suggested that its Red Data Book status be retained. The species is protected by provincial ordinances in both KwaZulu-Natal and the Free State (see Branch 1988c).

The two currently recognized subspecies of *P. melanotus* (including Northern and Southern *melanotus*, and *P. m. subviridis*) are both widespread (Fig. 5.1) and abundant (pers. obs.). However, there are at least two populations (Suikerbosrand, Gauteng; Nkandhla district, KwaZulu-Natal) of *P. m. melanotus* that should be afforded protection as they may be evolving as separate lineages. Fortunately the major part of the Gauteng population is situated within Suikerbosrand Nature Reserve where it enjoys a large measure of protection. This population is geographically isolated (Fig. 5.1) and forms a distinct monophyletic subclade within Southern *melanotus* (Fig. 4.2). It should be treated as an Evolutionarily Significant Unit (ESU). The population in Nkandhla district is protected in both the Nkandla and Qudeni State Forests. This population appears to be geographically isolated (Fig. 5.1) and there was a fixed allelic difference (shared with *P. transvaalensis*) between it and all other *P. m. melanotus* populations (Table 3.3). However, it did not differ significantly from other Southern *melanotus* in the mtDNA analysis (e.g. Fig. 4.2). The Nkandhla district population should nevertheless be considered a Management Unit (MU).

As mentioned in section 6.3, it has been suggested that global temperature changes may have devastating effects on *Pseudocordylus* populations. For example, slight decreases in temperature may bring about the extinction of the Suikerbosrand population of *P. m. melanotus*, whereas slight increases in temperature may cause the extinction of *P. nebulosus*.

Clade E consists of two reciprocally monophyletic subclades, one of which comprises the Hogsback (= Amatole-Winterberg) population and the other comprising Naude's Nek, S Lesotho and *P. microlepidotus* (Fig. 4.2). Together with the S Lesotho population, the Amatole population differs from other *P. m. subviridis* on account of a fixed allelic

difference (Table 3.3). The Amatole-Winterberg population is also geographically isolated (Fig. 5.1) and morphologically diagnosable (Fig. 5.45). It should be categorized as an ESU. This population is protected because a large part of its habitat lies within State Forests. The Naude's Nek and S Lesotho populations are apparently not geographically isolated from one another or from other Maloti-Drakensberg populations of *P. m. subviridis*, nor are they morphologically separable, but on account of their topology (Fig. 4.2) and the emphasis on genetic distinctiveness (Moritz 1994), this subclade should also be considered an ESU. *Pseudocordylus microlepidotus* is a distinct species, geographically and morphologically.

Apart from *P. spinosus*, here considered a distinct species, specimens in clades C and D are morphologically indistinguishable or very similar and populations are geographically either sympatric or at most parapatric. Specimens from the locality Qoqolosing were morphologically closest to *P. m. melanotus* and grouped with other *P. m. melanotus* in the allozyme analysis (Table 3.3; Appendix 3.1). However, in the mtDNA analysis, all but one (NMB R8361 = *melanotus*) grouped with *P. m. subviridis* (Fig. 4.2). NMB R8365 from Thibella was morphologically intermediate. It grouped with *P. m. melanotus* in the allozyme analysis, but with *P. m. subviridis* in the mtDNA analysis. The remaining specimens in clade C (excluding *P. spinosus*) were referable to *P. m. subviridis* both morphologically and with regard to the allozyme analysis (except for NMB R8567 not used in the allozyme study). All specimens in clade D (and E, excluding *P. microlepidotus*) were referable to *P. m. subviridis* in all three analyses (morphological, allozymes, mtDNA). All the analyzed specimens from Monontsha Pass and Organ Pipes Pass were referable to *P. m. subviridis* in the allozyme study (Table 3.3), but the mtDNA analysis showed that they were divisible into clades C and D (Fig. 4.2). Clades C (minus *P. spinosus*) and D should be considered ESUs.

*Pseudocordylus transvaalensis* occurs in three isolated populations in Limpopo Province. The Western and Eastern populations are known from only three quarter-degree units each, whereas the Central population is known from four units. Unlike the two subspecies of *P. melanotus*, *P. transvaalensis* is largely a solitary lizard usually found in large rocky outcrops and appears to be far less common even in good habitat (pers. obs. at sites in all three populations). The three allopatric populations of *P. transvaalensis* (Fig. 5.1) were morphologically distinguishable (Fig. 5.47). However, the two populations

(Western and Central) analyzed for allozymes (Chapter 3) and mtDNA (Fig. 4.2) did not form reciprocally monophyletic groups. The three populations should therefore be treated as MUs.

## 6.5 Future studies

### 6.5.1 Distribution surveys

An attempt should be made to survey the area between the Southern Berg and the Amatole Mountain range to determine whether any populations of *P. m. subviridis* remain in the region. The same applies to the areas between the three apparently allopatric populations of *P. transvaalensis*, and the areas between the two isolated *P. m. melanotus* populations (Suikerbosrand and Nkandhla district) and the main *P. m. melanotus* population (Southern *melanotus*). The recorded presence of an apparently isolated population of *P. m. melanotus* in northern Swaziland (see Fig. 5.1; Appendix 2.1) requires confirmation. The species was not recorded from this area by Boycott (1992). Also, a further attempt should be made to determine/confirm whether *P. m. melanotus* occurs on the farm Ceylon in the south-eastern Free State as recorded by De Waal (1978). This locality is situated about 200 km SSW of the nearest other *P. m. melanotus* locality, but is much closer to known *P. m. subviridis* sites. During the course of this study an attempt was made to locate specimens on this farm, but to no avail. A more determined effort should be made to locate specimens in the area. If collected, samples should be sequenced to establish the genetic relationship of this population to other *P. m. melanotus* and *P. m. subviridis*. The occurrence of *P. spinosus* in the Ixopo district of KwaZulu-Natal suggests that this species may have a much wider distribution than currently known. The area between the latter locality and the Drakensberg escarpment should be surveyed to determine the extent of its range and the extent of sympatry between *P. spinosus* and *P. m. subviridis*.

In a few areas there are zones of parapatry between taxa and these should be investigated and an attempt made to establish how extensive or narrow they are. This will help when attempting to reconstruct the biogeographical history of the complex. The areas include: Groot Winterhoek area where *P. melanotus subviridis* and *P. microlepidotus fasciatus* have been found parapatrically in very close proximity (Chapter 2); Qwa-Qwa where *P.*

*m. melanotus* and *P. m. subviridis* occur in parapatry and apparently even sympatry (Qoqolosing, Chapter 4); Kranskop-Nkandla area where *P. m. melanotus* and *P. m. subviridis* appear to be separated by the Tugela and Sundays River valleys; vicinity of Legalameetse Nature Reserve in the eastern escarpment where *P. m. melanotus* and *P. transvaalensis* apparently occur in parapatry (Chapter 2).

A start has already been made with regard to surveying the geographical extent and altitudinal distribution of *Pseudocordylus* in the Maloti-Drakensberg (M. Cunningham & M.F. Bates). The occurrence of the various forms in this mountain range will form part of a survey for the Maloti-Drakensberg Transfrontier Conservation and Development Project (MDTP) that began in April 2006. During this time it will be possible to determine whether or not *P. m. subviridis* and *P. spinosus* are not only sympatric, but possibly also microsympatric.

#### 6.5.2 Genetic studies

Better resolution of evolutionary relationships will be achieved if more populations are included in the analyses. Additional and more comprehensive sampling should be conducted within the range of Northern *melanotus*. At least one locality should be sampled in what may be an isolated northern Free State population. Samples from the Western population of *P. transvaalensis* should be sequenced so as to establish whether or not this species is monophyletic (the three populations are separable in discriminant analysis – see Chapter 5).

Sequence divergence values for the 16S rRNA gene appear to be low between taxa in some genera (*e.g.* scincids, see Daniels *et al.* 2002). An analysis of a more rapidly evolving gene such as CO1 may allow better resolution, in particular of relationships within the ingroup indicated in Fig. 4.2. Better resolution of evolutionary relationships within the complex may also be obtained if genetic variation is investigated using nuclear sequences, microsatellites or single nucleotide polymorphisms.

Although morphological changes may occur rapidly and genetic changes require much longer periods of time, it was surprising to find that *P. spinosus* was imbedded within *P. m. subviridis* according to the mtDNA phylogeny. A possible explanation is that the *P.*

*spinosus* population in question is of hybrid origin, *i.e.* derived from male *P. spinosus* and female *P. m. subviridis*, despite being entirely *P. spinosus*-like in external appearance, but it is more likely that *P. spinosus* is derived from *P. m. subviridis* and that genetic differentiation did not keep pace, so to speak, with morphological diversification (see section 4.4). To resolve this apparently confusing relationship and to establish whether or not the Goodoo Pass sample represents an isolated case of hybridization, it will be necessary to collect additional *P. spinosus* from areas more distant from Goodoo Pass, including areas where *P. m. subviridis* is not known to occur. It will also be informative to collect samples from the population in Ixopo district. The possibility of hybridization could also be investigated using allozymes as this will make it possible to directly identify heterozygotes.

The mtDNA phylogeny indicated that *P. microlepidotus* is embedded in the southern subclade of *P. m. subviridis*. Melville *et al.* (2005) also found that *P. microlepidotus* and *P. m. subviridis* were more closely related than either was to *P. m. melanotus*. This genetic similarity suggests that *P. microlepidotus* was derived from ancestral *P. m. subviridis*. The phylogeography of the *P. microlepidotus* species complex is currently being studied by M. Cunningham (2004; pers. comm.).

According to Rocek (1984), *Pseudolacerta lamandini* and related forms (early to middle Eocene, c. 50 million years ago) and *Palaeocordylus bohemicus* (Lower Miocene, c. 20 mya) are referable to the family Cordylidae, suggesting that cordylids inhabited Europe during the Jurassic-Miocene period. However, although he accepts that they are cordyliform lizards, Branch (1998) does not consider either *Pseudolacerta* or *Palaeocordylus* as being referable to the family Cordylidae. When there is a lack of fossil or biogeographic information useful for calibrating a molecular clock, various mitochondrial protein-coding gene calibrations determined for other related groups of animals – based on fossil or biogeographic information – have been used (*e.g.* BurrIDGE, Melendez & Dyer 2006). Although there are no African cordyliform fossils, Daniels, Mouton & Du Toit (2004) applied a molecular clock for a few *Cordylus* species based on mitochondrial protein-coding gene calibrations for various lizards, frogs and fish. To estimate molecular dates it is necessary to measure the genetic distance between species and then use a calibration rate (number of genetic changes per unit time) to convert the genetic distance to time (Bromham & Penny 2003). However, this is based on the idea

that molecular evolution occurs at a more-or-less uniform rate over time, which is considered controversial, so a relaxed Bayesian clock is sometimes preferred for estimating divergence times between clades (Bromham & Penny 2003; Kumar 2005; Tolley, Burger, Turner & Matthee 2006). However, even with a relaxed Bayesian clock at least one calibration point is needed, whether it be a dated fossil, a secondary point from a molecular analysis, or a geological calibration point. A relaxed molecular clock may be applied to the *P. melanotus* species complex in future studies in order to estimate divergence times of the various lineages.

### 6.5.3 Taxonomic description

This analysis indicated that the northern-most population of *P. m. melanotus* (Northern *melanotus*) represents an undescribed species. As noted above, Smith's (1838, 1843) descriptions of *P. melanotus* were based on specimens from the eastern Free State (Ficksburg district, *i.e.* "Southern *melanotus*") and therefore it is the northern population of *P. m. melanotus* ("Northern *melanotus*") that requires a new name. The latter should be formally described, but only following a detailed analysis of morphological characters in a larger sample of specimens from this area as well as additional mtDNA analyses based on more populations, as noted above.

## REFERENCES

- AMBROSE, D., TALUKDAR, S. & POMELA, E.M. (Eds.). 2000. *Biological Diversity in Lesotho: a Country Study*. Lesotho Government, National Environment Secretariat, Maseru.
- ANON, 2002. CITES standard reference for the species of *Cordylus* (Cordylidae, Reptilia) - prepared at the request of the CITES Nomenclatural Committee. Convention on International Trade in Endangered Species of Wild Fauna and Flora. Twelfth meeting of the Conference of the Parties – Santiago (Chile), 3-15 November 2002: Nomenclature Committee report. Pp. 1-12. ([www.cites.org/cop/12/ESF12i-14.PDF](http://www.cites.org/cop/12/ESF12i-14.PDF)). (Prepared by D.G. Broadley).
- ANON, 2003. *The Columbia Electronic Encyclopaedia*, 6th edition. Columbia University Press. ([www.infoplease.com/ce6/world/A0834778.html](http://www.infoplease.com/ce6/world/A0834778.html)).
- AVISE, J.C. 1974. Systematic value of electrophoretic data. *Syst. Zool.* **23**(4): 465-481.
- AVISE, J.C. & AQUADRO, C.F. 1982. A comparative summary of genetic distances in the vertebrates. *Evol. Biol.* **15**: 151-185.
- BAIRD, S.J.E. 2006. Phylogenetics: Fisher's markers of admixture. *Heredity* **97**: 81-83.
- BALAKRISHNAN, R. 2005. Species Concepts, Species Boundaries and Species Identification: A View from the Tropics. *Syst. Biol.* **54**(4): 689-693.
- BĂNĂRESCU, P.M. 1984. The concept of center of origin and allopatric speciation. *Rev. Roum. Biol. Biol. Anim.* **29**(2): 87-91.
- BATES, M.F. 1992a. *The herpetofauna of the Orange Free State – with special emphasis on biogeographical patterning*. Unpublished M.Sc. thesis, University of Natal, Durban.

- BATES, M.F. 1992b. The grid and locus code method for plotting locality records. *African Herp News* **18**: 21-23.
- BATES, M.F. 1996. New reptile distribution records for the Free State province of South Africa. *Navors. nas. Mus., Bloemfontein* **12**(1): 1-47.
- BATES, M.F. 2005. Taxonomic history and geographical distribution of the *Pseudocordylus melanotus* (A. Smith, 1838) and *P. microlepidotus* (Cuvier, 1829) complexes (Sauria: Cordylidae). *Navors. nas. Mus., Bloemfontein* **21**(4): 37-112.
- BAUER, A.M. 1999. Evolutionary scenarios in the *Pachydactylus* Group geckos of southern Africa: new hypotheses. *Afr. J. Herpetol.* **48**(1&2): 53-62.
- BAUER, A.M. & GOOD, D.A. 1996. Phylogenetic systematics of the day geckos, genus *Rhoptropus* (Reptilia: Gekkonidae) of southwestern Africa. *J. Zool.* **238**: 635-663.
- BAUER, A.M. & LAMB, T. 2002. Phylogenetic relationships among members of the *Pachydactylus capensis* group of southern African geckos. *Afr. Zool.* **37**(2): 209-220.
- BAUER, A.M. & LAMB, T. 2005. Phylogenetic Relationships of southern African geckos in the *Pachydactylus* Group (Squamata: Gekkonidae). *Afr. J. Herpetol.* **54**(2): 105-129.
- BAUER, A., GOOD, D.A. & BRANCH, W.R. 1997. The taxonomy of the southern African leaf-toed geckos (Squamata: Gekkonidae), with a review of Old World "*Phyllodactylus*" and the description of five new genera. *Proc. Calif. Acad. Sci.* **49**(14): 447-497.
- BAUER, A.M., LAMB, T. & BRANCH, W.R. 2006. A Revision of the *Pachydactylus serval* and *P. weberi* Groups (Reptilia: Gekkota: Gekkonidae) of Southern Africa, with the Description of Eight New Species. *Proc. Calif. Acad. Sci.* **57**(23): 595-709.
- BAUM, D.A. & DONOGHUE, M.J. 1995. Choosing among alternative "phylogenetic" species concepts. *Syst. Bot.* **20**: 560-573.

- BAVERSTOCK, P.R. & MORITZ, C. 1996. Sampling Design. Pp. 17-27. **In:** Hillis, D.M., Moritz, C. & Mable, B.K. (Eds), *Molecular Systematics*. Sinauer Associates Inc., Sunderland, Massachusetts.
- BERG, D.M., ELDERKIN, C.L., CHRISTIAN, A., METCALFE-SMITH, J.L., VAUGHN, C.C. & GUTTMAN, S.I. 2002. Hierarchical analysis of genetic variation in freshwater mussels: comparison of allozymes and DNA sequences. **In:** Population Distribution: Studies of Dispersal, Behavior, and Genetics II. Abstract of paper presented at the North American Benthological Society Annual meeting, Pittsburgh, Pennsylvania, 2002.
- BOELHOUWERS, J.C. & MEIKLEJOHN, K.I. 2002. Quaternary periglacial and glacial geomorphology of southern Africa: review and synthesis. *S. Afr. J. Sci.* **98**: 47-55.
- BOULENGER, G.A. 1885. *Catalogue of the lizards in the British Museum (Natural History)*. Vol. II. Iguanidae, Xenosauridae, Zonuridae, Anguidae, Anniellidae, Helodermatidae, Varanidae, Xantusiidae, Teiidae, Amphisbaenidae. 2nd edition, London, pp. i-xiii + 1-497, pls I-XXIV.
- BOULENGER, G.A. 1903. On a collection of Batrachians and Reptiles from the Interior of Cape Colony. *Ann. Mag. Nat. Hist. Seventh Series*, **12**: 215-217, pls XVI-XVII.
- BOULENGER, G.A. 1905. On a collection of Batrachians and Reptiles made in South Africa by Mr. C.H.B. Grant, and presented to the British Museum by Mr. C.D. Rudd. *Proc. Zool. Soc. Lond.* **2**: 248-255.
- BOULENGER, G.A. 1908. On a Collection of Fresh-water Fishes, Batrachians, and Reptiles from Natal and Zululand, with descriptions of New Species. *Ann. Natal Govt. Mus.* **1**(3): 219-235, figs 1-3, pls 15-16.
- BOURQUIN, O. 2004. Reptiles (Reptilia) in KwaZulu-Natal: I – diversity and distribution. *Durb. Mus. Novit.* **29**: 57-103.

- BOURQUIN, O. & CHANNING, A. 1980. Herpetofauna of the Natal Drakensberg: An annotated checklist. *Lammergeyer* **30**: 1-20.
- BOYCOTT, R.C. 1992. *A herpetofaunal survey of Swaziland*. Unpublished M.Sc. thesis, University of Natal, Durban.
- BRAIN, C.K. 1985. Temperature-induced Environmental Changes in Africa as Evolutionary Stimuli. Pp. 45-52. **In:** Vrba, E.S. (Ed.), *Species and speciation*. Transvaal Museum Monograph No. 4. Transvaal Museum, Pretoria.
- BRAIN, C.K. & MEESTER, J. 1964. Past climatic changes as biological isolating mechanisms in southern Africa. Pp. 332-340. **In:** Davis, D.H.S. (Ed.), *Ecological studies in southern Africa*. W. Junk, The Hague.
- BRANCH, W.R. 1981. An annotated checklist of the lizards of the Cape Province, South Africa. *Ann. Cape Prov. Mus. (nat. Hist.)* **13**(11): 141-167.
- BRANCH, B.[W.R.]. 1985. Cape lizards. VIII. Girdled lizards and their relatives. *The Naturalist* **29**(3): 10-18.
- BRANCH, B.[W.R.]. 1988a. *Field Guide to the Snakes and other Reptiles of Southern Africa*. Struik, Cape Town.
- BRANCH, W.R. (Ed.) 1988b. A provisional and annotated checklist of the herpetofauna of southern Africa. *J. Herpetol. Assoc. Africa* **34**: 1-19.
- BRANCH, W.R. 1988c. *Pseudocordylus langi*: species account. Pp. 163-164. **In:** W.R. Branch (Ed.), *South African Red Data Book – Reptiles and Amphibians*, *S. Afr. Natl. Sci. Programmes Rep.* **151**.
- BRANCH, W.R. 1988d. *Pseudocordylus spinosus*: species account. Pp. 165-166. **In:** W.R. Branch (Ed.), *South African Red Data Book – Reptiles and Amphibians*, *S. Afr. Natl. Sci. Programmes Rep.* **151**.

- BRANCH, W.R. 1990. The herpetofauna of the Cape Province, South Africa: New distribution records and zoogeography. *J. Herpetol. Assoc. Afr.* **37**: 17-44.
- BRANCH, B.[W.R.] 1998. *Field Guide to Snakes and other Reptiles of Southern Africa*. 3rd edition. Struik, Cape Town.
- BRANCH, W.R. 1999. Dwarf adders of the *Bitis cornuta-inornata* complex (Serpentes: Viperidae) in Southern Africa. *Kaupia* **8**: 39-63.
- BRANCH, W.R. 2006. Priorities for systematic studies on southern African reptiles. Pp. 2-20. **In:** Branch, W.R., Tolley, K.A., Cunningham, M., Bauer, A.M., Alexander, G., Harrison, J.A., Turner, A.A. & Bates, M.F. (Eds), A plan for phylogenetic studies of southern African reptiles: Proceedings of a workshop held at Kirstenbosch, February 2006. Biodiversity Series No. 5. South African National Biodiversity Institute, Pretoria.
- BRANCH, W.R. & BAUER, A.M. 1994. *Phyllodactylus peringueyi* (Reptilia, Gekkonidae): Its taxonomic history, rediscovery, and phylogenetic affinities. *Ann. S. Afr. Mus.* **104**(2): 13-30.
- BRANCH, W.R. & BAUER, A.M. 1995. Herpetofauna of the Little Karoo, Western Cape, South Africa with notes on life history and taxonomy. *Herpetol. Natl. Hist.* **3**(1): 47-89.
- BRANCH, W.R. & BAUER, A.M. 2005. The life and herpetological contributions of Andrew Smith. **In:** W.R. Branch & A.M. Bauer (Eds.), The herpetological contributions of Sir Andrew Smith: with an introduction, concordance of names, and annotated bibliography. Society for the Study of Amphibians and Reptiles, Villanova.
- BRANCH, W.R. & BURGER, M. 1991. Life history note: *Lamprophis guttatus*: Diet. *J. Herpetol. Assoc. Afr.* **39**: 24.
- BRANCH, W.R., BAUER, A.M. & GOOD, D.A. 1995. Species limits in the *Phyllodactylus lineatus* complex (Reptilia: Gekkonidae), with the elevation of two taxa

- to specific status and the description of two new species. *J. Herpetol. Assoc. Afr.* **44**(2): 33-54.
- BRANCH, W.R., BAUER, A.M. & GOOD, D.A. 1996. A review of the Namaqua gecko, *Pachydactylus namaquensis* (Reptilia: Gekkonidae) from southern Africa, with the description of two new species. *S. Afr. J. Zool.* **31**(2): 53-69.
- BRANCH, W.R., TOLLEY, K.A., CUNNINGHAM, M., BAUER, A.M., ALEXANDER, G., HARRISON, J.A., TURNER, A.A. & BATES, M.F. (Eds). 2006. A plan for phylogenetic studies of southern African reptiles: Proceedings of a workshop held at Kirstenbosch, February 2006. Biodiversity Series No. 5. South African National Biodiversity Institute, Pretoria.
- BREMER, K. & WANNTORP, H-E. 1979. Geographic Populations or Biological Species in Phylogeny Reconstruction? *Syst. Zool.* **28**(2): 220-224.
- BRISTOW, D. 1985. *Mountains of Southern Africa*. C. Struik Publishers, Cape Town.
- BROADLEY, D.G. 1962. On some reptile collections from the north-western and north-eastern districts of Southern Rhodesia 1958-61, with descriptions of four new lizards. *Occ. Pap. natn. Mus. S. Rhod. Ser. B*, **3**(26): 787-843.
- BROADLEY, D.G. 1964. A review of the crag lizards (Genus *Pseudocordylus*) of Natal. *Ann. Natal Mus.* **16**: 99-110.
- BROADLEY, D.G. 1966. *The herpetology of south-east Africa*. Unpublished Ph.D. thesis, University of Natal, Pietermaritzburg.
- BROADLEY, D.G. 1983. *FitzSimons' Snakes of southern Africa*. Delta Books, Johannesburg.
- BROADLEY, D.G. 2000. A review of the genus *Mabuya* in southeast Africa (Sauria: Scincidae). *Afr. J. Herpetol.* **49**(2): 87-110.

- BROADLEY, D.G. & BRANCH, W.R. 2002. A review of the small east African *Cordylus* (Sauria: Cordylidae), with the description of a new species. *Afr. J. Herpetol.* **51**(1): 9-34.
- BRODY, L.S., MOUTON, P. le F.N. & GRANT, W.S. 1993. Electrophoretic analysis of the *Cordylus cordylus* species group in the southwestern Cape, South Africa. *Amphibia-Reptilia* **14**: 19-33.
- BROMHAM, L. & PENNY, D. 2003. The modern molecular clock. *Nature Rev. Genet.* **4**: 216-224.
- BROOKS, D.R. & McLENNAN, D.A. 2002. *The Nature of Diversity: An Evolutionary Voyage of Discovery*. The University of Chicago Press, Chicago.
- BROWN, W.L. 1957. Centrifugal speciation. *Q. Rev. Biol.* **32**: 247-277.
- BRYGOO, E.-R. 1985. Les types de Cordylidés et de Dibamidés (Reptiles, Sauriens) du Muséum national d'Histoire naturelle Catalogue critique. *Bull. Mus. Natl. Hist. Nat.*, 4A, **7**: 249-265.
- BURRIDGE, C.P., MELÉNDEZ, C.R. & DYER, B.S. 2006. Multiple Origins of the Juan Fernandez Kelpfish Fauna and Evidence for Frequent and Unidirectional Dispersal of Cirrhitoid Fishes Across the South Pacific. *Syst. Biol.* **55**(4): 566-578.
- BUSACK, S.D. & LAWSON, R. 2006. Historical biogeography, mitochondrial DNA, and allozymes of *Psammodromus algirus* (Lacertidae): a preliminary hypothesis. *Amphibia-Reptilia* **27**(2): 181-193.
- BUSACK, S.D., LAWSON, R. & ARJO, W.M. 2005. Mitochondrial DNA, allozymes, morphology and historical biogeography in the *Podarcis vaucheri* (Lacertidae) species complex. *Amphibia-Reptilia* **26**(3): 239-256.

- CARLIN, J.L. 1997. Genetic and morphological differentiation between *Eurycea longicauda* and *E. guttolineata* (Caudata: Plethodontidae). *Herpetologica* **53**(2): 206-217.
- CHANNING, A. 2001. *Amphibians of Central and Southern Africa*. Protea Book House, Pretoria.
- COSTANDIUS, E., MOUTON, P. le F.N. & BOUCHER, C. 2006. Conservation status of the dwarf crag lizard, *Pseudocordylus nebulosus*, from the Hottentots Holland Mountains, South Africa. *S. Afr. J. Wild. Res.* **36**(2): 123-132.
- COYNE, J.A. & ORR, H.A. 2004. *Speciation*. Sinauer Associates, Inc., Sunderland, Massachusetts.
- CRACRAFT, J. 1983. Species concepts and speciation analysis. *Curr. Ornithol.* **1**: 159-187.
- CUNNINGHAM, M. 2001. *Vicariance, speciation and diversity in Australopapuan rainforest frogs*. Unpublished Ph.D. thesis, University of Queensland, Brisbane.
- CUNNINGHAM, M. 2004. Moving among montane islands: a comparative phylogeographic history of Cape Fold Mountain crag lizards (*Cordylus* 'Pseudocordylus' capensis group and *Cordylus* 'Pseudocordylus' microlepidotus). Pp. 13-14, Programme and Abstracts: 7<sup>th</sup> Herpetological Association of Africa Symposium: Bayworld, Port Elizabeth, South Africa, 6-9 October 2004.
- CUNNINGHAM, M. & BLOOMER, P. 2004. A taxonomic resolution of *Heleophryne* aided by mitochondrial DNA. P. 34, Programme and Abstracts: 7<sup>th</sup> Herpetological Association of Africa Symposium: Bayworld, Port Elizabeth, South Africa, 6-9 October 2004.
- CUNNINGHAM, M. & VENCES, M. 2006. From Berg to Karoo – A mitochondrial DNA perspective on speciation in *Vandijkophrynus*. Pp. 41-42. Programme &

Abstracts: 8<sup>th</sup> Herpetological Association of Africa Symposium, North-West University, Potchefstroom, 24-27 November 2006.

CUVIER, G. 1817. Le règne animal distribué d'après son organisation pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie comparée. Tome II, contenant les reptiles, les poissons, les mollusques et les annélides. Paris, Déterville, pp. i-xviii, 1-532, pls i-xv.

CUVIER, G. 1829. Le règne animal distribué d'après son organisation pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie comparée. Tome II, Les Reptiles. Paris, Déterville, pp. i-xvi, 1-406, pls i-xx.

DANIELS, S.R., MOUTON, P. le F.N. & DU TOIT, D.A. 2004. Molecular data suggest that melanistic ectotherms at the south-western tip of Africa are the products of Miocene climatic events: evidence from cordylid lizards. *J. Zool.* **263**: 373-383.

DANIELS, S.[R.], HEIDEMAN, N.[J.L.], HENDRICKS, M.[G.J.] & WILLSON, B. 2002. A molecular phylogeny for the South African limbless lizard taxa of the subfamily Acontinae (Sauria: Scincidae) with special emphasis on relationships within *Acontias*. *Mol. Phylogenet. Evol.* **24**(2): 315-323.

DANIELS, S.R., HEIDEMAN, N.J.L., HENDRICKS, M.G.J., MOKONE, M.E. & CRANDALL, K.A. 2005. Unraveling evolutionary lineages in the limbless fossorial skink genus *Acontias* (Sauria: Scincidae): are subspecies equivalent systematic units? *Mol. Phylogenet. Evol.* **34**(3): 645-654.

DARDA, D.M. 1994. Allozyme variation and morphological evolution among Mexican salamanders of the genus *Chiropterotriton* (Caudata: Plethodontidae). *Herpetologica* **50**(2): 164-187.

DEACON, J. & LANCASTER, N. 1988. *Late Quaternary Palaeoenvironments of Southern Africa*. Clarendon Press, Oxford.

DE GRAAFF, G. 1981. *The rodents of southern Africa*. Butterworths, Durban.

- DE QUEIROZ, A., LAWSON, R. & LEMOS-ESPINAL, J.A. 2002. Phylogenetic Relationships of North American Garter Snakes (*Thamnophis*) Based on Four Mitochondrial Genes: How Much DNA Sequence Is Enough? *Mol. Phylogenet. Evol.* **22**(2): 315-329.
- DE QUEIROZ, K. 1998. The General Lineage Concept of Species, Species Criteria, and the Process of Speciation: A Conceptual Unification and Terminological Recommendations. Pp. 57-75. **In:** D.J. Howard & S.H. Berlocher (Eds), *Endless Forms: Species and Speciation*. Oxford University Press, Oxford.
- DE QUEIROZ, K. 1999. The General Lineage Concept of Species and the Defining Properties of the Species Category. Pp. 49-89. **In:** R.A. Wilson (Ed.), *Species: New Interdisciplinary Essays*. MIT Press, Cambridge, Massachusetts.
- DE QUEIROZ, K. 2005. A Unified Concept of Species and Its Consequences for the Future of Taxonomy. *Proc. Calif. Acad. Sci.*, Series 4, **56** (Suppl. I) (18): 196-215.
- DE ROCHEBRUNE, A.-T. 1884. Faune de la Sénégambie. Reptiles. Octave Doin, Paris. Pp. 221.
- DE WAAL, S.W.P. 1978. The Squamata (Reptilia) of the Orange Free State, South Africa. *Mem. nas. Mus., Bloemfontein* **11**: +i-iii, 1-160.
- DOWLING, T.E., MORITZ, C., PALMER, J.D. & RIESEBERG, L.H. 1996. Nucleic Acids III: Analysis of Fragments and Restriction Sites. Pp. 249-320. **In:** Hillis, D.M., Moritz, C. & Mable, B.K. (Eds), *Molecular Systematics*. Sinauer Associates Inc., Sunderland, Massachusetts.
- DU TOIT, D.A., MOUTON, P. LE F.N., FLEMMING, A.F. & VAN NIEKERK, A. 2004. Environmental factors determining the presence/absence of generation glands in girdled lizards: A case study of the *Cordylus cordylus-niger-oelofseni* complex. P. 15, Programme and Abstracts: 7<sup>th</sup> Herpetological Association of Africa Symposium: Bayworld, Port Elizabeth, South Africa, 6-9 October 2004.

- DUMÉRIL, C.-A.-M. & BIBRON, G. 1839. *Erpétologie général ou Histoire naturelle complète des Reptiles*. Paris, Roret éd. [Nine volumes text, one volume plates], vol. 5, i-viii + 1-855, pls 43-58.
- ECHELLE, A.A. 1990. In defense of the phylogenetic species concept and the ontological status of hybridogenetic taxa. *Herpetologica* **46**(1): 109-113.
- ELDREDGE, N. & CRACRAFT, J. 1980. *Phylogenetic Patterns and the Evolutionary Process: Method and Theory in Comparative Biology*. Columbia University Press, New York.
- ENGSTROM, T.N., SHAFFER, H.B. & McCORD, W.P. 2004. Multiple Data Sets, High Homoplasy, and the Phylogeny of Softshell Turtles (Testudines: Trionychidae). *Syst. Biol.* **53**(5): 693-710.
- ESSEX, R. 1927. Studies in Reptilian Degeneration. *Proc. Zool. Soc., Lond.* **2**: 879-945, pls 1-7.
- ESTES, R.D. 1983. The fossil record and early distribution of lizards. Pp. 365-398. **In:** Rhodin, A. & Miyata, K. (Eds), *Advances in herpetology and evolutionary biology: Essays in honor of Ernest E. Williams*. Museum of Comparative Zoology, Harvard University.
- EXCOFFIER, L., SMOUSE, P.E. & QUATTRO, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**(2): 479-491.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.
- FERGUSON, J.W.H. 2002. On the use of genetic divergence for identifying species. *Biol. J. Linn. Soc.* **75**: 509-516.
- FITZSIMONS, V.[F.M.]. 1937. Notes on the reptiles and amphibians collected and described from South Africa by Andrew Smith. *Ann. Transvaal Mus.* **27**(4): 259-274.
- FITZSIMONS, V.F.M. 1943. The Lizards of South Africa. *Transvaal Mus. Mem.* **1**: i-xv, 1-528, pls 1-24.

- FITZSIMONS, V.[F.M.]. 1947. Descriptions of New Species and Subspecies of Reptiles and Amphibians from Natal, together with Notes on some other Little Known Species. *Ann. Natal Mus.* **11**(1): 111-137, pls I-III.
- FITZSIMONS, V.F.M. 1948. Notes on some reptiles and amphibians from the Drakensberg, together with a description of a new *Platysaurus* from northern Natal. *Ann. Transvaal Mus.* **21**(1): 73-80.
- FITZSIMONS, V. [F.M.]. 1958. A new *Cordylus* from Gorongosa, Mocambique. *Ann. Natal Mus.* **14**(2): 351-353.
- FLEMMING, A.F. 1996. An analysis of the *Agama atra* complex (Reptilia: Agamidae) in Namaqualand and southern Namibia. Unpublished Ph.D. thesis, University of Stellenbosch, Stellenbosch.
- FRIEDMANN, Y. & DALY, B. (Eds). 2004. *Red Data Book of the Mammals of South Africa: A Conservation Assessment*. CBSG Southern Africa, Conservation Breeding Specialist Group (SSC/IUCN), Endangered Wildlife Trust. South Africa.
- FROST, D.R. & HILLIS, D.M. 1990. Species in concept and practice: Herpetological applications. *Herpetologica* **46**(1): 87-104.
- FROST, D.R., KLUGE, A.G. & HILLIS, D.M. 1992. Species In Contemporary Herpetology: Comments On Phylogenetic Inference And Taxonomy. *Herpetol. Rev.* **23**(2): 46-54.
- FROST, D., JANIES, D., MOUTON, P. le F.N. & TITUS, T. 2001. A Molecular Perspective on the Phylogeny of the Girdled Lizards (Cordylidae, Squamata). *Am. Mus. Novit.* **3310**: 1-10.
- GABOR, C.R., RYAN, M.J. & MORIZOT, D.C. 2005. Character displacement in sailfin mollies, *Poecilia latipinna*: allozymes and behavior. *Environ. Biol. Fish.* **73**: 75-78.

- GEORGES, A. & ADAMS, M. 1996. Electrophoretic delineation of species boundaries within the short-necked freshwater turtles of Australia (Testudines: Chelidae). *Zool. J. Linn. Soc.* **118**: 241-260.
- GERGUS, E.W.A. 1998. Systematics of the *Bufo microscaphus* complex: Allozyme evidence. *Herpetologica* **54**(3): 317-325.
- GLOR, R.E., KOLBE, J.J., POWELL, R., LARSON, A. & LOSOS, J.B. 2003. Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). *Evolution* **57**(10): 2383-2397.
- GOEDBLOED, D. & CUNNINGHAM, M. 2006. Distribution and phylogeography of *Pseudocordylus langi*, a montane endemic lizard from the Drakensberg Mountains. P. 48. Programme & Abstracts: 8<sup>th</sup> Herpetological Association of Africa Symposium, North-West University, Potchefstroom, 24-27 November 2006.
- GOOD, D.A. & BAUER, A.M. 1995. The Namaqua day gecko revisited: Allozyme evidence for the affinities of *Phelsuma ocellata*. *J. Herpetol. Assoc. Afr.* **44**(1): 1-9.
- GRANT, W.S., DEMPSTER, Y.L. & DA SILVA, F.M. 1988. Use of protein electrophoresis in evolutionary systematics. *Trans. Roy. Soc. S. Afr.* **46**(4): 295-311.
- GRAY, J.E. 1831. Synopsis of the Species of the Class Reptilia. Pp. 1-110. **In:** Griffith, E. (Ed.), *The Animal Kingdom arranged in conformity with its organization by the Baron Cuvier*. London, Vol. 9, pp. 1-481 + 1-110, pls i-xlii.
- GRAY, J.E. 1838. Catalogue of the Slender-tongued Saurians, with Descriptions of many new Genera and Species. *Ann. Natl. Hist.* **1**, pp. 274-283 + 388-394.
- GRAY, J.E. 1845. *Catalogue of the specimens of lizards in the collection of the British Museum*. London, pp. i-xxviii + 1-289.

- GREEN, D.M., KAISER, H., SHARBEL, T.F., KEARSLEY, J. & McALLISTER, K.R. 1997. Cryptic Species of Spotted Frogs, *Rana pretiosa* Complex, in Western North America. *Copeia* **1997**(1): 1-8.
- GROBLER, J.H. & BRONKHORST, P.J. 1981. A preliminary checklist of reptiles and amphibians in the Mountain Zebra National Park. *Koedoe* **24**: 193-197.
- GUÉRIN-MÉNEVILLE, F.É. 1829-1844. Iconographie du règne animal de G. Cuvier; ou, Représentation d'après nature de l'une des espèces les plus remarquables, et souvent non encore figurées, de chaque genre d'animaux. Avec un texte descriptif mis au courant de la science. Ouvrage pouvant servir d'atlas à tous les traités de zoologie. Paris & London: J.B. Baillière, three volumes of colour plates.
- HARRIS, H. & HOPKINSON, D.A. 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*. Amsterdam, North Holland.
- HARTL, D.L. & CLARK, A.G. 1997. *Principles of Population Genetics*. Sinauer Associates, Inc., Sunderland, Massachusetts.
- HENNIG, W. 1966. *Phylogenetic Systematics*. University of Illinois Press, Champaign.
- HERSELMAN, Y.M., MOUTON, P. le F.N. & VAN WYK, J.H. 1992. The status of the races of the graceful crag lizard, *Pseudocordylus capensis*, from South Africa. *Amphibia-Reptilia* **13**(2): 109-119.
- HEWITT, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**(6789): 907-913.
- HEWITT, J. 1909. Description of a New Species of *Platysaurus* and notes on the Specific Characters of certain Species of *Zonuridae*, together with Synoptical Keys to all the known South African Species and a resume of our Knowledge on their distribution: and a key to the known Genera of South African Lizards. *Ann. Transvaal Mus.* **2**: 29-40.

- HEWITT, J. 1918. *A Guide to the Fauna of the Albany District: Part I – Vertebrates (Mammals, Birds, Reptiles, and Amphibians, but excluding Fish)*. Albany Museum, Grahamstown.
- HEWITT, J. 1927. Further descriptions of Reptiles and Batrachians from South Africa. *Rec. Alb. Mus.* **3**: 371-415, pls 10-14.
- HEWITT, J. 1937. *A Guide to the Vertebrate Fauna of the Eastern Cape Province, South Africa. Part II.-Reptiles, Amphibians, and Freshwater Fishes*. Albany Museum, Grahamstown.
- HIGHTON, R. 1997. Geographic protein variation and speciation in the *Plethodon dorsalis* complex. *Herpetologica* **53**(3): 345-356.
- HILLIS, D.M. 1987. Molecular versus morphological approaches to systematics. *Annu. Rev. Ecol. Syst.* **18**: 23-42.
- HILLIS, D.M., MABLE, B.K. & MORITZ, C. 1996. Applications of Molecular Systematics: The State of the Field and a Look to the Future. Pp. 515-543. **In:** Hillis, D.M., Moritz, C. & Mable, B.K. (Eds), *Molecular Systematics*. Sinauer Associates Inc., Sunderland, Massachusetts.
- HILLIS, D.M., MABLE, B.K., LARSON, A., DAVIS, S.K. & ZIMMER, E.A. 1996. Nucleic Acids IV: Sequencing and Cloning. Pp. 321-381. **In:** Hillis, D.M., Moritz, C. & Mable, B.K. (Eds), *Molecular Systematics*. Sinauer Associates Inc., Sunderland, Massachusetts.
- INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE. 1999. *International Code of Zoological Nomenclature*. 4th edition. The International Trust for Zoological Nomenclature, The Natural History Museum, London.
- JACOBSEN, N.H.G. 1989. *A herpetological survey of the Transvaal*. Ph.D. thesis, University of Natal, Durban.

- JOHNS, G.C. & AVISE, J.C. 1998. A Comparative Summary of Genetic Distances in the Vertebrates from the Mitochondrial Cytochrome *b* Gene. *Mol. Biol. Evol.* **15**(11): 1481-1490.
- KIRBY, P.R. 1939. *The diary of Dr. Andrew Smith, 1834-1836*. Vol. I. The Van Riebeeck Trust, Cape Town.
- KIRBY, P.R. 1940. *The diary of Dr. Andrew Smith, 1834-1836*. Vol. II. The Van Riebeeck Trust, Cape Town.
- KIRBY, P.R. 1965. *Sir Andrew Smith*. A.A. Balkema, Cape Town.
- KNOWLES, L.L. & MADDISON, W.P. 2002. Statistical phylogeography. *Mol. Ecol.* **11**(12): 2623-2635.
- KUMAR, S. 2005. Molecular clocks: four decades of evolution. *Nature Rev. Genet.* **6**: 654-662.
- KUMAR, S., TAMURA, K., JAKOBSEN, I.B. & NEI, M. 2001. *MEGA2: Molecular Evolutionary Genetics Analysis Software*. Arizona State University, Tempe, AZ.
- LAMB, T. & BAUER, A.M. 2000. Relationships of the *Pachydactylus rugosus* group of geckos (Reptilia: Squamata: Gekkonidae). *Afr. Zool.* **35**(1): 55-67.
- LAMB, T. & BAUER, A.M. 2001. Mitochondrial Phylogeny of Namib Day Geckos (*Rhoptropus*) Based on Cytochrome *b* and 16S rRNA Sequences. *Copeia* **2001**(3): 775-780.
- LAMB, T., MEEKER, A.M., BAUER, A.M. & BRANCH, W.R. 2003. On the systematic status of the desert plated lizard (*Angolosaurus skoogi*): phylogenetic inference from DNA sequence analysis of the African Gerrhosauridae. *Biol. J. Linn. Soc.* **78**: 253-261.

- LAMBIRIS, A.J.L. 1988. Annual report on the herpetological collection of A.J.L. Lambiris: 1988. *Ann. Rep. Lambiris Herp. Coll.* **8**(1): 1-73 + 2 pages (map + fig.).
- LAMBIRIS, A.J.L. 1989. Annual report on the herpetological collection of A.J.L. Lambiris: 1989. Supplement No. 1 to the full report of 1988. *Ann. Rep. Lambiris Herp. Coll.* **8**(2): 1-15 + 1 page (map).
- LANG, M. 1991. Generic relationships within Cordyliformes (Reptilia: Squamata). *Biologie* **61**: 121-188.
- LAUBE, M. & KUHN, R. 2006. Development of microsatellite markers for noninvasive DNA samples of the eastern green lizard *Lacerta viridis*. *Mol. Ecol. Notes* **6**(3): 731-733.
- LAZELL, J. 1992. Taxonomic Tyranny and the Exoteric. *Herpetol. Rev.* **23**(1): 14.
- LEISTNER, O.A. & MORRIS, J.W. 1976. Southern African Place Names. *Ann. Cape Prov. Mus. (nat. Hist.)* **12**: i-iv, 1-565.
- LEVENE, H. 1949. On a matching problem arising in genetics. *Ann. Math. Stat.* **20**: 91-94.
- LOSOS, J.B., MOUTON, P. le F.N., BICKEL, R., CORNELIUS, I. & RUDDOCK, L. 2002. The effect of body armature on escape behaviour in cordylid lizards. *Anim. Behav.* **64**(2): 313-321.
- LOVERIDGE, A. 1944. A revision of the African lizards of the family Cordylidae. *Bull. Mus. Comp. Zool. Harvard Univ.* **1**: 1-118, pls 1-12.
- LYE, W.F. 1975. *Andrew Smith's Journal of his expedition into the interior of South Africa / 1834 – 36*. A.A. Balkema, Cape Town.

- MAKOKHA, J.S., TOLLEY, K.A. & MATTHEE, C.A. 2004. MtDNA phylogeography of Burchell's Sand Lizard (*Pedioplanis burchelli*) in the Cape Fold Mountains. P. 40, Programme and Abstracts: 7<sup>th</sup> Herpetological Association of Africa Symposium: Bayworld, Port Elizabeth, South Africa, 6-9 October 2004.
- MARKERT, C.L. & FAULHABER, I. 1965. Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Zool.* **159**: 319-332.
- MATSCHIE, P. 1891 (?1890). Ueber eine kleine Sammlung von Reptilien und Amphibien aus Süd-Afrika. *Zool. Jahrb. Syst.* **5**: 605-611.
- MATTHEE, C.A. & FLEMMING, A.F. 2002. Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in Southern Africa. *Mol. Ecol.* **11**(3): 465-471.
- MATTHEE, C.A. & ROBINSON, T.J. 1996. Mitochondrial DNA differentiation among geographic populations of Smith's red rock rabbit, *Pronolagus rupestris* (Mammalia: Lagomorpha). *Heredity* **76**: 514-523.
- MATTHEE, C.A., TILBURY, C.R. & TOWNSEND, T. 2004. A phylogeographic review of the African leaf chameleons: genus *Rhampholeon* (Chamaeleonidae): the role of vicariance and climate change in speciation. *Proc. R. Soc. Lond., B*, **271**: 1967-1976.
- MAUSFELD, P., VENCES, M., SCHMITS, A. & VEITH, M. 2000. First data on the molecular phylogeography of the scincid lizards of the genus *Mabuya*. *Mol. Phylogenet. Evol.* **17**(1): 11-14.
- MAYR, E. 1942. *Systematics and the Origin of Species*. Reprinted 1982, Columbia University Press, New York.
- MAYR, E. 1963. *Animal species and evolution*. Harvard University Press, Cambridge, Massachusetts.

- MAYR, E. 1982. *The Growth of Biological Thought*. Harvard University Press, Cambridge, Massachusetts.
- McCONNACHIE, S., ALEXANDER, G.J. & WHITING, M.J. 2004. Between a rock and a cold place: Is low temperature a potential distribution limiter for *Cordylus melanotus*? P. 24, Programme and Abstracts: 7<sup>th</sup> Herpetological Association of Africa Symposium: Bayworld, Port Elizabeth, South Africa, 6-9 October 2004.
- MERREM, B. 1820. Versuch eines Systems der Amphibien. – Testamen systematis Amphibiorum. Marburg, 8 vols, pp. XV & 189, 1 pl.
- MINK, D.G. & SITES, J.W. 1996. Species limits, phylogenetic relationships, and origins of viviparity in the *scalaris* complex of the lizard genus *Sceloporus* (Phrynosomatidae: Sauria). *Herpetologica* **52**(4): 551-571.
- MINTER, L.R., BURGER, M., HARRISON, J.A., BRAACK, H.H., BISHOP, P.J. & KLOEPFER, D. (Eds). 2004. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. SI/MAB Series 9. Smithsonian Institute, Washington D.C.
- MONTANUCCI, R.R. 1992. Commentary on a Proposed Taxonomic Arrangement for Some North American Amphibians and Reptiles. *Herpetol. Rev.* **23**(1): 9-10.
- MORITZ, C. 1994. Defining 'Evolutionarily Significant Units' for conservation. *TREE* **9**(10): 373-375.
- MORITZ, C. & HILLIS, D.M. 1996. Molecular Systematics: Context and Controversies. Pp. 1-13. **In:** Hillis, D.M., Moritz, C. & Mable, B.K. (eds), *Molecular Systematics*. Sinauer Associates Inc., Sunderland, Massachusetts.
- MOUTON, P. le F.N. 1996. Amphibians and reptiles. **In:** Lesotho Highlands Water Project, final report. Contract no. 1008. Baseline Biology Survey and Reserve Development, phase 1B. Volume 3 – fauna. AfriDev Consultants [for Lesotho Highlands Development Authority], Darling, South Africa. Pp. 109-115.

- MOUTON, P. le F.N. 1997. Family Cordylidae. Pp. 19-23. **In:** Van Wyk, J.H. (Ed.), Proc. FitzSimons Commemorative Symposium, South African Lizards: 50 years of Progress and Third H.A.A. Symposium on African Herpetology. Herpetological Association of Africa, Stellenbosch.
- MOUTON, P. le F.N. & FLEMMING, A.F. 2001. Osteoderm function in the lizard family Cordylidae. Proceedings of the Sixth International Congress of Vertebrate Morphology, Jena, Germany, July 21–26, 2001. *J. Morphol.* **248**: 264.
- MOUTON, P. le F.N. & OELOFSEN, B.W. 1988. A model explaining patterns of geographic character variation in *Cordylus cordylus* (Reptilia; Cordylidae) in the south-western Cape, South Africa. *S. Afr. J. Zool.* **23**(1): 20-31.
- MOUTON, P. le F.N. & VAN WYK, J.H. 1993. Sexual dimorphism in cordylid lizards: a case study of the Drakensberg crag lizard, *Pseudocordylus melanotus*. *Can. J. Zool.* **71**: 1715-1723.
- MOUTON, P. le F.N. & VAN WYK, J.H. 1994. Taxonomic status of geographical isolates in the *Cordylus minor* complex (Reptilia: Cordylidae): A description of three new species. *J. Herpetol. Assoc. Afr.* **43**: 6-18.
- MOUTON, P. le F.N., GAGIANO, C. & SACHSE, B. 2005. Generation glands and sexual size dimorphism in the Cape Crag Lizard, *Pseudocordylus microlepidotus*. *Afr. J. Herpetol.* **54**(1): 43-51.
- MOUTON, P. le F.N., FLEMMING, A.F., EFFENBERGER, E., & VISAGIE, L. 2005. Spines versus speed in cordylid lizards: the armadillo lizard, *Cordylus cataphractus*, as an extreme case. Pp. 74-75. Programme & Abstracts: Fifth World Congress of Herpetology, Stellenbosch, 19-24 June 2005.
- MOUTON, P. le F.N., NIEUWOUDT, C.J., BADENHORST, N.C. & FLEMMING, A.F. 2002. Melanistic *Cordylus polyzonus* (Sauria: Cordylidae) populations in the Western Cape, South Africa: Relics or Ecotypes? *J. Herpetol.* **36**(3): 526-531.

- MUELLER, R.L. 2006. Evolutionary Rates, Divergence Dates, and the Performance of Mitochondrial Genes in Bayesian Phylogenetic Analysis. *Syst. Biol.* **55**(2): 289-300.
- MURPHY, R.W. & OTTLEY, J.R. 1980. A Genetic Evaluation of the Leafnosed Snake, *Phyllorhynchus arenicolus*. *J. Herpetol.* **14**(3): 263-268.
- MURPHY, R.W., SITES, J.W. Jr., BUTH, D.G. & HAUFLER, C.H. 1996. Proteins: Isozyme Electrophoresis. Pp. 51-120. **In:** Hillis, D.M., Moritz, C. & Maple, B.K. (Eds), *Molecular Systematics*. Sinauer Associates Inc., Sunderland, Massachusetts.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**(3): 583-590.
- NEI, M., MARUYAMA, T. & CHAKRABORTY, R. 1975. The bottleneck effect and genetic variability in populations. *Evolution* **29**: 1-10.
- NISHIKAWA, K., MATSUI, M. & TANABE, S. 2005. Biochemical phylogenetics and historical biogeography of *Hynobius boulengeri* and *H. stejnegeri* (Amphibia: Caudata) from the Kyushu region, Japan. *Herpetologica* **61**(1): 54-62.
- ODIERNA, G., CANAPA, A., ANDREONE, F., APREA, G., BARUCCA, M., CAPRIGLIONE, T. & OLMO, E. 2002. A Phylogenetic Analysis of Cordyliformes (Reptilia: Squamata): Comparison of Molecular and Karyological Data. *Mol. Phylogenet. Evol.* **23**(1): 37-42.
- PALUMBI, S.R., MARTIN, A., ROMANO, W., McMILLAN, O., STICE, L. & GRABOWSKI, G. 1991. *The Simple Fools Guide to PCR*, Version 2.0. Distributed by authors, University of Hawaii, Honolulu.
- PARRA-OLEA, G., GARCIA-PARIS, M., PAPENFUSS, T.J. & WAKE, D.B. 2005. Systematics of the *Pseudoeurycea bellii* (Caudata: Plethodontidae) species complex. *Herpetologica* **61**(2): 145-158.

- PARTRIDGE, T.C. & MAUD, R.R. 2000. *The Cenozoic of Southern Africa*. Oxford University Press, New York.
- POSADA, D. & CRANDALL, K.A. 1998. MODELTEST (version 3.06). Testing the model of DNA substitution. *Bioinformatics* **14**: 817.
- POYNTON, J.C. 1964. The Amphibia of Southern Africa: a faunal study. *Ann. Natal Mus.* **17**: 1-334.
- RAYMOND, M. & ROUSSET, F. 1995a. An exact test for population differentiation. *Evolution* **49**: 1280-1283.
- RAYMOND, M. & ROUSSET, F. 1995b. GENEPOP (version 1.2): a population genetics software for exact test and ecumenicism. *J. Hered.* **86**: 248-249.
- RIDGEWAY, G.J., SHERBURNE, S.W. & LEWIS, R.D. 1970. Polymorphism in the esterases of the Atlantic herring. *Trans. Am. Fish. Soc.* **99**: 147-151.
- RIDLEY, M. 1989. The cladistic solution to the species problem. *Biol. Phil.* **4**: 1-16.
- ROBINSON, J.P. & HARRIS, S.A. 1999. Amplified Fragment Length Polymorphisms and Microsatellites: A phylogenetic perspective. Chapter 12. **In**: Gillet, E.M. (Ed.), Which DNA marker for Which Purpose? Final Compendium of the Research Project Development, optimisation and validation of molecular tools for assessment of biodiversity in forest trees in the European Union DGXII Biotechnology FW IV Research Programme Molecular Tools for Biodiversity.
- ROCEK, Z. 1984. Lizards (Reptilia: Sauria) from the lower Miocene locality Dolnice (Bohemia, Czechoslovakia). *Rozpravy Ceskoslovenske akademie ved Rada matematickych a prirodnich ved* **94**(1): 1-69.
- RONQUIST, F. & HUELSENBECK, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**(12): 1572-1574.
- ROSE, W. 1926. Some notes on the Lizards of the Cape Peninsula. *Ann. S. Afr. Mus.* **20**(6): 491-494.

- ROSENBLUM, E.B., BELFIORE, N.M. & MORITZ, C. 2006. Anonymous nuclear markers for the eastern fence lizard, *Sceloporus undulatus*. *Mol. Ecol. Notes* **6**(4) (in press.).
- RYDER, O.A. 1986. Species Conservation and Systematics: the Dilemma of Subspecies. *TREE* **1**(1): 9-10.
- SAITOU, N. & NEI, M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**(4): 406-425.
- SCHLEGEL, H. 1834. Monographie van het geslacht *Zonurus*. *Tijdschr. Nat. Gesch. Phys.* **1**: 203-221, pl. vii.
- SCHNEIDER, S., ROESSLI, D. & EXCOFFIER, L. 2000. *Arlequin Version 2.000: A software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva.
- SCOTT, I.A.W., KEOGH, J.S. & WHITING, M.J. 2004. Shifting sands and shifty lizards: molecular phylogeny and biogeography of African flat lizards (*Platysaurus*). *Mol. Phylogenet. Evol.* **31**(2): 618-629.
- SEBA, A. 1735. *Locupletissimi rerum naturalium thesauri accurata descriptio, et iconibus artificiosissimis expressio, per universam physices historiam. Opus, cui, in hoc rerum genere, nullum par existit. Ex toto terrarum orbe collegit, digessit, et depingend. (Amstelaedami)*, vol. 2, pp. i-xxx + 1-154, pls. i-cxiv.
- SHAKLEE, J.B., ALLENDORF, F.W., MORIZOT, D.C. & WHITT, G.S. 1990. Gene nomenclature for protein-coding loci in fish. *Trans. Am. Fish. Soc.* **119**: 2-15.
- SHAW, C.R. & PRASAD, R. 1970. Starch gel electrophoresis of enzymes – a compilation of recipes. *Biochem. Genet.* **4**: 297-330.
- SKINNER, J.D. & CHIMIMBA, C.T. 2005. *The Mammals of the Southern African Subregion*. Cambridge University Press, New York.

- SLOWINSKI, J.B. & PAGE, R.D.M. 1999. How Should Species Phylogenies Be Inferred from Sequence Data. *Syst. Biol.* **48**(4): 814-825.
- SMITH, A. 1838. Contributions to South African Zoology. *Mag. Nat. Hist.* **2**(2): 30-33.
- SMITH, A. 1843. Illustrations of the Zoology of South Africa, Reptilia, pls 24-27, 30.
- SMITH, A. 1844. Illustrations of the Zoology of South Africa, Reptilia, pls 35-36.
- SMITH, A. 1849a. Illustrations of the Zoology of South Africa, 5. Reptilia.
- SMITH, A. 1849b. Illustrations of the Zoology of South Africa, 2. Mammalia.
- SOULE, M.E., YANG, S.Y., WEILER, M.G. & GORMAN, G.C. 1973. Island lizards: the genetic-phenetic variation correlation. *Nature* **242**(5394): 191-193.
- SPRACKLAND, R.G. & SWINNEY, G.N. 1997. The Lizard Collections of the National Museums of Scotland. *Herpetol. Review* **28**(3): 118-119.
- STANLEY, S.E., MOYLE, P.B. & SHAFFER, H.B. 1995. Allozyme Analysis of Delta Smelt, *Hypomesus transpacificus* and Longfin Smelt, *Spirinchus thaleichthys* in the Sacramento-San Joaquin Estuary, California. *Copeia* **1995**(2): 390-396.
- STEJNEGER, L. 1936. Types of the Amphibian and Reptilian Genera Proposed by Laurenti in 1768. *Copeia* **3**: 133-141.
- STEPIEN, C.A. & ROSENBLATT, R.H. 1996. Genetic Divergence in Antitropical Pelagic Marine Fishes (*Trachurus*, *Merluccius*, and *Scomber*) between North and South America. *Copeia* **1996**(3): 586-598.
- SWART, B.L., MATTHEE, C.A. & TOLLEY, K.A. 2004. The phylogeography of the Southern Rock Agama (*Agama atra*) in the Cape Fold Mountains, South Africa. P. 28. Programme and Abstracts: 7<sup>th</sup> Herpetological Association of Africa Symposium: Bayworld, Port Elizabeth, South Africa, 6-9 October 2004.

- Swofford, D.L. 2002. PAUP\* Phylogenetic Analysis Using Parsimony (and other methods), Version 4.10. Illinois Natural History Survey, Champaign, IL.
- Swofford, D.L. & Selander, R.B. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72**: 281.
- Templeton, A.R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Mol. Ecol.* **7**(4): 381-397.
- Templeton, A.R. 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. *Mol. Ecol.* **13**(4): 789-809.
- Templeton, A.R. & Sing, C.F. 1993. A Cladistic Analysis of Phenotypic Associations with Haplotypes Inferred from Restriction Endonuclease Mapping. IV. Nested Analyses with Cladogram Uncertainty and Recombination. *Genetics* **134**(2): 659-669.
- Templeton, A.R., Routman, E. & Phillips, C.A. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics* **140**(2): 767-782.
- Thompson, J.S. & Crotter, B.I. 1998. Allozyme Variation among Disjunct Populations of the Florida Green Watersnake (*Nerodia floridana*). *Copeia* **1998**(3): 715-719.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **24**: 4876-4882.
- Thorpe, J.P. 1982. The molecular clock hypothesis: Biochemical evaluation, genetic differentiation and systematics. *Annu. Rev. Ecol. Syst.* **13**: 139-168.

- TOLLEY, K.A. & BURGER, M. 2004. Distribution of *Bradypodion taeniabronchum* (Smith 1831) and other dwarf chameleons in the eastern Cape Floristic Region of South Africa. *Afr. J. Herpetol.* **53**(2): 123-133.
- TOLLEY, K.A., BURGER, M., TURNER, A.A. & MATTHEE, C.A. 2006. Biogeographic patterns and phylogeography of dwarf chameleons (*Bradypodion*) in an African biodiversity hotspot. *Mol. Ecol.* **15**: 781-793.
- TOLLEY, K.A., TILBURY, C.R., BRANCH, W.R. & MATTHEE, C.A. 2004. Phylogenetics of the southern African dwarf chameleons, *Bradypodion* (Squamata: Chamaeleonidae). *Mol. Phylogenet. Evol.* **30**(2): 354-365.
- VAN DEVENTER, T.R., LOWE, C.H., McCRYSTAL, H.K. & LAWLER, H.E. 1992. Viewpoint: Reconsider Suggested Systematic Arrangements For Some North American Amphibians and Reptiles. *Herpetol. Rev.* **23**(1): 10-14.
- VAN WYK, J.H. & MOUTON, P. le F.N. 1992. Glandular epidermal structures in cordylid lizards. *Amphibia-Reptilia* **13**(1): 1-12.
- VISSER, J. 1984. Akkedisse van Suider-Afrika. 39. Krans-akkedis: Hulle bewaar die geheim van die das-adders. *Landbouweekblad*, 14 December 1984: pp. 50-51, 53, 55.
- WAGNER, R.S., MILLER, M.P. & HAIG, S.M. 2006. Phylogeography and genetic identity of newly-discovered populations of torrent salamanders (*Rhyacotriton cascadae* and *R. variegatus*) in the central Cascades (USA). *Herpetologica* **62**(1): 63-70.
- WELCH, K.R.G. 1982. *Herpetology of Africa: A Checklist and Bibliography of the Orders Amphisbaenia, Sauria and Serpentes*. Robert E. Krieger Publishing Company, Malabar, Florida.
- WERMUTH, H. 1968. Liste der rezenten Amphibien und Reptilien. Cordylidae (Subfam. Cordylinae et Gerrhosaurinae). *Das Tierreich* **87**: ++, VIII-X, 1-30.

- WHITT, G.S. 1970. Developmental genetics of the lactate dehydrogenase isozymes of fish. *J. Exp. Zool.* **175**: 1-35.
- WIENS, J.J. & PENKROT, T.A. 2002. Delimiting Species Using DNA and Morphological Variation and Discordant Species Limits in Spiny Lizards (*Sceloporus*). *Syst. Biol.* **51**(1): 69-91.
- WIENS, J.J. & REEDER, T.W. 1995. Combining Data Sets with Different Numbers of Taxa for Phylogenetic Analysis. *Syst. Biol.* **44**(4): 548-558.
- WILEY, E.O. 1978. The evolutionary species concept reconsidered. *Syst. Zool.* **27**(1): 17-26.
- WILEY, E.O. 1981. *Phylogenetics: The Theory and Practice of Phylogenetic Systematics*. John Wiley & Sons, New York.
- WINSTON, J. 1999. *Describing Species: Practical Taxonomic Procedure for Biologists*. Columbia University Press, New York.
- YODER, A.D., OLSON, L.E., HANLEY, C., HECKMAN, K.L., RASOLOARISON, R., RUSSELL, A.L., RANIVO, J., SOARIMALALA, V., KARANTH, K.P., RASELIMANANA, A.P. & GOODMAN, S.M. 2005. A multidimensional approach for detecting species patterns in Malagasy vertebrates. *Proc. Natl. Acad. Sci. U.S.A.* **102** (Suppl. 1): 6587-6594.

## APPENDICES

**Appendix 2.1:** List of *Pseudocordylus* localities, comprising published and unpublished records. Names in inverted commas could not be located on maps. If a record was available in the form of a locality name (*e.g.* farm) only, the co-ordinates for the center of the area were determined, as was the range of elevations for the entire area. When exact collection localities (degrees, minutes and seconds) and (often) altitudes (meters above sea level) were determined this is indicated using an asterisk after the co-ordinates. Co-ordinates are listed as a series of numbers: the first two digits represent degrees, the second two digits represent minutes and the third two digits represent seconds. Asterisks accompanying catalogue numbers indicates that specimens were examined.

### *Pseudocordylus transvaalensis*

#### **Limpopo Province:**

##### Western population:

- A1. Farm: Groothoek (278), Waterberg Mtns/Sandrivierberg Mtns, Thabazimbi district (2429S, 273630E; 2427Bc3) (Jacobsen 1989, TM): TM 65248\*, 74388\*.
- A2. Farm: Hartbeestfontein (281), Waterberg Mtns, Thabazimbi district (2429S, 2741E; 2427Bc4) (Jacobsen 1989, TM): TM 74381\*, 74386\*; (242921S, 273758E\*; 2427Bc4): NMB R8430-44\*.
- A3. Farm: Rhenosterpoort (402), Waterberg district (1600 m; 2439S, 2809E; 2428Ca4) (Jacobsen 1989, TM): TM 74364-5\*; SARCA No. 115.
- A4. SW of Perdekop peak, Farm: Rhenosterpoort (283), Waterberg Mtn, Waterberg district (1600-1700 m; 242830S, 274930E; 2427Bd3) (SARCA No. 115).
- A5. Waterberg district (too coarse for grid) (Jacobsen 1989, NMZB, not examined; NMZB-UM as listed was identified by D.G. Broadley, pers. comm., 12 December 2001): NMZB-UM 10972.
- A6. NW of Warmbaths (vicinity of 2428CA): TM 33307\*, 33794\*.

##### Central population:

- A7. Farm: Makapansgat (39), Sugarloaf Hill, Potgietersrust district (240830S, 291030E; 2429Aa4) (Jacobsen 1989, TM): TM 33278\*, 57306\*.
- A8. Farm: Maribashoek (50), Maribashoekberg Mtns, Potgietersrust district (2413S, 2907E; 2429Aa3) ("Maribas Hoek": FitzSimons 1943, TM; Jacobsen 1989, TM): TM 11093\*.
- A9. Matlalas (= Mataias) Location, Seshego district (234530S, 290030E; 2329Cc1) (Jacobsen 1989, TM): TM 74390-3\*.

- A10. Farm: Oostenryk (92), Buffelshoekberg Mtns, Potgietersrust district (2417S, 2914E; 2429Ac2) (Jacobsen 1989, TM): TM 74377-9\*.
- A11. Percy Fyfe Nature Reserve, Potgietersrust district (2402S, 290930E; 2429Aa2) (Jacobsen 1989, TM): TM 42703\*, 74369\*, 74383\*.
- A12. Farm: Zandspruit (287), Potgietersrust district, near Waterberg Mtns (2413S, 2855E; 2428Bb4) (Jacobsen 1989, TM): TM 74372-5\*.
- A13. Farm: Helderfontein (6), Potgietersrust district (240130S, 2905E; 2429Aa1): NMB R8195-208\*.

Eastern population:

- A14. Farm: Diepgelegen (945), Pietersburg district (2351S, 295930E; 2329Dd2) (Jacobsen 1989, TM): TM 74370\*.
- A15. Farm: Flynn (217), Strydpoortberg Mtns, Pietersburg district (2404S, 295015E; 2429Bb1) (Jacobsen 1989, TM): TM 74366-8\*.
- A16. Houtbosdorp, 15 km NNW of Haenertsburg, Pietersburg district (234830S, 2954E; 2329Dd2) (Jacobsen 1989, TM): TM 74382\*, 74384-5\*, 74387\*.
- A17. Farm: Mphome (949), Pietersburg district (235030S, 295430E; 2329Dd2) (Jacobsen 1989, TM): TM 74371\*, 74394\*.
- A18. Farm: Paardevlei (201), Wolkberg Mtn, Strydpoortberg Mtns, Pietersburg district (240230S, 295530E; 2429Bb2) (Jacobsen 1989, TM): TM 74376\*.
- A19. Serala Mtn, Pietersburg/Letaba 1 districts (2401S, 300430E; 2430Aa1) ("Serala 5 KT": Jacobsen 1989): TM 74389\*.
- A20. Woodbush State Forest, Letaba 1 district (2349S, 295930E; 2329Dd2) ("Woodbush": FitzSimons 1943, TM, type series; Jacobsen 1989, TM): Type series: TM 1695\*, 1696 (now only skull), 1697\*, 1698 (transferred to Normal College, Johannesburg), 1699\*, 1700\*, 1701\* (= *Pseudocordylus melanotus melanotus*), 1954-5\*; TM 42326\*.
- A21. Iron Crown, 6 km S of Haenertsburg, Strydpoortberg Mtns, Pietersburg district (235950S, 2957E; 2329Dd4): TM 33795\*.
- A22. Farm: Acre (2), Pietersburg district (2401S, 3004E; 2430Aa1): TM 74380\*.
- A23. Farm: Spitskop (1011), Suikerboskoppie hill, Thaba-moopo district (1876 m; 2352S, 295330E; 2329Dd2): NMB R8041-2\*.
- A24. Farm: Klipspruit (908), Pietersburg district (1450 m; 234923S, 295303E; 2329Dd2): NMB R8546-7\*.
- A25. Farm: Tomason (950), Pietersburg district (1600-1800 m; 235113S, 295407E; 2329Dd2): NMB R8548\*.

- A26. Farm: Monte Christo (1011), Thabamooopo district (1700-1800 m; 235202S, 295328E; 2329Dd2): NMB R8549-51\*.
- A27. Mphome (= Kratzenstein) Mission Station (only ruins remain), near Haenertsburg, Pietersburg district (2329DD) (Matschie 1891, as *P. microlepidotus*).

*Pseudocordylus melanotus melanotus*

Northern *P. m. melanotus*:

**Limpopo Province:**

- B1. Ga-Selati River, probably the vicinity of Orrie Baragwanath Pass, Legalameetse Nature Reserve, E side of the Transvaal Drakensberg, Phalaborwa district (2430Ab3) (“Selati [River]”: FitzSimons 1943, TM, as *P. subviridis transvaalensis*; “Selati”: Loveridge 1944, MCZ, erroneously listed as “paratype” of *P. subviridis transvaalensis*; “Selati”: Jacobsen 1989, TM, as 2430AB on his map, but 2430BA in gazetteer for both “Selati” and “Selati River”): TM 168\*, 171-174\*.

**Mpumalanga:**

- B2. Farm: Boschhoek (36), Lydenburg district (250730S, 3019E; 2530Ab3) (Jacobsen 1989, TM): TM 74195-7\*.
- B3. Farm: De Kuilen (205), Drakensberg Mtns, Lydenburg district (2508S, 3036E; 2530Ba3) (Jacobsen 1989, TM): TM 74257\*.
- B4. Farm: Desire (563), Drakensberg Mtns, Pilgrim's Rest 2 district (2456S, 3047E; 2430Dd3) (Jacobsen 1989, TM): TM 74277\*, 74285\*.
- B5. Farm: Doornhoek (545), Drakensberg Mtns, Pilgrim's Rest 2 district (245330S, 304030E; 2430Dc4) (Jacobsen 1989, TM): TM 74231\*.
- B6. Farm: Dycedale (368), Barberton district (2547S, 310530E; 2531Cc1) (Jacobsen 1989, TM): TM 74247\*, 74251\*, 74256\*, 74264\*.
- B7. Farm: Elandsfontein (322), Belfast district (253430S, 3009E; 2530Ca2) (Jacobsen 1989, TM): TM 74191-2\*.
- B8. “God's Window [Paradise Camp]”, Blyde River Nature Reserve, 9 km NE of Graskop (town), Drakensberg Mtns, Pilgrim's Rest 2 district (2452S, 3054E; 2430Dd2) (Jacobsen 1989, TM): TM 38237\*.
- B9. Farm: Hartebeestvlakte (163), Drakensberg Mtns, Pilgrim's Rest district (2504S, 3040E; 2530Ba2) (Jacobsen 1989, TM): TM 45006-13\*, 45015\*, 53903-5, 55656\*, 55658-61\*, 55685\*.
- B10. Farm: Knapdaar (92), Drakensberg Mtns, Lydenburg district (2517S, 3017E; 2530Ad1) (Jacobsen 1989, TM): TM 74193-4\*.

- B11. Farm: Konigstein (625), Barberton district (254930S, 304730E; 2530Dd1) (Jacobsen 1989, TM): TM 74202\*.
- B12. Farm: Kranskloof (554), Lydenburg district (245730S, 303530E; 2430Dc3) (Jacobsen 1989, TM): TM 74255\*.
- B13. Farm: Langkloof (356), Belfast district (2537S, 2959E; 2529Db2) (Jacobsen 1989, TM): TM 74189\*, 74244\*, 74262\*.
- B14. Lisabon Falls, 2 km N of Nelspruit, Farm: Boschrand (283), Nelspruit district (2526S, 3058E; 2530Bd4) (Jacobsen 1989, TM, as 2530BB): TM 55784\*.
- B15. Farm: Kaffervoetpad (87), Lisabon State Forest, Lydenburg district (2515S, 3030E; 2530Bc1) (Jacobsen 1989, TM): TM 74230\*, 74265\*.
- B16. Farm: Lochiel (192), Eerstehoek district (2609S, 3050E; 2630Bb3) (Jacobsen 1989, TM): TM 74204\*.
- B17. Lochiel (small centre), Farm: Lochiel (192), Eerstehoek district (2609S, 3047E; 2630Bb3) (FitzSimons 1943, NMSA, as *P. subviridis transvaalensis*): TM 24371\*; NMSA 786.
- B18. Farm: Lochiel (192), Eerstehoek district (1650 m; 260855S, 305108E\*; 2630Bb3): NMB R8267-76\*.
- B19. Farm: Aankomst (191), Eerstehoek district (261003S, 305221E\*; 2630Bb3): NMB R8266\*.
- B20. Long Tom Pass, Farm: Lot C (204), Drakensberg Mtns, Pilgrim's Rest district (2509S, 3037E; 2530Ba3) (Jacobsen 1989, TM): TM 54342-4 ["Staircase": 2509S, 3038E; 2530Ba4]; 74238-9\*, 74242\*; JV 1813-4.
- B21. Farm: Loopfontein (298), Drakensberg Mtns, Belfast district (2531S, 3032E; 2530Da1) (Jacobsen 1989, TM): TM 74253\*, 74261\*.
- B22. "Magalieskop", Pilgrim's Rest 2 district (2430DB) (Jacobsen 1989, TM): TM 12285-8\*, 12357\*, 12359-61\*, 12362, 12363-4\*, 12374-5\*.
- B23. Mariepskop Mtn, Pilgrim's Rest 2 district (2432S, 3052E; 2430Db1) (FitzSimons 1943, TM, as *P. melanotus transvaalensis*; "Mariepskop 420KT": Jacobsen 1989, TM): TM 34761\*, 57946 (top; peak = 1944 m)\*, 74218-9\*, 74221-2\*; PEM R626-7.
- B24. Mount Anderson Mtn, Drakensberg Mtns, Lydenburg district (2505S, 3039E; 2530Ba2) (Jacobsen 1989, TM): TM 53903-6\*.
- B25. Nederhorst railway station, Farm: Palmietfontein (104), 6.5 km NNE of Dullstroom (town), Belfast district (2522S, 3008E; 2530Ac2) (Jacobsen 1989, TM): TM 57469\*.

- B26. Farm: Olifantsgeraamte (198), Pilgrim's Rest district (2509S, 304530E; 2530Bb3) (Jacobsen 1989, TM): TM 74232\*, 74205-6\*.
- B27. "Pilgrim's Pass", near Jock of the Bushveld Memorial, Farm: Doornhoek (545), Pilgrim's Rest 2 district (2452S, 3041E; 2430Dc2) (Jacobsen 1989, TM): TM 54340\*.
- B28. Farm: Pittville (197), Ermelo district (261230S, 3038E; 2630Ba4) (Jacobsen 1989, TM): TM 74235\*.
- B29. Farm: Rietvlei (375), Belfast district (2544S, 3016E; 2530Cb3) (Jacobsen 1989, TM): TM 74190\*, 74220\*.
- B30. Farm: The Brook (196), Ermelo district (2611S, 3041E; 2630Ba4) (Jacobsen 1989, TM): TM 2815\*, 74250\*.
- B31. Farm: Wanhoop (78), S of Steenkampsberg Mtns, Lydenburg district (2516S, 3008E; 2530Ac2) (Jacobsen 1989, TM): TM 74198-9\*, 74228\*, 74241\*, 74252\*.
- B32. Lydenburg, Lydenburg district (2506S, 3027E; 2530Ab2) (FitzSimons 1943, TM, as *P. subviridis transvaalensis*): TM 691-2\*.
- B33. "Mariepskop Forestry Reserve", Pilgrim's Rest 2 district (2430DD) ("Mariepskop": FitzSimons 1943, TM, as *P. subviridis transvaalensis*): TM 35566\*.
- B34. Farm: Doornkop (356), Belfast district (2529S, 2956E, 2529Bd4) ("Doornkop near Belfast": FitzSimons 1943, AM, as *P. subviridis subviridis*; Loveridge 1944, as *P. microlepidotus melanotus* and *P. langi* [the latter added with reserve]; referred to *P. melanotus melanotus* by Jacobsen 1989).
- B35. 24 km E of Lydenburg, between Farm: Ceylon (197) and Long Tom State Forest, Drakensberg Mtns, Pilgrim's Rest district (2530BA) (Jacobsen 1989, NMZB, not examined): NMZB-UM 2004-6.
- B36. Spitskop Mtn, 4 km SSW of Sabie, Drakensberg Mtns, Pilgrim's Rest district (2508S, 304530E; 2530Bb3) ("Spitskop, Sabie": Jacobsen 1989, NMZB, not examined): NMZB-UM 2007.
- B37. Sabie, Drakensberg Mtns, Pilgrim's Rest district (2506S, 3047E; 2530Bb1) (FitzSimons 1943, AM, as *P. subviridis transvaalensis*).
- B38. Sabie (Mundi Forestry Area), Farm: no. 196, Drakensberg Mtns, Pilgrim's Rest 2 district (250822S, 304540E\*; 2530Bb3): NMB R8242-50\*; (250822S, 304532E\*; 2530Bb3): NMB R8251-60\*; (250827S, 304542E\*; 2530Bb3): NMB R8261-4\*.
- B39. Fanie Botha Hiking Trail, 11 km NE of Graskop (town), Drakensberg Mtns, Pilgrim's Rest 2 district (245101S, 305344E; 2430Dd2): TM 81661-3.

- B40. Blyde River Canyon, 29 km NNE of Graskop (town), Blyde River Canyon Nature Reserve, Drakensberg Mtns, Pilgrim's Rest 2 district (243954S, 305157E; 2430Db3): TM 81664-8, 81672-3, 82389\*.
- B41. Farm: Wales (250), Bosebokrant, Drakensberg Mtns, Pilgrim's Rest 2 district (245101S, 310139E; 2431Cc1): TM 81669-71.
- B42. Farm: Rietfontein (255), Nelspruit district (2520S, 3045E; 2530Bd1): TM 55713\*.
- B43. Mount Sheba Mtn, Farm: no. 561, Pilgrim's Rest 2 district (2456S, 3043E; 2430Dc4): TM 55785\*.
- B44. Blyde River Canyon, Drakensberg Mtns, Pilgrim's Rest 2 district (2430Db): TM 74240\*.
- B45. Mac-Mac Pools, Pilgrim's Rest district (2501S, 3051E; 2530Bb1): AJL 2691; JV 4112.
- B46. Graskop (town), Drakensberg Mtns, Pilgrim's Rest 2 district (2456S, 305030E; 2430Dd3): NMSA 1421-3.
- B47. 16 km E of Lydenburg, Lydenburg district (2000 m; 2530Ba1): CAS 85843.

**Swaziland:**

- B48. Forbe's Reef (centre), between Silotwane Hills and Ntababovu (? hills), 18 km NNW of Mbabane (260930S, 310530E; 2631Aa3) (FitzSimons 1943, TM, as *P. subviridis transvaalensis*; Loveridge 1944, MCZ, as *P. microlepidotus melanotus*; Boycott 1992 [JV, TM, NMZB-UM], as *P. melanotus transvaalensis*): TM 2718-9\*, 47118\*; NMZB-UM 2014, 2016, 2018-9, 2186-7; JV 4607.
- B49. Near Forbes Reef, Ntababovu Mtns, 24 km N of Mbabane (2631AA): AMNH R-114358-9 (ex-NMZB-UM 2015 and 2017 respectively).
- B50. Mbabane (261903S, 310821E; 2631Ac2) (Boycott 1992, DNSM, TM, as *P. melanotus transvaalensis*): TM 19248\*, 47437\*; DMR 684; JV 2891.
- B51. Mdimba Mtn plateau, 15 km SE of Mbabane (2623S, 3116E; 2631Ad3) ("Mdzimba Hills": Visser 1984, as *P. melanotus transvaalensis*; "Mdzimba Hills (east)": Boycott 1992, TM, as *P. melanotus transvaalensis*): TM 47226\*, 47522\*, 47545-7\*.
- B52. Lomahasha (village), Lubombo Mtns, Lubombo district (2559S, 3159E; 2531Dd4) (Boycott 1992, TM as listed, considered by him as a dubious record for *P. melanotus melanotus*): TM 67396\*.
- B53. Ndlovulu, 6 km SSW of Motjane (centre), 14 km NW of Mbabane (261604S, 310010E; 2631Ac1) ("7 km S of Motshane": Boycott 1992, TM, as *P. melanotus transvaalensis*): TM 71826\*.

- B54. “Malolotja Nature Reserve”, 5 km NE of Forbe's Reef, Ntababovu Mtns (260836S, 310822E; 2631Aa4) (Boycott 1992, TM, as *P. melanotus transvaalensis*): TM 71795\*, 80823; (260823S, 310807E; 2631Aa4): DNSM 1611.
- B55. About 5 km NNE of Bulembu, Makhonjwa Mtns (255454S, 310915E; 2531Cc4): TM 80010\*.
- B56. Lilomuli (village), 7.5 km NW of Mbabane (261615S, 310511E; 2631Ac1): TM 80011\*.
- B57. “Hawane Falls”, Hawane (area), 10 km N of Mbabane (261354S, 310822E; 2631Aa4): TM 80803.
- B58. “Breytenbach, Mbabane district” (locality not traced): TM 24106\*, 24116\*.
- B59. Commissie Nek, Mdimba Hill (262002S, 311527E; 2631Ad1): DNSM 1610.

Southern *P. m. melanotus*:

**Mpumalanga:**

- B60. Farm: Buitenzorg (114), Wakkerstroom district (2717S, 3007E; 2730Ac1) (Jacobsen 1989, TM): TM 74287\*.
- B61. Farm: De Roodepoort (435), Ermelo district (263130S, 295430E; 2629Db2) (Jacobsen 1989, TM): TM 74279-80\*.
- B62. Farm: Doornhoek (577), Balfour district (1500-1550 m; 2644S, 2846E; 2628Db3) (Jacobsen 1989, TM): TM 74263\*.
- B63. Farm: Goedemoed (373), Amersfoort district (265830S, 3003E; 2630Cc3) (Jacobsen 1989, TM): TM 74271\*.
- B64. Farm: Goedgevonden (134), Wakkerstroom district (1845 m; 2718S, 3029E; 2730Ad2) (Jacobsen 1989, TM): TM 37396-7\*, 74215-7\*.
- B65. Greylingstad (town), Balfour district (1500-1550 m; 264430S, 284430E; 2628Da4) (Jacobsen 1989, TM, as 2628DB): TM 74273\*, 74288\*.
- B66. Farm: Kalkoenkrans (366), Amersfoort district (2656S, 3005E; 2630Cc3) (Jacobsen 1989, TM): TM 74254\*, 74291\*.
- B67. Kastrol Nek, 16 km ENE of Wakkerstroom, Wakkerstroom district (2717S, 3017E; 2730Ad1) (Loveridge 1944, MCZ, as *Pseudocordylus microlepidotus melanotus*; Jacobsen 1989, TM): TM 4413-9\*, 4421-30\*, 11190-3\*.
- B68. Farm: Klipfontein (241), Ermelo district (2619S, 2957E; 2629Bd2) (Jacobsen 1989, TM): TM 74249\*.
- B69. Farm: Kranspoort (248), Ermelo district (2622S, 2951E; 2629Bd1) (Jacobsen 1989, TM): TM 74259\*.

- B70. Farm: La Belle Esperance (191), Piet Retief district (2717S, 303230E; 2730Bc1) (Jacobsen 1989, TM): TM 74223-5\*.
- B71. Farm: Langfontein (84), Wakkerstroom district (about 1850-1900 m; 2714S, 300830E; 2730Aa4) (Jacobsen 1989, TM): TM 45737\*.
- B72. Farm: Leiden (340), Wakkerstroom district (2651S, 3017E; 2630Cd1) (Jacobsen 1989, TM): TM 74248, 74286\*.
- B73. Farm: Maviriestad (321), Ermelo district (264430S, 3013E; 2630Ca4) (Jacobsen 1989, TM, as "Maviriestad"): TM 42364\*.
- B74. Farm: Paardeplaats (101), Kwa-Mandlangampisi Mtn, Wakkerstroom district (271450S, 3029E; 2730Ab4) (Jacobsen 1989, TM): TM 74200-1\*, 74266-70\*, 74272\*, 74274\*, 74282\*.
- B75. Farm: Rolfontein (536), Amersfoort district (2657S, 2957E; 2629Dd4) (Jacobsen 1989, TM): TM 74281\*, 74283\*.
- B76. Farm: Smalkloof (122), Volksrust district (2719S, 2950E; 2729Bd1) (Jacobsen 1989, TM): TM 74236\*, 74289\*.
- B77. Farm: Tafelkop (126), Wakkerstroom district (2717S, 301515E; 2730Ad1) (Jacobsen 1989, TM): TM 74258\*.
- B78. Farm: Vaalkop (490), Ermelo district (265030S, 294030E; 2629Dc2) (Jacobsen 1989, TM): TM 74237\*.
- B79. Farm: Verkyk (88), Volksrust district (2711S, 2957E; 2729Bb4) (Jacobsen 1989, TM): TM 74209-13\*, 74214, 74290\*.
- B80. Wakkerstroom, Wakkerstroom district (2722S, 3008E; 2730Ac2) (FitzSimons 1943, TM, as *P. subviridis subviridis*; Jacobsen 1989, TM): TM 1373, 1374-5\*, 1379\*, 1426\*, 1499\*, 1573\*, 44886-8\*, 44891-2\*; BMNH 1905.3.7.97.
- B81. Farm: Welgedacht (82), Volksrust district (2711S, 294830E; 2729Bb3) (Jacobsen 1989, TM): TM 74233-4\*.
- B82. Farm: Welgemeend (206), Ermelo district (261130S, 295230E; 2629Bb4) (Jacobsen 1989, TM): TM 74284\*.
- B83. Farm: Zandkraal (99), Kwa-Mandlangampisi Mtn, Wakkerstroom district (2714S, 3026E; 2730Ab4) (Jacobsen 1989, TM): TM 74243\*.
- B84. Farm: Tolderia (128), 10 km SW of Lothair, Ermelo district (262630S, 3022E; 2630Ad3): TM 1064, 1065-6\*.
- B85. Ermelo, Ermelo district (2632S, 2959E; 2629Db2) (FitzSimons 1943, TM, as *P. subviridis subviridis*): TM 47290-1\*.

- B86. 2 km NNE of Farmstead: Welgevonden, Farm: Welgelegen (107), Ermelo district (1650 m; 2622S, 3005E; 2630Ac1): TM 63609\*.
- B87. Carolina, Carolina district (2604S, 3007E; 2630Aa1) (FitzSimons 1943, TM, as *P. subviridis transvaalensis*).
- B88. Farm: Kleinfontein (3), Elandsberg Mtn, Amersfoort district (270230S, 300430E; 2730Aa1): TM 74207-8\*.
- B89. Farm: Bergvliet (192), Wakkerstroom district (271730S, 3031E; 2730Bc1): TM 74188\*.
- B90. Farm: Zuurbron (132), 31 km ENE of Wakkerstroom, Wakkerstroom district (2717S, 302630E; 2730Ad2): BMNH 1905.3.7.94-6\*.
- B91. Farm: Mooibron (133), Wakkerstroom district (271930S, 302745E; 2730Ad2): TM 79578.
- B92. Farm: Hexrivier (634), Balfour district (265430S, 2842E; 2628Dc4) (Jacobsen 1989, TM): TM 74203\*, 74260\*.
- B93. Farm: Klipplaatdrift (504), Amersfoort district (1600 m; 265457S, 295310E\*; 2629Dd4): NMB R8278-86\*.

**Gauteng:**

- B94. Farm: Blesboklaagte (181), Suikerbosrant, Vereeniging district (2628S, 2808E; 2628Ac4) (Jacobsen 1989, TM): TM 74246\*.
- B95. Farm: Eendracht (185), Suikerbosrant, Heidelberg district (2628S, 281930E; 2628Ad3) (Jacobsen 1989, TM): TM 74226-7\*.
- B96. “Keyterskloof”, Suikerbosrand Nature Reserve, Suikerbosrant, Heidelberg district (2628Ca2) (Jacobsen 1989, TM): TM 30152\*.
- B97. Suikerbosrand Nature Reserve, Suikerbosrant, Heidelberg district (2632S, 281230E; 2628Ca2) (Jacobsen 1989, TM): TM 74229, 74245, 74275-6\*, 74278\*.
- B98. Farm: Valsfontein (183), Suikerbosrand Nature Reserve, Suikerbosrant, Heidelberg district (2630S, 2814E; 2628Ca2) (Jacobsen 1989, TM): TM 39950\*, 67653-4\*, 68180\*.
- B99. Irene, Pretoria district (2552S, 2813E; 2528Cc2) (FitzSimons 1943, TM, accepted with reserve as *P. microlepidotus fasciatus*).
- B100. Heidelberg, Heidelberg district (2631S, 2822E; 2628Cb1) (FitzSimons 1943, AM, accepted with reserve as *P. microlepidotus fasciatus*).
- B101. Pretoria District (Roux 1907, ZMA, as *P. microlepidotus*; Loveridge 1944, as *P. microlepidotus melanotus*).

- B102. 0.8 km NW of Springbok Overnight Hut, Suikerbosrand Nature Reserve, Suikerbosrant, Heidelberg district (1750 m; 263019S, 281204E\*; 2628Ca2): NMB R8419-21\*.
- B103. 1.7 km N of Springbok Overnight Hut, Suikerbosrand Nature Reserve, Suikerbosrant, Heidelberg district (1840-1860 m; 262941S, 281202E\*; 2628Ac4): NMB R8422-3\*.
- B104. Diepkloof, 0.8 km WSW of Visitors' Centre, Suikerbosrand Nature Reserve, Suikerbosrant, Heidelberg district (1690-1740 m; 262911S, 281203E\*; 2628Ac4): NMB R8424-5\*.
- B105. 1.7 km SW of Visitors' Centre, Suikerbosrand Nature Reserve, Suikerbosrant, Heidelberg district (1830-1850 m; 262943S, 281156E\*; 2628Ac4): NMB R8426-9\*.
- B106. 2.3 km WSW of Visitors' Centre, Suikerbosrand Nature Reserve, Suikerbosrant, Heidelberg district (1790-1810 m; 262914S, 281103E\*; 2628Ac4): NMB R8415-8\*.

**Free State:**

- B107. Farm: Allanvale (249), Vrede district (1780-2021 m; 274130S, 293930E; 2729Da4) (De Waal 1978, NMB): NMB R2989-95.
- B108. Farm: Bachelor's Home (800), Drakensberg Mtns, Harrismith district (280830S, 293130E; 2829Ba3) (De Waal 1978, NMB): NMB R1479-86.
- B109. Farm: Berlin (497), Bothasberg Mtn, Vrede district (1720-2025 m; 272930S, 290330E; 2729Ac3) (De Waal 1978, NMB): NMB R2415-8; (1980 m; 272909S, 2905E; 2729Ac3): NMB R8573\*; (1940 m; 272907S, 290500E\*; 2729Ac3): NMB R8574\*.
- B110. Farm: Bon Haven (1692), Harrismith district (1660-1962 m; 2829S, 2910E; 2829Ac4) (De Waal 1978, NMB): NMB R3062-9.
- B111. Farm: Ceylon (290), Wepener district (about 1500 m; 2948S, 2654E; 2926Dd2) (De Waal 1978, NMB): NMB R2723-4\*.
- B112. Farm: Elandsfontein (990), Elandsfonteinrant, Lindley district (1580-1680 m; 2757S, 280330E; 2728Cc3) (De Waal 1978, NMB): NMB R4238-40.
- B113. Farm: Falle Grange (632), Reitz district (1620-1660 m; 273230S, 2823E; 2728Cb2) (De Waal 1978, NMB): NMB R3400-6.
- B114. Farm: Grootkloof (251), Ficksburg district (1680-1740 m; 2838S, 2855E; 2827Db4) (De Waal 1978, NMB): NMB R941-7\*.
- B115. Farm: Grootkrans (71), Heilbron district (1500 m; 273730S, 275330E; 2727Db2) (De Waal 1978, NMB): NMB R3478-80.

- B116. Farm: Lange Hoek (352), Harrismith district (1707-1926 m; 2803S, 292330E; 2829Ab2) (De Waal 1978, NMB): NMB R1446-7.
- B117. Farm: Machbela (595), Harrismith district (2748S, 2922E; 2729Cd1) (De Waal 1978, NMB): NMB R3024-31.
- B118. Farm: Mooigelegen (863), Harrismith district (1600-1646 m; 280230S, 2851E; 2828Bb1) (De Waal 1978, NMB): NMB R4317-22\*.
- B119. Farm: Morgenzon (370), Harrismith district (275530S, 2913E; 2729Cc4) (De Waal 1978, NMB): NMB R2958-60.
- B120. Farm: Morgenzon (123), Senekal district (1620-1840 m; 2832S, 275230E; 2827Db1) (De Waal 1978, NMB): NMB R304-6.
- B121. Farm: Oever (645), Drakensberg Mtns, Harrismith district (1880-1971 m; 2754S, 294030E; 2729Dc4) (De Waal 1978, NMB): NMB R1433-6.
- B122. Farm: Parva Sed Mea (865), Harrismith district (1600-1640 m; 2810S, 285230E; 2828Bb4) (De Waal 1978, NMB): NMB R673-4\*.
- B123. Platberg Mtn, Farm: Harrismith Townlands (131), Harrismith district (about 2816S, 2911E; 2829Ac2) (De Waal 1978, NMB): NMB R2800-1, 4575; TM 54338, 55319; (near towers on summit: 2398 m; 281617S, 291247E\*; 2829Ac2): NMB R8533\*.
- B124. Farm: Rambouillet (396), Lindley district (ave. 1500-1540 m; 274930S, 2748E; 2727Dd1) (De Waal 1978, NMB): NMB R1836-8\*.
- B125. Reitz, Reitz district (about 1600-1700 m; 2748S, 2826E; 2728Cd2) (De Waal 1978, NMZB-UM): NMZB-UM 10933.
- B126. Farm: Stoffelfontein (407), Lindley district (1570-1615 m; 280620S, 275710E; 2827Bb2) (De Waal 1978, NMB): NMB R4260.
- B127. Farm: Sweet Waters (674), Vrede district (1760-1780 m; 2735S, 294315E; 2729Da2) (De Waal 1978, NMB): NMB R834.
- B128. Farm: Tafelberg (815) Harrismith district (1720-2286 m; 2813S, 291130E; 2829Aa4) (De Waal 1978, NMB): NMB R4286-92, 4297.
- B129. Farm: Tygerfontein (240), Vrede district (highest 2090 m; 2735S, 2927E; 2729Cb2) (De Waal 1978, NMB): NMB R2951-3.
- B130. Farm: Uitvlugt (227), W slopes of Gemsbokberg Mtn, Vrede district (1660-2060 m; 272730S, 292230E; 2729Ad4) (De Waal 1978, NMB): NMB R835-45.
- B131. Farm: Waterfall (1157), Harrismith district (2818S, 2925E; 2829Ad2) (De Waal 1978, NMB): NMB R3079-82, 3087-8, 3091.

- B132. Farm: Allemans Gras (611), Harrismith district (2749S, 290745E; 2729Cc2) (Bates 1996, TM as listed): TM 26229-31; CAS 106015.
- B133. Farm: Ark (1010), Harrismith district (about 1676 m; 282745S, 290245E; 2829Ac3) (Bates 1996, NMB as listed): NMB R6235-7, 6238\*, 6239-42, 6562-5, 6600, 6601-8\*.
- B134. Farm: Berlin (536), Rooiberg Mtns, Bethlehem district (282930S, 282915E; 2828Ad4) (Bates 1996, TM as listed; same specimen erroneously also listed under "Bethlehem" by Bates 1996): TM 50096.
- B135. Farm: Bosch Kloof (487), Drakensberg Mtns, Harrismith district (283140S, 285915E; 2828Db2) (Bates 1996, NMB as listed): NMB R6451-2\*.
- B136. Farm: Frazerfield (187), Harrismith district (about 1646 m; 2827S, 290130E; 2829Ac3) (Bates 1996, NMB, some as listed): NMB: R6225\*, 6226, 6227\*, 6228-29, 6230-2\*, 6233-4, 6252, 6273-4, 6275\*, 6276-7, 6278-9\*, 6285\*, 6286, 6299-300\*.
- B137. Farm: Grootfontein (153), Harrismith district (highest 1698 m; 281830S, 291415E; 2829Ac2) (Bates 1996, NMB as listed): NMB R6436.
- B138. Farm: Jagers Rust (383), Heilbron district (1680-1759 m; 273320S, 281045E; 2728Ca2) (Bates 1996, NMWN as listed): NMWN 3365.
- B139. Lindley, Lindley district (about 1500-1600 m; 275250S, 2755E; 2727Dd4) (Bates 1996, TM as listed): TM 47285-9; CAS 135486.
- B140. Farm: Louisas Mount (1064), Harrismith district (about 1680 m; 2810S, 290720E; 2829Aa3) (Bates 1996, NMB as listed): NMB R6320-38.
- B141. Farm: Mooi Hoek (130), Harrismith district (2812S, 290940E; 2829Aa4) (Bates 1996, NMB as listed): NMB R6311-9.
- B142. Farm: Mooihoek (556), Lindley district (1460-1522 m; 275430S, 2746E; 2727Dd3) (Bates 1996, TM as listed): TM 54769.
- B143. Petrus Steyn (town), Lindley district (ave. 1700 m, highest 1762 m; 2739S, 280745E; 2728Ca4) (Bates 1996, NMWN as listed): NMWN 3366.
- B144. Harrismith, Harrismith district (281630S, 290745E; 2829Ac2) (FitzSimons 1943, TM, as *P. subviridis subviridis*; referred to *P. melanotus melanotus* by De Waal 1978): TM 11245, 47549.
- B145. Farm: Nelsonshoek (229), Nelson's Kop Mtn, Harrismith district (2200 m; 2814S, 2927E; 2829Ab4): NMB R7871-4\*.
- B146. Farm: Uyshoek (1092), Harrismith district (1770 m; 281550S, 292045E\*; 2829Ad1): NMB R8170-92\*.

- B147. Farm: Tafelberg 'A' (1312), Tafelberg Mtn, Harrismith district (+2000 m; 281430S, 291115E\*; 2829Aa4): NMB R8503\*.
- B148. Qoqolosing, Farm: Witzieshoek (1815), Harrismith district (1900-1960 m; 283532S, 285423E\*; 2828Db2): NMB R8361\*.
- B149. Mabedlana Mtn, Farm: Sandhurst (333), Harrismith district (1900-2000 m; 281116S, 292409E\*; 2829Ab4): NMB R8507-11\*.
- B150. Farm: Dipka (200), Vrede district (1700 m; 273930S, 2901E; 2729Ca3): NMB R8522\*.

**KwaZulu-Natal:**

- B151. Farm: no. 11931, 1 km N of Ekombe (centre), Nkandhla district (283745S, 305330E; 2830Db4): TM 53529-30\*, 53531.
- B152. Farm: Corriedale (11630), 1 km N of Qudeni (centre), Nkandhla district (2836S, 3052E; 2830Db1): TM 53944\*.
- B153. Farm: Bloemhof (127), Vryheid district (2749S, 3059E; 2730Dd2): TM 56732.
- B154. Van Reenen (centre), Bergville district (1524 m; 282201S, 292218E; 2829Ad1) (FitzSimons 1943, DNSM, NM, as *P. subviridis subviridis*; Broadley 1964, NMSA, DNSM, as *P. subviridis transvaalensis*): DNSM R390; NMSA 552, 618 (two specimens), 620, 1330-1.
- B155. Nkonyane Mtn, Qudeni Forest, Nkandhla district (284005S, 305239E; 2830Db4): DNSM 391.
- B156. Muller's Pass, 23 km SW of Newcastle, Bergville district (2752S, 2943E; 2729Dc2) (Broadley 1964, NMSA, as *P. subviridis transvaalensis*): NMSA 898a-h\*.
- B157. Botha's Pass, 26 km NW of Newcastle, Drakensberg Mtns, Newcastle district (1817 m; 2738S, 2943E; 2729Da4) (Broadley 1964, NUM, NMZB-UM, as *P. subviridis transvaalensis*): NMZB-UM 2066.
- B158. Oliviershoek Pass, Bergville district (2834S, 2904E; 2829Ca1) ("Oliviershoek": photo in Visser 1984): JV 1591-2, 1599.
- B159. Farm: Engelbrechtsdrift (409), 12 km SSW of Wakkerstroom, Utrecht district (about 1750 m; 272730S, 300430E; 2730Ac3) ("Farm Engelbrecht's Drift, 10 km SW of Wakkerstroom": Lambiris 1988, AJL as listed, as *P. subviridis transvaalensis*): AJL 2263-72; TM 62996.
- B160. W side of Majuba Mtn summit, Newcastle district (1881 m; 272830S, 295055E; 2729Bd3): TM 71882.
- B161. "Ulumbe Camp", Ncandu Forest Reserve, Newcastle district (275401S, 294145E; 2729Dc4): TM 71887\*.

- B162. Ncandu Nature Reserve, Newcastle district (2754S, 2941E; 2729Dc4): TM 80077-8\*.
- B163. Babanango, Babanango district (1300 m; 282230S, 3105E; 2831Ac3): JV 2186.
- B164. N of Nkandla (town), Nkandhla district (2831Ca1): JV 47879 (two embryos), 47979 (embryo), 52079, 52279, 52779, 52779 (embryo), 52879 (embryo), 52979 (two embryos), 53079 (embryo); no number (? adult).
- B165. Hill at Vumanhlamvu village between Nkandla town and Nkandla Forest, Nkandhla district (about 1200-1300 m; 284200S, 310734E\*; 2831Ca4): NMB R8366-76\*.
- B166. Qudeni Forest, Nkandhla district (2830DB) (Broadley 1964, NMSA, *P. subviridis transvaalensis*): NMSA 997a-e\*.
- B167. Ntabayabesutu village, 10 km NNW of Qudeni village, Nkandhla district (about 1500 m; 283130S, 305018E\*; 2830Db1): NMB R8377-87\*.
- B168. Farm: Braet Mead (14238), 12 km SSE of Babanango, Nkandhla district (about 1100-1200 m; 282855S, 310604E\*; 2831Ac3): NMB R8388\*.
- B169. Farm: Legerplaats (634), Ngome Forest, Ngome Forest Reserve, Ngotshe district (2749S, 312510E; 2731Cd2) (Ezemvelo KZN [KwaZulu-Natal] Wildlife sight record - L. Raw, 9 October 1980).
- B170. Slang River near Groenvlei (centre), Utrecht district (?2730Ac4; not plotted) (recent TM *Pseudocordylus* according to Ezemvelo KZN [KwaZulu-Natal] Wildlife records).
- B171. Ngoye Forest area (highest 486 m; 2831Dc2) (Bourquin 2004, in reference to museum material collected in the period 1944-1970, as *Cordylus melanotus melanotus*; possibly in reference to PEM R2687-9 from “Ngoyo Mountains”, as *P. melanotus* in PEM catalogue; record needs confirmation - not plotted on map).

***Pseudocordylus melanotus subviridis***

**Free State:**

- C1. Farmstead Rydal Mount, Farm: Castle View (59), Harrismith district (about 1740 m; 2831S, 285130E; 2828Db1): USNM 153869.
- C2. Tsheseng, about 21 km SE of Witsieshoek, Harrismith district (2838S, 2852E; 2828Db3): CAS 125784-8.
- C3. 18 km SE of Kestell, Harrismith district (2828Bd3): CAS 126018.
- C4. Golden Gate Highlands National Park, Bethlehem district (2828Da1) (De Waal 1978, TM): TM 34618-9, 42814, 54653, 54659, 54662; NMB R5459-63\*.

- C5. Farm: Bramleys Hoek (52), Bethlehem district (2826S, 283045E; 2828Bc3) (De Waal 1978, NMB): NMB R3522-3\*.
- C6. Wodehousekop, Golden Gate Highlands National Park, Bethlehem district (282930S, 2838E; 2828Bc4) (Bates 1996, NMB as listed): NMB R6346\*.
- C7. Monontsha Pass, Farm: Witzieshoek (1815), Harrismith district (2377 m; 283515S, 284130E; 2828Da2) (as “Monontsa Pass”: De Waal 1978, NMB): NMB R653\*, 659-60\*, 662-3\*, 668-9\*, 670-1\*, 3298\*, 3300-5\*, 4607-11\*.
- C8. Monontsha Pass border post (South African side), Farm: Witzieshoek (1815), Harrismith district (2200 m; 283453S, 284154E\*; 2828Da2): NMB R8335-6\*.
- C9. Monontsha Pass, near “Kraal”, Farm: Woes Arabia (40), Harrismith district (2200 m; 283515S, 284123E\*; 2828Da2): NMB R8337-46\*.
- C10. Monontsha Pass, 0.5 km NE of Monontsa Pass border post (Lesotho side, near Ha Molisana), Farm: Woes Arabia (40), Harrismith district (2100 m; 283515S, 284108E\*; 2828Da2): NMB R8347\*.
- C11. Monontsha Pass, near “Kraal”, 1 km NE of Monontsa Pass border post (Lesotho side, near Ha Molisana), Farm: Woes Arabia (40), Harrismith district (2200 m; 283514S, 284131E\*; 2828Da2): NMB R8348-53\*.
- C12. 350 m W of Witzieshoek Mountain Resort, Farm: Witzieshoek (1815), Harrismith district (2220 m; 284109S, 285345E\*; 2828Db4): NMB R8562-3\*.
- C13. 1 km NW of Witzieshoek Mountain Resort, Farm: Witzieshoek (1815), Harrismith district (2200 m; 284055S, 285340E\*; 2828Db4): NMB R8354-57\*.
- C14. Sentinel Mtn, Farm: Witzieshoek (1815), Harrismith district (284420S, 285330E; 2828Db4) (De Waal 1978, NMB): NMB R3336-43\*, 4614 (cleared & stained), 4615-26\*, 6437-47\*, 6570-5\*.
- C15. Entrance to Sentinel Hiking Trail, Farm: Witzieshoek (1815), Harrismith district (2460-2500 m; 284339S, 285338E\*; 2828Db4): NMB R8358\*.
- C16. Sentinel Road, 2 km N of Sentinel Mtn peak, Farm: Witzieshoek (1815), Harrismith district (2517 m; 284323S, 285325E\*; 2828Db4): NMB R8526-7\*.
- C17. 50 m N of bottom of Chain Ladder, Sentinel Hiking Trail, Farm: Witzieshoek (1815), Harrismith district (2850 m; 284449S, 285253E\*; 2828Db4): NMB R8560\*.
- C18. 100 m N of Sentinel Hiking Trail car park, Farm: Witzieshoek (1815), Harrismith district (2520 m; 284349S, 285334E\*; 2828Db4): NMB R8567\*.
- C19. 100 m SE of Sentinel Hiking Trail car park, Farm: Witzieshoek (1815), Harrismith district (2460 m; 284352S, 285333E\*; 2828Db4): NMB R8561\*.

- C20. Qoqolosing, Farm: Witzieshoek (1815), Harrismith district (1900-1960 m; 283519S, 285612E\*; 2828Db2): NMB R8359\*; (1900-1960 m; 283532S, 285423E\*; 2828Db2): NMB R8360, 8362-64\*.
- C21. Thibella (village), 2 km N of Fika Patso Dam, Farm: Witzieshoek (1815), Harrismith district (1800-1900 m; 283915S, 285140E\*; 2828Db3): NMB R8365\*.
- C22. Nemahadi Pass, 2 km NNW of Mont-aux-Sources (peak), Farm: Witzieshoek (1815), Harrismith district (3096 m; 284517S, 285154E\*; 2828Dd1): NMB R8528-30\*.
- C23. Nemahadi Pass, old border post, 2 km NNW of Mont-aux-Sources (peak), Farm: Witzieshoek (1815), Harrismith district (3082 m; 284517S, 285200E\*; 2828Dd1): NMB R8531\*.
- C24. Fika Patso Dam resort, Farm: Witzieshoek (1815), Harrismith district (1960 m; 284018S, 285040E\*; 2828Db3): NMB R8532\*.

**KwaZulu-Natal:**

- C25. Balgowan (centre), 20 km NW of Howick, Lions River district (292330S, 300230E; 2930Ac3) (Boulenger 1908, as *P. microlepidotus*; Loveridge 1944, as *P. microlepidotus melanotus*): BMNH 1907.4.17.17.
- C26. Rietvlei (centre), near source of Mvoti River, Umvoti district (2930AB) (“Umvoti” [2930BA]: Boulenger 1910, as *P. microlepidotus*; “Umvoti (River)”: FitzSimons 1943, SAM; “Umvoti”: Loveridge 1944, as *P. microlepidotus melanotus*): SAM ZR 4215-6.
- C27. Mont-aux-Sources, Drakensberg Mtns, Bergville district (2846S, 2852E; 2828Dd1) (Hewitt 1925, as *P. microlepidotus*; Essex 1927, as *P. microlepidotus*; Hewitt 1927 [summit]; FitzSimons 1943, AM, NMSA, TM; “Mont-aux-Sources, Drakensberg, Basutoland”: Loveridge 1944, paratypes of *P. langi*, type locality, “11000 ft [= 3353 m], TM 1346-1347, 13849-13850; FitzSimons 1948, TM, including TM 1346-1347, 13849-13850; Broadley 1964, NMSA, TM; Bourquin & Channing 1980, TM, NMSA; Bourquin 1989, TM, as *P. melanotus*): NMSA 897 (summit; 34 specimens); TM 1346-7, 13849-50 (all paratypes of *P. langi*), TM 13848, 13851-3 (“Mont-aux-Sources area”), 37594; TM 3846-7 & 3849-50 (paratypes of *P. langi*); TM 66730; NMB R6836\* (“Natal side”; ex-AM 5302 which included one *P. spinosus*).
- C28. Giants Castle, Impendle district (2921S, 2929E; 2929Ad2) (FitzSimons 1943, NMSA, NMZB, TM; Loveridge 1944, as *P. microlepidotus melanotus*; Broadley 1964, NMSA, NMZB-UM, TM): NMZB-UM 5466; NMSA 553, 954-6; TM 2517-8, 2520, 2532 (2929AB), 55919 (all “Giants Castle area” - 2929AB/AD), 19376.
- C29. Little Tugela River valley, 31 km W of Escourt, Escourt district (2901S, 293230E; 2929Ba1) (FitzSimons 1943 [NM, “Little Tugela Valley”]).

- C30. Nottingham Road (centre), Lions River district (2922S, 295930E; 2929Bd2) (FitzSimons 1943, NMSA; Broadley 1964, NMZB-UM, as *P. subviridis transvaalensis*): NMSA 685\*; NMZB-UM 2075.
- C31. Farm: no. 2170, 2 km NNE of Nottingham Road (centre), Mooi River district (2920S, 3000E; 2930Ac1) ("near [E of] Nottingham Road": Yeadon 1991, RY 240, as *P. melanotus transvaalensis*): NMB R-RY 238-41\*, 367\*.
- C32. Pietermaritzburg, Pietermaritzburg district (2930CB) (FitzSimons 1943, NMSA; Broadley 1964, NMSA, as *P. subviridis transvaalensis*): NMSA 808\*.
- C33. Dargle (centre), Impendle district (2933S, 2958E; 2929Db2) (FitzSimons 1943, NMSA; Broadley 1964, NMSA, as *P. subviridis transvaalensis*): NMSA 809a-b\*.
- C34. "Bushmans Peak", near Giants Castle (?2929Cc) (FitzSimons 1943, NMSA [2591 m]; Broadley 1964, NMSA; Bourquin & Channing 1980, NMSA).
- C35. Farmstead: Willbrook on Farm: Weltevreden (1903), Escourt district (2908S, 295215E; 2929Bb3) ("Wellbrook": FitzSimons 1943, DNSM; "Willbrook": Broadley 1964, DM, as *P. subviridis transvaalensis*): DNSM R389; AMNH R-5888.
- C36. Polela (centre), 35 km ENE of Underberg, Polela district (294630S, 2952E; 2929Dd1) (FitzSimons 1943, DM).
- C37. Underberg (W part of town), Underberg district (294730S, 292930E; 2929Cd2) (FitzSimons 1943, TM; "Drakensberg near Underberg at 6000 ft" [= 1829 m]: Loveridge 1944, TM 20992, paratype of *P. langi*; "Drakensberg near Underberg": Broadley 1964, TM; "near Underberg": Yeadon 1991 [RY 264: 2947S, 2929E]); TM 20992; NMB-RY R263-6\*; 267 (lost); NMZB-UM 10934.
- C38. Royal Natal National Park, Bergville district (2842S, 285530E; 2828Db4) (Broadley 1964, NMZB-UM; Bourquin & Channing 1980, NMZB-UM): NMZB-UM 5322.
- C39. Cathkin Peak, Cathkin Peak Forest Reserve, Escourt district (2905S, 2921E; 2929Ab1) (Broadley 1964, NMSA): NMSA 747 (two specimens; 2134 m); TM 31231.
- C40. Champagne Castle, Bergville district (1676 m; 290530S, 2920E; 2929Ab1) (Broadley 1964, NMSA): NMSA 673.
- C41. Champagne Castle Hotel (also "Hostel"), Estcourt district (2903S, 292515E; 2929Ab2) (Bourquin & Channing 1980, NMSA): NMSA 542, 951-3, 1195.
- C42. Cathedral Peak Forest Reserve (or "Station"), Bergville district (2829Cc4) (Bourquin & Channing 1980, NMZB-UM, MCZ, TM): TM 49700, 49698-9, 50083-4, 51659-65, 51667.
- C43. Cathedral Peak State Forest, Bergville district (2929Aa2): TM 51644-6.

- C44. Cathedral Peak, Cathedral Peak Forest Reserve, Bergville district (285530S, 290730E; 2829Cc4) (Bourquin & Channing 1980, NMSA, TM): NMZB-UM 2163-9, 2171, 2173-4, 2177 (all 2286 m); AMNH R114356-7 (ex-NMZB-UM 2175 and 2176 respectively; both 2176 m); NMSA 1162 (2134 m); TM 29973.
- C45. Between Cathedral Peak and Organ Pipes Pass, Cathedral Peak Forest Station, Bergville district (2829Cc4): TM 48576-7.
- C46. Giants Castle Game Reserve, ?Estcourt district (2929AB) (Bourquin & Channing 1980, TM, NMSA, NMZB-UM): CAS 156445; NMSA 666 (seven specimens), 667, 1475.
- C47. Game Reserve Camp, Giants Castle Game Reserve, Estcourt district (2916S, 2931E; 2929Bc1) (Lambiris 1988, AJL as listed): AJL 2033.
- C48. “Injasuthi”, Farm: no. 7129, near Van Heyningens Pass, Giant’s Castle Game Reserve, Estcourt district (2907S, 2926E; 2929Ab2): TM 62995.
- C49. Farm: Cloudland (9039), Giant's Castle Game Reserve, Estcourt district (2911S, 2927E; 2929Ab4): TM 66745.
- C50. Swartkop Mtn, W of Pietermaritzburg, Pietermaritzburg district (2939S, 3010E; 2930Ca4) (“Swartkops”: Broadley 1964, NMSA, as *P. subviridis transvaalensis*): NMSA 1227.
- C51. Lidgetton (centre), 12.5 km WNW of Howick, Lions River district (2927S, 3006E; 2930Ac3) (Broadley 1964, NMSA, as *P. subviridis transvaalensis*): NMSA 1220.
- C52. Dansekop (2930AB: Leistner & Morris 1976) (Broadley 1964, NMZB-UM, as *P. subviridis transvaalensis*): NMZB-UM 2051-2.
- C53. Organ Pipes Pass, Bergville district (2901S, 291230E; 2929Aa2) (Broadley 1964, NMZB-UM, MCZ): NMZB-UM 2397-8, 2401-9 (all 2400-2700 m); NMSA 6515; TM 51647-8, 56394-5; TM 51649 (as “Organ Pipes Ridge”); TM 51650-6 (as “Organ Pipes Spur”); NMB R8151-68\*.
- C54. The Nek, Royal Natal National Park, Bergville district (284153S, 2854E; 2828Db4) (Lambiris 1988, AJL as listed): AJL 1230, 1273, 1290, 1354.
- C55. NE foot of Sentinel, Royal Natal National Park, Bergville district (284421S, 285340E; 2828Db4) (Lambiris 1988, AJL as listed): AJL 2292-4.
- C56. Inner Mnweni Needle, Bergville district (about 2853S, 2902E; 2829Cc3) (Lambiris 1989, AJL as listed): AJL 2859.
- C57. Gladstone’s Nose, Kamberg Nature Reserve, Mooi River district (2924S, 2941E; 2929Bc4) (Lambiris 1988, AJL as listed): AJL 2164; TM 62843.

- C58. “49 km along Himeville road from Nottingham Road”, Impendle district (2929Da1): CAS 156731.
- C59. Franklin (centre), Mount Currie district (3019S, 2927E; 3029Ad2): NMSA 883\*, 886\*; TM 38206-7\*.
- C60. Farm: Dartmoor (7421), Highmoor Forest Reserve, Mooi River district (2919S, 2937E; 2929Bc1): NMB R-RY R199-201\*.
- C61. Sani Pass, Underberg district (2935S, 291730E; 2929Cb1) (Yeadon 1991, RY 124): NMB R-RY R113\*, 120\*, 122 (lost), 123-4\*, 126\*; TM 29050-9, 30067.
- C62. South African Police Post, Sani Pass, Cobham State Forest, Underberg district (293630S, 292030E; 2929Cb1): TM 30068.
- C63. Farm: Hewitt (7446), Cobham State Forest, Mzimkulwana (area), Underberg district (294132S, 292538E; 2929Cb4): NMB R-RY R873\*.
- C64. Bushman's Nek Pass, Mzimkulwana Nature Reserve, Underberg district (2949S, 2911E; 2929Cc2): NMB R-RY R115.
- C65. “Farm: Borreray”, 21.5 km NNE of Himeville, Impendle district (2933S, 2933E; 2929Da1): NMB R-RY R392\*, 456-9\*, 460, 471\*.
- C66. Underberg (E part of town), Underberg district (2948S, 2930E; 2929Dc1): NMB-R-RY R284.
- C67. Farmstead: Mearns, Farm: no. 15142, Mooi River district (291407S, 295928E; 2929Bb4): NMB R-RY R824\*.
- C68. Kamberg Mtn, E of Kamberg Nature Reserve, Mooi River/Lions River districts (2922S, 2946E; 2929Bd1): NMB R-RY R626\*.
- C69. Spioenkop Mtn, 22 km SW of Nottingham Road (centre), Impendle district (292651S, 294753E; 2929Bd3): NMB R-RY R439-40\*, 441 (lost), 442-3\*, 444-5, 446-7\*.
- C70. Farmstead: Glamoor, Farm: Leeuwbosch (1275), 7 km SW of Nottingham Road (centre), Lions River district (2923S, 2956E; 2929Bd4): NMB R-RY R403\*, 405-8\*, 475-8\*.
- C71. Farm: Welton (2108), Nhlosane Mtn, 21.5 km SSW of Nottingham Road (centre), Impendle district (2933S, 295730E; 2929Db2): NMB R-RY R826-32\*, 901-2\*, 907\*, 910\*; TM 50916-7\*, 50918, 51626, 52364-5.
- C72. Johnstone's Kop (E part), 9 km NNE of Nottingham Road (centre), Mooi River district (291630S, 3000E; 2930Ac1): NMB R-RY R628-9\*.
- C73. Farm: Fordoun (14783), about 4 km NE of Nottingham Road (centre), Lions River district (2920S, 3002E; 2930Ac1): NMB R-RY R346-7\*, 348-9, 350\*, 377.

- C74. Farmstead: Easingwold, Farm: no. 14534, 8 km ENE of Nottingham Road (centre), Lions River district (2920S, 3003E; 2930Ac1): NMB R-RY R474\*.
- C75. Farm: Wahroonga (13458), Lions River district (293617S, 3008E; 2930Ca2): NMB R-RY R948\*.
- C76. Mooi River, Mooi River district (291230S, 295942E; 2929Bb4): TM 44113-4; DMR 1132, 1471-82.
- C77. Farm: "Treverton", about 2.5 km NNE of Mooi River, Farm: no. 12407, Mooi River district (291100S, 3000E; 2930Aa3): PEM R6409.
- C78. Wonder Valley, 4 km NE of Cathkin Peak, Cathkin Peak Forest Reserve, Estcourt district (2904S, 2923E; 2929Ab2): TM 21266, 21268, 24681, 55002.
- C79. Masongwane River valley, Farm: no. 11121, Bergville district (285730S, 291450E; 2829Cc4): TM 51634-7.
- C80. Farm: Baviaanskloof (12921), Loteni Nature Reserve, Impendle district (2926S, 2931E; 2929Bc3): TM 62997.
- C81. Farmstead: Sunnyside, Farm: no. 7821, Coleford Nature Reserve, Underberg district (2956S, 292630E; 2929Cd4): TM 63931.
- C82. Farm: Hartsease (32191), Estcourt district (2900S, 2930E; 2929Ba1): TM 63970.
- C83. Monk's Cowl area, Monk's Cowl Nature Reserve, Estcourt district (2929AB): TM 65141.
- C84. Highmoor Forest Reserve, Mooi River district (2929BC): TM 69061, 69063-4.
- C85. "Mdedelelo Wilderness Area", Drakensberg Mtns (2929AB/AD): TM 69154.
- C86. "The Twins", Drakensberg Mtns (3028Ab4 or 2829Cc3; not plotted): TM 34914.
- C87. Near Kokstad, Mount Currie district (3029AD) ("Kokstad": FitzSimons 1943; "Drakensberg near Kokstad": Loveridge 1944, paratype of *P. langii*): TM 21063\* (paratype of *P. langii*).
- C88. S of Kokstad, 1 km from Transkei border, Mount Currie district (3029CB): TM 53914\*.
- C89. Drakensberg Gardens, 27 km W of Himeville, Underberg district (2945S, 2914E; 2929Cc2): TM 20993.
- C90. "Mafeking, Natal" (locality not traced): SAM ZR 43779-80.
- C91. Farm: Welgevonden (969), Loskop Mtn, Lions River district (2921S, 3015E; 2930Ad1): TM 51627-32.

- C92. Hlabeni Mtn, Hlabeni State Land, 18 km SSW of Polela, Polela district (2958S, 2944E; 2929Dc4): TM 56737-41.
- C93. Arrochar Hill, 10 km ESE of Mooi River, Mooi River district (2913S, 3006E; 2930Aa3): TM 64708.
- C94. Farmstead: Fabers Hill, Farm: Good Hope (962), Impendle district (293930S, 295630E; 2929Db4): TM 69057-8.
- C95. Amphitheatre escarpment near Tugela Falls, Royal Natal National Park, Bergville district (2990 m; 284511S, 285340E\*; 2828Dd2): Tissue sample (M. Cunningham, pers. comm.).
- C96. Between Sentinel Caves and Chain Ladder, Bergville district (284448S, 285258E\*; 2828Db4): Sight record (M. Cunningham, pers. comm.).
- C97. 50 m E of top of Chain Ladder, Bergville district (3020 m; 284449S, 285254E\*; 2828Db4): NMB R8558\*.
- C98. 300 m SE of top of Chain Ladder, Bergville district (3000 m; 284458S, 285256E\*; 2828Db4): NMB R8559\*.
- C99. “Ntonjelane Pass”, Cathedral Peak area, Bergville district (2805 m; 285618S, 290540E\*; 2829Cc3): Sight record (also tissue sample from nearby: *e.g.* 2719 m, 285607S, 290538E\*) (M. Cunningham, pers. comm.).
- C100. Farm: Welton (2108), SW slopes of Nhlosane Mtn, Impendle district (2934S, 2957E; 2929Db2) (Ezemvelo KZN [KwaZulu-Natal] Wildlife sight record: O. Bourquin, 24 July 1977).
- C101. Farm: no. 7673, Mount Gilboa, Umvoti district (291715S, 301730E; 2930Ad1) (un-numbered University of Durban-Westville [WM] specimen recorded by Ezemvelo KZN [KwaZulu-Natal] Wildlife).

**Lesotho:**

- C102. Near or at the top of Menyameng Pass, Front Range, western part of the Maloti Mountains, north-western Lesotho (about 3100 m a.s.l.; 29°08'30"S, 28°13'30"E; 2928Aa4) (“top of the high mountainous range, which extends behind Kafferland and the country of Natal” – restricted type locality of *Cordylus* [*Pseudocordylus*] *subviridis*: Smith 1843, as *P. microlepidotus*; “Mountains behind Kaffirland”: Loveridge 1944, as *P. microlepidotus melanotus*).
- C103. Bokong (2150 m; 2920S, 282730E; 2928Ad2) (Bourquin 1989, TM, as *P. melanotus*): TM 21777-9.
- C104. Upper Bokong River valley (2950-3025 m; 2904-05S, 2826E; 2928Ab2) (“Bokong River valley”: Meakins *et al.* 1988, sight record, as *Cordylus giganteus*; “Upper Bokong Valley”: Mouton & Van Wyk 1993, NML & USEC as listed, as *P. melanotus*): NML 29-30; USEC-H2513, 2596-602 (USEC-H2598-9 given as west of Thaba Chitja – 2928Ac3 by Mouton 1996, as *P. melanotus*).

- C105. Near Ha Lejone (= Ha Sebotha) (2175 m; 2905S, 2829E; 2928Ab2) (Mouton & Van Wyk 1993, USEC as listed, as *P. melanotus*): USEC-H2481-2.
- C106. Lesotho Highlands Development Authority base, Ha Poli (2100-2150 m; 2908S, 2829-30E; 2928Ab4) (Mouton & Van Wyk 1993, NML as listed, as *P. melanotus*): NML 25, 27-8.
- C107. About 5 km W of Ha Ramanamane (2150 m; 2919S, 2829E; 2928Ad2) (Mouton & Van Wyk 1993, NML & USEC as listed, as *P. melanotus*): NML 51; USEC-H2604 (as “Bokong River to Ha Motoko” – 2928Ac3 in Mouton 1996, as *P. melanotus*); USEC-H2605.
- C108. 1.5 km S of Ha Seshote (2275 m; 2917S, 2833E; 2928Bc1) (Mouton & Van Wyk 1993, USEC as listed, as *P. melanotus*): USEC-H2511-2.
- C109. Katse Mtns, E of Ha Ramokoatsi (2400 m; 2921S, 2829E; 2928Ad2) (Mouton & Van Wyk 1993, USEC as listed, as *P. melanotus*): USEC-H2514-47, 2573-9, 2606-35.
- C110. Laitsoka Mtn (2550 m; 2912S, 2830E; 2928Ba3) (Mouton & Van Wyk 1993, USEC as listed, as *P. melanotus*): USEC-H2413-36.
- C111. Laitsoka Pass, viewpoint at summit (2500 m; 2912S, 2830E; 2928Ba3) (Mouton & Van Wyk 1993, USEC as listed, as *P. melanotus*): USEC-H2384-404, 2483-502, 2548-72.
- C112. Maliba-Matso River, 1 km SE of Ha Poli (2000 m; 2909S, 2829E; 2928Ab4) (Mouton & Van Wyk 1993, NML as listed, as *P. melanotus*): NML 26, 74.
- C113. Mountain SW of 'Mamohau mission station (2400 m; 2908S, 2827E; 2928Ab4) (Mouton & Van Wyk 1993, NML & USEC; as listed, as *P. melanotus*): NML 31-40; USEC-H2603 (as “Bokong River to Ha Motoko” – 2928Ac3 in Mouton 1996, as *P. melanotus*).
- C114. Tributary of Mokhoulane River, 5 km W of range management site (2650 m; 2907S, 2826E; 2928Ab2) (Mouton & Van Wyk 1993, USEC as listed, as *P. melanotus*): USEC-H2405-12, 2461-80, 2580-95.
- C115. 1 km SE of Mphorosane (2300 m; 2911S, 2830E; 2928Ba3) (Mouton & Van Wyk 1993, NML, USEC; as listed, as *P. melanotus*): NML 52-64; USEC-H2365-83, 2437-60, 2503-10.
- C116. 3 km S of Nkaiobee Pass (2300 m; 2918S, 2831E; 2928Bc1) (Mouton & Van Wyk 1993, USEC as listed, as *P. melanotus*): USEC-H2636-9.
- C117. Thabana-li-Mele (2050 m; 2909S, 2829E; 2928Ab4) (Mouton & Van Wyk 1993, NML as listed, as *P. melanotus*): NML 65.
- C118. Area along Jorotane [= Jorodane] River from Sehlabaneng [village] to the village of Maetsisa (2928Ac3): Sight record (Mouton 1996, as *P. melanotus*).

- C119. Area from Khoaba-lea-bua Mountain to Maetsisa (2928Ac3): Sight record (Mouton 1996, as *P. melanotus*).
- C120. Sehlabaneng (2250-2300 m; 292250S, 280120E; 2928Ac3): Sight record (Mouton 1996, as *P. melanotus*).
- C121. Black Mtn, between Sani and Mokhotlong, Masenkeng area (2789 m; 2929Ca2): TM 29072-6, 33778.
- C122. Black Mtn, 17 km NW of Sani Pass (3200 m; 2929Ac4) (Bourquin 1989, TM, as *P. melanotus*): TM 47920-1.
- C123. Blue Mtn Pass (2926S, 2758E; 2927Bd4): BMNH 1974.2259-76 (2713 m); TM 30285-98.
- C124. Cheche's Pass (2700 m; 2933S, 2813E; 2928Ca2) ("10 km E of Marakabeis": Bourquin 1989, TM as *P. melanotus*: TM 30315-6, 30318-9, 30321-3, 34691.
- C125. Ha Lechesa, 4 km NE of Semonkong (about 2400 m; 294945S, 280515E\*; 2928Cc1): NMB R7482-3.
- C126. 1 km NW of Ha Mamokae (2800 m; 2933S, 2913E; 2929Ca2): TM 47903.
- C127. 8 km W of Ha Marakabei on road to Maseru (2600 m; 2928Ca1): TM 30317, 30320, 30324-5.
- C128. Ha Mavuka (2150 m; 295341S, 290308E\*; 2929Cc3): PEM R639, 2541-2.
- C129. Hill above drift, Tsoelikane River near confluence with Leqooa River, at Ha Moshebi (= Ha Letsoala Makuta) (2164 m; 295551S, 290155E\*; 2929Cc3): NMB R6828-30\*.
- C130. Ha Sehlabathebe (2200 m; 295330S, 2903E; 2929Cc3) ("Sehlabathebe National Park entrance": Bourquin 1989, PEM, as *P. melanotus*): PEM 2533-6.
- C131. Sehlabathebe National Park (2929Cc3) (Bourquin 1989, NPB, as *P. melanotus*; Lambiris 1989, AJL as listed): AJL 2823.
- C132. Sehlabathebe National Park, between Park Lodge and Research Station (295215S, 290530; 2929Cc1): NMB R5793-800\*, 5803\*.
- C133. Hill above Tsoelikane River, about 300 m S of Tsoelikane Falls, Sehlabathebe National Park (2375-2400 m; 295350S, 290729E\*; 2929Cc3): NMB R6847\*.
- C134. Hillside flanking upper Tsoelikane River valley, about 0.5 km N of Kepising (hill) peak, Sehlabathebe National Park (2475 m [given as 2378 m]; 295246S, 290725E\*; 2929Cc3): NMB R6845-6\*.
- C135. Top of hill SSW of Agricultural Station, 2 km E of Ha Mavuka, Sehlabathebe National Park (2530 m; 295346S, 290434E\*; 2929Cc3): NMB R6831\*.

- C136. 2 km E of Ha Stefane, 2 km SE of Makuta (2350 m; 295917S, 290201E\*; 2929Cc3) (“15 km SW of Sehlabathebe National Park”: Bourquin 1989, as *P. melanotus*): PEM R2547-9.
- C137. St. Albans, near Ha Paulusi and Moshebi (2200 m; 295531S, 290208E\*; 2929Cc3) (“10 km SE of Ramas Gate” & ? “10 km SW of Sehlabathebe”: Bourquin 1989, PEM, as *P. melanotus*): PEM R2550-2.
- C138. 1 km S of Ha Tsoane (2932S, 280630E; 2928Ca1) (“5 km W of Marakabeis”: Bourquin 1989, TM, as *P. melanotus*): TM 42557-8.
- C139. Between Kotisephola and Matsoaing (2929Ac4): TM 29073 (2713 m), 29074-6.
- C140. Le Bihan Falls (= 'Maletsunyane Falls), Thusong (= Ha Moahloli) (about 2200 m; 2952S, 280330E; 2928Cc1) (“Malutsenyane Falls”: FitzSimons 1943, AM): NMB R6832-5 [ex-AM 5221, “Malutsenyane Falls”]; TM 30370; BMNH 1926.10.24.7-8 [“near Malutsenyane Falls”].
- C141. Lepaqaqa, Maloti Mtns (about 2200-2300 m; 2904S, 2828E; 2928Ab2): NMB R5902.
- C142. Likalaneng area (2928Ac3): TM 69221.
- C143. Mokoabong Pass, Central Range (about 2700 m; 2931S, 2829E; 2928Cb2): TM 69219-20.
- C144. Maloti Mtns, E of Pitseng (2928AB): NMB R5149.
- C145. Maseru (2927Bc1; locality dubious – G. Setaro, pers. comm., October 2004 – not plotted on map): UKNHM 209729-45.
- C146. Masoleng (2500-2600 m; 2928Bb1) (“50 km E of Oxbow”: Bourquin 1989, TM, as *P. melanotus*): TM 53378.
- C147. Kofa-Senqu River confluence, 1 km NW of Matsamaneng (1580 m; 300400S, 282445E\*; 3028Ab2): NMB R6738, 6748-9.
- C148. Menoaneng Pass (2975-3025 m; 292530S, 285730E; 2928Bd4): TM 69213-5.
- C149. Moteng (area) (2846S, 2832E; 2828Dc1) (“Moteng Pass”: Bourquin 1989, TM, as *P. melanotus*): TM 34962-4.
- C150. Mothae (3000 m; 2858S, 2848E; 2828Dd3): NMB R6418-21.
- C151. Top of Sani Pass (2743 m; 2935S, 2917E; 2929Cb1) (Bourquin 1989, TM, as *P. melanotus*): PEM R4723-4, 4725\*; 4726-9, 4730-3\*; TM 29060-3, 47900-2, 69212; TM 30066 (as “Sani Pass Top”).
- C152. Sani Flats, Sani River bank, 4 km W of Sani Gate (2652 m; 293515S, 291445E; 2929Ca2): TM 29064-9, 29071.

- C153. Sani Flats, near Sani River (3200 m; 2934S, 2914E; 2929Ca2) (Bourquin 1989, TM, as *P. melanotus*): TM 47904, 47907.
- C154. Sani River (2929CA): TM 47905-6.
- C155. Seate River (“Seati Pass”) (2928BB): TM 46199.
- C156. Sehonghong River valley, E of Mokhotlong (2929AC) (“Sehonghong River banks” & “16 km E of Makhotlong”: Bourquin 1989, as *P. melanotus*): TM 34913, 34973, 35528-30, 35691, 35705-6.
- C157. Semonkong (about 2200-2250 m; 295030S, 2804E; 2928Cc1): TM 69224.
- C158. Thaba-Putsoa Mtn (2944S, 2755E; 2927Db4) (Hewitt 1927): MMK/F/853 (“no. 5223”, four specimens); BMNH 1926.10.24.9 (“Ribaneng area”); TM 30365-9; NMB R6844 (ex-AM 5323; “Thaba Patsua”).
- C159. Morija (293745S, 2731E; 2927Da3) (Boulenger 1910, SAM, as *P. microlepidotus*; FitzSimons 1943, SAM; Loveridge 1944, as *P. langi*): SAM ZR 8654, 9002.
- C160. Nemahadi police camp (see Namahali Pass; may be in KwaZulu-Natal) (3000 m; 284530S, 2852E; 2828Dd1) (Hewitt 1925, AM, as *P. microlepidotus*; FitzSimons 1943, AM; Loveridge 1944, as *P. langi*): NMB R6838-43 (ex-AM 4972, six specimens).
- C161. ’Maletsenyane River (2928CC) (“Malutsenyane River”: Hewitt 1927; “Malutsenyane”, Loveridge 1944, as *P. langi*).
- C162. Ribaneng area (“Rebaneng Pass”) (2927Dc2) (“Rebaneng Pass”: Hewitt 1927; “Rebaneng Pass”: FitzSimons 1943, AM, MMK; Loveridge 1944, as *P. langi*): MMK/F/ 853 (“no. 5222”, “near Rebaneng Pass”).
- C163. Linakaneng River (2928Bd4): NMZB 5467.
- C164. 4 km E of Ha Batho (= Ha Monyane) (2390 m; 3001S, 2859E; 3028Bb2): PEM R2529-32.
- C165. Qachas Nek (centre), 28 km NNW of Matatiele, Drakensberg Mtns, Lesotho (300715S, 284130E; 3028Ba2): PEM R2554.
- C166. “27.4 km beyond Blue Mtn Pass” (?2286 m): BM 1974.2277-9.
- C167. “16 km E of Marakabeis” (not in TM catalogue – not plotted) (Bourquin 1989, TM, as *P. melanotus*).
- C168. 4 km WNW of Ongeluks Nek, Mabala (area) (301958S, 281307E\*; 3028Ac2): PEM R13363-4.

- C169. Lekhalong-la-Molimo-Nthuse (= God Help Me Pass) (292520S, 2755E; 2927Bd4): JV 4899b-k\*, 4899 (nine specimens), 4915-8\*, 4922-4\*, 4926\*, 4928\*, 5010\*.
- C170. 0.5 km SW of Thaba Chitja (= Ha Khanyetsi) village (2350-2400 m; 300550S, 281615E\*; 3028Ab1): NMB R8405-14\*.
- C171. Drakensberg Mtns on Lesotho side (10 000 ft = 3048 m; ? vicinity of Mont-aux-Sources) (“Drakensberg on Basutoland side”: Loveridge 1944, TM, as listed, paratypes of *P. langi*): TM 2531, 2533 (paratypes of *P. langi*; both “Drakensberg Mountains, Lesotho”).

**Eastern Cape - Drakensberg and associated areas:**

- C172. Ugie, Maclear district (3112S, 2814E; 3128Aa4) (FitzSimons 1943, AM; Loveridge 1944, as *P. langi*).
- C173. Herschel (centre), Witteberg Mtns, Transkei (3037S, 270930E; 3027Ca2) (FitzSimons 1943, NMSA; Broadley 1964, NMSA 551a [male], as *P. microlepidotus fasciatus* & NMSA 551b-c [“females”] as *P. subviridis subviridis*): NMSA 551a-c [previously all labeled as NMSA 551]\*.
- C174. 3 km S of Qacha’s Nek, Transkei (1950 m; 300916S, 284040E\*; 3028Ba4) (“4 km S of Qacha’s Nek” - 300930S, 284130E; 3028Ba4: Bourquin 1989, as *P. melanotus*): PEM R2537-40.
- C175. Prentjiesberg, about 10 km NE of Ugie, Maclear district (1900 m; 3108S, 2810E; 3128Aa4) (Branch & Burger 1991, PEM).
- C176. “Mvenyane Mission”, Farm: no. 202, 18 km SE of Matatiele, Transkei (302942S, 285615E; 3028Bd4): DNSM R392-3.
- C177. Naude’s Nek, Farm: no. 61, Drakensberg Mtns, Barkly East district (304342S, 280720E; 3028Ca3): DNSM R760; PEM R2487-9.
- C178. Near top of Naude’s Nek, Farm: no. 61, Barkly East district (2300-2450 m; 3044S, 2808E; 3028Ca4): NMB R8292-314\*.
- C179. Upper reaches of Bell River, Naudes Nek, Farm: no. 61, Drakensberg Mtns, Barkly East district (2500 m; 304329S, 280747E\*; 3028Ca4): PEM R2946.
- C180. Upper reaches of Bell River, Farm: New Pass (25), SE of Dooiemanskran, Drakensberg Mtns, Barkly East district (2550 m; 304229S, 280832E\*; 3028Ca4): PEM R2941, 2944, 2951, 2958.
- C181. Scobell’s Kop turn-off, Farm: Ben Moore (62), Drakensberg Mtns, Barkly East district (2730 m; 3046S, 280630E; 3028Cc1): PEM R2490.
- C182. Farm: Ben Moore (62), Barkly East district (304430S, 2807E; 3028Ca3): PEM R2491-5.

- C183. Ongeluks Nek, Drakensberg Mtns, Transkei (302030S, 281530E; 3028Ad1): PEM R2516-23, 2665-9.
- C184. Farm: New Pass (25), SE of Dooiemanskrans, Drakensberg Mtns, Barkly East district (2650 m; 304214S, 280741E\*; 3028Ca4): PEM R2940, 2959, 2973, 3060, 3079.
- C185. Otto du Plessis Pass, Drakensberg Mtns, Barkly East district (311445S, 2732E; 3127Ba3): TM 69280-3.
- C186. Farm: Klipplaat (353), Otto du Plessis Pass, plateau of Drakensberg Mtns, Barkly East district (2100 m; 311336S, 273035E\*; 3127Ba3): PEM R2938-9, 2943, 2945, 2947-9, 2955-7, 2960-3, 3071-8.
- C187. Lay-by, top of Otto du Plessis Pass, Drakensberg Mtns, Barkly East district (2100 m; 311402S, 273102E\*; 3127Ba3): PEM R3066-70.
- C188. Farm: no. 72, 2 km WSW of Tentkop Mtn, Maclear district (3055S, 2812E; 3028Cc4): PEM R6940.
- C189. Prentjiesberg Mtn, 10 km NW of Ugie, Farm: Montana (245), Maclear district (3108S, 280830E; 3128Aa4): PEM R6948, 7046, 7055.
- C190. Bottle Nek Pass, 3 km S of Farmstead: Kylemore, Farm: Morrision (340), Drakensberg Mtns, Barkly East district (2050 m; 311140S, 274835E\*; 3127Bb3): PEM R2942.
- C191. Buffalo Nek, 17 km NW of Mount Frere (town), Transkei (1650 m; 3048S, 285130E; 3028Dd1): PEM R2553, 2606.
- C192. Barkly East, Barkly East district (3058S, 273530E; 3027Dc3): TM 46100.
- C193. Farm: Avilion (159), Barkly East district (3057S, 2737E; 3027Dc3): TM 47502.
- C194. Rama's Gate border control post, 35 km NNE of Matatiele (3003S, 2856E; 3028Bb2) (Bourquin 1989, PEM, as *P. melanotus*): PEM R2543-6, 2555, 2608-13, 2633-5, 2661-4, 2690-2.
- C195. Farm: Hamilton (64), Drakensberg Mtns, Barkly East district (304455S, 2804E; 3028Ca3): NMB R6945-6.
- C196. "Ongeluksnek Nature Reserve", 0.5 km NW of Ongeluks Nek Border Control Post, Drakensberg Mtns (302020S, 281520E\*; 3028Ad1): PEM R13365.
- C197. "Weza", 2 km NNW of Mount Ntlontsane, 20 km SE of Kokstad, Transkei (304203S, 293136E\*; 3029Da3): PEM R3952.
- C198. 4.5 km NNW of Mount Ntlontsane, 18 km SE of Kokstad, Transkei (304104S, 293106E\*; 3029Da3): PEM R3964-6.

**Eastern Cape - Amatole Mtns and associated areas:**

- C199. Hogsback (centre), Amatole Mtns, Cathcart district (3236S, 265630E; 3226Db2) (Essex 1925, AM & 1927, as *P. microlepidotus*; FitzSimons 1943, AM, SAM; Loveridge 1944, as *P. langi*): PEM R4953-67; USEC-H2551.
- C200. Katberg Mtn, Didima Range, Stockenstrom district (3229S, 263730E; 3226Bc4) (summit: Hewitt 1937; FitzSimons 1943, AM; Loveridge 1944, as *P. langi*): TM 21758\*.
- C201. "Katberg Pass, Katberg Forestry Station", 1 km SSE of Katberg railway station, Stockenstrom district (3233S, 2641E; 3226Da2): PEM R12259-60.
- C202. Devil's Bellows Nek, Farm: no. 1, Katberg Mtn, Didima Range, Stockenstrom district (1400 m; 322549S, 263913E\*; 3226Bc4): PEM R3061.
- C203. Finella Falls, Farm: Finella Falls, Great Winterberg Mtns, Winterberg Mtns, Adelaide district (322230S, 2623E; 3226Ad4) ("Finella Falls, Great Winterberg": Hewitt 1937; "Fenella Falls": FitzSimons 1943, AM; "Great Winterberg": Loveridge 1944, as *P. langi*): PEM R8656-60\*.
- C204. Stutterheim, Stutterheim district (3234S, 272530E; 3227Cb2) (FitzSimons 1943, SAM): SAM ZR 11243\*, 11314\*.
- C205. Farm: Bold Point (178), E slopes of Elandsberg Mtn, Stockenstrom district (3233S, 265445E; 3226Db2): PEM R589-92, 6502; UKNHM 207983.
- C206. Farm: New Glenholm (182), E slopes of Elandsberg Mtn, Stockenstrom district (3234S, 265430E; 3226Db2): CAS 156374, 156381-5, 173019.
- C207. Devil's Bellows Nek, ?Farm: Umtwakazi no. 20, Didima Range, Hewu district (1600 m; 322432S, 264014E\*; 3226Bc4): PEM R2950, 2952-4, 3062-5.
- C208. Farm: Glamorgan (205), Great Winterberg Mtns, Tarkastad district (3222S, 2621E; 3226Ad1; not plotted) (as *P. melanotus subviridis* x *P. microlepidotus fasciatus* hybrid according to PEM catalogue and W.R. Branch [pers. comm., 1998]): PEM R8662.
- C209. "Tordoone", Amatole Mtns, Stutterheim district (3227CA): PEM R593-4.
- C210. Menziesberg Mtn, Stockenstrom district (3237S, 2652E; 3226Db1): TM 47628-9\*.
- C211. Amatole Mtns (summit: Hewitt 1937; Loveridge 1944, as *P. langi*; Branch 1985, as *P. melanotus melanotus*).
- C212. Farm: Waterfall (161), Amatole Mtns, Cathcart district (323425S, 265641E\*; 3226Db2): NMB R8212\*; (323400S, 265629E\*; 3226Db2): NMB R8215-24\*.
- C213. Farm: Moreson (162), Amatole Mtns, Cathcart district (323445S, 265930E\*; 3226Db2): NMB R8213-4\*.

C214. Farm: no. 32, Amatole Mtns, Cathcart district (323451S, 265644E\*; 3226Db2): NMB R8225-31\*, 8450-2\*.

*Pseudocordylus langi*

**KwaZulu-Natal:**

- D1. Organ Pipes Pass, Cathedral Peak Forest Reserve, Bergville district (2901S, 291230E; 2929Aa2) (Broadley 1964 NMZB-UM, MCZ, NMSA, TM; “Cathedral Peak Forest Reserve”: Bourquin & Channing 1980, NMZB-UM, MCZ, NMSA, TM): NMZB-UM 2411-2\*, 2414-5\*, 2417-20\*, 2444\*, 3012\*; NMSA 1471 (about 3048 m); TM 27448-9\*; TM 51657, TM 51658\* (both as “Organ Pipes Spur” in TM catalogue); AMNH R-114353 (2896-3048 m; ex-NMZB-UM 2413); NMB R8445-9\* (top of Pass).
- D2. Mont-aux-Sources, Bergville district (2846S, 2852E; 2828Dd1) (Broadley 1964, MCZ; Bourquin & Channing 1980, TM, MCZ; Bourquin 1989, TM): MCZ 46835 (“11000 ft” = 3353 m, *i.e.* possibly near peak, holotype); TM 67659\*.
- D3. “Ntonjelane Pass”, Cathedral Peak area, Bergville district (2805 m; 285618S, 290540E\*; 2829Cc3): NMB R8501\*.
- D4. Crow’s Nest Cave entrance, 1.7 km NE of Mont-aux-Sources peak, Bergville district (3155 m; 284535S, 285304E\*; 2828Dd2): NMB R8537\*.
- D5. Amphitheatre escarpment near Tugela Falls, Royal Natal National Park, Bergville district (2990 m; 284511S, 285340E\*; 2828Dd2): Tissue sample (M. Cunningham, pers. comm.).

**Free State:**

- D6. Between Sentinel Cave and Chain Ladder, Bergville district (284448S, 285258E\*; 2828Db4): Tissue sample (M. Cunningham, pers. comm.).
- D7. Chain Ladder, Sentinel Hiking Trail, Farm: Witzieshoek (1815), Harrismith district (284450S, 285253E; 2828Db4): NMB R8555-7\* (2900 m); NMB R8552\* (2970 m); NMB R8554\* (3000 m).
- D8. Near Chain Ladder, Mont-aux-Sources, Farm: Witzieshoek (1815), Harrismith district (2905 m; 284448S, 285252E\*; 2828Db4): NMB R8500\*.
- D9. 200 m SE of Vemvane River falls, Farm: Witzieshoek (1815), Harrismith district (3020 m; 284456S, 285243E\*; 2828Db4): NMB R8553\*.
- D10. Nemahadi Pass, 2 km NNW of Mont-aux-Sources peak, Farm: Witzieshoek (1815), Harrismith district (3096 m; 284517S, 285154E\*; 2828Dd1): NMB R8538\*.

**Lesotho:**

- D11. Cleft Peak (3048 m; 2901S, 2911E; 2929Aa2; may be in KwaZulu-Natal): NMZB-UM 2421\*.

*Pseudocordylus spinosus*

**KwaZulu-Natal:**

- E1. Cathkin Peak area, Monk's Cowl State Forest, Estcourt district (290430S,292030E; 2929Ab1) ("Cathkin Peak area": FitzSimons 1947, TM & NMSA as listed, as *P. spinosus*): TM 21262\*, 21263, 21264-5\* (paratypes); 21267 (holotype); NMSA 647, three specimens (paratypes, apparently erroneously as "Little Tugela Valley" in catalogue).
- E2. Giant's Castle area, Impendle district (2929Ad2 [?2929AB in G.C.G.R.]) (FitzSimons 1947, TM & NMSA as listed): TM 2521\* (paratype, as "Giant's Castle area"); NMSA 550, 555 (paratypes, as "Giant's Castle ; as "Giant's Castle Game Reserve" in catalogue).
- E3. Injasuti Nature Reserve, Giant's Castle Game Reserve, Estcourt district (2907S, 2926E; 2929Ab2): NMB R-RY R125\*.
- E4. Royal Natal National Park, Bergville district (2828Db4) (Broadley 1964, NUM 73): NMZB-UM 5323; TM 39687 (as 2843S, 2856E); AMNH R-57655.
- E5. Dooley Ridge (= Knoll), Royal Natal National Park, Bergville district (top is 2056 m; 284208S, 285550E; 2828Db4) (Broadley 1964): NMZB-UM 2057-8; TM 21698\*.
- E6. Goodoo Pass, 750 m ESE of Witzieshoek Mountain Resort, Royal Natal National Park, Bergville district (2100 m; 284113S, 285425E\*; 2828Db4): NMB R8568-71\*.
- E7. Goodoo Pass, 1 km ESE of Witzieshoek Mountain Resort, Royal Natal National Park, Bergville district (2000 m; 284113S, 285438E\*; 2828Db4): NMB R8572\*.
- E8. Mont-aux-Sources, Royal Natal National Park, Bergville district (2828Db4): TM 31240.
- E9. Mont-aux-Sources, Bergville district (2828DD): NMB R6837\*; NMZB-UM 2091-2, 2433.
- E10. Cathedral Peak, Cathedral Peak State Forest, Bergville district (2829CC) (Broadley 1964): NMSA 1161 (2134 m); NMZB-UM 2162 (1981 m); TM 21699-700 (?285530S, 290730E; 2829Cc4).
- E11. Cathedral Peak State Forest, Bergville district (2829CC): TM 50085-6\*, 50731, 51633, 51638-43, 51666, 52191-2, 52357.
- E12. Champagne Castle, Estcourt district (2929Ab1) (Broadley 1964): NMSA 675 (1676 m), 1469.
- E13. Champagne Castle Hostel, Bergville district (2903S, 2925E; 2929Ab2): NMSA 936.

- E14. Little Tugela Valley, ?Estcourt district (1829 m; 2901S, 2933E; 2929Ba1): NMSA 646 (? paratypes  $N = 3$ , therefore 647 as in FitzSimons 1947).
- E15. Farm: Eersteling (1370), Ixopo district (3012S, 3001E; 3030Aa3): TM 55302-3\*.
- E16. N slopes of Drakensberg Mtn: TM 34533.
- E17. Drakensberg Mtn: AMNH R-114354-5.

**Free State:**

- E18. “Sentinel [Mtn]” (probably on Sentinel Road), Farm: Witzieshoek (1815), Harrismith district (2439 m; about 2844S, 285330E; 2828Db4) (De Waal 1978, NMB): NMB R3357\*, 4612-3\*.
- E19. Sentinel Road, 2 km N of Sentinel Mtn peak, Farm: Witzieshoek (1815), Harrismith district (2517 m; 284323S, 285325E\*; 2828Db4): NMB R8534\*.
- E20. Sentinel Road, 250 m SW of Witzieshoek Mountain Resort, Farm: Witzieshoek (1815), Harrismith district (2254 m; 284106S, 285346E\*; 2828Db4): NMB R8535-6\*.

*Pseudocordylus microlepidotus microlepidotus*

**Western Cape:**

- F1. Cape Town, Cape district (335730S, 1829E; 3318Cd4) (Boulenger 1910, SAM).
- F2. Plumstead (suburb), Cape Town, Cape district (340130S, 182830E; 3418Ab2): TM 47209.
- F3. Table Mountain, Cape Town, Cape district (3358S, 1824E; 3318Cd4) (FitzSimons 1943, AM, MMK, SAM, TM; Loveridge 1944; Visser 1984): DMR 603; MMK/F/852; UKNHM 196008 (1070 m); TM 1703, 13600-1; PEM R608 (plateau), 624, 625\*, 1383, 1398-9\*, 1480; SAM ZR1115, 44985-6, 44990; NMZB-UM 5243\*; USEC-H2458-66 (860 m).
- F4. Near Maclear beacon, Table Mountain, Cape district (about 1086 m; 335824S, 182530E; 3318Cd4) (Visser 1984).
- F5. Riviersonderend(berg) Mtns, Caledon/Robertson/Worcester districts (3419BA/BB, not plotted) (“Rivierzondereinde Mtns”: FitzSimons 1943, SAM): SAM ZR18008.
- F6. Jonaskop Mtn, Riviersonderend Mtns, Riviersonderend State Forest, Worcester/Robinson/Caledon districts (1640 m; 335819S, 193025E\*; 3319Dc3): USEC-H870-1, 940, 944-6, 2398-408.
- F7. “Zuurbrak Peak”, 5 km N of Suurbraak (town) on Farm: Erf (1), Langeberg Mtns (highest 1507 m; 335730S, 2039E; 3320Dc4) (FitzSimons 1943, SAM): SAM ZR17392.

- F8. Grootvadersbos Forestry Station, 12 km SE of Barrydale (town), Langeberg Mtns, Heidelberg district (3359S, 2048E; 3320Dd3) (“Grootvadersbosch”: FitzSimons 1943, TM & 1946, TM, as listed, but also TM 19910, 20005): TM 19901, 19963, 19977-80, 20006-27.
- F9. Vaalrivier Kloof, “Grootvadersbos”, Langeberg Mtns, Heidelberg district (1200 m; 3357S, 2048E; 3320Dd3): USEC-H2528-42.
- F10. Pampoenkloof, 7 km N of Montagu, Farm: no. 84, Baden (area name), between Waboomsberg Mtns and Langeberg Mtns, Montagu district (3343S, 2007E; 3320Ca3): TM 55122, 55467, 69269.
- F11. “Dassieshoek Nature Reserve”, Farm: Dassie's Hoek (16), 1 km SE of Die Vensterbank, 7.5 km N of Robertson, Langeberg Mtns, Robertson district (3344S, 1953E; 3319Db4): TM 55340.
- F12. Pass at Sneeuberg Mtn, about 16 km NE of Citrusdal, Cederberg State Forest, Clanwilliam district (3231S, 1909E; 3219Ca2) (“Sneeuwberg Pass”: FitzSimons 1943, SAM; “Sneewgat Valley, Tulbagh”: SAM catalogue): SAM ZR14227a-b.
- F13. 1 km NE of Sneeuberg Hut, Sneeuberg Mtn, Cederberg Mtns, Cederberg State Forest, Clanwilliam district (1300 m; 322848S, 190919E\*; 3219Ac4): USEC-H624.
- F14. Matroosberg Mtn, Hexrivier Mtns, Ceres/Worcester districts (332230S, 1940E; 3319Bc4) (FitzSimons 1943, SAM): SAM ZR14340; PEM R3528\*, 3533\* (NW slope at ski huts), 3536.
- F15. Jonkersberg Mtn, Jonkersberg Forestry Station, Outeniqua Mtns, about 22 km ENE of George, George district (3356S, 2213E; 3322Cc4) (FitzSimons 1943, TM & 1946, TM as listed): TM 20158-64, 20207 (?not in TM catalogue), 20220-1.
- F16. Prince Alfred's Pass, S slope of Outeniqua Mtns, about 4 km S of Avontuur (centre), Langkloof Mtns, Uniondale district (about 3346S, 2310E; 3323Cc2) (FitzSimons 1943, TM & 1946, TM as listed): TM 20296; PEM R1024 (near N end), 1506 (east summit), 1620 (top)\*.
- F17. Dutoitskloof Pass, about 8 km E of Paarl, Dutoitskloof Mtns (about 3344S, 1906E; 3319Ca3) (“Du Toit's Kloof”: FitzSimons 1943, SAM, TM; Visser 1984, photographic record): SAM ZR 18930; JV 43280\*, 43380\*.
- F18. Farm: Anysberg West (262), Anysberg Mtn (top), Anysberg Nature Reserve, Laingsburg district (900 m; 333107S, 203340E\*; 3320Da1) (Burger 1993, USEC as listed): USEC-H1946.
- F19. Farm: Dyselberg (123), Dysselberg Mtn, Kammanassieberg Mtns, Oudtshoorn district (333530S, 223030E\*; 3322Da1) (Branch & Bauer 1995, PEM as listed): PEM R11075-6.

- F20. Farm: no. 61, E of Farm: Paardeberg (58), Uniondale district (333622S, 225138E\*; 3322Db1) (Branch & Bauer 1995, PEM as listed): PEM R11093.
- F21. Farm: no. 61, S edge of plateau, 1 km N of Buffelsberg Mtn, Kammanassieberg Mtns, Uniondale district (3337S, 225130E; 3322Db1) (Branch & Bauer 1995, PEM as listed): PEM R588, 634.
- F22. Farm: no. 61, Weather Station, N edge of main plateau, Buffelsberg Mtn, Kammanassieberg Mtns, Uniondale district (3337S, 2252E; 3322Db1) (Branch & Bauer 1995, PEM as listed): PEM R3557.
- F23. Mannetjiesberg Mtn, Kammanassieberg Mtns, Uniondale district (1576 m; 333621S, 225242E\*; 3322Db2): USEC-H2071-5.
- F24. Landsrivier (river) at track crossing on S slopes of Mannetjiesberg Mtn, Farm: Molen River (114), Kammanassieberg Mtns, Uniondale district (3337S, 225630E; 3322Db2) (Branch & Bauer 1995, PEM as listed): PEM R3551-3\*.
- F25. Campsite 4 km SW of Mannetjiesberg Mtn, Farm: no. 61, Kammanassieberg Mtns, Uniondale district (3337S, 2253E; 3322Db2) (Branch & Bauer 1995, PEM as listed): PEM R597, 633\*.
- F26. E edge of saddle, 1 km E of campsite, 4 km SW of Mannetjiesberg Mtn, Farm: no. 61, Kammanassieberg Mtns, Uniondale district (3337S, 225330E; 3322Db2) (Branch & Bauer 1995, PEM as listed): PEM R622-3, 3555.
- F27. Farm: Molen River (114), S slopes of Mannetjiesberg Mtn, Kammanassieberg Mtns, Uniondale district (900 m; 3339S, 225530E; 3322Db4) (Branch & Bauer 1995, PEM as listed): PEM R596\*.
- F28. Elandsvlakte, Kammanassieberg Mtns, George district (3338S, 2245E; 3322Db3) (sight record: Branch & Bauer 1995).
- F29. 200 m below summit of Rooiberg Mtn at Bailey Peak, Ladismith district (1279 m; 333754S, 212537E\*; 3321Cb4) (Branch & Bauer 1995, PEM as listed): PEM R8566.
- F30. 1 km below (E of) Bailey Peak, Farm: Hart (185), Rooiberg Mtn, Ladismith district (1434 m; 333748S, 212538E\*; 3321Cb4) (Branch & Bauer 1995, PEM as listed): PEM R8579\*.
- F31. Summit of Bailey Peak, Rooiberg Mtn, Ladismith district (1484 m; 333757S, 212517E\*; 3321Cb4) (sight record: Branch & Bauer 1995).
- F32. Bottom of saddle between Rooibergkop and Bailey Peak, Rooiberg Mtn, Ladismith district (1166 m; 333816S, 212622E\*; 3321Cb4) (sight record: Branch & Bauer 1995).

- F33. Swartberg Pass, about 14 km SSE of Prince Albert, Farm: no. 192, Great Swartberg Mtns, Prince Albert district (3320S, 2202E; 3322Ac1): TM 39732-3, 56416; PEM R585-6, 635\* (highest point, about 1950 m).
- F34. Farm: Dorps Rivier (191), N slopes of Oliewenberg Mtn, 13.5 km SSW of Prince Albert, Great Swartberg Mtns, Prince Albert district (3320S, 2206E; 3322Ac1): CAS 180369-70; PEM R637\*.
- F35. “6.5 km E of Gouekrans Hut”, Great Swartberg Mtns, Prince Albert district (3322AC): CAS 180374.
- F36. Farm: no. 192, Swartberg Pass, 12.5 km S of Prince Albert, near Voortrekker Gedenkteken, N slopes of Waboomsberg Mtn, Great Swartberg Mtns, Prince Albert district (332030S, 2202E; 3322Ac1): PEM R636\*.
- F37. Farm: Dorps Rivier (191), Swartberg Pass, 13 km S of Prince Albert, Great Swartberg Mtns, Prince Albert district (3321S, 220230E; 3322Ac1): PEM R638\*.
- F38. Farm: Paarde Vley (194), NW slopes of Waboomsberg Mtn, 16 km SSW of Prince Albert, Great Swartberg Mtns, Prince Albert district (3321S, 2158E; 3321Bd2): TM 66093-4.
- F39. “Forestry Station”, Gamkaskloof, between Gamkasberg and Osberg Mtns, Great Swartberg Mtns, Prince Albert district (3322S, 213730E; 3321Bc2): PEM R4414.
- F40. W side of Farm: Klein Valie (182), Mooikloof (bottom), Great Swartberg Mtns, Prince Albert district (3320S, 2218E; 3322Ad1): PEM R6733; (331953S, 221802E\*; 3322Ad1): 7882, 7890\*.
- F41. Farm: Albert Berg (4), Oliewenberg Mtn, Great Swartberg Mtns, Oudtshoorn district (3321S, 2204E; 3322Ac1): PEM R7831\*.
- F42. Farm: De Vlei (176), NE slopes of Blesberg Mtn, Great Swartberg Mtns, Prince Albert district (332428S, 224355\*E; 3322Bc4): PEM R7852\*.
- F43. Above “Die Top”, Swartberg Pass, Swartberg State Forest, Groot Swartberg Mtns, Prince Albert district (1676 m; 332115S, 220232E\*; 3322Ac1): NMB R8470\*.
- F44. Farm: Toverkop (56), Towerkop Mtn, Klein Swartberg Mtns, Ladismith district (854 m; 332719S, 211201E\*; 3321Ac4): NMB R8474\*; (737 m; 332733S, 211201E\*; 3321Ac4): NMB R8475\*.
- F45. Farm: De Poort (61), Seweweekspoort Mtn, Klein Swartberg Mtns, Ladismith district (1535 m; 332414S, 212304E\*; 3321Ad4): NMB R8485\*.
- F46. Langeberg/Swartberg: TM 52553.
- F47. Paarl, Paarl district (3344S, 1858E; 3318Db4): TM 3839.

- F48. Wemmershoekberg Mtns, 9 km ESE of Paarl, Paarl district (1120 m; 334531S, 190448E\*; 3319Cc1): USEC-H1273.
- F49. Farm: Uitvlug (517), plateau of Potberg Mtn, Bredasdorp district (3423S, 2034E; 3420Bc3): TM 55329-30.
- F50. Farmstead: Heuningvlei, Farm: Krakadouw Heights (180), Boontjieskloof, Krakadou Mtns in Cederberg Mtns, Clanwilliam district (3213S, 1905E; 3219Aa3): TM 79652.
- F51. N of Dutoitskloof Pass, Farm: De Poort van Du Toits Kloof (583), Hawequa Mtns, Paarl district (900 m; 3342S, 1906E; 3319Ca3): CAS 165654.
- F52. Vicinity of Betty's Bay, S of Platberg Mtn, Caledon district (342130S, 1857E; 3418Bd2): CAS 165760.
- F53. Hottentots Holland Nature Reserve, 8 km SW of Franschhoek, Olifantsberg Mtn in Great Drakensteinberg Mtns, Paarl district (335730S, 1903E; 3319Cc3): CAS 173336.
- F54. Verkykerskop Mtn, Hottentots Holland Mtns, Somerset West district (1200 m; 340608S, 185804E\*; 3418Bb2): USEC-H995.
- F55. Farmstead: Landdroskop, Hottentots Holland Mtns, Hottentots Holland Nature Reserve, Caledon district (1133 m; 340300S, 190022E\*; 3419Aa1): USEC-H2527.
- F56. 2 km N of Bergplaas (town) on road to Kleinplaat (town), Bergplaas Plantation, Outeniqua Mtns, George district (3353S, 2241E; 3322Dc4): PEM R1508.
- F57. Farm: Hoeks Berg (182), 7 km S of McGregor, Riviersonderend Mtns, Robertson district (3401S, 1950E; 3419Bb1): PEM R1618\*.
- F58. 1 km SE of Kleinplaat (town), Bergplaas Plantation, Outeniqua Mtns, George district (3352S, 2241E; 3322Dc2): PEM R1621-4.
- F59. Elandsvlakte, Kammanasieberg Mtns, George district (3339S, 224430E; 3322Da4): PEM R3220-1\*.
- F60. "Gamka Mountain Nature Reserve, W of Rhebuck neck" (3321DB): PEM R7285.
- F61. "Camferskloof", Camferskloof Mtn, Outeniqua Mtns, George district (3350S, 2223E; 3322Cd2): PEM R8655\*.
- F62. Montagu Pass, NNW of George, George district (3353S, 2226E; 3322Cd4): SAM ZR18333.
- F63. "Cape Peninsular" (about 3418AB) (Rose 1926).

- F64. Farm: Keur Kloof (278), Niekerksberg Mtn, Langkloof Mtns, Uniondale district (334719S, 232358\*E; 3323Cd2): NMB R8045-7\*.
- F65. Cradock's Pass, Farm: no. 142, NW of George, Outeniqua Mtns, George district (335430S, 222656E\*; 3322Cd4): USEC-H2578.
- F66. Farm: Groot Hoek (19), Donkerhoek (area), Outeniqua Mtns, "Outeniqua Nature Reserve", Mossel Bay district (500 m; 335211S, 220506E\*; 3322Cc1): USEC-H2588.
- F67. Dwarsberg Mtn, Outeniqua Mtns, Knysna district (3349S, 2257E; 3322Dd2): NMB R8048\*.
- F68. "Mountain Rose", Farm: no. 559, NE of Betty's Bay, Caledon district (140 m; 341939S, 185827E\*; 3418Bd2): USEC-H1456.
- F69. Shaw's Mountain Pass, 10 km SSW of Caledon (town), Caledon district (350 m; 341845S, 192420E\*; 3419Ad2): USEC-H1469-70.
- F70. Farmstead: Mont Rochelle, 3 km ENE of Franschoek, Franschoek Mtns, Paarl district (1120 m; 335335S, 190917E\*; 3319Cc4): USEC-H1540-1.
- F71. Farmstead: Franskraal, Farm: Fransche Kraal (708), 4 km ESE of Gansbaai, Franskraal se Berge (Mtns), Hermanus district (110 m; 343527S, 192325E\*; 3419Cb2): USEC-H1420-2.
- F72. "Mooihawe Jeugkamp", 8 km NE of Pringle Bay (town), Buffelstalberg Mtn, Kogelberg State Forest, Caledon district (20 m; 341753S; 185300E\*; 3418Bd2): USEC-H928.
- F73. "Middelburg Pass", Farm: no. 475, Middelburg Mtn, 13.5 km SE of Citrusdal (centre), Clanwilliam district (1100 m; 323753S, 190906E\*; 3219Ca4): USEC-H1157.
- F74. Farmstead: Tweede Tol, Limietberg Mtns, Hawequa State Forest, Worcester district (375 m; 333416S, 190807E\*; 3319Ca2): USEC-H1036.
- F75. Wolwekloof, 8 km SE of Gordon's Bay (town), Kogelberg State Forest, Koelberg Mtn, Caledon district (800 m; 341214S, 185548E\*; 3418Bb4): USEC-H1030.
- F76. Farm: no. 307, Koelberg Mtn, 7 km SE of Gordon's Bay (town), Strand district (500 m; 341040S, 185552E\*; 3418Bb4): USEC-H1027-8.
- F77. Simonsberg Mtn, about 8 km NE of Stellenbosch, Stellenbosch district (1300 m; 335305S, 185533E\*; 3318Dd4): USEC-H500.
- F78. Farm: Bosch Kloof (65), Riviersonderend Mtns, Caledon district (1000 m; 340024S, 192056E\*; 3419Ab1): USEC-H1552.

- F79. Farm: De Poort van Du Toits Kloof (583), Hawequa Mtns, 11 km NE of Paarl, Paarl district (1140 m; 334105S, 190542E\*; 3319Ca3): USEC-H919.
- F80. Rooiberg Mtn, Ladismith district (3321CB): JV 1497-8\*, 1501\*.
- F81. Farm: Grassy Mount (48), top of Naudesberg Mtn, Langeberg Mtns, Montagu district (1450 m; 333852S, 194454E\*; 3319Da4): USEC-H2467-526, 3267.
- F82. Farm: Rheeboek's Vlakte (45), Naudesberg Mtn, Langeberg Mtns, Montagu district (1267 m; 333755S, 194435E\*; 3319Da4): USEC-H2245-6.
- F83. "Boosmansbos", Platbosrivierkloof, Helderfontein area, Langeberg Mtns, Heidelberg district (1060 m; 335642S, 205135E\*; 3320Dd3): USEC-H1776-9.
- F84. Farm: Venster Bank (113), Koo River valley, Langeberg Mtns, Montagu district (1260 m; 334246S, 195302E\*; 3319Db4): USEC-H3172.
- F85. Robinson Pass, 1.5 km W of Ruitersberg Mtn peak, Mosselbaai district (300 m; 335253S, 220130E\*; 3322Cc3): USEC-H2047-53, 2056.
- F86. Farm: Uitvlug (517), W side of Potberg Mtn, De Hoop Nature Reserve, Bredasdorp district (350 m; 342214S, 203233E\*; 3420Bc1): USEC-H744.
- F87. Farm: no. 356, Matroosberg Mtn, Hexrivierberg Mtns, Ceres district (1920 m; 332230S, 193943E\*; 3319Bc4): USEC-H742.
- F88. Aasvoelkop Mtn, Kleinriviersberg Mtns, Hermanus district (820 m; 342231S, 191736E\*; 3419Ad3): USEC-H674.
- F89. Boosmansbos Wilderness Area, Swellendam district (1160 m; 335630S, 204849E\*; 3320Dd3): NMB R8453\*.
- F90. Farm: no. 164, Bantamskop Mtn, Witberg Mtns, Laingsburg district (1470 m; 331653S, 203031E\*; 3320Bc1): NMB R8502\*.
- F91. Groot-Wintershoek Protected Area, Groot-Wintershoekberg Mtns, Piketberg district (1011 m; 325856S, 190513E\*; 3219Cc3): Tissue sample (M. Cunningham, pers. comm.).
- F92. 1 km NNW of Farmstead: Perdevlei, Groot-Wintershoek Protected Area, Groot-Wintershoekberg Mtns, Piketberg district (1323 m; 330247S, 190849E\*; 3319Aa2): Tissue sample (M. Cunningham, pers. comm.).
- F93. Farm: no. 256, Cockscomb Mtn, Groot-Wintershoekberg Mtns, Uitenhage district (1550 m; 333406S, 244710E\*; 3324Db1): Tissue sample & photographic record (M. Cunningham, pers. comm.).
- F94. Farm: no. 5, 10 km NNW of Villiersdorp, Kroonlandpiek Mtn, Stettynsberg Mtns, Caledon district (995 m; 335408S, 191530E\*; 3319Cd3): Sight record (M. Cunningham, pers. comm.).

- F95. Farm: no. 91, “Suurlemoenkloof”, Cloete’s Pass, Cloetesberg Mtn, Attakwasberg Mtns, Mosselbaai district (444 m; 335511S, 214520E\*; 3321Dd3): Tissue sample (M. Cunningham, pers. comm.).
- F96. Smutsberg Mtn, Formosa State Forest, Kougaaberg Mtns, Joubertina district (1737 m; 333819S, 234733E\*; 3323Db3): Tissue sample & photographic record (M. Cunningham, pers. comm.).
- F97. About 32 km SW of Steytlerville, Baviaanskloofberg Mtns, Willowmore district (1195 m; 333115S, 240452E\*; 3324Ca1): To be accessioned into PEM collection (M. Cunningham, pers. comm.).
- F98. Farm: no. 368, Elandberg Mtns, Uitenhage district (780 m; 334433S, 250050E\*; 3325Ca3): Tissue sample (M. Cunningham, pers. comm.).
- F99. Salmonsdam Protected Area, Perdeberg Mtn, Hermanus district (527 m; 342526S, 193907E\*; 3419Bc4): Tissue sample (M. Cunningham, pers. comm.).
- F100. 1 km E of Mont Rochelle, Hottentots Holland Nature Reserve, Caledon district (335403S, 190950E; 3319Cc4) (SARCA No. 156).
- F101. Swartberg Pass, Swartberg State Forest, Oudtshoorn district (332126S, 220513E; 3322Ac1) (SARCA No. 158).

**Eastern Cape:**

- F102. Van Stadens River, near Vanstadensberg Mtn (3325CC): PEM R621.
- F103. Longmore Forest, Longmore Forest Reserve, Vanstadensberg Mtn, Hankey district (3325CC): PEM R598-601, 1507.
- F104. Suurberg Mtn (top at W end), Kirkwood district (3325AC): PEM R602.
- F105. Suurberg Mtn (top near TV tower), Kirkwood district (about 963 m; 3325AD): PEM R1741, 1761.
- F106. Top of Kareedouw Pass, 2 km SW of Kareedouw (town), between Kareedouwberg and Tsitsikamma Mtns, Humansdorp district (335530S, 2416E; 3324Cd3): PEM R3216-9.
- F107. Farm: no. 416, NE slopes of Vanstadensberg Mtn, about 16 km SW of Uitenhage, Port Elizabeth district (335148S, 251658E\*; 3325Cd1): PEM R6530.
- F108. “Bridgemead, Port Elizabeth”, Farm: no. 398, 11 km S of Uitenhage, Port Elizabeth district (3352S, 2524E; 3325Cd2): PEM R7054.
- F109. “Formosa Conservation Area”, Farm: no. 281, NE slopes of Niekerksberg Mtn, Langkloof Mtns, Joubertina district (3346S, 2326E; 3323Cd2): PEM R9339.
- F110. “Baviaanskloof Wilderness Area”, Farm: Klein Rivier (4), Baviaanskloof Mtns, Hankey district (333716S, 242506E\*; 3324Cb2): PEM R9342.

- F111. “Algoa Bay” = ?Addo Heights area, Kirkwood/Alexandria districts (3325DB; not plotted on map - too vague) (Smith 1843, restricted type locality of *P. algoensis*; Loveridge 1944): BMNH RR.1946.8.8.49 (57.6.13.88).
- F112. Port Elizabeth, Port Elizabeth district (3325DC; too vague - not plotted on map): BMNH 87.12.6.6, BMNH 90.2.26.13.
- F113. Elands River, E of Uitenhage, between Elandsberg and Great Winterhoek Mtns, Uitenhage district (3325Cd1) (FitzSimons 1943, PEM, as *P. microlepidotus fasciatus*).
- F114. Farm: Drinkwater’s Kloof (239), “Takkieskraal, Formosa Conservation Area”, Kougaaberg Mtns, Willowmore district (334420S, 241525E\*; 3324Cb3): USEC-H2566.
- F115. Farm: Marias Dal (171), “Formosa Conservation Area”, Kougaaberg Mtns, Joubertina district (334146S, 234121E\*; 3323Da4): USEC-H2572.
- F116. Farm: Rylstone (163), 3 km N of Otterford Forest Station, Otterford Forest Reserve, Elandsberg Mtns, Hankey district (334509S, 250120E\*; 3325Cc1): NMB R8044\*.
- F117. Lady’s Slipper, Van Stadensberg Mtn, Port Elizabeth district (335314S, 251551E\*; 3325Cd3): NMB R8456\*.
- F118. Vermaakskop, Groot-Winterhoekberg Mtns, Groendal Wilderness Area, Uitenhage district (950 m; 333739S; 251645E; 3325Cb3): NMB R8541\*; (965 m; 333735S; 251645E; 3325Cb3): NMB R8542\*.

***Pseudocordylus microlepidotus fasciatus***

**Northern Cape:**

- G1. Farm: Modderfontein (147), about 28 km SSE of Colesberg, Colesberg district (305830S, 250830E; 3025Cc4) (“Colesberg” = 3025Ca3: FitzSimons 1943, SAM): SAM ZR 18621a-b\*.
- G2. Deelfontein (centre), Richmond district (3059S, 2348E; 3023Dd3) (Boulenger 1903, as *P. microlepidotus*; Loveridge 1944): BMNH 1903.4.27.32-3\*.

**Western Cape:**

- G3. Farm: Quaggas Drift (108), Koueveld Mtns, Murraysburg district (about 3205S, 2403E; 3224Aa1): PEM R587.

**Eastern Cape:**

- G4. Farmstead: Abbotsbury, Farm: Paarde Kloof (140), 22 km WNW of Farm: Lets Kraal (154), Noodhoek Mtns, Graaff-Reinet district (3202S, 2436E; 3224Ba1) (FitzSimons 1943, AM; Loveridge 1944).

- G5. Coetzeesberg Mtns, Pearson/Somerset East district (3225AC) (FitzSimons 1943, AM; Loveridge 1944).
- G6. Dordrecht, Wodehouse district (312215S, 2703E; 3127Ac1) (FitzSimons 1943, AM; Loveridge 1944).
- G7. Grahamstown, Albany district (331830S, 2632E; 3326Bc1) (“rocky hills in vicinity of Graham’s Town”: Smith 1843, restricted type locality of *Cordylus* [*Pseudocordylus*] *fasciatus*; Essex 1927, as *P. microlepidotus*; FitzSimons 1943, AM, TM; Loveridge 1944, in reference to Smith): TM 175-7\*.
- G8. Farm: no. 305, Thomas Baines Nature Reserve, 9 km SW of Grahamstown, Albany district (460 m; 332230S, 262856E\*; 3326Ad4): USEC-H2547.
- G9. Farm: Schurfte Berg (4), Bankberg Mtns, Somerset East district (3220S, 2519E; 3225Ad1) (“Schurfteberg”: FitzSimons 1943, AM; “Schurfteberg”: Loveridge 1944).
- G10. Mountain Zebra National Park, Cradock district (?3225AB/AD) (Grobler & Bronkhorst 1981): TM 54660.
- G11. Witmos railway siding, Farm: Rivier View (49), Bedford district (323230S, 254445E; 3225Da2): TM 22790.
- G12. Wapadsberg Pass, Sneeu Berg Mtns, Graaff-Reinet district (315530S, 2453E; 3124Dd4): PEM R595\*, 3156.
- G13. Swaershoek Pass, Gannahoekberg Mtns, SW of Cradock, Cradock district (about 3218S, 253015E; 3225Bc1): PEM R603-7.
- G14. “Rufortskloof, Elands River” (?3126CC): PEM R609-10.
- G15. Farmstead: Doornplaats, Farm: Rust (126), N of Sneeu Berg Mtns, Graaff-Reinet district (about 320230S, 241730E; 3224Ab1): PEM R1509-14\*, 1619\*.
- G16. Farmstead: Thysfontein, Farm: Thysfontein (143), Stormberg Mtns, Wodehouse district (1800 m; 312446S, 265216E\*; 3126Bd3): PEM R2860-1, 2970, 4394\*.
- G17. Smuts Pass, Farm: Boshoffs Kraal (149), Stormberg Mtns, Wodehouse district (1850 m; 312340S, 264725E\*; 3126Bd3): PEM R2862.
- G18. Farm: Buffels Fontein (150), N of Stormberg Mtns, Wodehouse district (1800 m; 312212S, 264348E\*; 3126Bc2): PEM R2879-85\*.
- G19. Farm: Roman Fountain (87), Bamboesberg Mtn, Molteno district (1800 m; 313230S, 261515E\*; 3126Cb1): PEM R2863\*, 2864\*, 2869\*.
- G20. S part of Farm: Plessies Kraal (189), 1 km S of Jamestown, Aliwal North district (1550 m; 310734S, 264847E\*; 3126Bb3): PEM R2865, 2867\*, 2870.

- G21. Top of Penhoek Pass, Farm: Drooge Fontein (155), N slopes of Penhoekberg Mtn, Stormberg Mtns, Wodehouse district (1900 m; 312632S, 264136E\*; 3126Bc4): PEM R2866\*.
- G22. 10 km NW of Lady Frere, NE slopes of Mount Arthur peak, Mount Arthur Range, Transkei (1300 m; 313736S, 270937E\*; 3127Ca4): PEM R2868\*, 2873\*, 2877-8\*, 3082\*.
- G23. Between Dyobhudaka and Sidakeni, plateau of Mount Arthur Range, Transkei (1350 m; 313631S, 270949E\*; 3127Ca2): PEM R2874\*.
- G24. Between Dyobhudaka and Macubeni, plateau of Mount Arthur Range, Transkei (1350 m; 313545S, 271005E\*; 3127Ca2): PEM R2928\*, 2966-7\*, 2968, 2972\*.
- G25. About 21 km NNE of Cathcart, Intaba Etsola Mtn, Queenstown district (1000 m; 320750S, 271443E\*; 3227Aa4): PEM R2871\*, 2964\*.
- G26. Farm: Mooi Hoek (59), 12 km SSW of Jamestown, Wodehouse district (1650 m; 311352S, 264708E\*; 3126Bb3): PEM R2872.
- G27. Farm: Andover (15), Wodehouse district (1500 m; 310054S, 271349E\*; 3127Aa2): PEM R2875.
- G28. Farm: Riet Fontein (54), 6 km ESE of Jamestown, Wodehouse district (1600 m; 310817S, 265144E\*; 3126Bb3): PEM R2876.
- G29. Farm: Haisses Fountain (88), Bamboesberg Mtn, Molteno district (1850 m; 313223S, 261320E\*; 3126Ca2): PEM R2969\*.
- G30. Nkalweni, 2 km SE of Umhlanga railway siding, 11 km W of Indwe, Transkei (1500 m; 312825S, 271354E\*; 3127Ac4): PEM R2971\*.
- G31. Cibini (village), 8 km WSW of Indwe, Transkei (1500 m; 312903S, 271554E\*; 3127Ad3): PEM R3081\*.
- G32. "Pramkop area", Mountain Zebra National Park, Cradock district (3225AB/AD): PEM R3140\* (3225AD), 3329-30\* (3225AB).
- G33. "Rooiplaat", Mountain Zebra National Park, Cradock district (3225AB): PEM R3752.
- G34. Farm: Oxton (412), 10 km WSW of Sada, Queenstown district (3214S, 2643E; 3226Ba4): PEM R3277\*.
- G35. Tarka Pass (S part), Farm: Fouries Kraal (38), 26 km NNE of Somerset East, Somerset East district (900 m; 323015S, 2541E; 3225Da2): PEM R3326\*.
- G36. Tarka Pass (N part), Farm: Blomfontein (549), 12 km SSW of Mortimer (centre), Cradock district (1100 m; 3228S, 253930E; 3225Bc4): PEM R3327\*.

- G37. Witkrans Nek, Farm: Tavelberg (207), 28 km SE of Middelburg, Joubertsberg Mtns, Middelburg district (314130S, 251030E; 3125Ca4): PEM R3777, 8748.
- G38. Farm: Baviaanskrans (12), Kompasberg Mtn, Sneeu-berg Mtns, Graaff-Reinet district (314330S, 243030E; 3124Da3): PEM R3846.
- G39. Farm: Blaauwewater (67), SW slopes of Lootsberg Mtn, Sneeu-berg Mtns, Graaff-Reinet district (3148S, 2449E; 3124Dd1): TM 20357.
- G40. Farmstead: Compassberg, Farm: Krugers Baaken (146), 13 km N of Nieu-Bethesda, Kompasberg Mtn, Sneeu-berg Mtns, Middelburg district (314508S, 243303E\*; 3124Dc1): NMB R8049\*.
- G41. Farm: Strydfontein (512), Winterberg Mtns, Cradock district (about 3221S, 2556E; 3225Bd2): PEM R3847.
- G42. Farm: Waterval (513), Winterberg Mtns, Cradock district (3222S, 2555E; 3225Bd2): PEM R8749.
- G43. Farmstead: Process, Farm: Kingsmead (193), Winterberg Mtns, Tarkastad district (1900 m; 321714S, 262645E\*; 3226Ad2): PEM R3080\*.
- G44. Finella Falls, Farm: Finella Falls, Great Winterberg Mtn, Winterberg Mtns, Adelaide district (322230S, 2623E; 3226Ad4): PEM R8651-2\*.
- G45. Berghof/Weltevrede Rest Camps, Mountain Zebra National Park, W of Cradock, Cradock district (321530S, 2527E; 3225Ad2): PEM R3850.
- G46. Farm: Tweefontein (38), 30 km W of Nieu-Bethesda, Graaff-Reinet district (3155S, 241445E; 3124Cc4): PEM R8648.
- G47. Farmstead: Palmietfontein, Farm: no. 24, about 25 km NNW of Aberdeen, Kamdebooberg Mtns, Aberdeen district (321730S, 235630E; 3223Bd2): PEM R8649-50.
- G48. Kleinemonde (town), Bathurst district (333230S, 2702E; 3327Ca1; questionable locality, not plotted on map) (FitzSimons 1943, SAM, "Kleinmond River Mouth", as *P. microlepidotus microlepidotus*; SAM catalogue: "Kleinmond River Mouth, West bank", as *P. microlepidotus*): SAM ZR 18306a-f\*.
- G49. Farm: no. 91, Kromme River Valley, Cathcart district (1500 m; 322312S, 265240E\*; 3226Bd4): PEM R2965.
- G50. Farmstead: St. Olives, Farm: Dornfontyn (165), N of Ouberg Mtn, Graaff-Reinet district (440 m; 320452S, 242706E\*; 3224Ab2): USEC-H2549.
- G51. Farm: Winterhoek (269), "Karoo Nature Reserve", W of Graaff-Reinet, Graaff-Reinet district (3215S, 2429E; 3224Ad2): USEC-H2560.

- G52. East London, East London district (3327BB; questionable locality according to W.R. Branch [pers. comm.] – not plotted on map): JV un-numbered (one specimen)\*.
- G53. Valley of Desolation, about 5 km W of Graaff-Reinet, Graaff-Reinet district (about 3216S, 2428E; 3224Ad2): JV 20679\*.
- G54. Tarkastad, Tarkastad district (320030S, 150030E; 3226Ab1): NHMZ-UM 12919-20\*.
- G55. Olifantskop Pass, N of Farm: Sandvlakte (no number on map), Alexandria district (3325Bd4): NHMZ-UM 6682\*.
- G56. Lootsberg Mtn, Sneeu Berg Mtns, Middelburg district (1870 m; 315012S, 245123E\*; 3124Dd1): NMB R8462\*.
- G57. Albany district (Hewitt 1918, AM, as *P. microlepidotus*).
- G58. Tembuland (Essex 1927, as *P. microlepidotus*)

***Pseudocordylus microlepidotus namaquensis***

**Northern Cape:**

- H1. Farm: Steenkamps Vlake (416), W slopes of Steenkampsberg Mtn, Fraserburg district (3207S, 2136E; 3221Ba1): PEM R3797\*.
- H2. 100 km E of Sutherland (3221Bc4; vague - but plotted on map): JV 4114-5\*.
- H3. Komsberg Pass, N slopes of Skurwekop Mtn, Farm: Schietfontein (178), Komsberg Mtns, Sutherland district (1580 m; 324054S, 204517E\*; 3220Db3): USEC-H3380-1.
- H4. Bloukop Mtn, Farm: Steenkamps Hoek (444), N part of Nuweveldberg Mtns, Fraserburg district (1600 m; 320938S, 214302E\*; 3221Ba4) (“Nuweveldberg”: Mouton & Van Wyk 1994, USEC, as *P. microlepidotus*): USEC-H1922-3.

**Western Cape:**

- H5. Beaufort West, (N and W of) Nuweveldberg Mtns, Beaufort West district (3222S, 2235E; 3222Bc1) (FitzSimons 1943, SAM; Loveridge 1944; “Nieuweveldberg near Beaufort West”: Branch & Braack 1989): SAM ZR 1135, 1147-50, 18357 (all missing, discarded or donated to another institution).
- H6. “Nuweveldberge” (Mtns) (3122AB on PEM tag – locus code not given in catalogue; not plotted): PEM R3844\*.
- H7. Karoo National Park, Nuweveldberg Mtns near Beaufort West, Beaufort West district (3222BC) (“Nieuweveldberg near Beaufort West”: Branch 1985): TM 57323.

- H8. S part of Molteno Pass, Waterval area, Karoo National Park, N of Beaufort West, Nuweveldberg Mtns, Beaufort West district (321530S, 2234E; 3222Bc1) (“Karoo National Park, escarpment and middle plateau”: Branch & Braack 1989, PEM as listed]: PEM R3173\*, 3190, 3215\*, 3333-8\*.
- H9. Farmstead: Mountain View, Molteno Pass, Farm: Alwins Gate (186), Karoo National Park, Nuweveldberg Mtns, Beaufort West district (321530S, 223430E; 3222Bc1): BMNH 1988.563.
- H10. Between Beaufort West and Farm: Rhenosterkop (155), at Farm: Speelmans Kuil (154), Beaufort West district (900-950 m; taken as half-way point, about 3217S, 2243E; 3222Bc2) (FitzSimons 1943, TM): TM 13014-5.
- H11. Farmstead: Dunedin, Farm: Quagga Fontein (82), summit of Visserskop Mtn, Beaufort West district (3157S, 2225E; 3122Cd4): SAM ZR 44844-9.
- H12. Farm: Leeu Kloof (43), N slopes of Rooiberg Mtn, Beaufort West district (3152S, 2226E; 3122Cd2): PEM R3817, 3845.
- H13. Little Namaqualand (FitzSimons 1943, SAM; “Namaqualand”: Loveridge 1944]): 3 + 1 (missing) specimens: SAM ZR 859\*, 864\*, 872 (holotype?) (missing from collection), 873\*.

***Pseudocordylus microlepidotus* ssp. ("Transkei")**

**Eastern Cape (Transkei):**

- I1. Butterworth (321930S, 2809E; 3228Ac2) (FitzSimons 1943, AM, as *P. microlepidotus fasciatus*; Loveridge 1944, as *P. microlepidotus fasciatus*; “near Butterworth”: Visser 1984, as *P. microlepidotus fasciatus*): NMZB-UM 6499-501\*; PEM R2614-6\*.
- I2. Tsomo (town) (3202S, 2749E; 3227Bb1) (FitzSimons 1943, SAM, as *P. microlepidotus fasciatus*; Loveridge 1944, as *P. microlepidotus fasciatus*): PEM R2701\*; SAM ZR 2020-1\*.
- I3. Road from Tsomo (town) SE to Nqamakwe (village) then W to Hebehebe (village) (3227BB): PEM R2599, 2600\*, 2601-4, 2606\*.
- I4. Bridge over Tsomo River, 1 km SE of Tsomo (town) (800 m; 320230S, 274915E; 3227Bb1): PEM R2622\*.
- I5. Road between towns of Engcobo and Tsomo (3127DD): PEM R2623\*, 2702-4\*.
- I6. 14 km S of Tsomo (town) (3227BB): PEM R2625-8\*, 2699-700\*.

**Appendix 2.2:** Morphological characters used to distinguish between the three subspecies of *Pseudocordylus microlepidotus* as reported by Smith (1838, 1843), Hewitt (1927), FitzSimons (1943) and Loveridge (1944).

### **Position of the frontonasal**

*P. m. microlepidotus*: Frontonasal separated from rostral by a pair of supranasals in pl. 30, fig. 1, in contact with rostral in pl. 24, fig. 1 (Smith 1843: *C. montanus*); very narrowly excluded from, or very narrowly in contact with, rostral (pl. 24, fig. 2 and pl. 30, fig. 2) (Smith 1843: *C. algoensis*); in contact with rostral (Hewitt 1927); usually in contact with rostral (FitzSimons 1943); separated from, or rarely in contact with, rostral (Loveridge 1944).

*P. m. fasciatus*: Frontonasal in narrow contact with rostral in pl. 30, fig. 5, but apparently separated by a pair of supranasals in pl. 27, fig. 1 (Smith 1843: *C. fasciatus*); usually well separated (Hewitt 1927); usually separated by a pair of supranasals (FitzSimons 1943); separated from, or rarely in contact with, rostral (Loveridge 1944).

*P. m. namaquensis*: Frontonasal well separated from rostral (Hewitt 1927); well separated by a pair of supranasals (FitzSimons 1943); separated from rostral (Loveridge 1944).

### **Shape of the frontonasal**

*P. m. microlepidotus*: Frontonasal as long as wide (Smith 1843, pl. 30, fig. 1: *C. montanus*; FitzSimons 1943); distinctly wider than long (Smith 1843: pl. 30, fig. 2: *C. algoensis*); about as long as wide (Hewitt 1927); longer or shorter than wide (Loveridge 1944).

*P. m. fasciatus*: Frontonasal as long as wide (Smith 1843, pl. 30, fig. 5: *C. fasciatus*; Loveridge 1944).

*P. m. namaquensis*: Frontonasal wider than long (Hewitt 1927; FitzSimons 1943).

### **Colour pattern on the flanks**

*P. m. microlepidotus*: Dark dorsal bands extend onto the flanks, reaching the margin of the belly (Smith 1843, text and pl. 24, fig. 1: *C. montanus*); dark bands extend onto the flanks, but terminate well short of the belly (Smith 1843, pl. 24, fig. 2: *C. algoensis*); bands often extend to abdominal margin (FitzSimons 1943); black bars descend to the flanks (Loveridge 1944).

*P. m. fasciatus*: Dark dorsal bands apparently do not extend onto the flanks (Smith 1843: pl. 27, fig. 1: *C. fasciatus*); sides of body not vertically barred, except in specimens from near Butterworth in which the bands extend slightly onto the flanks (Hewitt 1927); dark dorsal colouration not, or but feebly, extending onto the flanks (FitzSimons 1943); flanks without vertical bars, or at most these encroach slightly onto the flanks (Loveridge 1944).  
*P. m. namaquensis*: Dark dorsal colouration not, or but feebly, extending onto the flanks (FitzSimons 1943).

**Texture of enlarged dorsal scales** (*i.e.* excluding narrow band of flat, smooth scales medially; and granular scales)

*P. m. microlepidotus*: Faintly carinated (Smith 1838: *C. montanus*); obtusely keeled (Smith 1843: pl. 24, fig. 1: *C. montanus*); Faint carina (Smith 1838, *C. algoensis*); keeled (Smith 1843: pl. 24, fig. 2: *C. algoensis*); keeled and striated (FitzSimons 1943); smooth or obtusely keeled (Loveridge 1944).

*P. m. fasciatus*: Small horny tubercle near the centre (Smith 1843, in text: *C. fasciatus*); apparently smooth (Smith 1843, pl. 27, fig. 1: *C. fasciatus*); smooth in adults, slightly keeled in juveniles (Hewitt 1927); mostly smooth, but may be slightly ribbed near periphery (FitzSimons 1943); smooth in adults, feebly keeled in juveniles (Loveridge 1944).

*P. m. namaquensis*: Finely ribbed, stellate towards periphery (Hewitt 1927); raised centers, ribbed towards periphery (FitzSimons 1943); keeled and striated (Loveridge 1944).

**Differentiation in size between median dorsal and dorsolateral scales**

*P. m. microlepidotus*: Dorsolateral scales larger than median dorsals, but differentiation in size not strongly marked (FitzSimons 1943).

*P. m. fasciatus*: Dorsolateral scales much larger than median dorsals, *i.e.* differentiation in size strongly marked (Smith 1843, text; FitzSimons 1943).

*P. m. namaquensis*: Dorsolateral scales larger than median dorsals, *i.e.* differentiation in size strongly marked (FitzSimons 1943).

### **Arrangement of lateral temporal scales**

*P. m. microlepidotus*: Lateral temporals in about two rows, each of mostly slightly elongated scales (Smith 1843: pl. 30, fig. 1a: *C. montanus*); scales of upper row elongated, those of the lower row square to round in shape (Smith 1843: pl. 24, fig. 1: *C. montanus*); irregularly arranged or in roughly 3-4 rows, only a few being slightly elongated (Smith 1843: pl. 24, fig. 2 and pl. 30, fig. 2a: *C. algoensis*); usually in three horizontal series (FitzSimons 1943); two rows, those of the upper row relatively small and polygonal, at most one or two vertically elongate (Loveridge 1944).

*P. m. fasciatus*: Lateral temporals in three rows of mostly slightly elongated scales (pl. 27, fig. 1); only the scales of the middle of the three rows mostly distinctly elongated (pl. 30, fig. 5a) (Smith 1843: *C. fasciatus*); small and polygonal (Hewitt 1927); irregularly arranged or in three rows of which the middle is the largest (FitzSimons 1943); polygonal, generally not vertically elongate (Loveridge 1944).

*P. m. namaquensis*: Lateral temporals in two rows, those of the upper row enlarged and somewhat elongated vertically (Hewitt 1927; Loveridge 1944); irregularly arranged or in about two horizontal rows, the upper being slightly elongated vertically (FitzSimons 1943).

### **Projection of lowermost enlarged temporal spine**

*P. m. microlepidotus*: Lowermost temporal spine “projecting” (FitzSimons 1943).

*P. m. fasciatus*: Lowermost temporal spine projecting feebly outwards (FitzSimons 1943).

*P. m. namaquensis*: Lowermost temporal spine projecting strongly outwards (Hewitt 1927; FitzSimons 1943).

### **Posterior infralabial keeled or smooth**

*P. m. microlepidotus*: Posterior infralabial keeled (Smith 1843: pl. 30, fig. 1a: *C. montanus*); smooth in pl. 24, fig. 2, but keeled in pl. 30, fig. 2a (Smith 1843: *C. algoensis*); usually keeled (FitzSimons 1943).

*P. m. fasciatus*: Posterior infralabial strongly keeled (in text), apparently smooth (Smith 1843, pl. 30, fig. 5a: *C. fasciatus*); without a projecting horizontal ridge, smooth or moderately keeled (in key), with compressed horizontal keel (in text) (FitzSimons 1943).

*P. m. namaquensis*: Posterior infralabial with a strongly compressed projecting horizontal ridge (FitzSimons 1943).

### **Gular markings**

*P. m. microlepidotus*: Livid blue (Smith 1843, text: *C. montanus*); livid blue (Smith 1843, text: *C. algoensis*); black (Rose in Loveridge 1944); chin and throat blue, sometimes restricted to area between rami [ramus = ascending, more-or-less vertical part of the lower jaw that makes a joint at the temple] (FitzSimons 1943).

*P. m. fasciatus*: Throat uniformly bluish (FitzSimons 1943).

*P. m. namaquensis*: Throat without infuscation [= darkening] (Hewitt 1927; Loveridge 1944); throat immaculate or with an elongate 8-shaped dark bluish marking (FitzSimons 1943).

### **Shape of the gular (throat) scales**

*P. m. microlepidotus*: Gular scales small and elongate, becoming smaller, rounded and subgranular towards base of throat (FitzSimons 1943); median gulars more-or-less slightly elongate like the lateral gulars (Loveridge 1944).

*P. m. fasciatus*: Median gulars more-or-less squarish, not even slightly elongate like the lateral gulars (Loveridge 1944).

*P. m. namaquensis*: Median gulars more-or-less rounded or squarish (Hewitt 1927); median gulars more-or-less squarish or subcircular, not even slightly elongate like the lateral gulars (Loveridge 1944).

**Appendix 3.1:** Localities, sample sizes and museum accession numbers of specimens used in the allozyme electrophoretic analysis of the *Pseudocordylus melanotus* species complex. The name used in the text to refer to any particular population is underlined: specimens assigned to a particular population were sometimes collected from more than one locality.

*Pseudocordylus transvaalensis*

1. Thabazimbi (15 specimens): Farm: Hartbeestfontein (281), Thabazimbi district, Limpopo Province (24°29'21"S, 27°37'58"E; 2427Bc4): NMB R8430-44.
2. Mokopane (14 specimens): Farm: Helderfontein (6KS), Potgietersrus district, Limpopo Province (24°01'30"S, 29°05'E; 2429Aa1): NMB R8195-208.

*Pseudocordylus melanotus melanotus*

3. Sabie (22 specimens): Sabie (Mundi forestry area), Farm no. 196, Pilgrim's rest 2 district, Mpumalanga Province (25°08'22"S, 30°45'40"E; 2530Bb3): NMB R8242-50; (25°08'22"S, 30°45'32"E; 2530Bb3): NMB R8251-60; (25°08'27"S, 30°45'42"E; 2530Bb3): NMB R8261-4.
4. Lochiel (11 specimens): Eerstehoek district, Mpumalanga Province: Farm: Aankomst (191) (26°10'03"S, 30°52'21"E; 2630Bb3): NMB R8266; Farm Lochiel (192) (26°08'55"S, 30°51'08"E; 2630Bb3): NMB R8267-76.
5. Amersfoort (9 specimens): Farm: Klipplaatdrift (504), Amersfoort district, Mpumalanga Province (26°54'57"S, 29°53'10"E; 2629Dd4): NMB R8278-86.
6. Suikerbosrand (15 specimens): Suikerbosrand Nature Reserve, Heidelberg district, Gauteng Province: 2.3 km WSW of Visitors' Centre (26°29'14"S, 28°11'03"E; 2628Ac4): NMB R8415-8; 0.8 km NW of Springbok Overnight Hut (26°30'19"S, 28°12'04"E; 2628Ca2): NMB R8419-21; 1.7 km N of Springbok Overnight Hut (26°29'41"S, 28°12'02"E; 2628Ac4): NMB R8422-3; Diepkloof, 0.8 km WSW of Visitors' Centre (26°29'11"S, 28°12'03"E; 2628Ac4): NMB R8424-5; 1.7 km SW of Visitors' Centre (26°29'43"S, 28°11'56"E; 2628Ac4): NMB R8426-9.
7. Harrismith (20 specimens): Farm: Uyshoek (1092), Harrismith district, Free State Province (28°15'50"S, 29°20'45"E; 2829Ad1): NMB R8170-89.
8. Qwa-Qwa (7 specimens): Farm: Witzieshoek (1815), Harrismith district, Free State Province: Qoqolosing village (28°35'19"S, 28°56'12"E; 2828Db2): NMB R8359; (28°35'32"S, 28°54'23"E; 2828Db2): NMB R8360-4; Thibella village, 2 km N of Fika Patso Dam (28°39'15"S, 28°51'40"E; 2828Db3): NMB R8365.
9. Nkandla (23 specimens): Nkandhla district, KwaZulu-Natal: Vumanhlamvu village between Nkandla town and Nkandla Forest (28°42'00"S, 31°07'34"E; 2831Ca4): NMB R8366-76; Ntabayabesutu, 10 km NNW of Qudeni village (28°31'30"S,

30°50'18"E; 2830Db1): NMB R8377-87; Farm: Braet Mead (14238), 12 km SSE of Babanango (28°28'55"S, 31°06'04"E; 2830Ac3): NMB R8388.

*Pseudocordylus melanotus subviridis*

10. Qwa-Qwa (24 specimens): Harrismith district, Free State Province: Monontsa Pass Border Post (R.S.A. side), Farm: Witzieshoek (1815) (28°34'53"S, 28°41'54"E; 2828Da2): NMB R8335-6; Monontsha Pass, near "Kraal", Farm: Woes Arabia (40) (28°35'15"S, 28°41'23"E; 2828Da2): NMB R8337-46; Monontsha Pass, 0.5 km NE of Monontsa Pass Border Post, Farm: Woes Arabia (40) (28°35'15"S, 28°41'08"E; 2828Da2): NMB R8347; Monontsha Pass, near "Kraal", 1 km NE of Monontsa Pass border post (Lesotho, near Ha Molisana), Farm: Woes Arabia (40) (28°35'14"S, 28°41'31"E; 2828Da2): NMB R8348-53; 1 km NW of Witzieshoek Mountain Resort, Farm: Witzieshoek (1815) (28°40'55"S, 28°53'40"E; 2828Db4): NMB R8354-7; Entrance to Sentinel Hiking Trail, Farm: Witzieshoek (1815) (28°43'39"S, 28°53'38"E; 2828Db4): NMB R8358.
11. Organ Pipes (15 specimens): Organ Pipes Pass, Bergville district, KwaZulu-Natal (29°01'S, 29°12'30"E; 2929Aa2): NMB R8151, 8153-64, 8166-7.
12. S Lesotho (10 specimens): 0.5 km SW of Thaba Chitja village, Lesotho (30°05'50"S, 28°16'15"E; 3028Ab1): NMB R8405-14.
13. Naude's Nek (23 specimens): Near top of Naudes Nek, Farm no. 61, Barkly East district, Eastern Cape Province (30°44'S, 28°08'E; 3028Ca4): NMB R8292-314.
14. Hogsback (20 specimens): Cathcart district, Eastern Cape Province: Farm: Waterfall (161) (32°34'25"S, 26°56'41"E; 3226Db2): NMB R8212; (32°34'00"S, 26°56'29"E; 3226Db2): 8215-24; Farm: Moreson (162) (32°34'45"S, 26°59'30"E; 3226Db2): NMB R8213-4; Farm no. 32 (32°34'51"S, 26°56'44"E; 3226Db2): NMB R8225-31.

*Pseudocordylus langi*

11. Organ Pipes (5 specimens): Top of Organ Pipes Pass, Bergville district, KwaZulu-Natal (29°01'S, 29°12'30"E; 2929Aa2): NMB R8445-9.

**Appendix 4.1:** Localities, sample sizes and museum accession numbers of specimens used in the mtDNA analysis of the *Pseudocordylus melanotus* species complex. The name used in the text to refer to any particular population is underlined: specimens assigned to a particular population were sometimes collected from more than one locality.

*Pseudocordylus transvaalensis*

1. Thabazimbi (5 specimens): Farm: Hartbeestfontein (281), Thabazimbi district, Limpopo Province (24°29'21"S, 27°37'58"E; 2427Bc4): NMB R8430, 8433, 8438-40.
2. Mokopane (5 specimens): Farm: Helderfontein (6KS), Potgietersrus district, Limpopo Province (24°01'30"S, 29°05'E; 2429Aa1): NMB R8195, 8197, 8200, 8204, 8206.

*Pseudocordylus melanotus melanotus*

3. Sabie (5 specimens): Sabie (Mundi forestry area), Farm no. 196, Pilgrim's rest 2 district, Mpumalanga Province (25°08'22"S, 30°45'40"E; 2530Bb3): NMB R8245, 8247; (25°08'22"S, 30°45'32"E; 2530Bb3): NMB R8251, 8256; (25°08'27"S, 30°45'42"E; 2530Bb3): NMB R8262.
4. Lochiel (5 specimens): Eerstehoek district, Mpumalanga Province: Farm: Aankomst (191) (26°10'03"S, 30°52'21"E; 2630Bb3): NMB R8266; Farm Lochiel (192) (26°08'55"S, 30°51'08"E; 2630Bb3): NMB R8267-8, 8274-5.
5. Amersfoort (5 specimens): Farm: Klipplaatdrift (504), Amersfoort district, Mpumalanga Province (26°54'57"S, 29°53'10"E; 2629Dd4): NMB R8278-9, 8281-3.
6. Suikerbosrand (5 specimens): Suikerbosrand Nature Reserve, Heidelberg district, Gauteng Province: 2.3 km WSW of Visitors' Centre (26°29'14"S, 28°11'03"E; 2628Ac4): NMB R8418; 0.8 km NW of Springbok Overnight Hut (26°30'19"S, 28°12'04"E; 2628Ca2): NMB R8420-1; 1.7 km SW of Visitors' Centre (26°29'43"S, 28°11'56"E; 2628Ac4): NMB R8427-8.
7. Vrede (2 specimens): Farm: Berlin (497), Bothasberg Mtn, Vrede district, Free State Province (1980 m; 27°29'09"S, 29°05'00"E; 2729Ac3): NMB R8573; (1940 m; 27°29'07"S, 29°05'00"E; 2729Ac3): NMB R8574.
8. Harrismith (5 specimens): Farm: Uyshoek (1092), Harrismith district, Free State Province (28°15'50"S, 29°20'45"E; 2829Ad1): NMB R8170, 8172, 8178, 8180, 8189.
9. Qoqolosing (1 specimen): Qoqolosing village, Farm: Witzieshoek (1815), Harrismith district, Free State Province: (28°35'32"S, 28°54'23"E; 2828Db2): NMB R8361.
10. Qudeni (2 specimens): Ntabayabesutu, 10 km NNW of Qudeni village, Nkandhla district, KwaZulu-Natal (28°31'30"S, 30°50'18"E; 2830Db1): NMB R8377.

11. Nkandla (3 specimens): Vumanhlamvu village between Nkandla town and Nkandla Forest, Nkandhla district, KwaZulu-Natal (28°42'00"S, 31°07'34"E; 2831Ca4): NMB R8366, 8368, 8371; Farm: Braet Mead (14238), 12 km SSE of Babanango, Nkandhla district (about 1100-1200 m; 28°28'55"S, 31°06'04"E; 2831Ac3): NMB R8388.

*Pseudocordylus melanotus subviridis*

9. Qoqolosing (3 specimens): Farm: Witzieshoek (1815), Harrismith district, Free State Province: Qoqolosing village (28°35'19"S, 28°56'12"E; 2828Db2): NMB R8359; (28°35'32"S, 28°54'23"E; 2828Db2): NMB R8360, 8363.
12. Monontsha Pass (3 specimens): Harrismith district, Free State Province: Monontsha Pass, 0.5 km NE of Monontsa Pass Border Post, Farm: Woes Arabia (40) (28°35'15"S, 28°41'08"E; 2828Da2): NMB R8347; Monontsha Pass, near "Kraal", 1 km NE of Monontsa Pass border post (Lesotho, near Ha Molisana), Farm: Woes Arabia (40) (28°35'14"S, 28°41'31"E; 2828Da2): NMB R8348, 8550.
13. Thibella (1 specimen): Thibella village, 2 km N of Fika Patso Dam, Harrismith district, Free State Province (28°39'15"S, 28°51'40"E; 2828Db3): NMB R8365.
14. Witzieshoek (3 specimens): Farm: Witzieshoek (1815), Harrismith district, Free State Province: 1 km NW of Witzieshoek Mountain Resort, (28°40'55"S, 28°53'40"E; 2828Db4): NMB R8354; Entrance to Sentinel Hiking Trail (28°43'39"S, 28°53'38"E; 2828Db4): NMB R8358; 100 m N of Sentinel Hiking Trail car park (2520 m; 28°43'49"S, 28°53'34"E; 2828Db4): NMB R8567.
15. Organ Pipes (5 specimens): Organ Pipes Pass, Bergville district, KwaZulu-Natal (29°01'S, 29°12'30"E; 2929Aa2): NMB R8156-7, 8160, 8166, 8168.
16. S Lesotho (4 specimens): 0.5 km SW of Thaba Chitja village, Lesotho (30°05'50"S, 28°16'15"E; 3028Ab1): NMB R8405, 8407, 8409-10.
17. Naude's Nek (5 specimens): Near top of Naudes Nek, Farm no. 61, Barkly East district, Eastern Cape Province (30°44'S, 28°08'E; 3028Ca4): NMB R8293, 8295-6, 8298, 8300.
18. Hogsback (5 specimens): Cathcart district, Eastern Cape Province: Farm: Waterfall (161) (32°34'25"S, 26°56'41"E; 3226Db2): NMB R8212; (32°34'00"S, 26°56'29"E; 3226Db2): NMB R8215-24; Farm: Moreson (162) (32°34'45"S, 26°59'30"E; 3226Db2): NMB R8213-4; Farm no. 32 (32°34'51"S, 26°56'44"E; 3226Db2): NMB R8225-31.

*Pseudocordylus langi*

14. Chain Ladder (1 specimen): 200 m SE of Vemvane River falls, Farm: Witzieshoek (1815), Harrismith district, Free State Province (3020 m; 28°44'56"S, 28°52'43"E; 2828Db4): NMB R8553.

15. Organ Pipes (2 specimens): Top of Organ Pipes Pass, Bergville district, KwaZulu-Natal (29°01'S, 29°12'30"E; 2929Aa2): NMB R8448-9.

*Pseudocordylus spinosus*

14. Goodoo Pass (5 specimens): Goodoo Pass, Royal Natal National Park, Bergville district, KwaZulu-Natal: 750 m ESE of Witzieshoek Mountain Resort (2100 m; 28°41'13"S, 28°54'25"E; 2828Db4): NMB R8568-71; 1 km ESE of Witzieshoek Mountain Resort (2000 m; 28°41'13"S, 28°54'38"E; 2828Db4): NMB R8572.

*Pseudocordylus microlepidotus microlepidotus*

Vermaakskop (1 specimen): Vermaakskop, Groot-Winterhoekberg Mtns, Groendal Wilderness Area, Uitenhage district, Eastern Cape (950 m; 33°37'39"S, 25°16'45"E; 3325Cb3): NMB R8541.

*Cordylus breyeri*

Thabazimbi (1 specimen): Farm: Hartbeestfontein (281), Thabazimbi district, Limpopo Province (24°29'53"S, 27°39'57"E; 2427Bc4): NMB R8539.

*Cordylus vandami*

Chuniespoort (1 specimen): Farm no. 359, Mogodhmo Mtn, Strydpoortberg Mtns, Thabamooop district, Limpopo province (24°13'S, 29°31'30"E; 2429Ba3): NMB R8543-5.

*Platysaurus intermedius intermedius*

Houtbosdorp (1 specimen): Between Houtbosdorp and Farm: Bonny Brae (959), Pietersburg district (23°52'S, 29°56-59'E; 2329Dd2): Photographic record.

**Appendix 4.2:** 16S rRNA sequences for 23 alleles in the *Pseudocordylus melanotus* and *P. microlepidotus* complexes. Sequences for outgroup taxa used in the mtDNA analysis are also shown (Platint = *Platysaurus i. intermedius*; Cbreyvan = *Cordylus breyeri* and *C. vandami*).

	1	2	3	4	5	
[	12345678901234567890123456789012345678901234567890					]
PA	ATGAATGGTTAAATGAGGATAGACCTGTCTCTTATGGGAAATCAGTGAAA					[ 50 ]
PB	.....					[ 50 ]
PC	.....					[ 50 ]
PD	.....					[ 50 ]
PE	.....A.....					[ 50 ]
PF	.....A.....					[ 50 ]
PG	.....A.....A.....					[ 50 ]
PH	.....A.....G.....					[ 50 ]
PI	.....A.....					[ 50 ]
PJ	.....A.....					[ 50 ]
PL	.....T.....					[ 50 ]
PM	.....T.....					[ 50 ]
PN	.....A.....					[ 50 ]
PO	.....A.....					[ 50 ]
PP	.....T.....A.....					[ 50 ]
PQ	.....T.....					[ 50 ]
PR	.....A.....A.G.....					[ 50 ]
PS	.....A.G.....					[ 50 ]
PT	.....G.....A.G.....					[ 50 ]
PU	.....A.G.....					[ 50 ]
Pmic	?????????.....?.....G.....					[ 50 ]
PlW	.....T.....A.....					[ 50 ]
PlX	.....T.....A.....					[ 50 ]
Platint	.....C.....G.A.....T.GA.A.....G.....					[ 50 ]
Cbreyvan	.....A.....C.A.....GC.....G.....					[ 50 ]

	5	6	7	8	9	0	1
[	123456789012345678901234567890123456789012345678901234567890						]
PA	CTGAACTTCCAGTACAAATGCTGGAATATATACACAAGACGAGAAGACCC						[ 100 ]
PB	.....						[ 100 ]
PC	.....						[ 100 ]
PD	.....						[ 100 ]
PE	.....C.....A.G.....T.....						[ 100 ]
PF	.....C.....A.G.....T.....						[ 100 ]
PG	.....C.....						[ 100 ]
PH	.....C.....						[ 100 ]
PI	.....C.....						[ 100 ]
PJ	.....C.....						[ 100 ]
PL	.....C.....T.....						[ 100 ]
PM	.....C.....T.....						[ 100 ]
PN	.....C.....T.....						[ 100 ]
PO	.....C.....AG.....T.....						[ 100 ]
PP	.....C.....T.....						[ 100 ]
PQ	.....C.....T.T.....						[ 100 ]
PR	.....C.....G.....						[ 100 ]
PS	.....C.T.....G.....						[ 100 ]
PT	.....C.T.....G.....						[ 100 ]
PU	.....C.....						[ 100 ]
Pmic	.....C.....G.....						[ 100 ]
PlW	.....C.....						[ 100 ]
PlX	.....C.....						[ 100 ]
Platint	.....T.....TA.T.T.....						[ 100 ]
Cbreyvan	.....C.C.....						[ 100 ]

```

[          1          ]
[          0          1          2          3          4          5]
[ 12345678901234567890123456789012345678901234567890 ]

PA      TGCGGAGCTTTTAAACCCCTCAAACAATAAATTGAGCATGTGTTTTCA [150]
PB      .....A..... [150]
PC      .....C.A..... [150]
PD      .....-.....A..... [149]
PE      .....--..... [148]
PF      .....T.....--..... [148]
PG      .....A..... [150]
PH      .....A..... [150]
PI      .....A..... [150]
PJ      .....A..... [150]
PL      .....T-.....C.....T...G..... [149]
PM      .....T-.....C.....T...G..... [149]
PN      .....-.....T...AA..... [149]
PO      .....A.....AA..... [150]
PP      .....-.....T...AA..... [149]
PQ      .....T-.....C.....T...G..... [149]
PR      .....A.....C.A..... [150]
PS      .....C.A..... [150]
PT      .....C.A..... [150]
PU      .....C.A..... [150]
Pmic    .....C.A..... [150]
PlW     .....-.....T.T.....TGC.-..... [148]
PlX     .....-.....T.T.....TGC.-..... [148]
Platint ..T.....-.....TT.....AT.AAA.....T. [149]
Cbreyvan.....C.....A.....-.....CC.-..... [148]

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[          1          2]
[          5          6          7          8          9          0]
[ 12345678901234567890123456789012345678901234567890 ]

PA      GTTGGGGCGACTTCGGAACGAAACAACGCTTCCGAGCA--AAGAGACCTC [198]
PB      .....--..... [198]
PC      .....--..... [198]
PD      .....--..... [197]
PE      .....A.....CA..... [198]
PF      .....A.....CA..... [198]
PG      .....--..... [198]
PH      .....--.....G.... [198]
PI      .....--..... [198]
PJ      .....--..... [198]
PL      .....A.....CA.....T... [199]
PM      .....A.....CA.....T... [199]
PN      .....CA.....T... [199]
PO      .....CA.....T.C. [200]
PP      .....G.....CA.....T... [199]
PQ      .....A.....CA.....T... [199]
PR      .....TA.....T... [200]
PS      .....TA.....T... [200]
PT      .....TA.....T... [200]
PU      .....TA.....T... [200]
Pmic    .....TA.....T.C. [200]
PlW     .....A...T.A.....CA..... [198]
PlX     .....A...T.A.....CA..... [198]
Platint .....A..A..A-.....TA.ACC....CA.CA [198]
Cbreyvan.....A..A..A.....CC.....C. [198]

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[	2	]	
[	0	1	2
[	1234567890123456789012345678901234567890123456789012345678901234567890]		
PA	TCTTGCAGAGGCCAAACAGGCCAAAGCACTAATAAACATTGACCCGGATAA	[ 248 ]	
PB	.....	[ 248 ]	
PC	.....	[ 248 ]	
PD	...C.....	[ 247 ]	
PE	.....A.....A.....CT.	[ 248 ]	
PF	.....A.....A.....CT.	[ 248 ]	
PG	.....A.....C.....C.....	[ 248 ]	
PH	.....A.....C.....T.....	[ 248 ]	
PI	.....A.....C.....C.....T.....	[ 248 ]	
PJ	.....A.....C.....T.....	[ 248 ]	
PL	.....T.....C.....TG	[ 249 ]	
PM	.....T.....C.....TG	[ 249 ]	
PN	.....T.GGG.....??.....T.....	[ 249 ]	
PO	.....T.....T.....	[ 250 ]	
PP	.....C.....T.....	[ 249 ]	
PQ	.....T.....C.....TG	[ 249 ]	
PR	.....T.....C.G.....T.....	[ 250 ]	
PS	.....T.G.....C.G.....T.....	[ 250 ]	
PT	.....T.....C.G.....T.....	[ 250 ]	
PU	.....T.....G.....T.....	[ 250 ]	
Pmic	.....T.....G.....T.....	[ 250 ]	
PlW	.....C..C...A.....TA.....T...-...A...T.....	[ 247 ]	
PlX	.....C..C...A.....TA.....C...-...A...T.....	[ 247 ]	
Platint	CTCAAG.A.CGGCCT.CAAGCC.AT.AC.....C.....C.....	[ 248 ]	
Cbreyvan	.....AC...C...A.....TC..C...CA.....TT	[ 248 ]	

[	2	3]	
[	5	6	7
[	123456789012345678901234567890123456789012345678901234567890]		
PA	-ACCCGATTAACGAACCAAGTTACCCAGGGATAACAGCGCTATCTTCTT	[ 297 ]	
PB	-.....	[ 297 ]	
PC	-.....	[ 297 ]	
PD	-.....	[ 296 ]	
PE	-.....	[ 297 ]	
PF	-.....	[ 297 ]	
PG	-.....	[ 297 ]	
PH	-.....	[ 297 ]	
PI	-.....	[ 297 ]	
PJ	-.....	[ 297 ]	
PL	-.....	[ 298 ]	
PM	-.....	[ 298 ]	
PN	-.T.....	[ 298 ]	
PO	-.T.....	[ 299 ]	
PP	-.T.....	[ 298 ]	
PQ	-.....	[ 298 ]	
PR	-.....	[ 299 ]	
PS	-.....	[ 299 ]	
PT	-.....	[ 299 ]	
PU	-.....G.....	[ 299 ]	
Pmic	-.....	[ 299 ]	
PlW	-.T.T.....	[ 296 ]	
PlX	-.T.T.....	[ 296 ]	
Platint	-.T.....A.....C.....C.....	[ 297 ]	
Cbreyvan	C.T.....	[ 298 ]	

```

[           3                               ]
[           0           1           2           3           4           5]
[ 123456789012345678901234567890123456789012345678901234567890 ]

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PA      TAAGAGTCCATATCAACAAGTAAGGCTTACGACCTCGATGTTGGATCAGG [ 347 ]
PB      ..... [ 347 ]
PC      ..... [ 347 ]
PD      ..... [ 346 ]
PE      ..... [ 347 ]
PF      ..... [ 347 ]
PG      ..... [ 347 ]
PH      ..... [ 347 ]
PI      ..... [ 347 ]
PJ      ..... [ 347 ]
PL      ..... [ 348 ]
PM      .....T.A..... [ 348 ]
PN      ..... [ 348 ]
PO      ..... [ 349 ]
PP      ..... [ 348 ]
PQ      ..... [ 348 ]
PR      ..... [ 349 ]
PS      ..... [ 349 ]
PT      ..... [ 349 ]
PU      ..... [ 349 ]
Pmic    ..... [ 349 ]
PlW     .....-..... [ 345 ]
PlX     .....-..... [ 345 ]
Platint .....G.-..... [ 346 ]
Cbreyvan .....C..... [ 348 ]

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[           3                               4]
[           5           6           7           8           9           0]
[ 123456789012345678901234567890123456789012345678901234567890 ]

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PA      ACACCCAAATGGTGCAGCCGCTATTAATGGTTTCGTTTGTTC AACGATTAA [ 397 ]
PB      ..... [ 397 ]
PC      ..... [ 397 ]
PD      ..... [ 396 ]
PE      ..... [ 397 ]
PF      ..... [ 397 ]
PG      ..... [ 397 ]
PH      .....C..... [ 397 ]
PI      ..... [ 397 ]
PJ      ..... [ 397 ]
PL      ..... [ 398 ]
PM      ..... [ 398 ]
PN      ..... [ 398 ]
PO      ..... [ 399 ]
PP      ..... [ 398 ]
PQ      ..... [ 398 ]
PR      ..... [ 399 ]
PS      ..... [ 399 ]
PT      ..... [ 399 ]
PU      ..... [ 399 ]
Pmic    ..... [ 399 ]
PlW     .....A..... [ 395 ]
PlX     .....A..... [ 395 ]
Platint .....C..... [ 396 ]
Cbreyvan ..... [ 398 ]

```

[	4	]	
[	0	1	2 ]
[	123456789012345678901]		
PA	CAGTCCTACGTGATCTGAGTT	[ 418 ]	
PB	.....	[ 418 ]	
PC	.....	[ 418 ]	
PD	.....	[ 417 ]	
PE	.....	[ 418 ]	
PF	.....	[ 418 ]	
PG	.....	[ 418 ]	
PH	.....	[ 418 ]	
PI	.....	[ 418 ]	
PJ	.....	[ 418 ]	
PL	.....	[ 419 ]	
PM	.....	[ 419 ]	
PN	.....	[ 419 ]	
PO	.....	[ 420 ]	
PP	.....	[ 419 ]	
PQ	.....	[ 419 ]	
PR	.....	[ 420 ]	
PS	.....	[ 420 ]	
PT	.....	[ 420 ]	
PU	.....	[ 420 ]	
Pmic	.....	[ 420 ]	
PlW	T.....	[ 416 ]	
PlX	T.....	[ 416 ]	
Platint	.....	[ 417 ]	
Cbreyvan	.....	[ 419 ]	

**Appendix 5.1:** Localities and specimens used in the morphological analysis of the *Pseudocordylus melanotus* species complex (numbers as used in Figs 5.3 to 5.5). Map co-ordinates are presented as a series of numerical values (degrees and minutes; or degrees, minutes and seconds). An asterisk after the co-ordinates indicates that an accurate determination to the level of minutes was possible; in other cases the center of a farm or town was used. One asterisk after a catalogue number(s) indicates that the specimen(s) was used in the allozyme electrophoretic study; two asterisks indicate that the specimen was used in both allozyme and mtDNA analyses; whereas a superscripted D means that the specimen was included in the mtDNA study.

*Pseudocordylus transvaalensis*

**Limpopo Province** (Western population):

1. Farm: Groothoek (278), Waterberg Mtns/Sandrivierberg Mtns, Thabazimbi district (2429S, 273630E; 2427Bc3): TM 65248, 74388.

Farm: Hartbeestfontein (281), Waterberg Mtns, Thabazimbi district (2429S, 2741E; 2427Bc4): TM 74381, 74386.

Farm: Hartbeestfontein (281), Waterberg Mtns, Thabazimbi district (242921S, 273758E\*; 2427Bc4): NMB R8430\*\*, 8431-2\*, 8433\*\*, 8434-7\*, 8438-40\*\*, 8441-4\*.

2. NW of Warmbaths (c. 2428Ca1): TM 33307, 33794.

Farm: Rhenosterpoort (402), Waterberg district (1600 m; 2439S, 2809E; 2428Ca4): TM 74364-5.

**Limpopo Province** (Central population):

3. Matlalas (= Mataias) Location, Seshego district (234530S, 290030E; 2329Cc1): TM 74390-3.

4. Farm: Helderfontein (6), Potgietersrust district (240130S, 2905E; 2429Aa1): NMB R8195\*\*, 8196\*, 8197\*\*, 8198-9\*, 8200\*\*, 8201-3\*, 8204\*\*, 8205\*, 8206\*\*, 8207-8\*.

Percy Fyfe Nature Reserve, Potgietersrust district (2402S, 290930E; 2429Aa2): TM 42703, 74369, 74383.

5. Farm: Zandspruit (287), near Waterberg Mtns, Potgietersrust district, (2413S, 2855E; 2428Bb4): TM 74372-5.

6. Farm: Maribashoek (50), Maribashoekberg Mtns, Potgietersrust district (2413S, 2907E; 2429Aa3): TM 11093.

Farm: Makapansgat (39), Sugarloaf Hill, Potgietersrust district (240830S, 291030E; 2429Aa4): TM 33278, 57306.

Farm: Oostenryk (92), Buffelshoekberg Mtns, Potgietersrust district (2417S, 2914E; 2429Ac2): TM 74377-9.

**Limpopo Province** (Eastern population):

7. Woodbush State Forest, Letaba 1 district (2349S, 295930E; 2329Dd2): TM 1695 (holotype); TM 1697, 1699-700, 1954-5 (paratypes); TM 42326.

Farm: Diepgelegen (945), Pietersburg district (2351S, 295930E; 2329Dd2): TM 74370.

Houtbosdorp, 15 km NNW of Haenertsburg, Pietersburg district (234830S, 2954E; 2329Dd2): TM 74382, 74384-5, 74387.

Farm: Mphome (949), Pietersburg district (235030S, 295430E; 2329Dd2): TM 74371, 74394.

Farm: Spitskop (1011), Suikerboskoppie hill, Thaba-moopo district (1876 m; 2352S, 295330E; 2329Dd2): NMB R8041-2.

Farm: Klipspruit (908), Pietersburg district (1450 m; 234923S, 295303E; 2329Dd2): NMB R8546-7.

Farm: Tomason (950), Pietersburg district (1600-1800 m; 235113S, 295407E; 2329Dd2): NMB R8548.

Farm: Monte Christo (1011), Thabamoopo district (1700-1800 m; 235202S, 295328E; 2329Dd2): NMB R8549-51.

8. Iron Crown, 6 km S of Haenertsburg, Strydpoortberg Mtns, Pietersburg district (235950S, 2957E; 2329Dd4): TM 33795.

Farm: Flynn (217), Strydpoortberg Mtns, Pietersburg district (2404S, 295015E; 2429Bb1): TM 74366-8.

Farm: Paardevlei (201), Wolkberg Mtn, Strydpoortberg Mtns, Pietersburg district (240230S, 295530E; 2429Bb2): TM 74376.

Serala Mtn, Pietersburg/Letaba 1 districts (2401S, 300430E; 2430Aa1): TM 74389.

Farm: Acre (2), Pietersburg district (2401S, 3004E; 2430Aa1): TM 74380.

*Pseudocordylus melanotus melanotus*

**Northern melanotus**

**Limpopo Province:**

9. Ga-Selati River, probably the vicinity of Orrie Baragwanath Pass, Legalameetse Nature reserve, E side of Transvaal Drakensberg, E of Leydsdorp, Phalaborwa district (2430Ab3): TM 168, 171-174.

**Swaziland:**

10. Lomahasha (village), Lubombo Mtns, Lubombo district, Swaziland (2559S, 3159E; 2531Dd4): TM 67396.

**Mpumalanga Province:**

11. Sabie (Mundi Forestry Area), Farm: no. 196, Drakensberg Mtns, Pilgrim's Rest 2 district (250822S, 304540E\*; 2530Bb3): NMB R8242-4\*, 8245\*\*, 8246\*, 8247\*\*, 8248-50\*; (250822S, 304532E\*; 2530Bb3): NMB R8251\*\*, 8252-5\*, 8256\*\*, 8257-9\*; 8260 (250827S, 304542E\*; 2530Bb3): NMB R8261\*, 8262\*\*, 8263\*, 8264.

12. Farm: Lochiel (192), Eerstehoek district (1650 m; 260855S, 305108E\*; 2630Bb3): NMB R8267-8\*\*, 8269-73\*, 8274-5\*\*, 8276\*.

Farm: Aankomst (191), Eerstehoek district (261003S, 305221E\*; 2630Bb3): NMB R8266\*\*.

**Southern melanotus**

**Gauteng Province:**

13. 2.3 km WSW of Visitors' Centre, Suikerbosrand Nature Reserve, Heidelberg district (1790-1810 m; 262914S, 281103E\*; 2628Ac4): NMB R8415-7\*, 8418\*\*.

1.7 km N of Springbok Overnight Hut, Suikerbosrand Nature Reserve, Heidelberg district (1840-1860 m; 262941S, 281202E\*; 2628Ac4): NMB R8422-3\*.

Diepkloof, 0.8 km WSW of Visitors' Centre, Suikerbosrand Nature Reserve, Heidelberg district (1690-1740 m; 262911S, 281203E\*; 2628Ac4): NMB R8424-5\*.

1.7 km SW of Visitors' Centre, Suikerbosrand Nature Reserve, Heidelberg district (1830-1850 m; 262943S, 281156E\*; 2628Ac4): NMB R8426\*, 8427-8\*\*, 8429\*.

0.8 km NW of Springbok Overnight Hut, Suikerbosrand Nature Reserve, Heidelberg district (1750 m; 263019S, 281204E\*; 2628Ca2): NMB R8419\*, 8420-1\*\*.

**Mpumalanga Province:**

14. Farm: Klipplaatdrift (504), Amersfoort district (1600 m; 265457S, 295310E\*; 2629Dd4): NMB R8278-9\*\*, 8280\*, 8281-3\*\*, 8284-6\*.

15. Farm: Zuurbron (132), 31 km ENE of Wakkerstroom, Wakkerstroom district (2717S, 302630E; 2730Ad2): BMNH 1905.3.7.94-6.

**KwaZulu-Natal:**

16. Muller's Pass, 23 km SW of Newcastle, Bergville district (2752S, 2943E; 2729Dc2): NMSA 898a-h.

17. "Ulumbe Camp", Ncandu Forest Reserve, Newcastle district (275401S, 294145E; 2729Dc4): TM 71887.

Ncandu Nature Reserve, Newcastle district (2754S, 2941E; 2729Dc4): TM 80077-8.

**Free State:**

18. Farm: Berlin (497), Bothasberg Mtn, Vrede district: (1980 m; 272909S, 2905E; 2729Ac3): NMB R8573<sup>D</sup>; (1940 m; 272907S, 290500E\*; 2729Ac3): NMB R8574<sup>D</sup>.

19. Farm: Mooigelegen (863), Harrismith district (1600-1646 m; 280230S, 2851E; 2828Bb1): NMB R4317-22.

Farm: Parva Sed Mea (865), Harrismith district (1600-1640 m; 2810S, 285230E; 2828Bb4): NMB R673-4.

20. Farm: Uyshoek (1092), Harrismith district (1770 m; 281550S, 292045E\*; 2829Ad1): NMB R8170\*\*, 8171\*, 8172\*\*, 8173-7\*, 8178\*\*, 8179\*, 8180\*\*, 8181-8\*, 8189\*\*, 8190-2.

21. Farm: Ark (1010), Harrismith district (about 1676 m; 282745S, 290245E; 2829Ac3): NMB R6238, 6564, 6601-6, 6608.

Farm: Frazerfield (187), Harrismith district (about 1646 m; 2827S, 290130E; 2829Ac3): NMB: R6225, 6227, 6230-2, 6275, 6278-9, 6285, 6299-300.

22. Farm: Bosch Kloof (487), Drakensberg Mtns, Harrismith district (283140S, 285915E; 2828Db2): NMB R6451-2.

Qoqolosing, Farm: Witzieshoek (1815), Harrismith district (1900-1960 m; 283532S, 285423E\*; 2828Db2): NMB R8361\*\*.

23. Farm: Rambouillet (396), Lindley district (c. 1500-1540 m; 274930S, 2748E; 2727Dd1): NMB R1836-8.

24. Farm: Grootkloof (251), Ficksburg district (1680-1740 m; 2838S, 2855E; 2827Db4): NMB R941-7.

25. Farm: Ceylon (290), Wepener district (about 1500 m; 2948S, 2654E; 2926Dd2): NMB R2723-4.

**KwaZulu-Natal (Nkandhla district):**

26. Farm: Braet Mead (14238), 12 km SSE of Babanango, Nkandhla district (about 1100-1200 m; 282855S, 310604E\*; 2831Ac3): NMB R8388\*.

27. Farm: Corriedale (11630), 1 km N of Qudeni (tiny "center"), Nkandhla district (2836S, 3052E; 2830Db1): TM 53944.

Ntabayabesutu village, 10 km NNW of Qudeni village, Nkandhla district (c. 1500 m; 283130S, 305018E\*; 2830Db1): NMB R8377-8\*\*, 8379-87\*.

Qudeni Forest, Nkandhla district (2830Db) (Broadley 1964, NM, *P. subviridis transvaalensis*): NMSA 997a-e.

Farm: no. 11931, 1 km N of Ekombe (tiny “center”), Nkandhla district (283745S, 305330E; 2830Db4): TM 53529, 53531.

28. Hill at Vumanhlamvu village between Nkandla town and Nkandla Forest, Nkandhla district (c. 1200-1300 m; 284200S, 310734E\*; 2831Ca4): NMB R8366\*\*, 8367\*, 8368\*\*, 8369-70\*, 8371\*\*, 8372-6\*.

*Pseudocordylus melanotus subviridis*

**Drakensberg:**

**Free State:**

22. Qoqolosing, Farm: Witzieshoek (1815), Harrismith district (1900-1960 m; 283519S, 285612E\*; 2828Db2): NMB R8359\*\*, (1900-1960 m; 283532S, 285423E\*; 2828Db2): NMB R8360\*\*, 8362\*, 8363\*\*, 8364\*.

Thibella (village), 2 km N of Fika Patso Dam, Farm: Witzieshoek (1815), Harrismith district (1800-1900 m; 283915S, 285140E\*; 2828Db3): NMB R8365\*\*.

29. Sentinel Mtn, Farm: Witzieshoek (1815), Harrismith district (284420S, 285330E; 2828Db4): NMB R3336-43, 4615-26, 6437-47, 6570-5.

1 km NW of Witzieshoek Mountain Resort, Farm: Witzieshoek (1815), Harrismith district (2200 m; 284055S, 285340E\*; 2828Db4): NMB R8354\*\*, 8355-7\*.

Entrance to Sentinel Hiking Trail, Farm: Witzieshoek (1815), Harrismith district (2460-2500 m; 284339S, 285338E\*; 2828Db4): NMB R8358\*\*.

Mont-aux-Sources, Bergville district, KwaZulu-Natal (2846S, 2852E; 2828Dd1): NMB R6836.

30. Monontsha Pass, Farm: Witzieshoek (1815), Harrismith district (2377 m; 283515S, 284130E; 2828Da2): NMB R653, 659-60, 662-3, 668-9, 670-1, 3298, 3300-5, 4607-11.

Monontsha Pass border post, Farm: Witzieshoek (1815), Harrismith district (2200 m; 283453S, 284154E\*; 2828Da2): NMB R8335-6\*.

Monontsha Pass, near “Kraal”, Farm: Woes Arabia (40), Harrismith district (2200 m; 283515S, 284123E\*; 2828Da2): NMB R8337-46\*.

Monontsha Pass, 0.5 km NE of Monontsa Pass border post (Lesotho, near Ha Molisana), Farm: Woes Arabia (40), Harrismith district (2100 m; 283515S, 284108E\*; 2828Da2): NMB R8347\*\*.

Monontsha Pass, near “Kraal”, 1 km NE of Monontsa Pass border post (Lesotho, near Ha Molisana), Farm: Woes Arabia (40), Harrismith district (2200 m; 283514S, 284131E\*; 2828Da2): NMB R8348\*\*, 8349\*, 8350\*\*, 8351-3\*.

31. Farm: Bramleys Hoek (52), Bethlehem district (2826S, 283045E; 2828Bc3): NMB R3522-3.

Wodehousekop, Golden Gate Highlands National Park, Bethlehem district (282930S, 2838E; 2828Bc4): NMB R6346.

Golden Gate Highlands National Park, Bethlehem district (2828Da1): NMB R5459-63.

**Lesotho:**

32. Lekhalong-la-Molimo-Nthuse (= God Help Me Pass) (292520S, 2755E; 2927Bd4): JV 4899b-k, 4915-8, 4922-4, 4926, 4928, 5010.

33. 0.5 km SW of Thaba Chitja (Ha Khanyetsi) village (2350-2400 m; 300550S, 281615E\*; 3028Ab1): NMB R8405\*\*, 8406\*, 8407\*\*, 8408\*, 8409-10\*\*, 8411-4\*.

34. Sehlabathebe National Park, between Park Lodge and Research Station (295215S, 290530; 2929Cc1): NMB R5793-800, 5803.

Hill above Tsoelikane River, about 300 m S of Tsoelikane Falls, Sehlabathebe National Park (2375-2400 m; 295350S, 290729E\*; 2929Cc3): NMB R6847.

Hillside flanking upper Tsoelikane River valley, about 0.5 km N of Kepising (hill) peak, Sehlabathebe National Park (2475 m; 295246S, 290725E\*; 2929Cc3): NMB R6845-6.

Top of hill SSW of Agricultural Station, 2 km E of Ha Mavuka, Sehlabathebe National Park (2530 m; 295346S, 290434E\*; 2929Cc3): NMB R6831.

Hill above drift, Tsoelikane River near confluence with Leqooa River, at Ha Moshebi (= Ha Letsoala Makuta) (2164 m; 295551S, 290155E\*; 2929Cc3): NMB R6828-30.

**KwaZulu-Natal:**

35. Organ Pipes Pass, Bergville district (2901S, 291230E; 2929Aa2): NMB R8151\*, 8152, 8153-5\*, 8156-7\*\*, 8158-9\*, 8160\*\*, 8161-4\*, 8165, 8166\*\*, 8167\*, 8168<sup>D</sup>.

36. Nottingham Road (small centre), Lions River district (2922S, 295930E; 2929Bd2): NMSA 685.

Farm: no. 2170, 2 km NNE of Nottingham Road, Mooi River district (2920S, 3000E; 2930Ac1): NMB R-RY 238-41, 367.

Farm: Fordoun (14783), about 4 km NE of Nottingham Road, Lions River district (2920S, 3002E; 2930Ac1): NMB R-RY 346-7, 350.

Farmstead: Easingwold, Farm: no. 14534, 8 km ENE of Nottingham Road, Lions River district (2920S, 3003; 2930Ac1): NMB R-RY 474.

Johnstone's Kop (E part), 9 km NNE of Nottingham Road, Mooi River district (291630S, 3000E; 2930Ac1): NMB R-RY 628-9.

37. Pietermaritzburg, Pietermaritzburg district (2930CB): NMSA 808.

38. Dargle (small centre), Impendle district (2933S, 2958E; 2929Db2): NMSA 809a-b.

Farm: Welton (2108), Nhlosane Mtn, 21.5 km SSW of Nottingham Road (small centre), Impendle district (2933S, 295730E; 2929Db2): TM 50916-7.

39. "Farm: Borreray", 21.5 km NNE of Himeville, Impendle district (2933S, 2933E; 2929Da1): NMB R-RY 392, 456-9, 471.

40. Near Kokstad, Mount Currie district (3029AD) ("Drakensberg near Kokstad": Loveridge 1944 as paratype of *P. langi*): TM 21063.

Franklin (centre), Mount Currie district (3019S, 2927E; 3029Ad2): NMSA 883, 886; TM 38206-7.

S of Kokstad, 1 km from Transkei border, Mount Currie district (3029Cb4): TM 53914.

#### **Eastern Cape:**

41. Near top of Naude's Nek, Farm: no. 61, Barkly East district (2300-2450 m; 3044S, 2808E; 3028Ca4): NMB R8292\*, 8293\*\*, 8294\*, 8295-6\*\*, 8297\*, 8298\*\*, 8299\*, 8300\*\*, 8301-14\*.

42. Herschel (small centre), Witteberg Mtns, Transkei (3037S, 270930E; 3027Ca2): NMSA 551a-c.

#### **Amatole Mountains (Eastern Cape):**

43. Katberg Mtn, Didima Range, Stockenstrom district (3229S, 263730E; 3226Bc4): TM 21758.

44. Menziesberg Mtn, Stockenstrom district (3237S, 2652E; 3226Db1): TM 47628-9.

Farm: Waterfall (161), Amatole Mtns, Cathcart district (323425S, 265641E\*; 3226Db2): NMB R8212\*; (323400S, 265629E\*; 3226Db2): NMB R8215-24\*.

Farm: Moreson (162), Amatole Mtns, Cathcart district (323445S, 265930E\*; 3226Db2): NMB R8213-4\*.

Farm: no. 32, Amatole Mtns, Cathcart district (323451S, 265644E\*; 3226Db2): NMB R8225-31\*, 8450-2.

45. Stutterheim, Stutterheim district (3234S, 272530E; 3227Cb2): SAM ZR 11243\*, 11314.

*Pseudocordylus langi*

**Free State:**

46. Near Chain Ladder, Mont-aux-Sources, Harrismith district (2905 m; 284448S, 285252E\*; 2828Db4): NMB R8500.

Chain Ladder, Sentinel Hiking Trail, Farm: Witzieshoek (1815), Harrismith district (284450S, 285253E; 2828Db4): NMB R8555-7 (2900 m); NMB R8552 (2970 m); NMB R8554 (3000 m).

200 m SE of Vemvane River falls, Farm: Witzieshoek (1815), Harrismith district (3020 m; 284456S, 285243E\*; 2828Db4): NMB R8553<sup>D</sup>.

**KwaZulu-Natal:**

Mont-aux-Sources, Bergville district (2846S, 2852E; 2828Dd1): TM 67659.

47. "Ntonjelane Pass", Cathedral Peak area, Bergville district (2805 m; 285618S, 290540E\*; 2829Cc3): NMB R8501.

48. Organ Pipes Pass, Cathedral Peak Forest Reserve, Bergville district (2901S, 291230E; 2929Aa2): NMZB-UM 2411-2, 2414-5, 2417-21, 2444, 3012; TM 27448-9; TM 51658 (both as "Organ Pipes Spur" on TM list); NMB R8445-7\*, 8448-9\*\* (top of Pass).

*Pseudocordylus spinosus*

**Free State:**

49. Sentinel Mtn, Farm: Witzieshoek (1815), Harrismith district (284420S, 285330E; 2828Db4): NMB R3357, 4612-3.

**KwaZulu-Natal:**

Goodoo Pass, 750 m ESE of Witzieshoek Mountain Resort, Royal Natal National Park, Bergville district (2100 m; 284113S, 285425E\*; 2828Db4): NMB R8568-71<sup>D</sup>.

Goodoo Pass, 1 km ESE of Witzieshoek Mountain Resort, Royal Natal National Park, Bergville district (2000 m; 284113S, 285438E\*; 2828Db4): NMB R8572<sup>D</sup>.

Dooley Ridge (= Knoll), Royal Natal National Park, Bergville district (284208S, 285550E; 2828Db4): TM 21698.

Mont-aux-Sources, Bergville district (c. 2828Dd1): NMB R6837.

50. Cathedral Peak State Forest, Bergville district (2829CC): TM 50085-6.

Cathkin Peak area, Monk's Cowl State Forest, Estcourt district (290430S, 292030E; 2929Ab1): TM 21262, 21264-5 (paratypes).

Injasuti Nature Reserve, Giant's Castle Game Reserve, Estcourt district (2907S, 2926E; 2929Ab2): NMB R-RY 125.

Giant's Castle, Impendle district (2929Ad2): TM 2521 (paratype, as "Giant's Castle area").

51. Farm: Eersteling (1370), Ixopo district (3012S, 3001E; 3030Aa3): TM 55302-3.

*Pseudocordylus microlepidotus fasciatus*

**Eastern Cape:**

52. Farm: Buffels Fontein (150), N of Stormberg Mtns, Wodehouse district (1800 m; 312212S, 264348E\*; 3126Bc2): PEM R2879-85.

**Appendix 5.2:** External characters examined on material referable to the *Pseudocordylus melanotus* species complex.

### **Mensural characters**

(Measurements were determined on the left side of the head, or on the left limb, if the right side was damaged or twisted.)

1. Length of head

(Measured from the upper, anterior edge of the tympanic opening [usually in line with or slightly behind the back end of the posterior upper temporal; behind the back ends of the posterior parietals] to the tip of the snout, on the right side of the head.)

2. Width of head

(Measured across the widest part of the head, slightly anterior to the tympanic openings, but excluding the temporal spines.)

3. Depth of head

(Measured over the deepest part of the head [*i.e.* over the middle of the lateral temporal region], from under the lower jaws anterior to the ear openings to the highest part of the 2nd [middle] upper temporal. When there was a slight difference in depth on either side of the head, the measurement for the right side was used.)

4. Length of right forelimb

(Measured on the outstretched arm from the anterior insertion/juncture with the body to the distal end of the terminal lamella of the 4th finger. If the forelimb could not be stretched because of fixation, the sum of the length of the upper forelimb [from anterior insertion to bend of elbow] plus the length of the lower forelimb [bend of elbow to distal end of terminal lamella of fourth finger] was calculated.)

5. Length of right hindlimb

(Measured on the outstretched leg from the anterior insertion/juncture with the body to the distal end of the terminal lamella of the 4th toe. If the hindlimb could not be stretched because of fixation, the sum of the length of the thigh [from anterior insertion to bend of knee] plus the length of the lower forelimb [bend of knee to distal end of terminal lamella of fourth toe] was calculated.)

6. Length of 4th (longest) toe on right foot  
(Measured under magnification from the posterior part of the first scale entirely or largely [ $> 60\%$ ] anterior to the junction between third and fourth toes, to the junction between terminal lamella and claw.)
7. Length of tail  
(Measured ventrally from vent to tip of tail, in a straight line.)
8. Snout-vent length  
(Measured ventrally from tip of snout to vent, with lizard pressed flat, or straightened and held by hand if fixed in a curved position.)

**Qualitative characters** (See Figs 5.17 to 5.20)

(Left and right sides examined unless otherwise indicated.)

1. Shape of the frontonasal (width vs length)  
(Width of frontonasal superior, equal, or inferior to length. When this was not clearly evident upon visual examination, the greatest width and length were measured with vernier calipers.)
2. Frontonasal completely, partly, or not divided by median longitudinal suture; or fragmented or absent
3. Presence or absence of a small scale posterior to the frontonasal, in contact with the prefrontals and sometimes also the frontal
4. Supranasals in contact or separated by the frontonasal (*i.e.* frontonasal in contact with rostral)
5. Frontonasal in contact with, or separated from, the loreal on either side of the head
6. Large subocular below middle of eye (*i.e.* "median subocular") reaching or not reaching the lip

7. Presence or absence of an anterior frontal  
(Small to large scale anterior to the frontal, in contact with the prefrontals.)
8. One or both anterior parietals divided or partly divided (*e.g.* divided anteriorly only, or suture poorly defined) into two scales diagonally, or not divided
9. Texture of posterior infralabial: smooth (*i.e.* without a distinct ridge or keel), strongly compressed with a slightly projecting horizontal ridge, or distinctly keeled
10. Dorsolaterals larger or smaller than median dorsals
11. Size of average median (paravertebral) dorsal scale in middle of back in relation to size of average dorsolateral scale in this area (*i.e.*  $>0.5$  or  $\leq 0.5$ )
12. Size of average lateral scale in the middle of back as a proportion of average dorso-lateral scale in this area ( $\geq 0.75$  or  $< 0.75$ )
13. Size of granular interspaces between longitudinal rows of dorso-laterals in relation to the largest associated dorsolaterals on either side: equal to larger,  $>0.5$  but not equal,  $\leq 0.5$ , enlarged scales in contact, or granules only and in contact
14. Texture of lateral dorsal scales: spinose or non-spinose
15. Femoral pores distinct, deep and with yellowish secretion; or tiny, shallow and pit-like, lacking secretion
16. Colour pattern on the throat  
(Entirely black, black anteriorly with a black median longitudinal band posteriorly, mainly pale with a median pair of dark longitudinal stripes.)
17. Ventrals smooth or not

**Meristic characters** (See Figs 5.17 to 5.20)

(Left and right sides examined and total count for both sides used.)

1. Number of upper temporals

(Scales in broad contact with parietals on either side of the head, separated from enlarged lateral temporals by one or two rows of small, sometimes elongated, scales. The anterior upper temporal is the longest and is in contact with the posterior supraocular and posterior supraciliary on either side. The second upper temporal is also elongated, but the third or posterior upper temporal - which may be confused as an outer or lateral occipital, but is much larger - is not always elongate but often triangular and always in contact with the outer corner of the posterior parietal.)

2. Number of horizontal rows of enlarged lateral temporals (See Fig. 5.30)

(Approximate number of horizontal rows formed by the enlarged lateral temporals. When two rows are present, the scales of the upper row are all or mostly longer [more elongate] than those below. When three rows are present; the same applies, but the middle row consists of scales that are always or mostly larger than those of the lowermost row. A middle and/or lower row is recognised only if at least two scales - distinctly larger than those below - are present, even if they are not in contact.)

3. Number of supraoculars

(Large scales situated immediately above a row of much smaller supraciliaries; anterior supraocular in contact with frontal, prefrontal and large preocular; posterior supraocular in contact with frontoparietal, anterior parietal and anterior upper temporal.)

4. Number of supraciliaries

(Small, elongate scales above the eye, in contact with supraoculars. The anterior supraciliary is usually in contact with the large preocular, but sometimes separated by one or two tiny granules or thin, flat, elongate scales [not counted], whereas the posterior supraciliary is in contact with the anterior upper temporal and at least one postocular. One or two granular scales or thin elongate scales sometimes present between the posterior supraciliary and the supraciliary anterior to it, and much smaller than either scale, are not counted as supraciliaries. Granular scales [less than half the

size of adjacent scales] sometimes present between supraciliaries and supraoculars, and supraciliaries and postoculars, are also not counted.)

5. Number of suboculars anterior to the median  
(Suboculars are moderate to large scales situated below the orbit. The anterior subocular is in contact with the large preocular.)
6. Number of suboculars posterior to the median  
(The posterior subocular is in contact with the small scales/granules separating postoculars and lateral temporals. The most posterior scale is considered a subocular only if it is at least one-third the side of the scale anterior to it. The posterior subocular may be situated more-or-less behind the orbit. If it is the second scale posterior to the median subocular [situated below the middle of the eye, narrowed below and reaching the lip] it may be separated from the supralabials, unlike the other suboculars.)
7. Number of supralabials anterior to median subocular  
(All scales bordering the upper lip and situated between the rostral and median subocular were counted. There is always at least one additional and distinct supralabial posterior to the median subocular, and often one or more, usually much smaller, additional “supralabials” posterior to it that are usually also in contact with the lip.)
8. Number of infralabials  
(All scales in contact with the lower lip, excluding the mental. The most posterior infralabial is the large [often keeled] scale situated at least partly below the downward slope beyond the corner of the mouth, below the anterior lateral temporals; it does not lie directly below the posterior supralabials.)
9. Number of sublabials  
(Enlarged scales bordering infralabials below. The most posterior sublabial is the scale situated below, and largely in contact with, the posterior infralabial.)

10. Number of gulars in contact with anterior sublabials

(Scales [often elongated] in contact with one or both of the large, paired anterior sublabials; excluding scales [medially and/or laterally] less than one-eighth the size of adjacent gulars.)

11. Number of gulars transversely between posterior sublabials

(Gular scales [often elongated], usually forming longitudinal rows, between the hind ends of the posterior [usually 5th] sublabials. The first row counted on either side is the one extending to the anterior end of the posterior sublabial. All scales situated on an imaginary line between the hind ends of the posterior sublabials are included in the count, even if they do not form part of a distinct row continuing anteriorly or posteriorly. The first one or two rows of laterals counted on either side consist of reduced scales, but extremely small scales on either side that do not clearly form rows are not counted.)

12. Number of small scales posterior to the interparietal

(When present, at least some of these scales are in contact with the posterior half of the interparietal.)

13. Number of occipitals (= nuchals)

(All scales behind the posterior parietals, situated between [*i.e.* excluding] the posterior upper temporals [which could be mistaken for greatly enlarged lateral occipitals]. Occipitals are usually enlarged and most [or all] are larger than the average scale in the row behind. The smallest scales considered as occipitals are at least one-quarter the average size of other occipitals [excluding the lateral and median occipitals which are often larger than other occipitals] and in broad contact with the occipitals on either side of them. Other tiny scales or granules anterior to, or even between, the larger scales are not considered as occipitals; nor are small scales if they are followed by scales similar in size to, and in broad contact with, other enlarged occipitals. Large, somewhat elongated scales partially separating the posterior parietals behind are not considered occipitals if they are followed by a scale/s similar in size and appearance to adjacent occipitals.)

14. Number of transverse rows of enlarged dorsals

(Counted from the first row behind the posterior part of the forelimb insertion to the row immediately anterior to the vent [when followed around to the ventral side]. Because transverse scale rows are often irregular in nature, they were counted on the [right] dorso-lateral part of the body, where rows are fairly regular; incomplete rows in this region were not counted.)

15. Number of longitudinal rows of enlarged dorsals

(Enlarged dorsal, dorso-lateral and lateral scales [excluding granules - *i.e.* scales less than half the size of adjacent enlarged dorsals] counted across the widest part of the body more-or-less midway between fore- and hindlimbs. Paravertebral scales, which are often reduced in size, are included. Lateral dorsals are often similar to lateral ventrals, but are smaller, more-or-less round and not flattened.)

16. Number of transverse rows of ventrals

(Counted on the left half of the body from the first row [which curves anteriorly] behind the posterior part of the forelimb insertion to the row [which curves posteriorly] immediately in front of the anterior part of the hindlimb insertion [*i.e.* scale rows between axilla and groin].)

17. Number of longitudinal rows of ventrals

(Plate-like scales counted across the widest part of the body, more-or-less midway between fore- and hindlimbs. Lateral ventrals on either side are rectangular, quadrangular or occasionally somewhat round, smooth [weakly keeled in one juvenile only, namely NMB R947], more-or-less flattened, usually at least one-third [occasionally one-quarter] the size of adjacent ventrals, and distinctly larger than adjacent dorsals. The lateral rows disappear anteriorly and posteriorly.)

18. Number of lamellae under 4th finger of right hand

(Counted from the first scale entirely or largely [ $> 60\%$ ] anterior to the junction between 3rd and 4th fingers, to the scale behind the claw. Incomplete lamellae, *i.e.* those that do not extend to either side, were excluded.)

19. Number of lamellae under 4th toe of right foot

(Counted from the first scale entirely or largely [ $> 60\%$ ] anterior to the junction between 3rd and 4th toes, to the scale behind the claw. Incomplete lamellae, *i.e.* those that do not extend to either side, were excluded.)

20. Number of femoral pores

(Indicated by shallow pits, or secretions from pore-bearing scales with underlying femoral glands, found anteriorly on the ventral aspect of each thigh. Each scale appears to contain a single pit or pore. The combined number of pores for both thighs is given.)

21. Number of differentiated glandular femoral scales

(Modified, swollen, cream to yellow generation gland scales posterior to the pit- or pore-bearing femoral scales. The combined number of scales for both thighs is given.)

22. Number of generation glands in pre-cloacal region

23. Number of generation glands on the dorsum