Species boundaries and conservation of the velvet worm genus *Peripatopsis* in South Africa

By
Dane E. McDonald

Thesis presented in partial fulfilment of the requirements for the degree Master of Science in Zoology at Stellenbosch University

Supervisor: Prof. Savel R. Daniels
Faculty of Science
Department of Botany and Zoology

December 2016

Declaration

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2016

Dane E. McDonald

Abstract

This study examined biogeographical and taxonomic relationships within the genus *Peripatopsis* with a special emphasis on the *Peripatopsis capensis* species complex using phylogenetic analyses, population genetics, divergence time estimation, ancestral area reconstruction, scanning electron microscopy, and gross morphological analysis. A total of 104 specimens were collected from 21 localities from the known distribution of the *Peripatopsis capensis* species complex in the Western Cape, while 54 specimens from prior studies on the *Peripatopsis* genus from the Western Cape, Eastern Cape, and KwaZulu-Natal were used for the analysis of the genus. Two partial gene fragments were selected for this study, namely the mitochondrial DNA cytochrome c oxidase one subunit (*COI*) and the *18S rRNA* nuclear DNA gene locus. DNA sequences were analysed using phylogenetic methods and divergence time dating. The *P. capensis* species complex population genetic analyses were conducted on *COI*, while ancestral area inference for *Peripatopsis* was carried out using *COI* and *18S*. For the *P. capensis* species complex geographic boundaries amongst cryptic lineages were inferred using Phylogeobuilder and evolutionary drivers of cladogenesis were determined for the latter as well as for the genus. A conservation assessment was carried out on described species using the IUCN red list framework and phylogenetic diversity indexes. Phylogenetic analysis reveals that *P. capensis* is a species complex comprised three geographically discrete and statistically well supported clades. *Peripatopsis* was retrieved as a genus comprising 12 statistically well supported and taxonomically described species, with three species being retrieved as non-monophyletic. Divergence time analysis results revealed Plio/Pleistocene diversification for the *P. capensis* species complex and an early Miocene diversification for the *Peripatopsis* genus coincident with climatic amelioration during the time period. The ancestor of the latter originated in the geomorphic province delineated as the Syntaxis Cape Fold Mountain region. While gross morphological characters for *P. capensis* such as leg pair number was invariant, scanning electron microscopy (SEM) yielded differentiating characters, albeit limited (e.g. dermal papillae and male

genital pads). Two new *Peripatopsis* species are described in the present study. According to the IUCN framework all described species within the *Peripatopsis* genus require red listing and phylogenetic diversity indexes have highlighted the Cape Peninsula and the Central Cape Fold mountains as regions with a rich evolutionary history and high evolutionary potential.

Acknowledgements

I would like to take this opportunity to thank my supervisor Professor Savel R. Daniels for guidance and support throughout this endeavour. I would also like to thank Prof Hilke Ruhberg, Prof Georg Mayer, and Dr Ivo Sena Oliveira for guidance and input at key stages of this project.

The following people are thanked for assistance with specimen collection: Anzio Abels, Francois Van Zyl, Hanlie Engelbrecht. Dawn Larson from the Iziko Museum in Cape Town is acknowledged for providing specimens for morphological evaluation. Cape Nature and South African National Parks (SANParks) are thanked for issuing collection permits.

Ilse Kotzee is thanked for assistance with GIS and map design, Madeleine Frazenburg for assistance with scanning electron microscopy (SEM). I would also like to thank Fawzia Gordon and Nicholas Solomons for support during this project. This work was funded by the National Research Foundation (NRF) with a grant to Professor Savel R. Daniels. I am also grateful to the NRF and the Lawrence Memorial Grant (via the Zoological Society of South Africa) for providing a bursary.

Lastly, I would like to thank the Evolutionary Genomics Group (EGG), Department of Botany and Zoology, and the Council for Scientific and Industrial Research (CSIR) 'sweatshop' for their assistance and helpful discussions.

Special thanks to:

- Marie Minnaar-McDonald for believing in me despite all odds and teaching me tenacity and persistence.
- Neil McDonald who started me on the path of scientific inquiry. RIP (1953-2012)
- Ilse Kotzee for loyal support throughout this endeavour.
- My close family and friends for endless encouragement.

Table of Contents

List of Figures

List of Tables

Chapter 1

1. General introduction

1.1 Preamble

The presence of cryptic species poses a challenge to taxonomists and conservationists alike (Daniels *et al*., 2003; Bickford *et al.*, 2007). Cryptic species are defined as two or more distinct lineages that are erroneously classified under a single morphological species (Bickford *et al*., 2007). In these instances speciation is not always accompanied by obvious morphological changes. Historically most species were delineated and diagnosed on morphological characteristics potentially resulting in an underestimation of taxonomic diversity (Goldstein *et al*., 2000; Bickford *et al.*, 2007). Inherently this presents a problem which extends to conservation authorities who have to assign conservation status to poorly defined taxa (Daniels *et al*., 2003). This problem is further confounded by the large emphasis placed on species or subspecies as the unit of conservation (Daniels *et al.*, 2003).

The development of modern molecular systematic techniques has enhanced the ability of scientists to document the presence of cryptic species. These molecular techniques have been used with great success to distinguish cryptic species across a wide variety of organisms (Avise, 2004). DNA sequencing is one of the most widely applied methods in delineating cryptic species, currently fuelled by the DNA bar coding of life initiative (Savolainen, 2005). However, DNA-based data should not be regarded as a substitute for studying and understanding systematic relationships at the whole organism level, since DNA data also presents challenges (Will & Rubinoff, 2004). The cost and accessibility may also be an obstacle for many developing countries, which possess high levels of biodiversity. This is evident in so called megadiverse countries, which are primarily poor and

developing, and contain 60-70% of the world's diversity (Roe & Elliott, 2005). Africa comprises four of these countries: namely, Democratic Republic of Congo, Kenya, Madagascar, and South Africa. Despite the limitations of DNA data, it provides evidence for defining species that are important and are most powerful when used in conjunction with other types of data such as morphology, behaviour, ecology, and biogeography (Ruhberg, 1992; Will & Rubinhoff, 2004, Bickford *et al.*, 2007).

The invertebrate diversity in South Africa is poorly studied (Herbert *et al.*, 2001). For example, only one third to one half of our insect species has been described (Sholtz & Chown, 1995). This paucity of data extends to investigations of cryptic invertebrate taxa, which have received limited attention. For example, taxonomic studies on freshwater macro crustaceans in South Africa revealed substantial levels of hidden diversity. South African freshwater amphipods initially comprised only four species (Barnard, 1916). Recent systematic efforts have led to the discovery of twenty six species and two genera (Thurston, 1973; Griffiths, 1981; Stewart & Griffiths, 1992a, b, c; Stewart, 1992; Stewart *et al*., 1994; Stewart & Griffiths, 1995). Until recently the South African freshwater isopod genus, *Mesamphisopus*, comprised four species. A subsequent revision by Gouws (2008) yielded six additional novel species, increasing the total number of described species to ten. Similarly, recent systematic endeavours using both morphological and genetic methods on the widely distributed South African freshwater crab species yielded 11 new species of *Potamonautes* (Daniels *et al.*, 1998, 2001; Stewart *et al.*, 1995; Stewart, 1997; Stewart & Cook, 1998; Gouws *et al.*, 2000, 2001 respectively; Phiri & Daniels, 2014; Daniels *et al.*, 2014). Evidently, several South African macro crustacean groups exhibit remarkable diversity that is often masked by conservative morphology and lack of diagnostic features.

Velvet worms (Onychophora) are a prime example of a group that is morphologically conservative (Sherbon & Walker, 2004). The frequent occurrence of intraspecific variation has resulted in a limited understanding of species boundaries, particularly among widely distributed species. These

widely distributed species may be prone to cryptic speciation and require systematic investigation. Onychophorans are soft-bodied terrestrial invertebrates (Reinhard & Rowell, 2005). They are vulnerable to desiccation and typically inhabit moist, humid, microhabitats (e.g. inside rotting logs, under stones, in leaf litter, caves and in soil) that make the group prone to allopatric speciation (Hamer *et al.*, 1997).

The Onychophora are divided into two families. The Peripatidae (Evans, 1901) which has a circumtropical distribution and occurs in the Neotropics, Mesoamerica, southeast Asia, and west equatorial Africa (Monge-Najera, 1995). The Peripatopsidae (Bouvier, 1907), has a circumaustral distribution occurring in Australasia, Chile, and southern Africa (South Africa, Swaziland, Mozambique) (Hamer *et al.*, 1997; Daniels *et al.*, 2009). Monge-Najera (1995) concluded that the Peripatopsidae have a Gondwanan origin. Interestingly, Peripatopsidae have not been recorded from the remaining Gondwanan remnants of New Caledonia, Madagascar, and the Seychelles (Newlands & Ruhberg, 1978). Hence vicariance is suggested to have played a major role in the current Onychophoran distribution (Ruhberg, 1985; Monge-Najera, 1995). According to Monge-Najera (1995) dispersal by means of rafting on logs or within vegetation could also have contributed. The paraphyly and divergence time estimations of the South African genera suggest that velvet worm stem lineages were present on Gondwana prior to continental fragmentation (Allwood *et al.*, 2010)

The Peripatopsidae currently comprise 42 genera and approximately 115 species (Reid, 1996, 2000a, b, 2002; Sherbon & Walker, 2004; Ruhberg & Hamer, 2005; Daniels *et al.*, 2009). There is a general consensus among velvet worm taxonomists that traditional taxonomic characteristics (e.g. morphological) may have underestimated the diversity within the group. This was demonstrated in several Australian and New Zealand studies (indicated below) where modern molecular systematic techniques (e.g. DNA sequencing, cytogenetics, allozyme electrophoresis, and scanning electron microscopy) were used. For example, initially, six genera and nine species (Ruhberg *et al.*, 1988) were known from Australia. However renewed recent research suggests the velvet worm diversity is

currently to 38 genera and 81 species (Briscoe & Tait, 1995; Reid *et al.*, 1995; Rowell *et al.*, 1995; Tait *et al*., 1995; Reid, 1996, 2000a, b, Gleeson *et al.*, 1998; Tait & Norman, 2001). In New Zealand, Trewick (1998) used allozyme electrophoresis to show that *Peripatoides novaezealandiae* was a species complex comprised of five genetically distinct species. Trewick's (2000) findings based on mtDNA sequence data supported this. Three additional lineages were also identified. These were based on samples from sites that were not included in that previous study (Trewick, 1998). Furthermore Rockman *et al.* (2001) observed strong congruence between the male head structures of fourteen putative *Planipapillus* species and the phylogeny based on *COI*, *12S rRNA*, and a nuclear intron from the *fushi tarazu* gene. This provides strong evidence for defining new species.

In South Africa the Peripatopsidae are comprised of 24 species in two genera; *Peripatopsis* (containing 17 described species) and *Opisthopatus* (containing seven described species). All South African Onychophora are legally 'protected' (New, 1995). Redlisted species include *O. roseus* (Vulnerable), *P. alba* (Vulnerable), *P. clavigera* (Vulnerable), and *P. leonina* (Critically Endangered) (IUCN, 2003). A recent study of the *Peripatopsis* of South Africa by Daniels *et al.* (2009) using a combination of the aforementioned modern molecular systematic techniques (DNA sequencing and SEM) revealed widespread cryptic speciation within the genus. Tentatively the species diversity doubled, from eight to 16 operational taxonomic units. The identification of so called 'umbrella species' or species complexes that comprise multiple novel allopatric operational taxonomic units (*P. balfouri, P. capensis,* and *P. moseleyi*) necessitated further examination. Both *P. balfouri* and *P. capensis* exhibit intraspecific morphological variability (Hamer, 1997). Traditional morphological characters such as number of legs and colour are highly variable in the two species. Daniels *et al.* (2009) found that *P. balfouri* comprised six evolutionary units; *P. capensis* comprised three evolutionary units; and *P. moseleyi* two evolutionary units. Daniels & Ruhberg (2010) subsequently conducted comprehensive geographic sampling focused on detecting

cryptic speciation in *P. moseleyi*. The same authors found that the *P. moseleyi* complex comprised five genetically distinct clades and subsequently described four new species (Ruhberg & Daniels, 2013). These findings were based on DNA sequence data (i.e. mt- and nDNA), general allopatry, the lack of shared haplotypes amongst any of the five novel lineages, and marked levels of *COI* sequence divergence. Furthermore it represents a more than four-fold increase in the complex's diversity. Daniels *et al.* (2013) uncovered a further three-fold increase of diversity within the *P. balfouri* species complex using the aforementioned molecular and morphological techniques. Similarly, there is evidence for cryptic speciation in the most widespread South African velvet worm species, *O. cinctipes* (Daniels *et al.*, 2016) using DNA sequencing, SEM, and morphological methods. Based on genealogical exclusivity, geographical concordance, and good statistical support; seven evolutionary lineages where identified within the *O. cinctipes* species complex. The latter authors have subsequently described five novel species. These results suggest that widespread velvet worm species are often comprised of cryptic species and that taxa benefit from molecular systematic scrutiny.

Evolutionary relationships within *P. capensis* remain unquantified, and species boundaries ill-defined. Intra and interspecific variation in leg number and colour is widely reported in the taxon (Hamer et al., 1997). The number of leg pairs in *P. capensis* range from 17 to 19 with highly variable colour ranging from orange brown, slate blue to violet black (Daniels *et al.*, 2009). The species has a wide distribution across the western and south-western Cape (Brinck, 1957). *Peripatopsis* generally occurs in fynbos, forests or in bushy ravines along mountain slopes (Hamer *et al.*, 1997). However, Endrody-Younga & Peck (1983) reported the occurrence of *P. capensis* and *P. moseleyi* in mesic grasslands. *P. balfouri* and *P. capensis* survive in habitats located within Western Cape Afrotemperate forest and southern Cape Afrotemperate forest in fynbos and the Afrotemperate forest biomes respectively (Mucina & Rutherford, 2006).

Divergence time estimates by Daniels *et al.* (2009) suggest that major cladogenic activity occurred during the Miocene. Tectonic activity raised the interior plateaus of southern Africa by 1000m (King, 1978). Hence forest patches were isolated; this limited conspecific forest fauna to high lying relictual areas (King, 1978). Suitable forest habitat was further reduced by the development of the proto-Benguela current on the west coast of southern Africa (Siesser, 1985). This cold Atlantic current caused marked xeric climatic conditions resulting in increased aridity and hence fire frequency (Mucina & Rutherford, 2006; Daniels *et al.*, 2009). According to Mucina & Rutherford (2006) this contributed to the development of fire-adapted woodlands, shrublands (e.g. fynbos), and grasslands which facilitated the fragmentation of forests along fire pathways. Hence the persistence of forest in fire refugia (e.g. ravines and wind shadow areas). It was the establishment of these disjunct forest areas that most likely limited the dispersal capabilities of allopatric populations. Interestingly, Daniels *et al.* (2009) found close phylogenetic relationships between the Cederberg (*Peripatopsis*) populations at the extreme north-west of the distribution and taxa from the southern Cape. This suggests that these Afrotemperate forest patches may have been historically linked. In the same study a similar observation was made for southwestern Cape Afromontane forest populations and the Cape Peninsula Afrotemperate forest populations, further reinforcing evidence for a historical link between these areas.

Peripatopsis capensis complex populations are generally allopatric making them candidate taxa for cryptic speciation (Daniels *et al.*, 2009). Traditional morphological characters (e.g. number of leg pairs, size of the last pair of legs, shape of the dorsal body papillae etc.) prove to have limited taxonomic value among taxa that are closely related (Hamer *et al.*, 1997, Daniels *et al.*, 2009). Limited geographic sampling as well as a lack of samples from crucial areas further precluded Daniels *et al.* (2009) from unravelling the systematic diversity and describing the novel taxa within the species complexes.

Using the latest findings, a phylogeny for all the novel described evolutionary units for the three species complexes and existing species will be reconstructed. Phylogenetic diversity (PD) indices will be applied to the tree topology to assess the conservation status of South African *Peripatopsis* species. In contrast to species or genus richness indices, which assign the same taxonomic value to all conservation units, PD takes the phylogenetic relationships between species or evolutionary history into account (Rodrigues & Gaston, 2002; Perez-Losada & Crandall, 2003). A phylogenetic index was successfully employed by Beenaerts *et al.* (2009) to show unexpectedly high levels of phylogenetic diversity amongst the Sri Lankan lowland freshwater carcinofauna. Such information may have been overlooked if the conservation assessment was based purely on species richness information. Hence Beenaerts *et al.* (2009) and Rodrigues & Gaston (2002) argue for the inclusion of PD in conservation assessments. The dubious conservation status and cryptic nature of *Peripatopsis* merits the use of PD as a conservation assessment tool.

The general aims of this study include 1) determining the boundaries between lineages present in the *P. capensis* species complex, 2) to describe the novel lineages, 3) undertaking a comprehensive re-evaluation of phylogenetic relationships of *Peripatopsis*, and examining the conservation status of taxa in the genus.

1.2 Research Problem and Objectives

This study aims to examine the biogeographical and evolutionary relationships within the *Peripatopsis* genus, with a specific focus *P. capensis* populations in the Western Cape using DNA sequence data (mtDNA and nDNA), scanning electron microscopy (SEM), and gross morphological analysis. Two partial gene fragments, cytochrome oxidase one subunit (*COI*) and the nuclear small subunit ribosomal *18S rDNA*, will be used to derive sequence data. The specific objectives of the study are as follows:

- To examine biogeographical and evolutionary relationships within/among *P. capensis* populations in the Western Cape using DNA sequence data.
- To apply SEM and gross morphological analysis to the phylogeny to investigate potential species discriminating characters and hence describe the species.
- To assign conservation priorities within the genus *Peripatopsis* according to the International Union for the Conservation of Nature (IUCN) redlisting framework and using phylogenetic diversity indexes.
- To understand the paleological and biogeographic relationships between the genus *Peripatopsis* and its environment.

Chapter 2

2. Phylogeography of the Cape velvet worm (Onychophora: *Peripatopsis capensis*) reveals the impact of Plio/Pleistocene climatic oscillations on Afromontane forest in the Western Cape, South Africa

*This work formed the basis of a peer reviewed paper published in the *Journal of Evolutionary Biology*, 25, 824-835.

JOURNAL OF Evolutionary Biology eseb

doi: 10.1111/j.1420-9101.2012.02482.x

Phylogeography of the Cape velvet worm (Onychophora: *Peripatopsis capensis*) reveals the impact of Pliocene/Pleistocene climatic oscillations on Afromontane forest in the Western Cape, South Africa

D. E. McDONALD & S. R. DANIELS

Department of Botany and Zoology, University of Stellenbosch, Matieland, South Africa

2.1 Introduction

South Africa harbours three biodiversity hotspots (Cape Floristic Region, Succulent Karoo, Maputaland-Pondoland-Albany) (Cowling *et al.*, 1992; Lombard *et al.*, 1999, Lahaye *et al.*, 2008). Among these, the Cape Floristic Region (CFR) is considered the smallest (Myers *et al.*, 2000). The CFR has the highest floristic diversity of any similar sized area on the globe and is characterized by exceptional endemism of vascular plants (Goldblatt & Manning, 2002; Cowling *et al.*, 2003). The interaction of several historical biotic and abiotic factors operational across varying spatial and temporal scales are thought causal in the cladogenesis of the CFR's rich floristic biodiversity. A large number of recent molecular phylogenetic studies, coupled with divergence time estimates of clades suggest that the majority of cladogenesis in the flora occurred since the onset of the early

Oligocene to mid Miocene epochs (Schnitzler *et al*., 2011; Verboon *et al.*, 2009). During the early Oligocene epoch a continental ice-sheet developed over East Antarctica, the expansion of which caused a sea level drop in the region of 500 m below the current shoreline (Linder, 2003; Cowling *et al.*, 2009). This marine regression spanned the entire Oligocene and early Miocene (Siesser & Dingle, 1981). Furthermore a dry climate prevailed with fossil flora indicating sclerophyllous vegetation (Axelrod & Raven, 1978).

The early Miocene CFR was characterized by a dramatic climatic shift from warm, tropical conditions supporting subtropical forest flora to drier more seasonal conditions that became progressively more pronounced during the Pliocene (Linder, 2003; Cowling *et al.*, 2009). The climatic shift can be attributed to the late Miocene development of the Benguela upwelling system along the west coast of southern Africa (Siesser, 1980). Furthermore, this was a period when the CFR underwent a phase of geomorphic evolution with tectonic uplift (Partridge & Maud, 2000). Dramatic geotectonic uplift (600-900 m) in eastern southern Africa increased the east-west rainfall gradient reinforcing western CFR aridity by intercepting a greater proportion of rainfall from the warm Agulhas current (Tyson, 1986). This effectively divided the CFR into a western "winter rainfall zone" (WRZ) and an eastern "year-round rainfall zone" (YRZ). In the western CFR, seasonal drought provided an environment conducive to regular lightning-induced fires. The CFR fire regime is suggested to have become established less than 6-8 million years ago, during the late Miocene (Bytebier *et al.*, 2011). This prompted the evolution of the pyrophytic Cape fynbos vegetation (Cowling *et al.*, 2009; Swart *et al.*, 2009; Mucina & Rutherford, 2006).

Aridification became increasingly more marked during the Plio/Pleistocene epochs with moderate marine transgression and regression. It is argued that in comparison with the western CFR, the eastern part of the CFR became progressively more arid during the Pleistocene with pronounced climatic instability, thereby reducing the extent of the Cape vegetation, increasing extinction rates, and disrupting the potential for speciation (Cowling *et al.*, 1992, 1997; Dynesius & Jansson, 2000).

In comparison speciation and extinction histories suggest a more reliable rainfall regime and greater climatic stability in the western CFR (Cowling & Lombard, 2002). These developments resulted in considerable habitat fragmentation among forested areas, resulting in the contraction of the habitats to high lying areas.

Within the CFR, the Afromontane forests represent the smallest biome and are comprised of two subtypes namely; southern Afrotemperate and southern coastal forest (Castley & Kerley, 1996; Mucina & Rutherford, 2006). These forest patches generally occur below 1000 m, in areas where rainfall exceeds 600 mm (Rutherford & Westfall, 1986; Mucina & Rutherford, 2006). The impacts of historical climatic amelioration on forested areas remain largely unexplored in the absence of phylogeogeographic studies on forest dwelling taxa. However, it would be reasonable to assume that the increased aridification experienced during the Miocene/Pliocene has fragmented forest habitats. It has been demonstrated that habitat specialists such as soft bodied invertebrates (e.g. land planarians and springtails) can be effectively employed to reconstruct the biogeographic patterning of forested areas (Garrick *et al.*, 2007; Carnaval *et al.*, 2009; Alvarez-Presas *et al.*, 2011). Their dependence on stable microenvironments and highly restricted dispersal ability make them suitable for reconstructing biome affinities (Garrick, 2007; Alvarez-Presas *et al.*, 2011). Onychophora, commonly known as velvet worms, are known to be habitat specialists, restricted to moist environments like closed canopy forests where they typically inhabit saproxylic environments (Hamer *et al.*, 1997; Daniels & Ruhberg, 2011). Their habitat specificity and general physiological intolerance to desiccation coupled with their low dispersal capabilities render these organisms ideal to test the contraction and expansions of their habitats (Hamer *et al.*, 1997; Alvarez-Presas *et al.*, 2011). The Cape velvet worm, *Peripatopsis capensis* Grube, 1866, has a relatively wide distribution in the Western Cape of South Africa where conspecific populations are confined to discontinuous, Afromontane forest areas and adjacent fynbos along the Cape Fold Mountains (Brinck, 1957; Hamer *et al.*, 1997). This taxon provides the ideal template organism with which to explore the

impact of climate and topography on Afromontane forest in the Western Cape. Daniels *et al.* (2009) demonstrated that *P. capensis* comprises three distinct clades corresponding to three distinct biogeographical regions in the Western Cape. The latter study further suggested a historical link between the southwestern Cape and the Cape Peninsula forests based on the close phylogenetic relationships of the respective sampling localities; however, limited geographic coverage of the species distribution precluded biogeographic inferences.

In the present study, *P. capensis* was extensively sampled from Afromontane forests throughout the Western Cape province of South Africa. The following three hypothesis are explored; 1) Due to its habitat specificity, *P. capensis* will display a genetic history which mirrors the palaeogeography of CFR forests in the light of climatic and geological perturbations, 2) populations of *P. capensis* are isolated due to the inhospitality of low lying coastal plains and the absence of low lying forests, 3) Increased levels of genetic differentiation should be observed along a west to south-easterly trajectory since the southeastern parts of the Cape Fold Mountain chain harboured larger fragments of forest patches, more pronounced habitat heterogeneity, and have historically received higher levels of rainfall.

2.2 Materials and Methods

2.2.1 Taxon sampling

A total of 104 *Peripatopsis capensis* specimens were collected from 21 localities in the western and south-western Cape regions, of the Western Cape province of South Africa (Fig. 2.1) (Table 2.1). Sample sizes ranged between a minimum of one and a maximum of 10. Samples were hand-collected from forest understory including rotten logs, leaf-litter, moss and beneath ravine rocks. Locality coordinates were recorded with a handheld global positioning system (Garmin-Trek Summit). Samples were killed by submergence in absolute ethanol. Specimens were preserved in absolute ethanol and stored at 4˚C in a refrigerator. Samples were identified using the dichotomous key provided by Sherbon & Walker (2004).

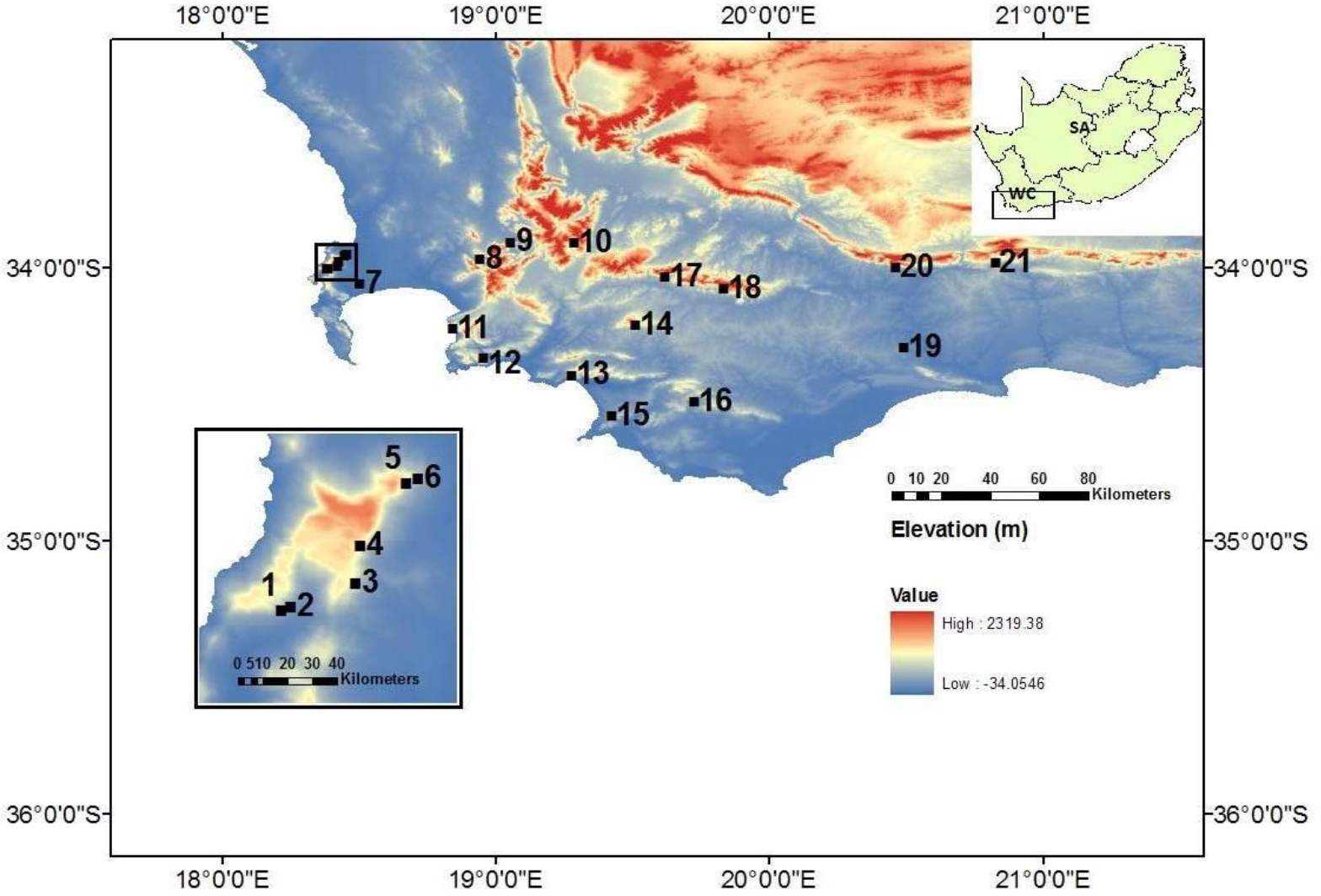


Figure 1: Map of the Western Cape (WC), South Africa (SA) showing the localities where *Peripatopsis capensis* was sampled. Sample localities: 1, Myburgh Ravine; 2, Orangekloof; 3, Cecelia Forest; 4, Skeleton Gorge; 5, Newland's Ravine; 6, Rhode's Memorial; 7, Rondevlei Nature Reserve; 8, Jonkershoek; 9, Bergriver Dam, Franschoek; 10, High Noon; 11, Dappat se Gat; 12, Kogelberg Biosphere Reserve; 13, Fernkloof Nature Reserve; 14, Caledon; 15, Grootbos Private Reserve; 16, Napier; 17, Greyton; 18, Oubos; 19, De Hoop Nature Reserve; 20, Marloth Nature Reserve; and 21, Grootvadersbosch Nature Reserve.

Table 1: Samples of *Peripatopsis capensis* collected from the Western Cape, South Africa during the present study and by Daniels et al. (2009). Pop *N* corresponds to the population number as indicated on the map (Fig. 1).

Pop *N*	Sample locality	*n*^	*n**	Coordinates
1	Myburgh Ravine	4	-	34˚00' 19.28"S 18˚22' 41.96"E
2	Orangekloof	4	-	34˚00' 15.69"S 18˚22' 58.64"E
3	Cecelia Forest	4	3	33˚59' 75.00"S 18˚24' 99.00"E
4	Skeleton Gorge	3	1	33˚58' 48.00"S 18˚25' 12.00"E
5	Newland's Ravine	-	3	33˚57' 15.50"S 18˚26' 41.03"E
6	Rhode's Memorial	6	-	33˚57' 09.73"S 18˚27' 04.66"E
7	Rondevlei Nature Reserve	1	-	34˚03' 37.50"S 18˚29' 59.76"E
8	Jonkershoek	4	-	33°58' 05.76"S 18°56' 24.65"E
9	Berg River Dam	4	-	33˚54' 25.14"S 19˚03' 04.48"E
10	High Noon	-	4	33˚54' 29.23"S 19˚16' 56.05"E
11	Dappat se gat	5	-	34˚13' 25.84"S 18˚50' 24.16"E
12	Kogelberg Biosphere Reserve	2	-	34˚19' 58.51"S 18˚57' 05.34"E
13	Fernkloof Nature Reserve	4	1	34˚23' 37.00"S 19˚16' 34.00"E
14	Caledon	1	-	34°12' 43.00"S 19°30' 23.00"E
15	Grootbos Private Reserve	4	5	34˚55' 05.00"S 19˚41' 37.00"E
16	Napier	6	-	34˚29' 23.72"S 19˚43' 20.25"E
17	Greyton	-	3	34˚02' 01.52"S 19˚36' 57.32"E
18	Oubos	4	-	34˚04' 34.33"S 19˚49' 43.76"E
19	Potberg-De Hoop Nature Reserve	5	5	34˚17' 35.00"S 20˚29' 19.00"E
20	Marloth Nature Reserve	7	3	33˚59' 51.84"S 20˚27' 27.68"E
21	Grootvadersbosch Nature Reserve	3	5	33˚58' 55.00"S 20˚49' 23.00"E
	Total number of specimens	71	33	

^present study
*Daniels et al. (2009)

2.2.2 DNA extraction, PCR, and sequencing

DNA was extracted from tissue samples using a Qiagen DNEasy kit, following the manufacturer's protocol. Extracted DNA was stored in a refrigerator until required for PCR. Prior to use, a 1µl DNA in 19µl water dilution was performed. Two partial gene fragments were selected for this study. These were the mtDNA cytochrome oxidase one subunit (*COI*) and the *18S* nuclear gene locus. The selection of these loci was based on the fact that they exhibit varying mutational rates, represent both the mtDNA and nDNA genomes respectively, and have been used with great success to reconstruct evolutionary relationships in velvet worm and other invertebrate groups (Allwood *et al.*, 2010; Gleeson *et al.*, 1998; Trewick, 2000; Daniels *et al.*, 2009, 2010). The following primer pairs were used; Folmer's *et al.* (1994) LCOI-1490 and HCOI-2198 for a partial fragment of the *COI* locus, and the primer pair 5F and 7R for *18S* after Giribet *et al.* (1996). Polymerase chain reactions (PCRs) were performed on a GeneAmp PCR System 2700 Thermocycler (Applied Biosystems, Foster City, CA, USA). For *COI*, standard PCR conditions were employed using 1-3µL template DNA (Daniels *et al.*, 2006, 2009; Daniels & Ruhberg, 2010). Standard protocols were used to cycle sequence purified PCR products. Sequencing was performed on an ABI 3730 XL automated machine. All specimens were sequenced for *COI*, whereas for *18S* a single representative specimen for each locality was sequenced.

2.2.3 Phylogenetic analyses

Sequence Navigator (Applied Biosystems) was used to check for base ambiguities and compute a consensus sequence from forward and reverse strands. Sequence alignment was performed in CLUSTAL X (Thompson *et al.*, 1997). Evolutionary relationships were determined using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference approaches. For the MP analysis of the *COI* dataset, trees were generated using the heuristic search option with tree bisection and reconnection (TBR) branch swapping using 100 random taxon stepwise additions and gaps were executed as characters. The phylogenetic confidence in nodes recovered from MP and ML analysis was obtained from bootstrap analysis of 1000 pseudo-replicates of data sets (Felsenstein, 1985). Bootstrap values >75% were treated as strongly supported. MP and ML analysis were conducted on the reduced, combined mtDNA and nDNA (*COI* and *18S*) data matrix using a single representative sample per locality and taxon for both genes. Uncorrected sequence divergence values were calculated for the *COI* locus using PAUP.

MRBAYES 3.0b4 (Ronquist & Huelsenbeck, 2003) was used to investigate optimal tree space using Bayesian inferences for the *COI* dataset. Akaike information criteria (AIC) (Akaike, 1973) were used to determine the best-fit maximum likelihood score. MODELTEST was used to determine the best-fit substitution model for each gene locus for the Bayesian analysis. For each analysis ten Markov Chains (MCMC) were run, starting from a random tree for five million generations, sampling from every 1000th tree. A 50% majority rule consensus tree was generated from the trees retained. After burn-in, trees were discarded. Posterior probabilities (p*P*) for each node were estimated by the percentage of time the node was recovered. Posterior probability values <0.95 were regarded as poorly resolved. The analysis was conducted on the reduced, combined mtDNA and nDNA (*COI* and *18S*) data matrix again using a single representative sample per locality and taxon. Phylogenetic data indicate that *Peripatopsis* (Pockock, 1894) is a monophyletic group (Daniels *et al.*, 2009). *P. moseleyi* Wood-Mason, 1879 and *P. sedgwicki* Purcell, 1899 are sister species to *P. capensis* (Daniels *et al.*, 2009). Hence two *P. segdwicki* specimens from Diepwalle (Western Cape) and Port Elizabeth (Eastern Cape) and two *P. moseleyi* specimens from Karkloof (KwaZulu-Natal) and Hogsback (Eastern Cape) respectively were selected as outgroup taxa.

2.2.4 Phylogeographic analysis

A haplotype network was constructed (for each individual clade obtained) using TCS 1.21 (Clement *et al.*, 2000). Population genetic structure analysis was performed exclusively on the *COI* mtDNA locus using ARLEQUIN version 3.5.1.2 (Excoffier *et al.*, 2010). This is the most rapidly evolving marker used in the present study for which the most comprehensive geographic sequencing was undertaken (Brower, 1994). Standard diversity indices, including number of haplotypes (*Nh*), haplotypic diversity (*h*), and nucleotide diversity (π) were used. To examine hierarchical population structure, analysis of molecular variance (AMOVA) was performed by pooling the sample localities from different locations into geographic clades evident from the preliminary phylogenetic analyses. Deviations in allele frequencies were investigated using Fu's *F* statistic (Fu, 1997). This test has proven to be especially sensitive to a departure from population equilibrium as in the case of a population expansion (Excoffier, 2010). GeoPhylobuilder v1.0 (Kidd & Liu, 2007) for ArcGIS was used to test the geographical concordance of the *COI* MP phylogram.

2.2.5 Divergence time estimation

To infer divergence times between the *Peripatopsis capensis* clades, the total evidence DNA sequence data (*COI* and *18S*) was used. According to Thorne & Kishino (2002) a multigene approach yields more information than single-gene data. Divergence time estimation was performed using a Bayesian framework; this employs a probabilistic model to define rates of molecular sequence evolution of lineages over time and uses the Markov Chain Monte Carlo (MCMC) method to derive clade ages as executed in the programme BEAST v1.5.1 (Drummond & Rambaut, 2007). A relaxed molecular clock was employed (Drummond *et al.*, 2006). For *COI* the suggested mutation rates of 1.5% - 2.3% per million years for arthropods were used (Brower, 1994; Farrell, 2001; Trewick & Wallis, 2001; Boyer, 2007). The *18S* mutation rate was estimated using a non-informative (1/x) prior. A multiple coalescent model was used for *P. capensis* (Heled & Drummond, 2010). MODELTEST was used to obtain the most likely substitution model and parameters for the combined (*COI* and *18S*) dataset. Twelve independent MCMC chains were run for 10 million generations with sampling carried out every 1000 generations. The convergence of the 12 combined chains was determined by EES for each parameter in Tracer after appropriate burnin cut-off. The trees comprising the 12 chains were combined using LogCombiner and were assessed using TreeAnnotator. FigTree was used to construct a chronogram (v.1.2.3.1, Rambaut, 2009).

2.3 Results

2.3.1 Phylogenetic analyses

A 638 bp fragment of the *COI* locus was amplified and sequenced for 108 specimens, which included 104 ingroup specimens and four outgroup (two *P. moseleyi* and two *P. sedgwicki*) specimens. Using the AIC criteria, the GTR+I+G (-1nL = 2714.76; AIC = 5449.52) substitution model was selected (Akaike, 1973). The base pair frequencies were A = 26.04%, C = 12.24%, G = 16.34%, and T = 45.37%. Similar results have been reported for velvet worms in other studies (Gleeson et al., 1998; Daniels et al., 2009, Daniels & Ruhberg, 2011). The rate matrix was R(a) [A-C] = 1.22, R(b) [A-G] = 21.56, R(c) [A-T] = 3.13, R(d) [C-G] = 0.59, R(e) [C-T] = 8.70, R(f) [G-T] = 1.00, while the proportion of invariable sites was 0.29, with a gamma shape distribution of 0.32. Analytical methods (MP and BI) revealed congruent tree topologies; hence, only the Bayesian tree

is shown and discussed (Fig. 2.2). MP analyses retrieved a total of 129 parsimony informative characters and yielded a total of 353 trees, with a tree length of 291 steps. The consistency index (CI) was 0.56 while the retention index (RI) was 0.95. The tree topologies generally retrieved the same clades with good statistical support. The analyses retrieved a statistically well-supported monophyletic group for *P. capensis* (>83%/1.00 *pP*). Three clades were retrieved with strong statistical support (>83%/1.00 *pP*). The Cape Peninsula clade (A) comprised specimens from Newlands, Skeleton Gorge, Cecelia Forest, Orangekloof, Rhode's Memorial and Myburgh's Ravine with strong statistical support (100%/1.00 *pP*). The Overberg clade (B) included specimens from Marloth Nature Reserve, De Hoop Nature Reserve, and Grootvadersbosch Nature Reserve with strong statistical support (100%/1.00 *pP*). The Theewaterskloof-Overstrand clade (C) comprised specimens from Jonkershoek, Rondevlei, Dappat se gat, Kogelberg Biosphere Reserve, Franschhoek, High Noon, Fernkloof Nature Reserve, Caledon, Greyton, Oubos, Grootbos Private Reserve, and Napier with strong statistical support (>89%/1.00 *pP*). Marked uncorrected sequence divergence values between the three clades were retrieved for the *COI* locus. For example clade A (Cape Peninsula) differed from clades B (Overberg) and C (Theewaterskloof-Overstrand) by 8.93% respectively. Furthermore clades B and C displayed a maximum sequence divergence value of 7.84%. Sequence divergence within clades was comparably low with 2.35% for clade A, 3.91% for clade B, and 3.13% for clade C. The three clades were geographically concordant, thus corresponding to the Cape Peninsula (clade A), Overberg (clade B), and the Theewaterskloof-Overstrand (clade C) regions as obtained from GeoPhyloBuilder and revealed two low lying barriers to gene flow (Fig. 2.3).

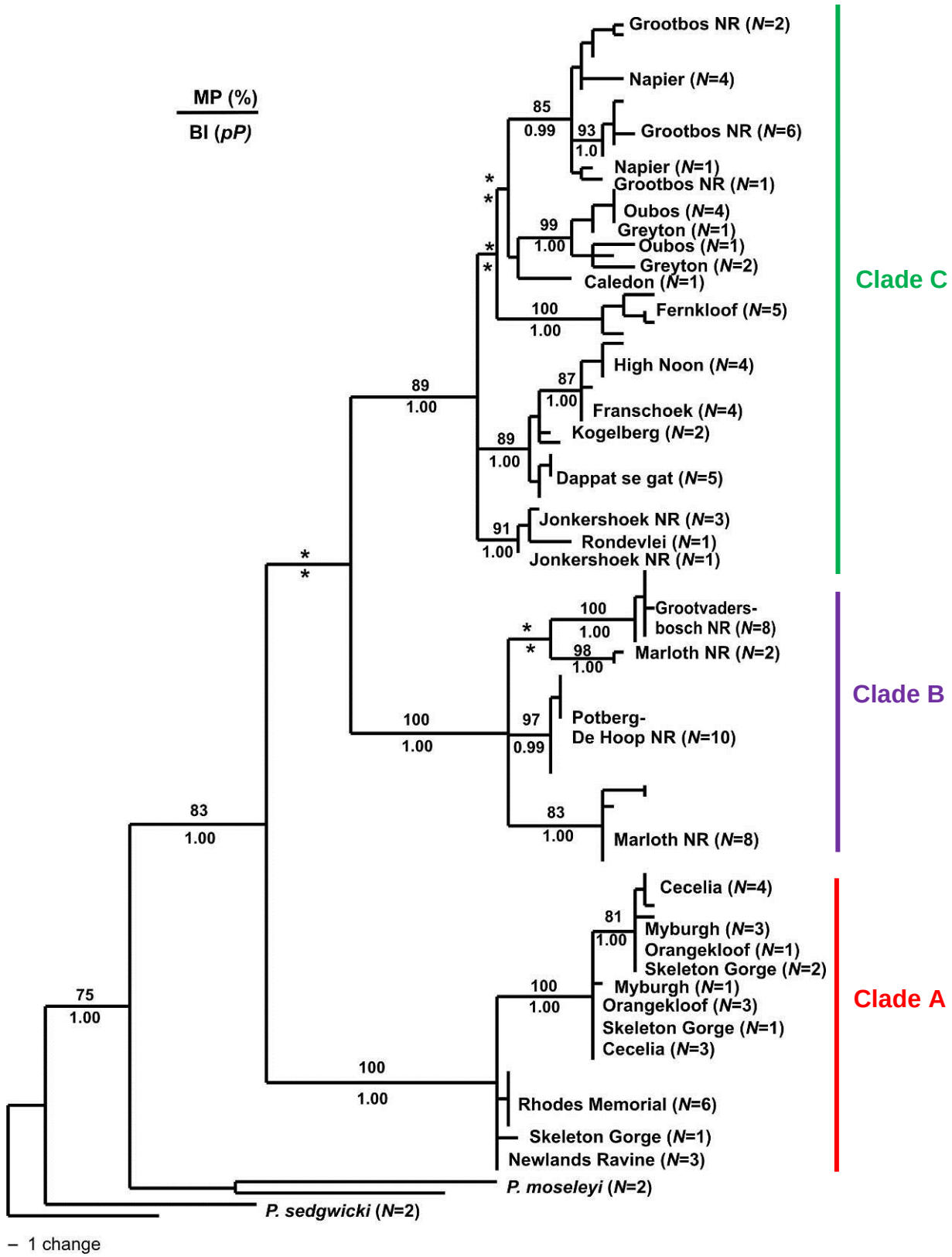


Figure 2: A Bayesian phylogram derived from the analyses of the *COI* mtDNA loci among the 21 *Peripatopsis capensis* sample sites across the Western Cape, South Africa. The values above each node represent bootstrap values (%) for parsimony (MP), while the values below each node represent the posterior probability (*p*P) value derived from the Bayesian inference analyses. Asterisk (*) indicate nodes that are statistically unsupported (<75% and <0.95 *p*P). Clades are labelled A (Cape Peninsula), B (Overberg), and C (Theewaterskloof-Overstrand).

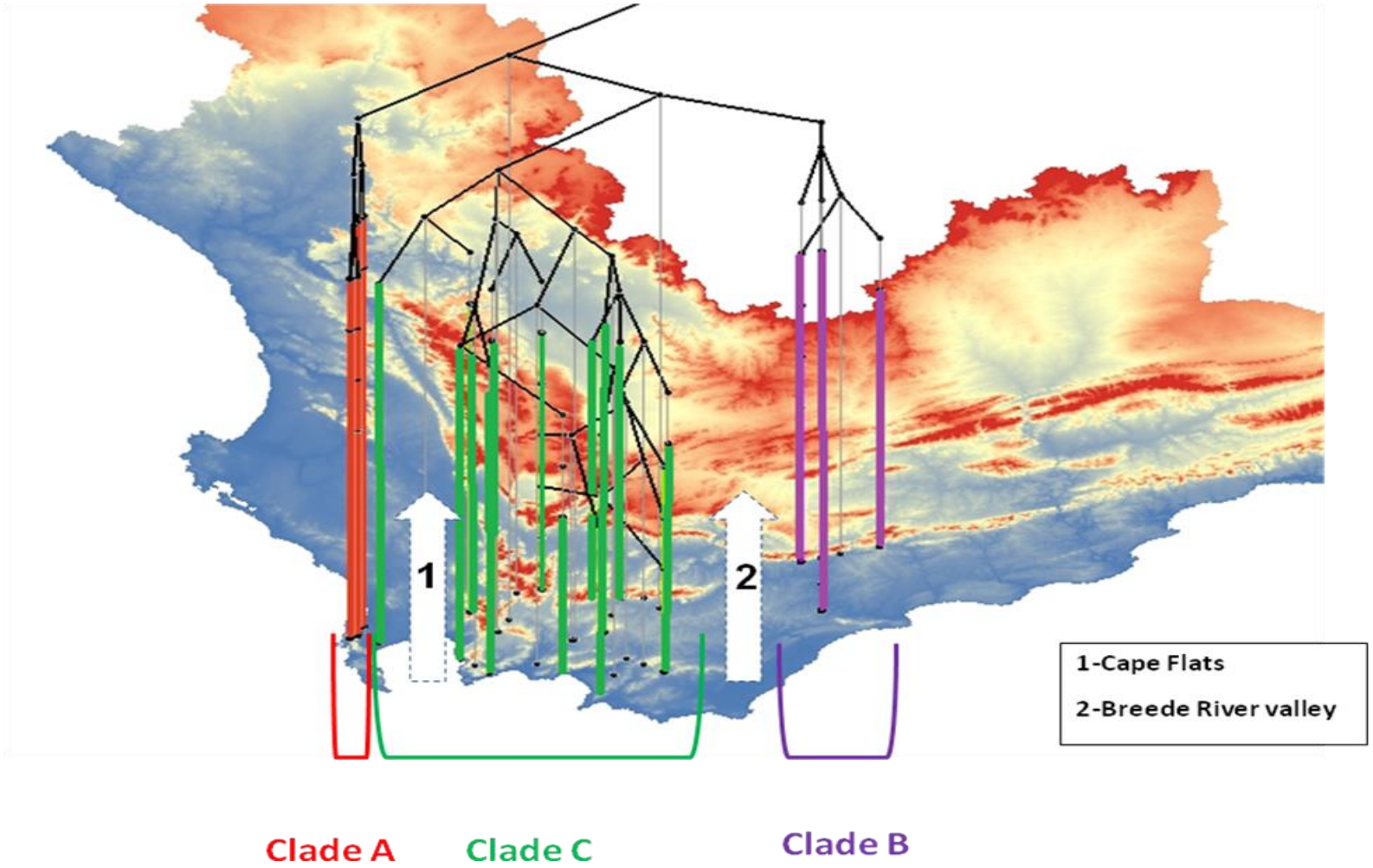


Figure 3: A map indicating the geographical concordance of clades. Dropnodes from the maximum parsimony (MP) phylogram corresponds to various sample localities. Clades correspond to three geographical regions namely; Cape Peninsula (Red), Theewaterskloof-Overstrand (Green), and Overberg (Purple).

2.3.2 Population genetics

The haplotype network for the *COI* locus corroborates the phylogenetic analyses and retrieved three clades (Fig. 4). A total of 54 haplotypes were retrieved, with clades A (Cape Peninsula) and B (Overberg) each comprised of ten haplotypes while clade C (Theewaterskloof-Overstrand) was comprised of 34 haplotypes (Appendix 1). The Cape Peninsula displayed the most shared haplotypes indicating gene flow between localities. For example, haplotypes five and two were each found at three different localities in clade A (Appendix 1). According to the AMOVA analysis, clade A had the highest levels of genetic variation within localities followed by clade C and B respectively. Clade B displayed the highest levels of genetic variation among localities followed by clades C and A respectively. In summary, AMOVA revealed limited genetic variation among localities on the Cape Peninsula, with the highest levels among the Overberg localities (Table 2.2). F_{ST} values within clades were statistically highly significant indicating substantial genetic structuring (Table 2.2, Appendix 1). Nucleotide diversity (π_n) and haplotype diversity (h) within clades are summarized in Table 2.3. The highest nucleotide diversity (π_n) was retrieved for the Theewaterskloof-Overstrand clade followed by the Overberg clade. The highest *h*-values were found for Theewaterskloof-Overstrand with the Cape Peninsula and Overberg having lower but similar values. Fu's *F*s were not statistically significant thus limiting any inferences from this analysis.

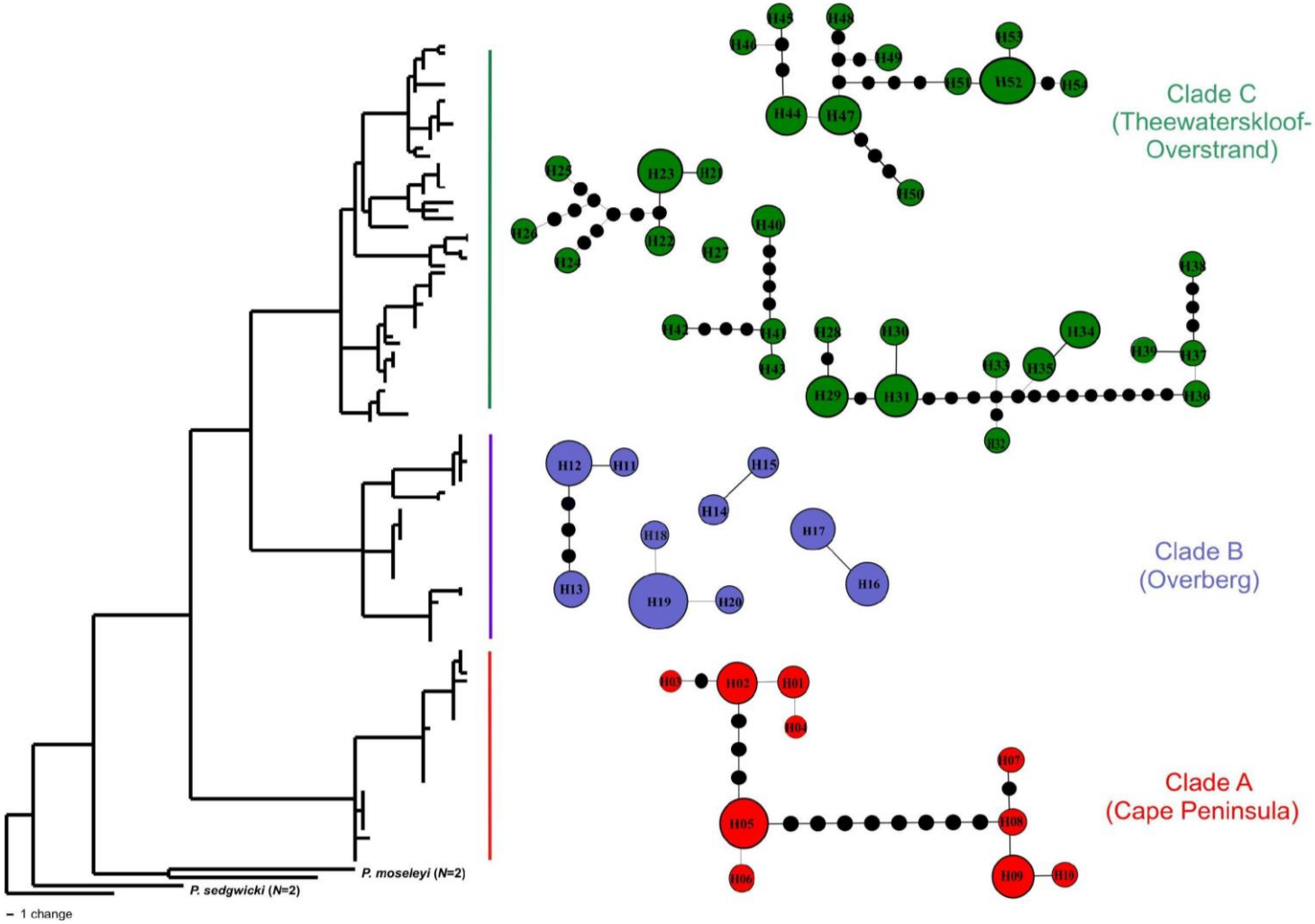


Figure 4: Haplotype networks derived from all samples sequenced for *COI* demonstrating three distinct haplogroups corresponding to the phylogram in Figure 2. The numbers inside the circles correspond with the haplotypes in Appendix 2. The closed black circles represent missing or unsampled haplotypes.

Table 2: Results of the AMOVA for the mtDNA analysis of the three clades detected within *Peripatopsis capensis*. All highlighted values are statistically significant (*P*<0.001).

Clades	F_{ST}	(V_a)	(V_b)	Fu's F_s
Clade A (Cape Peninsula)	**0.63298**	**63.30%**	**36.70%**	1.27
Clade B (Overberg)	**0.82683**	**82.68%**	**17.32%**	0.84
Clade C (Theewaterkloof-Overstrand)	**0.80952**	**80.95%**	**19.05%**	0.40

Table 3: Diversity measures for *Peripatopsis capensis* with population numbers (Pop *N*) corresponding to those in Figure 1, sample size (*N*), number of haplotypes (*Nh*), number of polymorphic sites (*Np*), haplotype diversity (*h*) and nucleotide diversity (π_n).

Pop *N*	Localities	*N*	*Nh*	*Np*	*h*	π_n
1	Myburgh Ravine	4	3	7	0.8333 ± 0.2224	0.005486 ± 0.004195
2	Orangekloof	4	2	4	0.5000 ± 0.2652	0.003135 ± 0.002630
3	Cecelia Forest	7	3	6	0.7143 ± 0.1267	0.004926 ± 0.003318
4	Skeleton Gorge	4	3	14	0.8333 ± 0.2224	0.011755 ± 0.008312
5	Newland's Ravine	3	1	0	0.0000	0.0000
6	Rhode's Memorial	6	2	0	0.3333 ± 0.2152	0.0000
7	Rondevlei Nature Reserve	1	1	0	1.0000	0.0000
8	Jonkershoek	4	3	2	0.8333 ± 0.2224	0.001567 ± 0.001553
9	Berg River Dam	4	2	1	0.5000 ± 0.2652	0.000784 ± 0.000972
10	High Noon	4	2	2	0.5000 ± 0.2652	0.001567 ± 0.001553
11	Dappat se gat	5	2	1	0.6000 ± 0.1753	0.000940 ± 0.001030
12	Kogelberg Biosphere Reserve	2	2	3	1.0000 ± 0.5	0.004702 ± 0.005430
13	Fernkloof Nature Reserve	5	4	9	0.9000 ± 0.1610	0.007210 ± 0.004971
14	Caledon	1	1	0	1.0000	0.0000
15	Grootbos Private Nature Reserve	9	7	16	0.9167 ± 0.0920	0.008447 ± 0.005098
16	Napier	6	3	5	0.8000 ± 0.1217	0.004180 ± 0.002980
17	Greyton	3	3	9	1.0000 ± 0.2722	0.009927 ± 0.008070
18	Oubos	4	3	7	0.7000 ± 0.2184	0.004389 ± 0.003241
19	Potberg-De Hoop Nature Reserve	10	2	1	0.5556 ± 0.0745	0.000871 ± 0.000876
20	Marloth Nature Reserve	10	5	17	0.7556 ± 0.1295	0.010658 ± 0.006205
21	Grootvadersbosch Nature Reserve	8	3	2	0.4643 ± 0.2000	0.000784 ± 0.000842

2.3.3 Combined DNA analyses (COI mtDNA and 18S rDNA)

The combined sequence data yielded a total of 1052 bp of which *18S* included 414 bp. Using the AIC criteria, the GTR+G (-1nL = 2204.02; AIC = 4426.04) model was selected for *COI* whereas the JC+I (-1nL = 665.52; AIC = 1333.05) model was selected for *18S*. For MP, 105 characters were parsimony informative producing a single tree with a tree length of 227 steps, CI= 0.57, and RI= 0.78. All three analytical methods (MP, ML and BI) produced near identical tree topologies (Fig. 2.3). The tree topology retrieved a statistically well-supported monophyletic group for *P. capensis* (>93%/97%/1.00 *pP*). Clade A (Cape Peninsula) formed a monophyletic group with strong statistical support (100%/99%/1.00 *pP*). Furthermore the monophyly of both clades B and C were also well supported (97%/99%/1.00 *pP* and 84%/80/1.00 *pP* respectively).

Divergence time analyses

Cladogenesis within *P. capensis* occurred 3.14 Mya (95% confidence interval: 2.13-4.38 Mya). Divergence between clade B (Overberg) and clade C (Theewaterskloof-Overstrand) occurred 2.39 Mya (95% confidence interval: 1.55-3.3 Mya). These results suggest a Pliocene/Pleistocene divergence among the three *P. capensis* clades. Divergence times within clade A range from 0.59-0.02 Mya. Clades B and C are characterized by divergence times that range between 0.92-0.75 Mya and 1.16-0.28 Mya respectively.

2.4 Discussion

Peripatopsis capensis formed a well-supported monophyletic group comprised of three statistically well supported, deeply divergent, and genetically distinct clades. Phylogenetic analysis reveals that the clades are characterised by short terminal branches, and long internal branches (Fig. 2). These clades correspond to three discrete geographical regions namely; the Cape Peninsula (clade A), Overberg (clade B), and Theewaterskloof-Overstrand (clade C). Two phylogeographic breaks were identified namely; Cape Flats and the Breede River valley (Fig. 4). Both areas are low lying and characterized by nutrient-poor, acidic soils, and characterised by sparse low rainfall regimes thus preventing the establishment of forest flora (Mucina & Rutherford, 2006). *P. capensis* diverged during the Plio/Pleistocene epoch. This epoch was characterised by increasing aridification which further facilitated the cladogenesis of the fire-dependent fynbos flora (Linder, 2003; Cowling *et al*., 2009; Schnitzler *et al*., 2011; Bytebier *et al.*, 2011). As a result CFR forests experienced marked contraction and fragmentation (Mucina & Rutherford, 2006). Additionally this period corresponds to varying cycles of marine transgressions and regressions coupled with geotectonic uplift in the south east (Linder, 2003; Siesser & Dingle, 1981; Cowling *et al.*, 2009). *P. capensis* displays deep phylogeographic structure due to its habitat specificity and low vagility. Clade A has low levels of genetic variation due to the historical isolation of the Cape Peninsula associated with an absence of forest at low lying areas and Plio/Pleistocene changes in the CFR environment. Increasing levels of genetic variation on an axis extending eastwards as far as the Langeberg Mountains, highlight the impact of climatic and geological factors on the dynamics of forest contraction and expansion. Due to its dependence on the stable microenvironment provided by CFR forests, *P. capensis* has delineated the historical dynamics of the latter with high fidelity.

2.4.1 Historical biogeography

The Cape Peninsula (clade A) is an isolated mountainous area surrounded by the Atlantic Ocean in the west and the Cape Flats in the east, thus isolating the velvet worm fauna. Interestingly Skeleton Gorge, which represents the most central sampled locality in the present study, contained the highest haplotypic diversity. This raises the possibility of Skeleton Gorge acting as a refugial area. The gorge is characterised by dense forest (McDonald pers. obs) and a perennial stream creating a moist and humid environment (Prain, 1913). This conforms to the mainland-island metapopulation pattern where, as a result, genetic divergences among populations are low (Mosblech *et al.*, 2011). Accordingly, clade A is characterized by the lowest levels of genetic variation compared to the other clades. In contrast, genetic variation within localities was comparably high indicating considerable genetic substructure and further evidence for an unstable metapopulation system where low quality forest patches could present an obstacle to gene flow within localities (Mosblech *et al.*, 2011). For the most part, *P. capensis* is generally restricted to forested palaeorefugia occupying sheltered ravines and gorges. Contemporary and historical forest habitat in this region could be the main driver of the genetic system observed. The prevailing climate during the Plio/Pleistocene would have limited the expansion of forest habitat. Linder (2003) suggests that low sea levels may have led to a loss of rainfall at mountain regions. These marine regressions are associated with lower absorption of incoming solar radiation due to a decrease in the area covered by the oceans. This results in decreased evaporation and, consequently decreased precipitation with an overall effect of increased aridity on-land (Theron, 1983). Lower temperatures further detract from past (Miocene) tropical conditions (Deacon, 1985; Daniels *et al.*, 2001).The Pliocene decrease in forest habitat was further exacerbated by prehistoric pastoralists (1500-2000 B.P) who used fire to manage the vegetation (Brain & Sillen, 1988; Deacon *et al.*, 1983). Hence the increasingly limited forest cover has a reduced capacity to maintain high levels of genetic diversity within the *P. capensis* Cape Peninsula clade.

Biogeographically the Cape Peninsula (Clade A) is separated from the Theewaterskloof-Overstrand region (Clade C) by a phylogeographic barrier of approximately 60km called the Cape Flats (Wishart & Hughes, 2001). The Cape Flats is assumed to have emerged after the closure of the 'Cape Strait', which once united Table Bay and False Bay (Schalke, 1973). Subsequent to the Pliocene lowering of sea levels around southern Africa and a probable rise in the underlying basement geology was the deposition of aeolian sands of marine origin (Walker, 1952; Siesser & Dingle, 1981; Adelana *et al.*, 2010). Thus the Cape Flats is characterised by nutrient-poor and calcareous alkaline soils supporting the shrub-dominated Cape Flats Dune Strandveld (Mucina & Rutherford, 2006). Several phylogeographic studies on mountain living invertebrates have identified the Cape Flats as a barrier to gene flow between the Cape Peninsula and the Theewaterskloof-Overstrand region. These include mountain living river crabs (Daniels et al., 2001), mountain living net-winged midges (Wishart & Hughes, 2001, 2003), and mountain living freshwater isopods (Gouws *et al.*, 2004, 2010). Interestingly the Rondevlei locality falls within the Cape Flats phylogeographic break. Yet, the Rondevlei specimen clustered with the Theewaterskloof-Overstrand clade despite being geographically closer to the Cape Peninsula. More specifically the phylogenetic trees retrieved indicated that it has a closer genetic affinity with Jonkershoek taxa suggesting that a historical corridor of suitable habitat may have connected these localities in the past. This can be explained by the Pleniglacial periods between 33 000 and 45 000 B.P during the Pleistocene where mixed *Podocarpus* forest were present in the central Cape Flats region (Schalke, 1973; Chase & Meadows, 2007).

The highest levels of genetic variation were observed within the Overberg clade (B). High genetic variation and a lack of shared haplotypes (Fig. 2.4) suggest that gene flow is limited among localities at the Overberg. However, within clade B, two specimens from Marloth were nested within the Grootvadersbosch subgroup suggesting the possibility of a historical connection between

the two localities. Both these localities occur along the sheltered slopes at the middle and upper reaches of the west-east trending southern Langeberg mountain range (McDonald *et al.*, 1996). These areas receive between 600mm and 1200mm of rainfall annually. According to McDonald & Cowling (1995) the latter lies within the non-seasonal rainfall zone of the CFR where rainfall is associated with circumpolar westerly fronts and postfrontal conditions related to the advection of cool moist air above the warm Indian Ocean. Gene flow between Potberg-De Hoop and the former localities are currently limited by an area colloquially known as the "Rûens", which is an undulating landscape straddled by the Potberg-De hoop mountain in the south and the Langeberg Mountains in the north. The Rûens is characterised by a highly dissected landscape, relatively low rainfall, and shallow soils supporting Rûens Silcrete Renosterveld (Schloms *et al.*, 1983; Rebelo *et al.*, 2006). Divergence within clade B is dated between 0.75-0.92Mya in the mid-Pleistocene. The late Pleistocene period was characterised by lower sea levels (100-160m below current sea levels) and a drier climate (Deacon, 1983; Cowling & Lombard, 2002).

Clades B and C are separated by the Breede River valley basin. According to Theron (1983) the elevated coastal topography in the southern Cape facilitated the establishment of larger rivers being deeply entrenched in steep-sided valleys as a result of marine regressions. The Breede River valley lies between the Riviersonderend Mountains in the south and the Langeberg Mountains in the north (Kirchner *et al.*, 1997). The valley displays unique abiotic characteristics relative to the latter montane regions. It is characterised as semi-arid and receives approximately 270mm of rainfall per annum. The valley comprises a variety of soil types (sandy, aeolian, acidic, alluvial, clay, and loam) supporting different variants of fynbos respectively (Mucina & Rutherford, 2006). These abiotic factors are unsuitable for the establishment of forests.

Clade C (Theewaterskloof-Overstrand) represents an area which forms the boundary between the winter rainfall zone (WRZ) of the western-most CFR and the year-round rainfall zone (YRZ) in the

south-east. In addition to high climatic variability, the Theewaterskloof-Overstrand sub region also forms a large part of the Cape Fold Mountains and hence represents a landscape characterized by high topographic heterogeneity. As a result clade C displayed three unique haplotypes, and had the highest haplotypic (*h*) and nucleotide (π_n) diversity. These findings suggest that clade C has had a long evolutionary history in large, stable populations (Fitzpatrick *et al.*, 2009).

2.4.2 Taxonomic considerations

Due to the geologically recent divergence of *P. capensis*, the rapidly evolving *COI* marker proved to be useful for elucidating population structure (Avise, 2004). Using the same marker, Hebert *et al.* (1991) employed a 3.3% sequence divergence to show that the Jamaican velvet worm species, *Plicatoperipatus jamaicensis*, comprised two distinct lineages. Similarly, Rockman *et al.* (2001) obtained low sequence divergence values ranging from 1.1-11.6% for morphologically distinct *Planipapillus* (Reid, 1996) species. Sequence divergence values in the present study represent an increase from the 6% sequence divergence obtained by Daniels *et al.* (2009) for the three clades and could be a result of increased sample size. In accordance with the findings of Daniels *et al.* (2009), the present study shows that velvet worm diversity associated with CFR forests were underestimated. The presence of three putative species, of which two are novel and undescribed, and associated with isolated forest patches, underpins the urgent need for a review of the group's conservation status. Despite the comparably high divergence values retrieved for *P. capensis* (7.84-8.93%) we exercise caution before basing our classification on a single molecular marker (Will & Rubinoff, 2004). The inclusion of the nuclear *18S* rDNA marker to create a combined topology was congruent with the data based on *COI* alone. We prefer to use the phylogenetic species concept as a starting hypothesis for species description (Cracraft, 1989). Preliminary gross morphological analyses have revealed several potential diagnostic characters including ventral coloration and the number of antennal rings. However, the most promising source of diagnostic morphological

characters is provided by scanning electron microscopy (SEM) (See Daniels *et al.*, 2009, Oliveira *et al.*, 2010, 2011). A forthcoming study aims to describe these two novel lineages.

Chapter 3

3. Two new velvet worm species from the Western Cape province of South Africa, *P. lawrencei sp. nov.* and *Peripatopsis overbergiensis sp. nov.* (Onychophora: Peripatopsidae)

*This work formed the basis of a peer reviewed paper published in *Zootaxa*, 3380, 55-68.

Zootaxa 3380: 55–68 (2012)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Two new *Peripatopsis* species (Onychophora: Peripatopsidae) from the Western Cape province, South Africa

DANE E. McDONALD[1], HILKE RUHBERG[2] & SAVEL R. DANIELS[1, 3]
[1] *Department of Botany and Zoology, University of Stellenbosch, Private Bag X 1, Matieland, 7602, South Africa*
[2] *Biozentrum Grindel und Zoologisches Museum, Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany*
[3] *Corresponding author. E-mail: srd@sun.ac.za*

3.1 Introduction

Onychophora have a notoriously conservative morphology and certain species are characterised by high intraspecific variability in diagnostic morphological features. Collectively these factors have led to an underestimation of species diversity in Australia, New Zealand, and more recently South Africa and Brazil (Briscoe and Tait, 1995; Reid *et al.*, 1995; Rowell *et al.*, 1995; Tait *et al.*, 1995; Reid, 1996, 2000a, b; Gleeson *et al.*, 1998; Tait and Norman, 2001; Daniels *et al.*, 2009; Lacorte *et al.*, 2011; Oliveira *et al.*, 2011). Daniels *et al.* (2009) using a combination of modern systematic techniques conducted the first molecular DNA study, in combination with morphological data and revealed widespread cryptic speciation within the South African genus *Peripatopsis* Pockock, 1894. Tentatively the species diversity doubled, from eight to sixteen operational taxonomic units (Daniels *et al.*, 2009). These results further suggest marked levels of localised endemicity. The identification of species complexes that comprise multiple novel operational taxonomic units

(*Peripatopsis balfouri* (Sedgwick, 1885), *P. capensis* (Grube, 1866), and *P. moseleyi* (Wood-Mason, 1879)) necessitated further examination. Subsequently, a genetic study was initiated to resolve the taxonomy within each of the three species complexes. Daniels and Ruhberg (2010) found that *P. moseleyi* was a complex comprised of five genetically distinct clades. Similarly, a systematic study on *P. capensis sensu lato* using the mitochondrial *COI* and *18S* genetic markers, detected three statistically well-supported monophyletic clades (McDonald & Daniels, 2012). The clades were allopatric, geographically exclusive and restricted to the Cape Peninsula (Clade A), Overberg (Clade B), and the Theewaterskloof-Overstrand (Clade C) (Fig. 3.1). Clades were further characterised by the absence of gene flow and marked sequence divergence at the *COI* gene. The latter authors concluded that three putative species were nested within *P. capensis*, two of which are novel and undescribed.

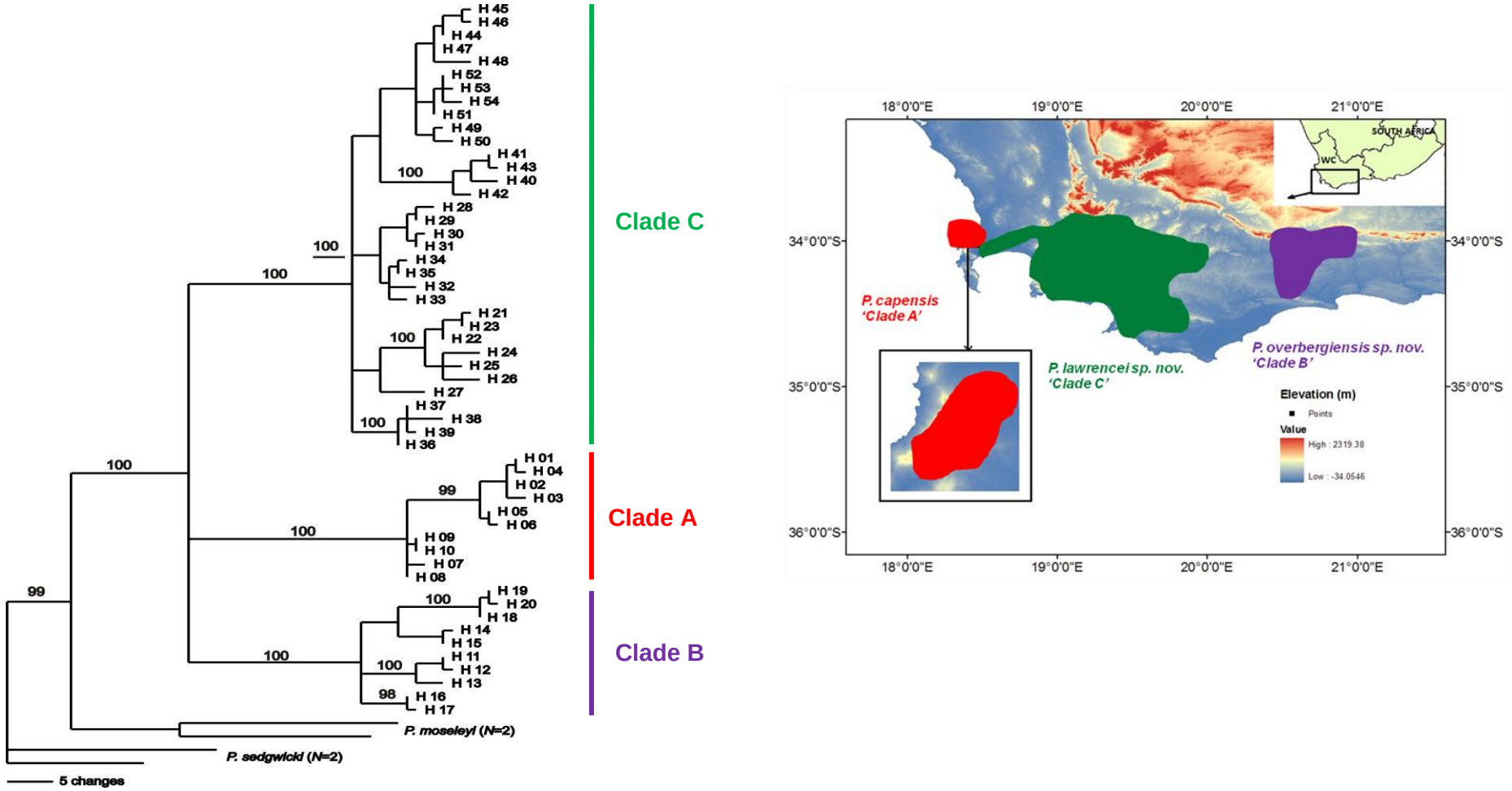


Figure 5: A Bayesian phylogram derived from the analysis of *COI* mtDNA loci among 21 *Peripatopsis capensis sensu lato* sample sites across the Western Cape, South Africa alongside a map indicating the geographical concordance of clades (= species). *P. capensis sensu strictu*, Clade A (red); *P. overbergiensis* sp. nov., Clade B (purple); *P. lawrencei* sp. nov., Clade C (green).

The Cape Peninsula, more specifically the Rhodes Memorial area, represents the type locality for *P. capensis*. However, since all the Cape Peninsula samples formed a distinct statistically well supported clade, all samples in this clade are referred to as *P. capensis*. Based on the latter study (McDonald & Daniels, 2012) we prefer to employ the phylogenetic species concept as a starting hypothesis (Carcraft, 1989). Accordingly the general lineage concept equates species with separately evolving metapopulation (i.e. an inclusive population made up of connected subpopulations) lineages where multiple lines of evidence support lineage separation (de Queiroz, 2007). In addition, morphological and geographic information will be used to establish discontinuities.

The present study aims to describe the two novel species nested within *P. capensis sensu lato* namely; *P. lawrencei* sp. nov. and *P. overbergi* sp. nov. using gross morphological and SEM observations. Conservation implications are discussed.

3.2 Materials and methods

3.2.1 Collection and preparation of specimens

Collected specimens were separated into two groups: 1) to be preserved in 100% ethanol for DNA analysis (McDonald & Daniels, 2012), and 2) preservation in 70% ethanol for SEM analysis (present study). A total of thirteen males representing each of the three clades identified in (McDonald & Daniels, in press) were selected for SEM analysis. A further 270 specimens (which included) samples from the present study, Daniels *et al.* (2009), and additional samples (*P. capensis sensu lato*) from the South African Museum (Iziko) were used for gross morphological analysis. Samples were hand-collected from forest understory including rotten logs, leaf-litter, moss, and beneath ravine rocks. Locality coordinates were recorded with a handheld global positioning system (Garmin-Trek Summit). All samples were refrigerated at 4˚C prior to analysis. Samples were identified using the dichotomous key provided by Sherbon and Walker (2004).

3.2.2 Gross morphological analyses

All specimens were observed with a Leica ES2 stereomicroscope. Holotypes and paratypes were measured for length. The following gross morphological characters were observed: number of leg pairs, claws on the last leg pair, dorsal and ventral body colour, dorsal patterning, antennal coloration or patterning, and presence of male crural papillae. Most museum specimens were depigmented and could thus not be accurately sexed. Voucher material has been deposited in the South African Museum of Natural History, Iziko Museums of Cape Town (SAM). Male and Females are designated by ‘M’ and ‘F’ respectively.

3.2.3 Scanning Electron Microscopy (SEM)

In preparation for SEM, specimens were subjected to an ethanol gradient of decreasing strength to substitute ethanol with water. Subsequently specimens were fixed in 4% formalin overnight. Hereafter specimens were subjected to an ethanol gradient of increasing strength from water to 100% ethanol. Specimens were dissected into five sections namely; ventral anterior region (legs 1-6), ventral mid-section (legs 7-12), ventral posterior (legs 13-17(+1)), and a 4mm^2 integument-cutting from the dorsal midsection. Dissected parts were dehydrated, critical-point dried, and gold coated. Mounted specimens were analysed in a Zeiss® Evo MA15 scanning electron microscope. Morphological characters associated with the dorsal papillae, spinous footpads, feet, antennae, and the male genital area were investigated.

3.2.4 DNA sequence data

The phylogenetic results from McDonald & Daniels (2012) were used for species diagnosis in the present study. In addition, DNA sequence accession numbers from Daniels *et al.* (2009) were downloaded from GENBANK.

List of abbreviations

SAM ENW – South African Museum, Cape Town – Entomological Collection (Iziko Museums of Cape Town)
ZMH – Zoological Museum Hamburg, Germany
SEM – scanning electron microscopy
M – male specimens
F – female specimens
n/a – not applicable
ICZN – International Code for Zoological Nomenclature
n – number of specimens

Table 4: Morphological variation in male *Peripatopsis capensis sensu lato*

Sample locality	*n*	Diagnostic Clade	Number of pregenital leg pairs	Primary dermal papillae shape	Dermal papillae arrangement	Dorsal coloration	Crural tubercle shape
Myburgh Ravine	1	Clade A (Cape Peninsula)	17	Cylindrical/Dome-shaped	Densely spaced	Slate black-brown	n/a
Orangekloof	4					Slate black	
Skeleton Gorge	1					Brown-black	
Rhodes Memorial	3					Dark brown/Indigo	
Potberg-De Hoop Nature Reserve	10	Clade B (Overberg)	18 (*n*=2; 17)	Semicircular/Dome-shaped	Widely spaced	Dark brown (*n*=9, white head collar)	Semicircular proximally/cylindrical distally
Marloth Nature Reserve	6		18 (*n*=1; 17)			Dark brown-black/rust orange/indigo	
Grootvadersbosch Nature Reserve	17		18 (*n*=4; 17)			Dark brown-black/rust orange	
Jonkershoek	3	Clade C (Theewaterskloof-Overstrand)	17	Conical/semicircular	Moderately spaced	Rust brown-orange	Distal section of tubercle not strongly demarcated
Berg River Dam	4					Dark rust-brown	
Dappat se gat	6					Dark brown-black	
Kogelberg Biosphere Reserve	3					Rust brown-orange	
Fernkloof Nature Reserve	4					Dark brown/Indigo-black	
Caledon	3					Rust brown	
Grootbos Private Reserve	5					Dark brown-indigo	
Napier	2					Dark brown-black	
Oubos	6					Rust brown-orange	
Total number of specimens	78						

3.3 Taxonomy

Peripatopsis capensis *sensu lato*

Principal specific characters (modified after Purcell, 1899, Ruhberg, 1985, and Hamer *et al.*, 1997):

Colour and patterning: Dorsal coloration: 1) Dark brown/slate black to olive green/bluish black, 2) Rust orange. Dark dorsal midline with orange or light lateral band above legs along the entire body. Creamy white to orange ventral coloration.

Legs: 17+1, seldom 18+1 (Last leg pair strongly reduced lacking claws and pads). Dorsal foot surfaces with ridges in most cases. 3-4 complete spinous footpads. Distinct crural tubercle present on last pregenital leg (Table 3.1).

Integument: 7-8 plicae between feet. Generally semicircular primary dermal papillae with 4-7 scale ranks. Antennae brown/black to dark indigo. 5-8 rows of chemoreceptors at antennal tips. Mouth surrounded by 13-17 oral lips.

Male genital area: Gonopore cruciform or with "horizontal arms" pointing slightly towards anus. Anterior genital pads larger than posterior pads.

Behaviour: Does not spiral when initially exposed (Compared to *P. balfouri*; which curls up spirally, Hamer *et al.*, 1997)

Habitat: Afromontane forest patches and surrounding fynbos (sometimes within or under pine logs, e.g. Rhodes Memorial). Collected under decaying wood logs, leaf litter, and stones, generally close to streams.

Distribution: Southern parts of the Western Cape province including the Cape Peninsula, Theewaterskloof-Overstrand, and Overberg regions.

Peripatopsis capensis *sensu stricto*

(Cape Peninsula or "Clade A", McDonald & Daniels, 2012)

Bouvier (1907) ascertained that the type material of *P. capensis* (Grube, 1866) has been lost. (Ruhberg, 1985). Consequently Ruhberg (1985) established a neotype to stabilize the taxon (Article 75, IUCN).

Neotype. Rhodes Memorial, Table Mountain, Cape Peninsula, Western Province, South Africa: 1 M collected 1975 by S. and M. Kiselowa. See Ruhberg (1985: 94) for full description. Zoological Museum Hamburg, Germany (ZMH A25/80)

Additional material examined. **SAM-ENW-C6438;** Myburgh Ravine, Table Mountain, Cape Peninsula, Western Cape, South Africa, 1 M and 3 F collected 2010 by McDonald and Abels. **SAM-ENW-C6439;** Orangekloof, Table Mountain, Cape Peninsula, Western Cape, South Africa, 2 M and 6 F collected 2010 by McDonald and Abels, **SAM-ENW-C6440;** 3 M and 2 F collected 2010 by McDonald and Abels. **SAM-ENW-C6441;** Cecelia Forest, Table Mountain, Cape Peninsula, Western Cape, South Africa, 2F and 2 (n/a) specimens collected 2006 by Picker, **SAM-ENW-C6442;** 1 F and 4 (n/a) specimens collected 2006 by Picker, **SAM-ENW-C6443;** 4 F collected 2010 by McDonald and Abels. **SAM-ENW-C6444;** Skeleton Gorge, Table Mountain, Cape Peninsula, Western Cape, South Africa, 3 (n/a) collected 2010 by McDonald. **SAM-ENW-C6445;** 1 F collected 2006 by S.K. **SAM-ENW-C6446;** Rhodes Memorial, Table Mountain, Cape Peninsula, Western Cape, South Africa, 1 M and 4 F collected 2010 by McDonald and Abels. **SAM-ENW-C6447;** Rhodes Memorial, Table Mountain, Cape Peninsula, Western Cape, South Africa, 1 M and 4 F collected 2010 by McDonald and Abels. **SAM-ENW-X7267;** Kirstenbosch, Table Mountain, Cape Peninsula, Western Cape, South Africa, 1 M collected 1898 by Treleaven, 1 (n/a) collected 1900 by Treleaven. **SAM-ENW-X6384;** Platteklip Ravine, Table Mountain, Cape Peninsula, Western Cape, South Africa, 1 M collected by Purcell 1900, **SAM-ENW-X6371;** 2 (n/a) collected by Purcell 1900, **SAM-ENW-X1061;** 1 (n/a)

collected 1896 by Lightfoot and Purcell, **SAM-ENW-7275;** 7 juveniles collected 1901 by Purcell, **SAM-ENW-C2010;** 1 (n/a) collected 1981 by Car. **SAM-ENW-X6386;** Newlands, Table Mountain, Cape Peninsula, Western Cape, South Africa, 1 M and 1 (n/a) collected 1900 by Lightfoot and Purcell, **SAM-ENW-X1064;** 1 (n/a) collected 1898 by Purcell, **SAM-ENW-X4014;** 1 (n/a) collected 1898 by Treleaven. **SAM-ENW-X1060;** Mowbray, Cape Town, Western Cape, South Africa, 1 (n/a) collected 1877 by Bright. **SAM-ENW-X1063;** St. James, Kalk Bay, Cape Peninsula, Western Cape, South Africa, 4 (n/a) collected 1898 by Purcell, **SAM-ENW-X1065;** 1 (n/a) collected 1896 by Beard, **SAM-ENW-B738;** 1 (n/a) collected 1909 by Godfrey, **SAM-ENW-X6383;** 3 M (date and collector not specified). **SAM-ENW-X7279;** Bergvliet, Constantia, Cape Peninsula, Western Cape, South Africa, 1 F collected 1901 by Purcell. **SAM-ENW-7289;** Unknown locality, Cape Peninsula, Western Cape, South Africa, 3 (n/a) collected 1903 by French.

Diagnosis

COI: Myburgh Ravine: **JN798075-JN798076**, Orangekloof: **JN798079-JN798082**, Rhodes Memorial: **JN798083-JN798088**, Skeleton Gorge: **EU855330, JN798089-JN798091**, Cecelia Forest: **EU855329-EU855331, JN798092-JN798095**

P. capensis sensu stricto forms a genetically distinct and well-supported monophyletic grouping (Fig. 3.1). Sequence divergence values between *P. capensis senso stricto* and (*P. lawrencei* sp. nov. and *P. overbergiensis* sp. nov.) are 8.1% and 8.6% respectively.

18S: Myburgh Ravine: **JN798147**, Orangekloof: **JN798148**, Rhodes Memorial: **JN798158**, Skeleton Gorge: **JN798150**, Cecelia Forest: **JN798143**

The *18S* topology was congruent with that of *COI* where *P. capensis sensu stricto* forms a genetically distinct and well-supported monophyletic grouping (McDonald & Daniels, 2012).

Description

Colour and patterning: Dark brown/slate black/indigo and seldom rust orange pigmentation. Dark dorsal midline with light lateral band above legs along the entire body (Table 3.1).

Legs: 17+1 leg pairs. Dorsal foot ridges absent. Three complete spinous footpads with the fourth being completely fragmented (Table 3.1)

Integument: Densely spaced primary dermal papillae with relatively large accessory papillae (fig. 3.2c, Table 1), cylindrical or dome-shaped primary dermal papillae with 6-9 scale ranks (fig. 3.3c, Table 1). 6-8 rows of chemoreceptors at the antennal tips. Mouth surrounded by 16-17 oral lips.

Male genital area: Gonopore cruciform. Genital pads covered with sensory spines.

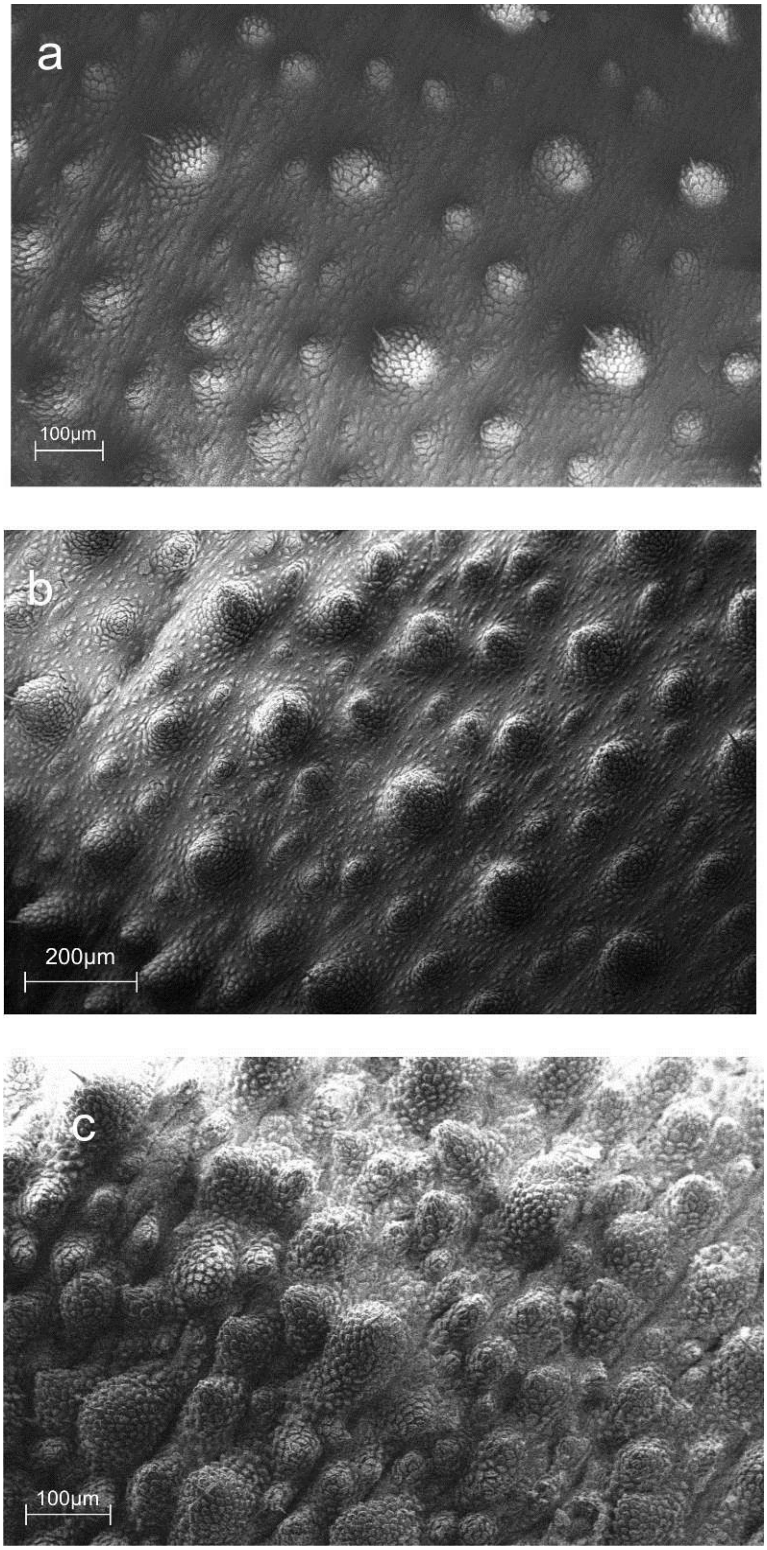


Figure 6: Scanning electron microscopy images illustrating the arrangement of the primary dermal papillae. a, *P. overbergiensis* sp. nov., widely spaced primary dermal papillae with 2 intermittent accessory papillae, b, *P. lawrencei* sp. nov., moderately spaced primary dermal papillae with 2-3 intermittent accessory papillae, c, *P. capensis*, densely spaced primary dermal papillae with 3-5 intermittent accessory papillae.

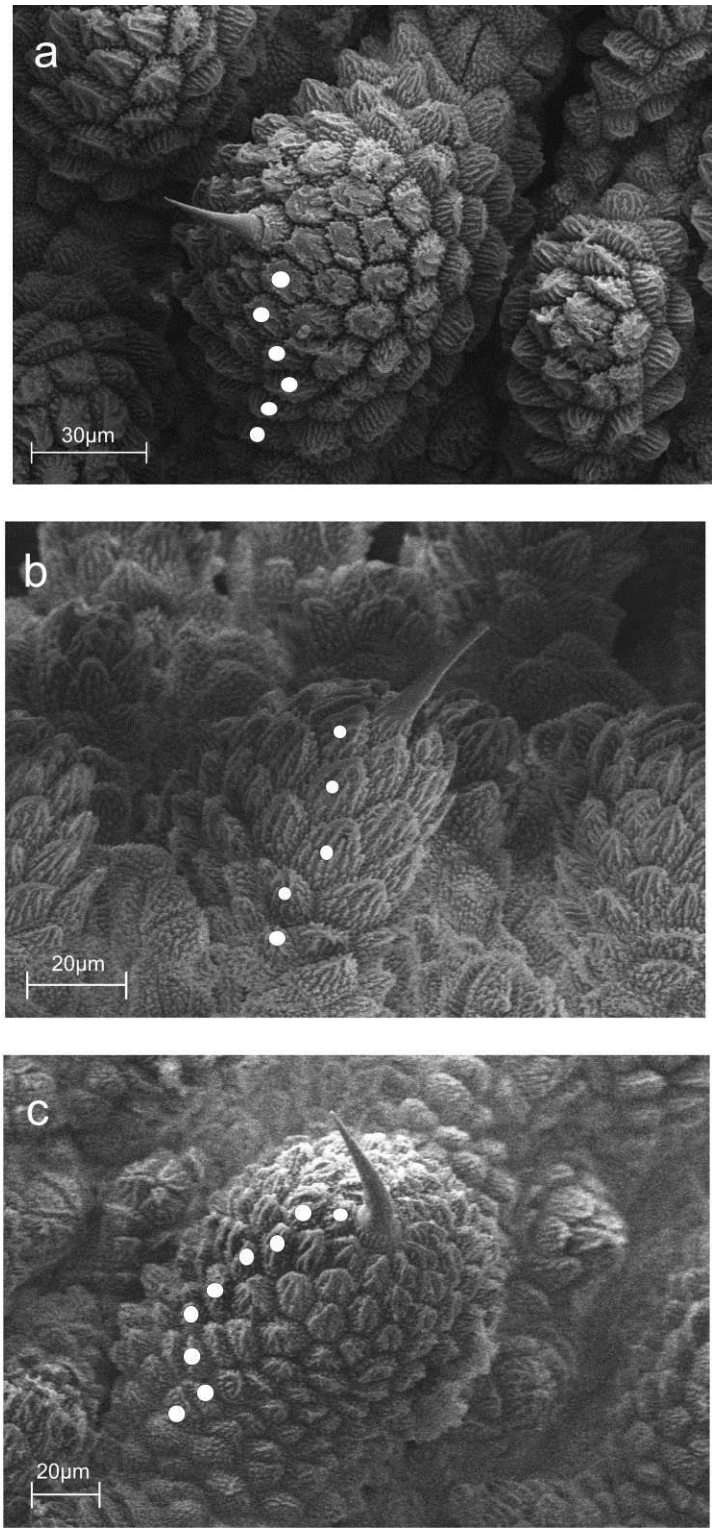


Figure 7: Scanning electron microscopy images illustrating morphological variation in the primary dermal papillae. a, *P. overbergiensis* sp. nov., semicircular or dome-shaped primary dermal papillae with 6 scale ranks, b, *P. lawrencei* sp. nov., conical or semicircular primary dermal papillae with 5 scale ranks, c, *P. capensis*, semicircular or dome-shaped primary dermal papillae with 9 scale ranks.

Remarks

P. capensis differs from *P. lawrencei* sp. nov. and *P. overbergiensis sp. nov.* in the primary dermal papillae structure as well as the dermal papillae arrangement (fig. 3.2 & 3.3). *P. capensis* is also characterised by the absence of ridges on the dorsal surface of the foot. Sequence divergence between *P. capensis* and the two novel lineages is marked (8.1% and 8.6% for *P. lawrencei* sp. nov. and *P. overbergiensis* sp. nov. respectively).

Habitat

Afromontane forest patches in the southwestern part of the Western Cape province. Collected under decaying wood logs, leaf litter, and stones generally close to streams. Specimens were also found within and beneath rotting pine logs from old pine plantations (e.g. Rhodes Memorial)

Distribution

Restricted to the Cape Peninsula Mountains.

Etymology

See original description.

***Peripatopsis lawrencei* sp. nov**

(Theewaterskloof-Overstrand or "Clade C", McDonald & Daniels, 2012)

Material examined

Holotype. **SAM-ENW-C6468a;** Oubos, 34˚04' 34.33"S, 19˚49' 43.76"E, Riviersonderend, Western Cape Province, South Africa, 1 M collected 20 October 2010 by McDonald and Abels.

Paratypes. **SAM-ENW-C6468b;** Oubos, 34˚04' 34.33"S, 19˚49' 43.76"E, Riviersonderend, Western Cape Province, South Africa, 4 M collected 20 September 2010 by McDonald and Abels.

Additional material examined. **SAM-ENW-C6457;** Fernkloof Nature Reserve, 34˚23' 37.00"S, 19˚16' 34.00"E, Hermanus, Western Cape Province, South Africa, 1 F collected 2006 by Daniels and van den Worm, **SAM-ENW-C6458a and b;** 3 M and 3 F collected 2010 by McDonald and Abels. **SAM-ENW-C6455;** Dappat se gat, 34˚13' 25.84"S, 18˚50' 24.16"E, Gordon's Bay, Western Cape Province, South Africa, 6 M and 3 F collected 2011 by Van Zyl. **SAM-ENW-C6456a and b;** Kogelberg Biosphere Reserve, 34˚19' 58.51"S, 18˚57' 05.34"E, Kleinmond, Western Cape Province, South Africa, 1 M and 3 F collected 2011 by McDonald and Abels. **SAM-ENW-C6462;** Grootbos Private Reserve, 34˚55' 05.00"S, 19˚41' 37.00"E, Gansbaai, Western Cape Province, South Africa, 1 F and 3 (n/a) collected 2006 by Daniels, **SAM-ENW-C6463a and b;** 5 M and 6 F collected 2010 by McDonald and Abels. **SAM-ENW-C6464;** Napier, 34˚29' 23.72"S, 19˚43' 20.25"E, Western Cape Province, South Africa, 5 F collected 2010 by Van Zyl, **SAM-ENW-C6465**; 2 M and 10 F collected 2006 by Daniels and Picker. **SAM-ENW-C6454;** High Noon, 33˚54' 29.23"S 19˚16' 56.05"E, Villiersdorp, Western Cape Province, South Africa, 9 juveniles collected 2006 by Picker, Cowlin, and Merl. **SAM-ENW-C6453;** 5 (n/a) collected 2006 by Picker and Cowlin. **SAM-ENW-C6466,** Greyton, 34˚02' 01.52"S 19˚36' 57.32"E, Western Cape Province, South Africa, 3 (n/a) (date and collector not specified). **SAM-ENW-C6448;** Jonkershoek Nature Reserve, 33°58' 05.76"S, 18°56' 24.65"E, Stellenbosch, Western Cape Province, South Africa, 1 M and 3 F collected 2011 by McDonald and Abels. **SAM-ENW-C6449;** 2 M and 2 F collected 2011 by McDonald and Abels. **SAM-ENW-X7290;** unspecified locality, Stellenbosch, Western Cape Province, South Africa, 1 (n/a) collected 1905 by Brown. **SAM-ENW-C6488;** Rondevlei, 34˚03' 37.50"S 18˚29' 59.76"E, Cape Flats, Western Cape Province, South Africa, 1 (n/a) collected by Picker (date not specified). **SAM-ENW-C6467;** Oubos, 34˚04' 34.33"S, 19˚49'

43.76"E, Riviersonderend, Western Cape Province, South Africa, 5 juveniles collected 2010 by McDonald and Abels. **SAM-ENW-C6461;** Klein Swartberg, 34°12' 43.00"S, 19°30' 23.00"E, Caledon, Western Cape Province, South Africa, 1 F collected 2011 by McDonald and Abels; **SAM-ENW-C6460;** 3 M and 4 F collected 2011 by Diedericks and Broeckhoven, **SAM-ENW-X6390;** 15 (n/a) collected by Watermeyer and Purcell (date not specified). **SAM-ENW-C6450, C6451, and C6452;** Berg River Dam, 33˚54' 25.14"S, 19˚03' 04.48"E, Franschoek, Western Cape Province, South Africa, 4 M and 2 F collected 2010 by Van Zyl. SAM-ENW-X6388; Houw Hoek, Western Cape Province, South Africa, 5 (n/a) collected 1900 by Purcell. Sir Lowry's Pass, Western Cape Province, South Africa, **SAM-ENW-X4024;** 1 (n/a) collected 1899 by Purcell.

Diagnosis:

COI: High Noon, Villiersdorp: **EU855288-EU855291**, Grootbos Private Nature Reserve: **EU855344-EU85548**, Greyton: **EU855340-EU855342**, Fernkloof Nature Reserve: **EU855336, JN798123-JN798126**, Dappat se gat: **JN798096-JN798100**, Caledon: **JN798101**, Jonkershoek: **JN798102-JN79805**, Kogelberg: **JN798106-JN79807**, Oubos: **JN798108-JN798111**, Rondevlei: **JN798112**, Napier: **JN798113-JN798118**

P. lawrencei sp. nov. forms a genetically distinct and well-supported monophyletic grouping (Fig. 3.1). Sequence divergence values between *P. lawrencei* sp. nov. and (*P. capensis* and *P. overbergiensis* sp. nov.) are 8.1% and 7.8% respectively.

18S: High Noon, Villiersdorp: **JN798146**, Greyton: **JN798153**, Fernkloof Nature Reserve: **JN798145**, Dappat se gat: **JN798151**, Caledon: **JN798142**, Jonkershoek: **JN798161**, Kogelberg: **JN798162**, Oubos: **JN798157**, Rondevlei: **JN798159**, Napier: **JN798160**, Grootbos Private Nature Reserve: **JN798154**

The *18S* topology is congruent with that for *COI* where *P. lawrencei* sp. nov. again forms a genetically distinct and well-supported monophyletic grouping (McDonald & Daniels, 2012).

Description

Measurements: Holotype (M): Length: 20mm, Paratypes (M adults, *n*=4): 12-19mm

Colour and patterning: Dark brown/slate black/indigo to rust orange pigmentation. Dark dorsal midline with light lateral band above legs along the entire body (Table 1).

Legs: 17+1 leg pairs. Dorsal foot surface with ridges (fig. 3.4a). Three complete spinous footpads with the fourth being completely to partially fragmented. Crural tubercle semicircular proximally and cylindrical distally (Table 1).

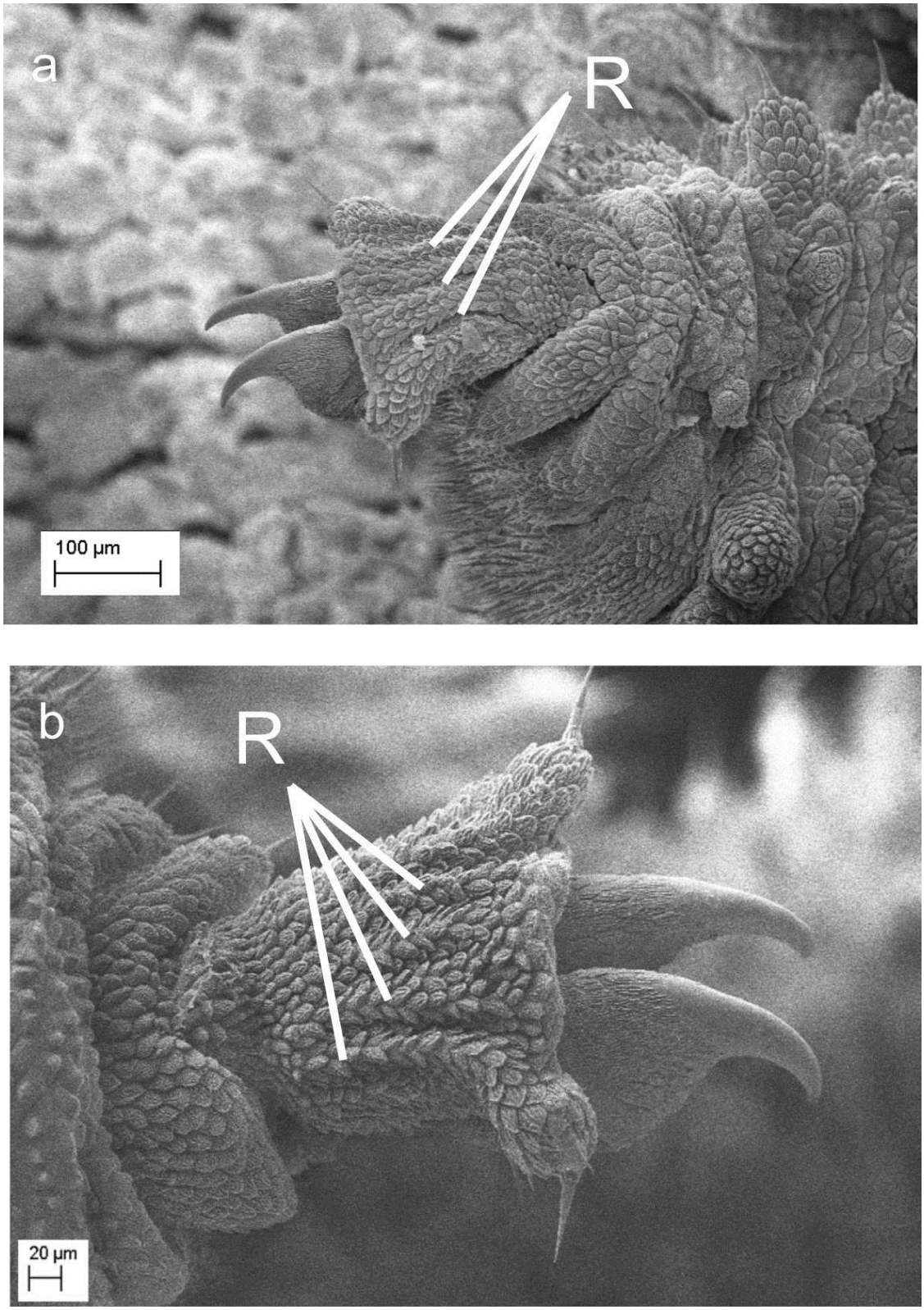


Figure 8: Scanning electron microscopy images illustrating the dorsal foot surface with ridges in: a, *P. lawrencei* sp. nov. and b, *P. overbergiensis* sp. nov.

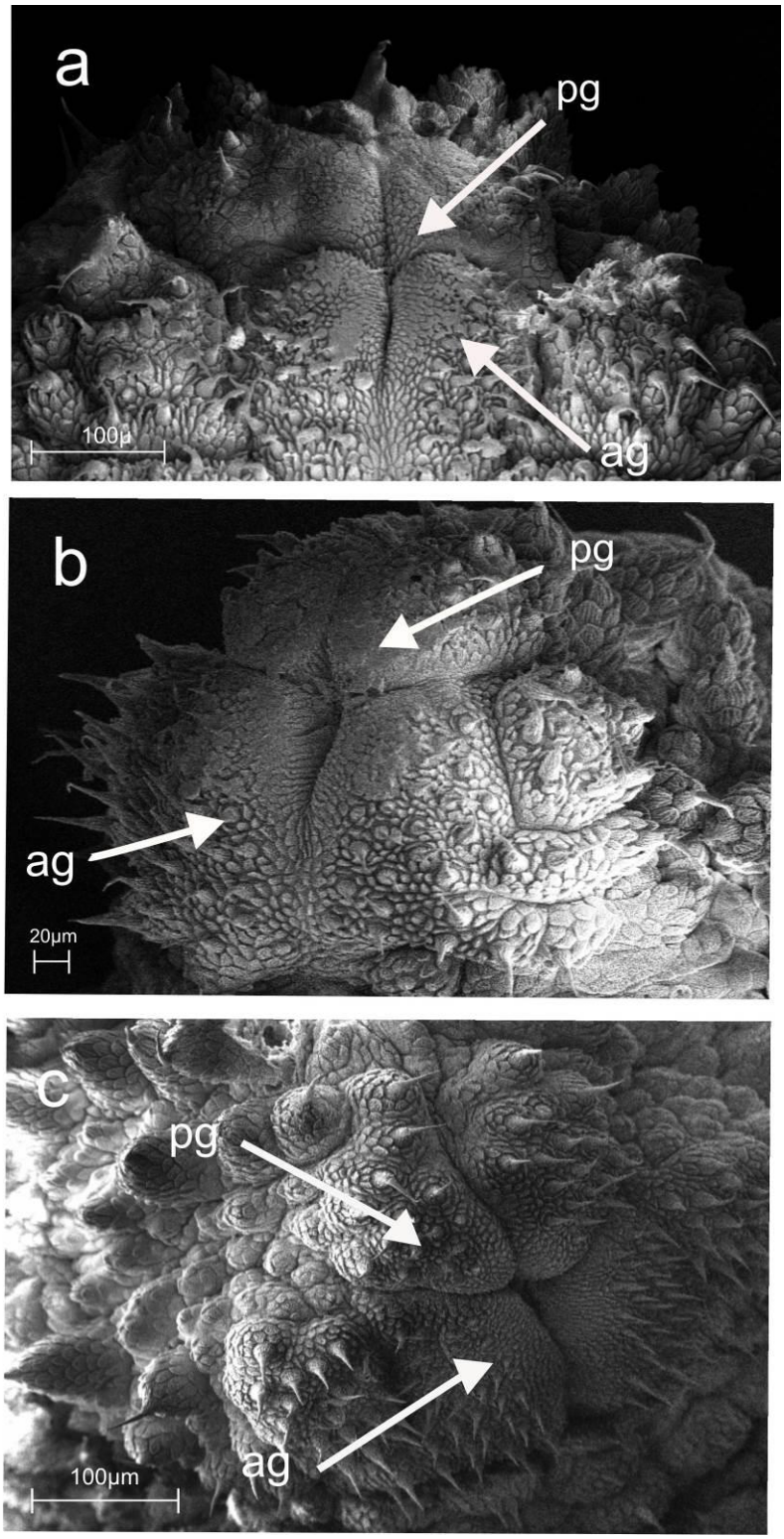


Figure 9: SEM images illustrating morphological variation in the genital area. a, *P. overbergiensis* sp. nov., genital area with posterior genital (pg) pads smaller (with fewer sensory spines) than anterior genital (ag) pads, b, genital area with posterior genital (pg) pads smaller (with fewer sensory spines) than anterior genital (ag) pads, c, genital

area with the posterior genital (pg) pads similar in size (and with same amount of sensory spines) as anterior genital (ag) pads.

Integument: Moderately spaced primary dermal papillae with 2-3 intermittent accessory papillae (fig. 3.2b, Table 1), conical or semicircular dermal papillae with 4-7 scale ranks (fig. 3.3b, Table 1). 5-7 rows of chemoreceptors at antennal tips. Mouth surrounded by 15-16 oral lips.

Male genital area: Gonopore cruciform with "horizontal arms" pointing slightly towards anus. Posterior genital pads smaller and with fewer sensory spines than anterior pads (fig. 3.5b).

Remarks

P. lawrencei sp. nov. differs from *P. capensis* in the structure of the male genital pads (compare Figs. 5b & 5c) as well as the number of scale ranks on the primary dermal papillae (figs. 4b &4c). Furthermore *P. lawrencei* sp. nov. is superficially similar to *P. overbergiensis* sp. nov. with slight differences in the dermal papillae arrangement and structure. However, sequence divergence values for the two species are 7.8%, indicating considerable genetic variation. Characters associated with the antennae (e.g. number of antennal rings) were generally unreliable due to preservation artefacts and were hence omitted from the analysis.

Habitat

Afromontane forest patches in the southwestern part of the Western Cape province. Collected under decaying wood logs, leaf litter, and stones generally close to streams.

Distribution

Theewaterskloof-Overstrand region: *P. lawrencei* sp. nov. collected from areas at and surrounding the Hottentots-Holland mountains including the Riviersonderend Mountains and Rondevlei being isolated on the Cape Flats.

Etymology

Named after the late Dr. Reginald Frederick Lawrence, a respected South African invertebrate systematist with a long-standing interest in velvet worms.

***Peripatopsis overbergiensis* sp. nov.**

(Overberg or "Clade B", in McDonald & Daniels, 2012)

Material examined

Holotype. **SAM-ENW-C6480a;** Grootvadersbosch Nature Reserve, 33˚58' 55.00"S, 20˚49' 23.00"E, Langeberg, Western Cape Province, South Africa, 1 M collected 5 October 2010 by McDonald and Abels.

Paratypes. **SAM-ENW-C6480b;** Grootvadersbosch Nature Reserve, 33˚58' 55.00"S, 20˚49' 23.00"E, Langeberg, Western Cape Province, South Africa, 2 M and 1 F collected 5 October 2010 by McDonald and Abels.

Additional material examined. **SAM-ENW-C6482, C6483, C6484, C6485, C6486, and C6487;** Grootvadersbosch Nature Reserve, 33˚58' 55.00"S, 20˚49' 23.00"E, Langeberg, Western Cape Province, South Africa, 17 M and 41 F collected 2006 by Daniels, Fortuin, and Gordon, **SAM-ENW-C6330;** 11 (n/a) collected by Prins (date not specified). **SAM-ENW-C6475, C6476, C6477, C6478, C6479;** Duiwelsbos, 33˚59' 51.84"S, 20˚27' 27.68"E, Marloth Nature Reserve, Swellendam, Western Cape Province, South Africa, 6 M, 3 F, and 9 sectioned specimens collected 2006 by Daniels, Fortuin, and Gordon; **SAM-ENW-C6472, C6473, C6474;** 1 M, 7 F and 3 juveniles collected 2010 by McDonald and Abels. **SAM-ENW-X6391;** Unspecified locality, Swellendam, Western Cape Province, South Africa, 2 M and 1 (n/a) collected 1900 by Fry, **SAM-ENW-X6389;** 8 (n/a) collected 1900 by Purcell. **SAM-ENW-B6386;** Tradouws Pass, Swellendam, Western Cape Province, South Africa, 1

(n/a) (date and collector not specified), **SAM-ENW-B6837;** 1 (n/a) collected (date not specified) by Barnard. **SAM-ENW-C6471;** De Hoop Nature Reserve, 34˚17' 35.00"S, 20˚29' 19.00"E, Potberg, Western Cape Province, South Africa, 3 M, 1 F, and 3 (n/a) collected 2006 by Daniels and Fortuin; **SAM-ENW-C6469 and C6470;** 6 M and 4 F collected 2010 by Daniels, McDonald, Engelbrecht, and Gordon.

Diagnosis

18+1, seldom (*n*=6) 17+1 leg pairs.

COI: Potberg-De Hoop Nature Reserve: **EU855316-EU855320, JN798137-798141**, Grootvadersbos Nature Reserve: **EU855311-EU855315, JN798127-JN798129**, Marloth Nature Reserve: **EU855284-EU855287, JN798130-JN79813033**

P. overbergiensis sp. nov. forms a genetically distinct and well-supported monophyletic grouping (Fig. 1). Sequence divergence values between *P. overbergiensis* sp. nov. and (*P. capensis* and *P. lawrencei* sp. nov.) are 8.6% and 7.8% respectively.

18S: Potberg-De Hoop Nature Reserve: **JN798144**, Grootvadersbos Nature Reserve: **JN798155**, Marloth Nature Reserve: **JN798156**

The *18S* topology is congruent with that for *COI* where *P. overbergiensis* sp. nov. again forms a genetically distinct and well-supported monophyletic grouping (McDonald & Daniels, 2012)

Description

Measurements: Holotype (M): length: 26mm, Paratypes: (M, *n*= 2) 14-23mm, (F, *n*= 1) 32mm

Colour and patterning: Dorsal coloration: two colour morphs present; 1) Dark brown/slate black to olive green/bluish black, 2) Rust orange. Dark dorsal midline with orange or light lateral band above

legs along the entire body. Creamy white or orange ventral coloration. De Hoop specimens (*n*=17) with distinct white antennal base (Table 1).

Legs: 18+1, seldom 17+1 leg pairs. Dorsal foot surface with ridges (Fig. 3.4b). Four complete or partially fragmented (last footpad) spinous footpads. Distal section of the crural tubercle is not strongly demarcated (Table 1).

Integument: Primary dermal papillae widely spaced with two intermittent accessory papillae (Fig. 3.2a, Table 1), semicircular or dome-shaped primary dermal papillae with 5-6 scale ranks (Fig. 3.3a, Table 1). 6-8 rows of chemoreceptors at antennal tips. Mouth surrounded by 13-16 oral lips.

Male genital area: Gonopore cruciform with "horizontal arms" pointing slightly towards anus. Posterior genital pads smaller with fewer sensory spines than anterior pads (Fig. 3.5a).

Remarks

P. overbergiensis is genetically distinct and forms a well-supported monophyletic grouping (Fig. 3.1). This species is distinct from *P. capensis* and *P. lawrencei* sp. nov in terms of the number of pregenital legs. Specimens having 17 pregenital leg pairs are associated with dark (dirty cream) ventral coloration. Some specimens (De Hoop locality only) are characterised by a white antennal base. *P. overbergiensis* sp. nov. is further characterised by four complete spinous footpads (Fig. 3.4a). Characters associated with the antennae (e.g. number of antennal rings) were generally unreliable due to preservation artefacts and were hence omitted from the analysis.

Habitat

Afromontane forest patches in the southeastern part of the Western Cape province. Collected under decaying wood logs, leaf litter, and stones generally close to streams.

Distribution

Overberg region: *P. overbergiensis* sp. nov has been collected from the Marloth and Grootvadersbosch nature reserves which form part of the Swellendam district and is located on the lower reaches of the Langeberg Mountain ranges. Specimens have also been collected from De Hoop nature reserve at the Potberg Mountain.

Etymology

Named after the Overberg biogeographic region.

3.4 Discussion

The analysis of DNA sequence data, scanning electron microscopy, and gross morphology provide compelling evidence for the presence of three distinct species nested within *P. capensis sensu lato.* The *P. capensis* species-complex is unified by a few superficial characters that have underestimated diversity within the group (Daniels *et al.*, 2009). Traditional gross morphological characteristics such as dorsal coloration and number of leg pairs were of limited utility in species diagnosis (Daniels & Ruhberg, 2010). However, number of leg pairs proved to be a partially useful diagnostic character for *P. overbergiensis* sp. nov. suggesting that certain characters should be evaluated on a case by case scenario. The use of scanning electron microscopy to explore fine scale morphological characters yielded more success (Read, 1984; Daniels *et al.*, 2009; Oliveira *et al*., 2011). However, the presence of only subtle diagnostic characters underscores the recent divergence within the *P. capensis* species-complex (3.14 Mya) (McDonald & Daniels, 2012). As a result, the designation of the two new species is largely based on DNA sequence data and geographical information (Fig. 3.1). DNA-based species descriptions are slowly gaining acceptance and have been successful in a variety of cryptic taxa (e.g. skipper butterflies) including Onychophora (e.g. *Peripatoides novaezealandiae* (Hutton, 1876), *Epiperipatus spp.*, and *Peripatopsis moseleyi*) (Hebert *et al.*, 2003; Cook *et al.*, 2010; Trewick, 1998; Oliveira *et al.*, 2011; Ruhberg & Daniels, 2013).

In accordance with de Queiroz's (2007) proposition of a unified species concept we have identified *P. lawrencei* sp. nov. and *P. overbergiensis* sp. nov. as "separately evolving lineages". For the *COI* gene, three distinct, well-supported monophyletic clades were characterized by long internal branches (McDonald & Daniels, 2012). Furthermore sequence divergence values ranged between 7.8%-8.6% among the three taxa. Previous studies on the Onychophora have employed considerably lower values in delineating new species (e.g. 3.3%, *Plicatoperipatus jamaicensis* (Clark, 1913); and 1.1-11.6%,

Planipapillus spp.) (Hebert *et al.*, 1991; Rockman *et al.*, 2001). Despite having a slower rate of evolution the *18S* rDNA marker displayed a similar tree topology providing further evidence for separately evolving lineages (Zhang & Hewitt, 2003). The DNA sequence data is further corroborated by the geographic distribution where *P. capensis*, *P. lawrencei* sp. nov. and *P. overbergiensis* sp. nov. are restricted to the Cape Peninsula, Theewaterskloof-Overstrand, and Overberg regions respectively and further characterised by the absence of geneflow (McDonald & Daniels, 2012). This provides a strong case for geographic isolation as additional operational criteria (de Queiroz, 2007; Cook *et al.*, 2010).

3.4.1 Conservation implications

The description of two new species within the *P. capensis* species complex has important implications for endemicity within the group. *P. capensis* and *P. overbergiensis* sp. nov. are now restricted to the Cape Peninsula and the Overberg regions respectively. Similarly, *P. lawrencei* sp. nov. has a narrow distribution and is limited to the Theewaterskloof-Overstrand region. *Peripatopsis* species are largely restricted to the forested, mountainous regions of the Western Cape (Hamer *et al.*, 1997). Indigenous forests are currently threatened by encroachment from alien invasive flora, the establishment of new timber plantations, and fire events (Hamer *et al.*, 1997; Alston & Richardson, 2006; Daniels, 2011). Decision-makers need to consider indigenous forest when developing fire management strategies for the surrounding fynbos vegetation. In the absence of adequate fire management, fynbos could develop high fuel loads resulting in very intense fires that damage the forest margin (Van Wilgen *et al.*, 1990). The latter authors suggest average fire frequencies that would allow tree species to establish and grow large enough to survive fires of moderate intensity allowing forest patches to expand.

A population genetic study undertaken by Daniels (2011) on *Opistopatus roseus* in the Ngele forest (KwaZulu-Natal) revealed that saproxylic habitats provided by indigenous forest potentially harbour high levels of genetically distinct individuals. Similar small-scale population studies could reveal valuable information on the intrapopulation diversity within *Peripatopsis*. Similar studies for other low dispersing invertebrate taxa have also revealed high levels of genetic diversity associated with Afromontane regions (e.g. montane isopods) (Gouws *et al.*, 2010). These areas should be prioritized for conservation. Since all three clades in the present study occur in high lying areas that are under nature conservation protection, all three species are currently well protected.

Chapter 4

4. The phylogenetics, biogeography, and conservation of the genus *Peripatopsis*

4.1 Introduction

Despite commitments by global leaders in 2002 to limit biodiversity loss through the Convention on Biological Diversity, several indicators have demonstrated that by 2010 biodiversity loss was in a continued state of decline and that there was no significant reduction or reversal in losses (Butchart *et al.*, 2010). Of the 5-9 million animal species globally, circa 11 000 to 58 000 species are being lost annually (Dirzo *et al.*, 2014). According to Cardoso *et al.* (2011) invertebrate groups comprise the vast majority of the latter global loss with 40% of the group threatened with extinction (Dirzo *et al.*, 2014). Yet, according to the IUCN Red List of threatened species <0.3% of invertebrates are known to have been evaluated leaving large gaps in our current knowledge (Baillie *et al.*, 2004). Mirroring the global conservation scenario invertebrates are severely under-represented in studies of southern African diversity and present unique taxonomic challenges given their high diversity and endemicity (Slotow & Hamer, 2000; McGeoch *et al.*, 2011). It follows that the conservation status of most South African invertebrate groups are poorly studied with the exception of butterflies, dragonflies, trapdoor and baboon spiders, terrestrial molluscs, one genus of millipede, and Onychophora (McGeoch *et al.*, 2011; Dippenaar-Schoeman, 2002; Henning & Henning, 1989; Samways, 2006; Scholtz & Chown, 1995; Daniels & Ruhberg, 2010; Daniels *et al.*, 2013; Daniels *et al.*, 2009; Daniels, 2011; McDonald *et al.*, 2012; Myburgh & Daniels, 2015; Ruhberg & Daniels, 2013).

Onychophora, more commonly known as ‘velvet worms’, are habitat specialists comprising an ancient relictual invertebrate lineage that has fascinated evolutionary biologists (Murienne *et al.*, 2014). The

organisms have limited vagility, exhibit a low desiccation tolerance, and are usually restricted to saproxylic environments within forests including decaying wood logs and leaf litter (Clusella-Trullas & Chown, 2008; Manton & Ramsay, 1937; Newlands & Ruhberg, 1978). Furthermore, in South Africa the group displays a close affinity with relictual Afromontane forest habitats (Daniels *et al*., 2009; Brinck, 1957; Hamer *et al.*, 1997; Enrody-Younga & Peck, 1983). Afromontane forest represents the smallest biome in South Africa and comprise <0.3% of the country's land area (Mucina & Rutherford, 2006). The biome is highly fragmented and most of the patches occupy less than 1km² generally occurring below 1000m, in areas where rainfall exceeds 600 mm (Rutherford & Westfall, 1986; Mucina & Rutherford, 2006). The current distribution of forest patches in Southern Africa is hypothesized to have arisen due to the synergy of several factors leading to climatic deterioration since the mid Miocene (~10 Mya), before which coastal margins and adjacent inland areas harboured extensive sub-tropical vegetation under warmer and moister conditions (Cowling *et al.* 2009; Coetzee 1983; Linder, 2003). Factors leading to the contraction of closed/forest habitat included aridity associated with the development of the Benguela upwelling system on the west coast of southern Africa (Siesser, 1980), geotectonic uplift across the sub-continent from the Miocene to Plio/Pleistocene (King, 1978), and marine regressions and transgressions (Hendey, 1983). Furthermore the uneven uplift of the Great Escarpment in the Pliocene (Partridge & Maud, 2000) led to the raising of eastern margins higher than western margins. As a result western Cape Floristic Region (CFR) aridity was strengthened due to greater interception of rainfall from the warm Agulhas current on the east coast. The latter resulted in the division of the southern African coastal margin into a western "seasonal rainfall zone" and an eastern "all year rainfall zone" (Cowling & Lombard, 2002). According to Linder (2003) the west-east axis may have a dominant influence on plant distribution patterns where the western CFR has twice as many species as the eastern CFR and double the endemism, suggesting climate variation as a driver in the west. A further feature of the interplay between western CFR species diversity and

climatic conditions was the development of fire-prone/pyrophytic fynbos habitat during the late Miocene (Linder, 2005; Bytebier *et al.*, 2011) which established fire pathways and restricted forest habitat to high lying areas and sheltered ravines. Hence these factors have led to the development of forest patches as palaeorefugia which continue to play a critical role in the survival and evolution of ancient invertebrate biota like Onychophora during climatic perturbations (Stuckenberg, 1962; Deacon, 1983; Keppel *et al.*, 2012). Presently the biome faces several threats including encroachment from alien invasive flora, habitat destruction, the establishment of new timber plantations, and to a lesser extent fire events and climate change (Eeley *et al.*, 1999; Daniels, 2011; Alston & Richardson, 2006; Hamer *et al.*, 1997).

Two velvet worm genera occur in South Africa, *Peripatopsis* and *Opisthopatus* (Hamer *et al.*, 1997). The genus *Peripatopsis* (Pockock, 1894) has a broad distribution across the latter mesic regions in the Western Cape, Eastern Cape, and KwaZulu-Natal provinces of South Africa. Historically *Peripatopsis* was characterised by eight species, with several species thought to be widely distributed, while the taxonomy was based on several highly variable morphological features (Hamer *et al.*, 1997). The most recent conservation assessment of South African velvet worm genera was undertaken by Hamer *et al.* (1997) and submitted to the IUCN. According to the latter author three *Peripatopsis* species required Red List status (IUCN, 1994). The point endemic *P. alba* (Lawrence, 1931), known only from the Wynberg cave system in the Western Cape, was categorised as 'vulnerable'. *P. clavigera* (Purcell, 1899) was categorised as 'vulnerable' considering that the forest habitat in which they occur was largely protected, while *P. leonina* (Purcell, 1899) was categorised as 'critically endangered' (Hamer *et al.*, 1997) due to the species 'not being reliably discovered for over 90 years', despite exhaustive searches. The latter species may have gone extinct following an absence of recollections in the last 100 years, further stressing the need for effective conservation of this ancient faunal group. The genus

Peripatopsis was recently subjected to considerable modern systematic scrutiny using DNA sequence data and scanning electron microscopy in an attempt to delineate species boundaries (Daniels *et al.*, 2009; Daniels & Ruhberg, 2010, McDonald & Daniels, 2012, McDonald *et al.*, 2012; Daniels *et al.*, 2012, Ruhberg & Daniels, 2013). Daniels *et al.* (2009) identified three main species complexes that required further taxonomic scrutiny (*P. balfouri* Sedgewicki, 1885; *P. capensis* Grube, 1866; and *P. moseleyi* Wood-Mason, 1879). *Peripatopsis capensis* was shown to comprise three evolutionary distinct units (McDonald & Daniels, 2012), two of which were described as new species (McDonald *et al.*, 2012). Within the *P. balfouri* complex three distinct lineages represented novel species, while *P. stelliporata* was synonymised with *P. balfouri* (Daniels *et al.*, 2013). Similarly, five novel lineages were identified within *P. moseleyi*, leading Ruhberg & Daniels (2013) to describe four new species. Consequently species diversity within *Peripatopsis* increased from nine (Hamer *et al.*, 1997) to sixteen described species. A large number of the novel described species are characterised by narrow endemic distribution ranges, rendering them sensitive to potential extinction in the wild. In light of the increase in species diversity among *Peripatopsis* observed as a result of these recent studies a reappraisal of the group's conservation status is required. The assessment of conservation status and category of threat requires continued revision as new information becomes available. According to Samways (2002) IUCN assessments generally need to be undertaken at a ten year minimum interval. Considering that the last formal conservation assessment of *Peripatopsis* was undertaken by Hamer *et al.* (1997) prior to the results from the subsequent systematic studies, a new assessment is now warranted.

Conservation assessments have traditionally focused on species richness as a metric to assign conservation priorities and hence protect biodiversity (Hidasi-Neto *et al.*, 2013). While species richness measures the number of species in an area it is unable to quantify the 'feature' diversity contributed by a species or set of taxa. Features represent possible future uses and benefits (or option value) and

includes functional diversity and evolutionary potential (Faith, 2013; Winter *et al.*, 2013). According to Faith (1992) taxonomically distinct species are likely to contribute more to the diversity of a given subset of species through its contribution of different features. The emergence of phylogenetic diversity (PD) indexes as an additional tool to inform conservation priority setting has added the loss of 'evolutionary information' to conservation considerations (Winter *et al.*, 2013). Phylogenetic diversity is measured as the sum of all the branch lengths which represent a species or set of taxa on a phylogenetic tree (Faith, 1992). Hence the measure calculates the total contribution of evolutionary history by a species or set by adding its branch lengths across the minimum spanning path of a tree from root to tips (Faith *et al.*, 2004; Laity *et al.*, 2015). Whilst some studies found species richness to be a good surrogate for phylogenetic diversity (Rodrigues *et al.*, 2005, Polasky *et al.*, 2001), other studies found the two metrics to be decoupled (Forest *et al.*, 2007) providing a strong motivation to employ both measures. Another measure which has emerged as a useful tool for assigning conservation priority is evolutionary distinctiveness (ED). ED scores for each species is calculated by dividing the total phylogenetic diversity of a clade amongst its members (Isaac *et al.*, 2007). Species with few close relatives will retrieve high ED values suggesting 'relicts', while species with low ED scores are suggestive of recent radiations or 'cradles of diversity' (Isaac *et al.*, 2007).

The first objective of this study is to gain a better understanding of how palaeoclimatic events shaped the distribution and evolutionary relationships within the *Peripatopsis* genus. Secondly, we will determine how phylogenetic diversity (PD), species richness (SR) and evolutionary distinctiveness (ED) is distributed within the genus. Finally, we conduct an assessment of the conservation status of the genus *Peripatopsis* based on the IUCN Red listing framework. We predict that species' threat status has changed since the last assessment considering the findings of recent taxonomic endeavours which have led to the near doubling of taxonomic diversity.

4.2 Materials and Methods

4.2.1 Samples and data

The genus *Peripatopsis* contains 16 species and is concentrated in Afromontane regions of the Western Cape, Eastern Cape, and KwaZulu-Natal in South Africa (Fig. 1). Sampled localities fell within four geomorphic provinces. These were the Cape Fold Mountains, Southern Coastal Platform, East London Coastal Hinterland, Southern Coastal Hinterland (Partridge *et al.*, 2010). The Cape Fold Mountains is further divided into 2 sub-provinces namely Atlantic and Syntax Zone. According to Nel (2007) representative spatial units are needed to help set conservation targets and goals. Geomorphic provinces are based on criteria including geomorphic history, geological structure, climate, location, and altitude. The latter attributes form the basis of Stuckenberg's (1962) zoogeographical delineation of the montane palaeogenic element in the South African invertebrate fauna. In Stuckenberg (1962) the distribution pattern of *Peripatopsis* is broadly classified into the Cape Fold Ranges and the Eastern Highlands, which is of limited value for identifying priority conservation areas. Hence for the purposes of this study Partridge et al.'s (2010) geomorphic provinces will be used to classify and delineate conservation priorities within *Peripatopsis*.

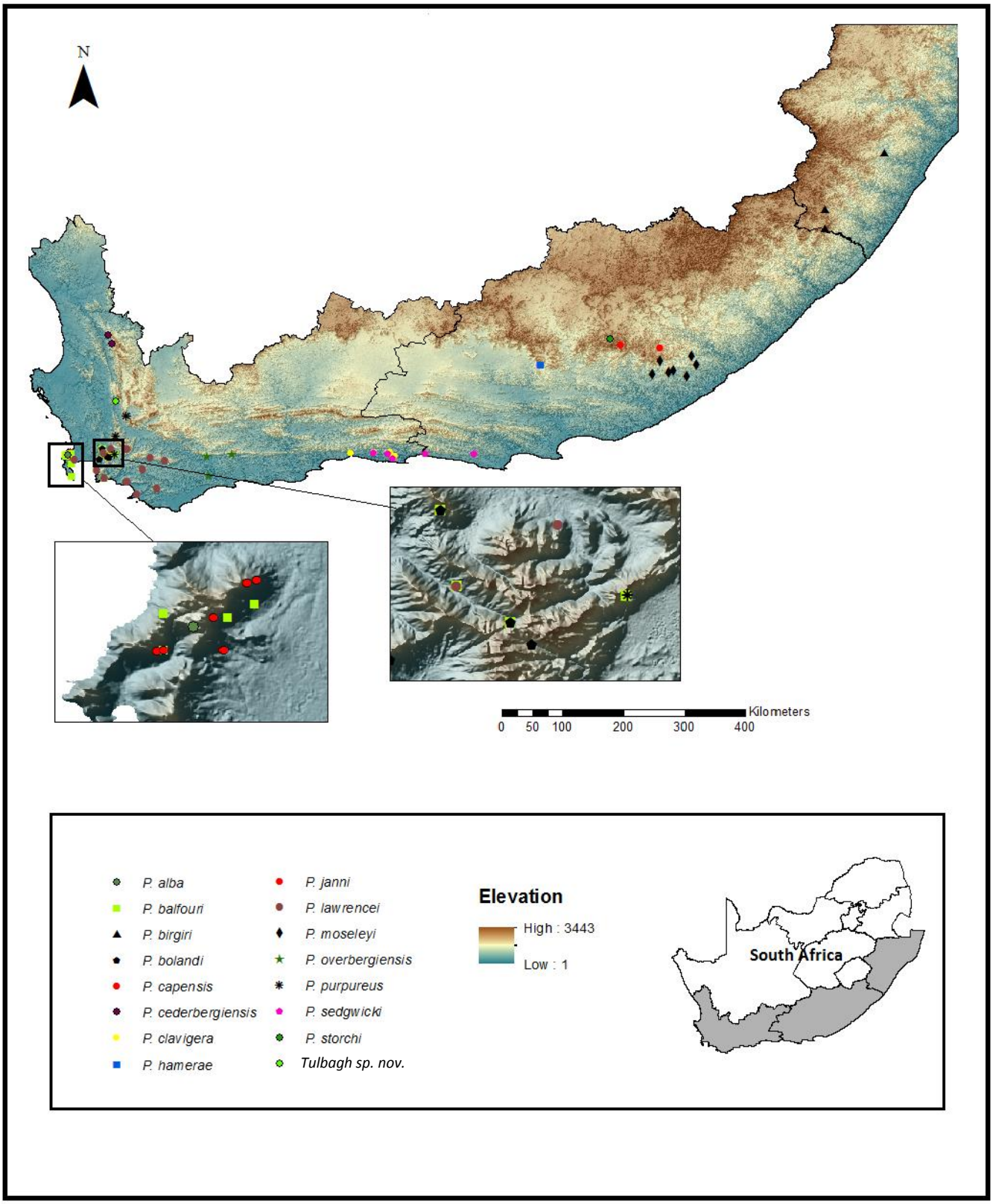


Figure 10: **Map of South Africa detailing the localities where the 15 *Peripatopsis* species and one putative species were sampled.**

Table 5: Sample localities and GENBANK accession numbers of *Peripatopsis* species collected throughout the Western and Eastern Cape and KwaZulu-Natal in South Africa. (n/a – not uploaded to GENBANK)

Species	**Locality**	**Genbank accession #**		**Reference study**	
		COI	***18S***	***COI***	***18S***
Peripatopsis moseleyi	Pirie Forest	HM210108	HM210137	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis storchi	Katberg	HM210122	HM210138	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis janni	Amathole	HM210118	HM210135	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis birgeri	Karkloof Falls	EU855276	HM210143	Daniels et al. (2009)	Daniels & Ruhberg (2010)
Peripatopsis birgeri	Mount Currie	HM210103	HM210145	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis hamerae	Groot Bruintjieshoogte	HM210133	HM210146	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis moseleyi	Seymour	n/a	HM210140	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis moseleyi	Amathole	n/a	HM210136	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis janni	Hogsback	n/a	HM210134	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis moseleyi	Hogsback	n/a	HM210141	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis moseleyi	Keiskammahoek	n/a	HM210142	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis birgeri	Swartkop Forest	n/a	HM210144	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis moseleyi	Quacu	n/a	HM210139	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis moseleyi	Stutterheim	n/a	n/a	Daniels & Ruhberg (2010)	Daniels & Ruhberg (2010)
Peripatopsis balfouri	Kirstenbosch	EU855343	KC766069	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis bolandi	Simonsberg	KC766103	KC766073	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis balfouri	Simonsberg	KC766100	KC766070	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis bolandi	Kogelberg	KC766115	KC766087	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis bolandi	Heldeberg	KC766110	KC766072	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis balfouri	Booi se skerm	KC766120	KC766074	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis balfouri	Newlands	KC766107	KC766075	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis balfouri	Slangolie	KC766113	KC766076	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis balfouri	St James	KC766123	KC766077	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis purpureus	Du Toit	KC766127	KC766078	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis alba	Wynberg Caves	KC766128	KC766079	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis balfouri	Orangekloof	KC766129	KC766080	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis balfouri	Du Toit	KC766132	KC766081	Daniels *et al.* (2013)	Daniels *et al.* (2013)
Peripatopsis cederbergiensis	Boschkloof	EU855280	KC766082	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis cederbergiensis	Helskloof	EU855296	KC766083	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis bolandi	Landroskop	EU855306	KC766088	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis bolandi	Jonkershoek 1	EU855324	KC766085	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis bolandi	Fernkloof	EU855333	KC766086	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis balfouri	Kogelberg	EU855339	KC766071	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis bolandi	Landroskop	EU855349	KC766084	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis purpureus	Bainskloof	EU855357	KC766090	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis purpureus	Mitchell's Pass	EU855358	KC766091	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis sedgwicki	Port Elizabeth	EU855367	n/a	Daniels *et al.* (2009)	n/a

Peripatopsis sedgwicki	Diepwalle	EU855253	n/a	Daniels *et al.* (2009)	n/a
Peripatopsis sedgwicki	Lady's Slipper	EU855366	n/a	Daniels *et al.* (2009)	n/a
Peripatopsis lawrencei	Fernkloof	JN798126	JN798145	McDonald & Daniels (2012)	McDonald & Daniels (2012)
Peripatopsis lawrencei	Dappat se Gat	JN798100	JN798151	McDonald & Daniels (2012)	McDonald & Daniels (2012)
Peripatopsis capensis	Skeleton Gorge	JN798091	JN798150	McDonald & Daniels (2012)	McDonald & Daniels (2012)
Peripatopsis capensis	Cecelia Forest	JN798095	JN798143	McDonald & Daniels (2012)	McDonald & Daniels (2012)
Peripatopsis lawrencei	Oubos	JN798110	JN798157	McDonald & Daniels (2012)	McDonald & Daniels (2012)
Peripatopsis lawrencei	High Noon	EU855288	JN798146	Daniels *et al.* (2009)	McDonald & Daniels (2012)
Peripatopsis lawrencei	Greyton	EU855340	JN798153	Daniels *et al.* (2009)	McDonald & Daniels (2012)
Peripatopsis overbergiensis	Grootvadersbosch	JN798129	JN798155	McDonald & Daniels (2012)	McDonald & Daniels (2012)
Peripatopsis overbergiensis	Marloth	JN798136	JN798156	McDonald & Daniels (2012)	McDonald & Daniels (2012)
Peripatopsis overbergiensis	Potberg De Hoop	JN798141	JN798144	McDonald & Daniels (2012)	McDonald & Daniels (2012)
Peripatopsis clavigera	Garden of eden	EU855375	KC766092	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis clavigera	Diepwalle	EU855254	KC766093	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis clavigera	Homtini	EU855267	KC766094	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis clavigera	Wilderness	EU855292	KC766095	Daniels *et al.* (2009)	Daniels *et al.* (2013)
Peripatopsis sp.	Tulbagh	n/a	n/a	n/a	n/a

Specimens used in this study were hand-collected during the period 2008-2012 (Table 1). We sampled multiple populations for all known species of the genus *Peripatopsis* with the exception of *P. leonina*, which is considered extinct following numerous collection attempts (Daniels *et al.*, 2013). We included one putative *Peripatopsis* species from the Tulbagh region. One specimen from each locality was included for phylogenetic analysis.

4.2.2 Phylogenetic analyses

Nucleotide sequence data for cytochrome oxidase one subunit (*COI*) region of the mitochondrial DNA and *18S rRNA* nuclear DNA gene locus were obtained for all species within the genus *Peripatopsis*. *COI* sequences were obtained from GENBANK following prior taxonomic work on the genus (Daniels & Ruhberg, 2010; Daniels *et al.*, 2013, 2009; McDonald & Daniels, 2012) while for the *18S* locus new sequences were generated since the earlier studies used a different primer pair combination. The new primer pairs 5F and 7R were obtained from Giribet *et al.* (1996). Sequence alignment was performed in CLUSTAL X (Thompson *et al.*, 1997). A phylogeny for the combined *COI* and *18S* haplotypes was estimated using a maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. A single representative sample per locality and taxon was used. MP was determined using MEGA v.7 (Kumar *et al.*, 2016). For the latter analysis trees were generated with Tree-Bisection-Regrafting (TBR) algorithm using 100 random additions of sequences and gaps were treated as characters. Nodal support for the MP analysis was obtained from a bootstrap test of 1000 replicates of datasets (Felsenstein, 1985). RAxML Blackbox (Stamatakis *et al.*, 2008) was used to conduct an ML analysis on the combined dataset. Akaike information criteria (AIC) (Akaike, 1973) were used to determine the best-fit maximum likelihood score. For the ML analyses, heuristic searches with TBR branch swapping and

100 random additions of taxa were performed. For the MP and ML analyses bootstrap values >75% were treated as strongly supported. MRBAYES 3.0b4 (Ronquist & Huelsenbeck, 2003) was used to investigate optimal tree space using Bayesian inferences for the combined dataset. JModelTest2 (Darriba *et al.*, 2012) was used to determine the best-fit substitution model for each gene locus for the Bayesian analysis. For each analysis, ten Markov Chain Monte Carlo (MCMC) simulations were run, starting from a random tree for five million generations, sampling from every 1000th tree. A 50% majority rule consensus tree was generated from the trees retained. After burnin, trees were discarded. Posterior probabilities (p*P*) for each node were estimated by the percentage of time the node was recovered. Posterior probability values < 0.95 were regarded as poorly resolved. The genus *Peripatopsis* was found to be sister to the Chilean genus *Metaperipatus* (Allwood *et al.*, 2010). Hence two *Metaperipatus species, M. inae and M. blainsvilli* were included as outgroups.

4.2.3 Divergence time estimation

Divergence times within the genus *Peripatopsis* were inferred using both the *COI* and *18S rDNA* partitions for the 56 taxa dataset. Divergence time estimation was performed using a Bayesian framework which employs a probabilistic model to define rates of sequence evolution of lineages over time. The latter uses the Markov Chain Monte Carlo (MCMC) method to derive clade ages as executed in the programme BEAST version 1.8.2 (Drummond *et al.*, 2012). A lognormal uncorrelated relaxed clock model was employed. For the *COI* and *18S rDNA* loci a mean mutation rate of 2% per Myr was assumed with a 95% interval of 1.4-2.6% per Myr, covering a wide range of published panarthropod rates (Knowlton & Weigt, 1998; Schubart *et al.*, 1998; Projecto-Garcia *et al.*, 2009). Despite the large credibility intervals retrieved by the latter approach, Daniels *et al.* (2016) states that the latter approach is necessary considering the large uncertainty of molecular clock calibration and represents a

conservative workaround to the dubious practice of biogeographic-based calibration. We applied the Yule process to the partitions as a tree prior because it is a simple model of speciation that is generally more appropriate when considering sequences from different species (Drummond & Bouckaert, 2015). Furthermore the GTR+Γ substitution model was employed. Two chains of 100 million generations were run on the dataset, with sampling being carried out every 10 000 generations. Stationary and effective sampling size (ESS) of parameters were investigated in Tracer 1.6 (Rambaut *et al.*, 2014); the initial 25% of samples were discarded as burnin, and sufficient ESS values > 300 were obtained for all parameters.

4.2.4 Ancestral area reconstruction

To determine a hypothesis of the geographical ancestral origins of the genus *Peripatopsis*, ancestral character state reconstruction for biogeographic areas based on South African geomorphic provinces (Partridge *et al.*, 2010) was carried out in Mesquite v3.04 (Maddison & Maddison, 2015). The present area of occurrence was coded for each taxon and corresponded to the following five geomorphic provinces: Atlantic Cape Fold Mountains, Syntax Zone Cape Fold Mountains, Southern Coastal Platform, East London Coastal Hinterland, Southern Coastal Hinterland (Partridge *et al.*, 2010). A likelihood reconstruction method was used to trace character history across the ultrametric BEAST tree in Mesquite. We used a Markov k-state 1 parameter model where the single parameter is the rate of change (Lewis, 2001). Character states were considered unequivocal when proportional likelihoods were ≥0.95.

4.2.5 Phylogenetic diversity, Evolutionary Distinctiveness, and Species Richness

Methods for estimating phylogenetic diversity have been divided in to two categories (Krajewski, 1994) whereby distance-based methods can be distinguished from node-based methods. Node-based methods are sensitive to phylogenetic branching order within a monophyletic group and the diversity contribution of a species is weighted by the inverse number of the nodes between that species and the root of the tree (Vane-Wright *et al.*, 1991). In contrast distance-based methods utilise phylogenetic trees with scaled branch-lengths, where the diversity of a subset of species is the length of the minimal spanning subtree that includes those species (Faith, 1992). We used the locality data for *Peripatopsis* in South Africa and the group's Bayesian phylogeny to perform a spatial analysis in BIODIVERSE (Laffan *et al.*, 2010). Species richness and phylogenetic diversity were calculated and displayed in ArcGIS 10.3. A one-way Analysis of Variance (Anova) was conducted for Faith's PD and an independent samples t-test was conducted for species richness values under the null hypothesis that mean PD/SR values of the five geomorphic provinces are equal. To assess the levels of taxonomic distinctness within *Peripatopsis* we measured Evolutionary Distinctness (ED) using the Tuatara v. 1.01 package for MESQUITE (Maddison & Mooers, 2007). The assessment was conducted using the ultrametric Beast tree. The measure was proposed by Isaac *et al.* (2007) and measures the length of the species' terminal branch plus its species-weighted shares of ancestral branches. A one-way Analysis of Variance (Anova) was conducted for the ED values under the null hypothesis that mean ED values of the five geomorphic provinces are equal.

4.2.6 Extent of occurrence, Area of occupancy, Extent of suitable habitat

Locality data were plotted onto a map using ArcGIS 10.3. Extent of occurrence (EOO) is defined as the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon (IUCN, 2012). EOO was

measured with a minimum convex polygon using the IUCN EOO calculator tool in ArcGIS 10.3 (IUNC spatial data resources). The minimum convex polygon (MCP) is the smallest polygon in which no internal angle exceeds 180° and which contains all the sites of occurrence. Hence the calculation is not possible for species with <3 localities.

Area of occupancy is defined as the area within its EOO which is occupied by a taxon. According to the IUCN (2012) the measure reflects the fact that a taxon will not usually occur throughout the area of its EOO, which may contain unsuitable or unoccupied habitats. Area of occurrence was calculated using a 4km² grid as recommended by the IUCN (2014) in ArcGIS 10.3. Extent of suitable habitat was calculated using the EOO minimum convex polygons for each species. Forest areas falling within the EOO were clipped and calculated using the South African National Vegetation Map (BGIS, 2012).

4.3 Results

4.3.1 Combined DNA sequence analyses (COI+18S rDNA)

The combined data yielded a total of approximately 1.2kb. The substitution model selected for the ML analyses using the AIC criteria (Akaike, 1973) was GTR+I+G (-lnL = 8118.86; AIC = 16477.71). The base frequency for the combined gene sequence was A = 23.15%, C = 20.05, G = 23.05, T = 33.75. The rate matrix was R(a)[A-C] = 0.53, R(b)[A-G] = 21.53, R(c)[A-T] = 8.84, R(d)[C-G] = 5.13, R(e)[C-T] = 7.59, R(f)[G-T] = 1, while the proportion of invariable sites (I) = 0.62, with a gamma shape distribution α=0.69. The substitution model for the *COI* locus using the AIC criteria was TPM3uf+I+G (-lnL = 6026.98; AIC = 12287.96). The base frequency for the gene fragment was A = 0.35%, C = 0.4%, G = 0.09%, T = 0.52%. The rate matrix was R(a)[A-C] = 4.59, R(b)[A-G] = 31.42, R(c)[A-T] = R(f)[G-T] = 1, R(d)[C-G] = 4.59, R(e)[C-T] = 31.42, while the proportion of invariable sites (I) = 0.46, with a gamma shape distribution α = 0.4. The substitution for the 18S rDNA locus

using the AIC criteria was TIM2+I+G (-InL = 1647.98; AIC = 3531.96). The base frequency for the gene fragment was A = 0.2%, C = 0.28%, G = 0.30%, T = 0.23%. The rate matrix was R(a)[A-C] = 0.41, R(b)[A-G] = 0.56, R(c)[A-T] = 0.41, R(d)[C-G] = R(f)[G-T] = 1, R(e)[C-T] = 1.59, and the proportion of invariable sites (I) = 0.64, with a gamma shape distribution $\alpha = 0.43$. The determination of evolutionary history using MP yielded two most parsimonious trees with a tree length of 1269 steps. The CI and RI for parsimony informative sites were 0.35 and 0.68 respectively. The three analytical methods (MP, ML, and BI) generated congruent tree topologies and supported the same clades in most cases. Hence the combined BI tree is shown and discussed (Fig. 2). The monophyly of *Peripatopsis* was well supported (1.00 p*P*/100%/100%). The two *P. clavigera* specimens from the Garden of Eden and Homtini localities formed a basal clade (0.97 p*P*/97%/99%) that was sister to a large clade comprising the remainder of the *Peripatopsis* genus.

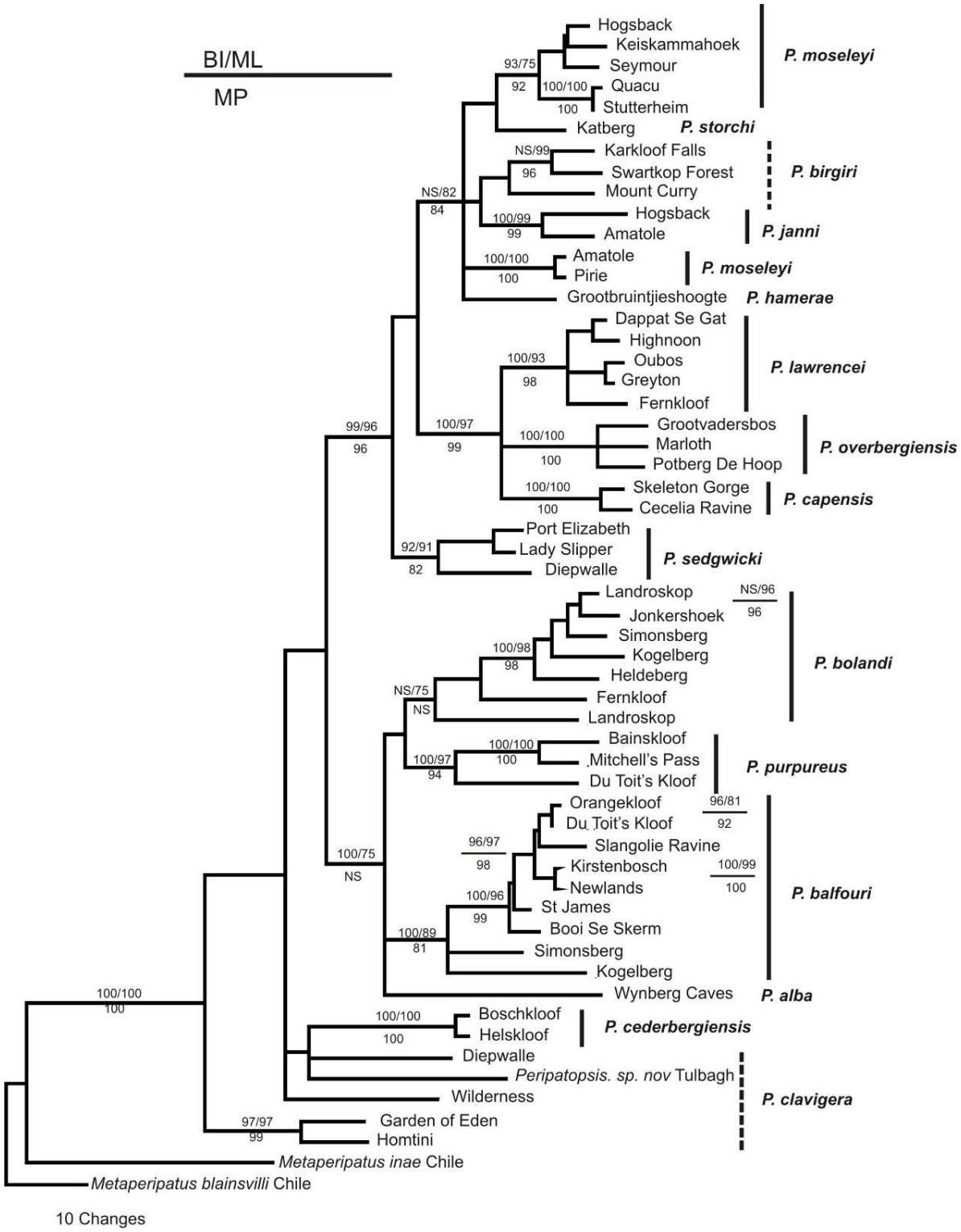
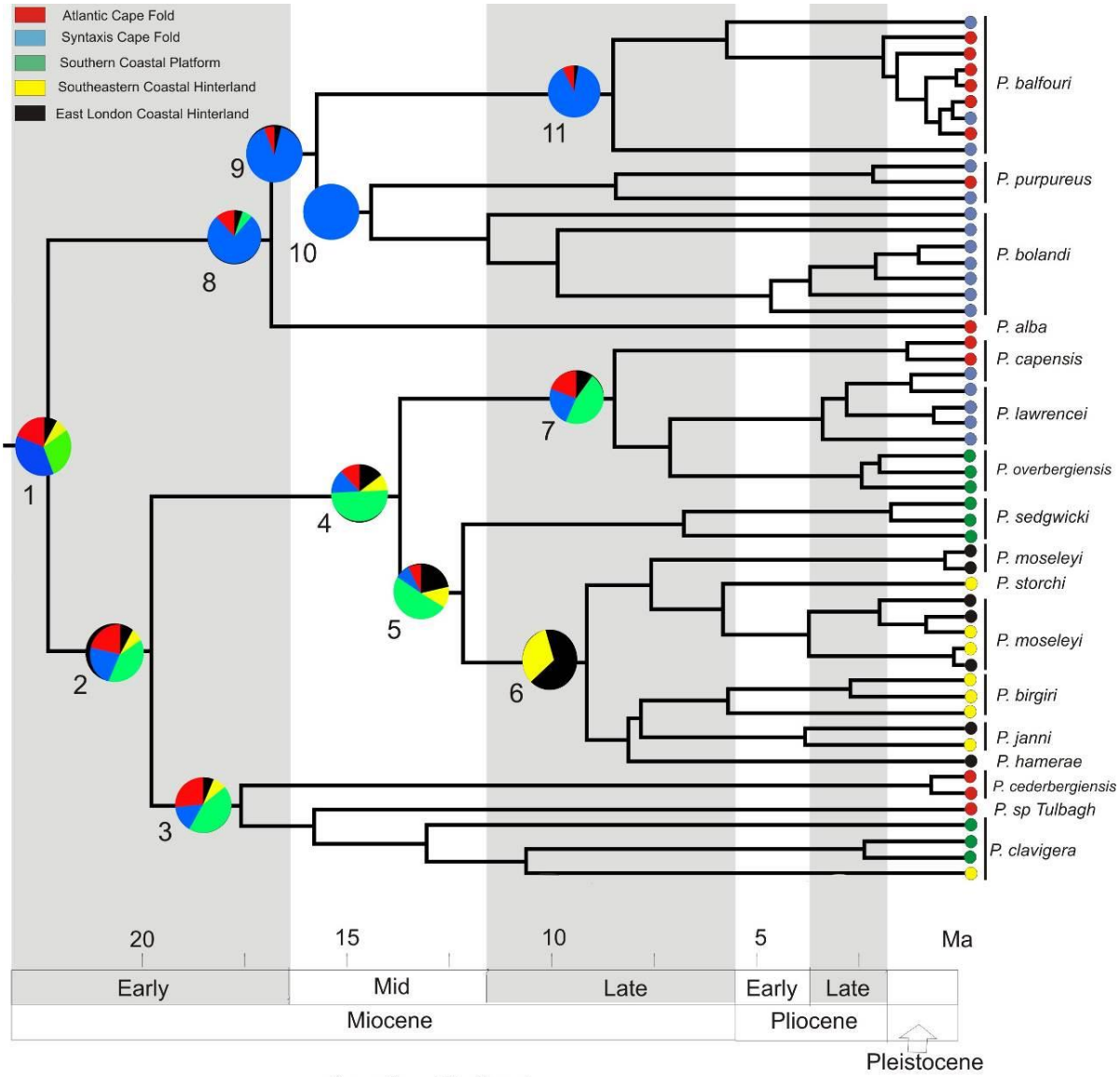


Figure 11: Bayesian inference topology for the combined DNA analyses amongst *Peripatopsis* species. The values above each node represent the posterior probability (*pP*) value derived from the Bayesian inference analyses and bootstrap values (%) for maximum likelihood (ML), while the values below each node represent those for maximum parsimony (MP). NS indicated nodes that were not statistically well supported (<75% and <0.95 pP).

The latter clade was unresolved and included a *P. clavigera* specimen from the Wilderness sister to an unresolved clade including *P. cederbergiensis* (Boschkloof and Helskloof, 1.00 p*P*/100%/100%), a *P. clavigera* specimen from Diepwalle, and the putative species from the Secret Falls locality in Tulbagh; also sister to the weakly supported major clade including the remainder of the *Peripatopsis* species (*P. alba, P.balfouri, P. purpureus, P. bolandi, P. sedgwicki, P. capensis, P. overbergiensis, P. lawrencei, P. hamerae, P. moseleyi, P. janni, P. birgiri,* and *P. storchi*). The latter included a well supported clade (1.00 p*P*/75%/NS) comprising the troglobitic *P. alba* retrieved as equidistant relative to *P. balfouri* (1.00 p*P*/89%/81%) and a weakly supported clade comprising *P. bolandi* (NS/75%/NS) and *P. purpureus* (1.00 p*P*/97%/94%). The second clade (1.00 p*P*/96%/96%) within the aforementioned major clade included a basal *P. sedgwicki* clade retrieved with good statistical support (0.92 p*P*/91/82) sister to a weakly supported clade comprising a further two clades representing Western Cape and Eastern Cape/KwaZulu-Natal species respectively. The Western Cape clade was retrieved with strong statistical support (1.00 p*P*/97%/99%) and included deeply divergent clades representing *P. capensis* (1.00 p*P*/100%/100%), *P. overbergiensis* (1.00 p*P*/100%/100%), and *P. lawrencei* (1.00 p*P*/93%/98%). The Eastern Cape/KwaZulu-Natal clade retrieved good support (NS/82%/84%) but internal clades were unresolved. With respect to the latter a *P. moseleyi* clade (1.00 pP/100%/100%) comprising a specimen from the Amatole Mountains together with a specimen from Pirie Forest was equidistant to and fell outside of a second *P. moseleyi* clade (0.93 p*P*/75%/92%) (Hogsback, Keiskammahoek, Seymour, Quacu, Stutterheim). The clade comprising *P. birgiri* specimens (Karkloof Falls, Swartkop Forest, Mount Curry) was not well supported.

4.3.2 Biogeographic analysis

The reconstruction of ancestral areas for the *Peripatopsis* genus suggests that the group originated in the Syntaxis Cape Fold Mountains geomorphic province during the early Miocene (Fig. 3).



Proportional likelihoods

Node number	ACF	SCF	SCP	SCH	ELCH	Age (Ma)	Lower 95%	Upper 95%
1	0.168	0.448	0.228	0.078	0.076	22	17.65	27.78
2	0.215	0.168	0.455	0.082	0.078	19.6	15.4	24.5
3	0.264	0.117	0.474	0.081	0.061	17.45	13.23	22.17
4	0.119	0.114	0.528	0.094	0.142	13.6	10.59	16.93
5	0.073	0.07	0.532	0.121	0.201	12.1	9.36	15.22
6	0.006	0.006	0.034	0.325	0.625	9.11	7.16	11.44
7	0.193	0.223	0.488	0.04	0.053	8.45	6.27	10.99
8	0.107	0.794	0.049	0.024	0.024	16.7	12.76	21.31
9	0.051	0.917	0.015	0.008	0.007	15.6	12.1	19.68
10	0.024	0.962	0.005	0.003	0.003	14.32	11.05	18.3
11	0.07	0.905	0.008	0.007	0.007	8.46	5.8	11.63

Figure 12: Ultrametric tree showing lineage diversification for the genus *Peripatopsis*. Likelihood optimisation probabilities for the ancestral areas indicated by pie charts at the major nodes. Highest likelihood for nodes 1-11 highlighted in the divergence time table. Terminal taxa are colour coded according to the area in which they occur.

The latter proportional likelihood was low (0.44) and the result should be considered equivocal. During this period the common ancestor diverged into two lineages occupying the Syntaxis Cape Fold and Southern Coastal Platform provinces. The Southern Coastal Platform lineage hence diverged into the ancestors of the contemporary clades comprising (*P. cederbergiensis*, *P. clavigera*, and the putative species from Tulbagh) and (*P. capensis, P. lawrencei, P. overbergiensis*, *P. sedgwicki, P. moseleyi, P. storchi, P. birgiri, P. janni*, and *P. hamerae* – hereafter called major south eastern clade). Near the boundary between the early-mid Miocene *P. cederbergiensis* diverged together with the Syntaxis Cape Fold lineage, which gave rise to the *P. alba* ancestor. During the mid-Miocene the putative Tulbagh species diverged from the *P. clavigera* complex, followed by the divergence of the main Syntaxis Cape Fold clade into the ancestor of *P. balfouri* and the ancestor of *P. purpureus* and *P. bolandi* both being restricted to the Syntaxis Cape Fold. Subsequently the latter ancestor diverged into the contemporary species clades.

This period also saw the divergence of the major south eastern clade into two lineages, one with contemporary species clades east of the ancestral area (Southern Coastal Platform) and the other with contemporary species clades within Southern Coastal Platform and west of the Southern Coastal Platform (Syntaxis Cape Fold and Atlantic Cape Fold). Towards the end of the mid-Miocene the ancestor of *P. clavigera* diverged followed by divergence of the clade east of the ancestral Southern Coastal Platform into the *P. sedgwicki* ancestor and the eastern clades. Several contemporary lineages emerged in the late Miocene. These included *P. balfouri*, *P. purpureus*, *P. bolandi*, *P. capensis, P. lawrencei, P. overbergiensis, P. sedgwicki, P. moseleyi, P. storchi, P. birgiri, P. jannie, and P. hamerae.* From the early Pliocene-Pleistocene there was an increase of diversification events across all taxa.

4.3.3 Phylogenetic Diversity, Evolutionary Distinctiveness, and Species Richness

To determine phylogenetic diversity (PD) we performed a spatial analysis on the Bayesian phylogenetic tree using locality data. A high level of phylogenetic diversity was retrieved for the Cape Peninsula region of the Atlantic and the Syntaxis sub-provinces of the Cape Fold Mountains in the Western Cape and to a lesser extent at the boundary between the East London Coastal Hinterland and the Southeastern Coastal Hinterland in the Eastern Cape (Fig. 4). Low to intermediate levels of phylogenetic diversity were recovered for localities falling between the Cape Fold Mountains and the Eastern Cape centres. Phylogenetic diversity was low in the north Atlantic sub-province of the Cape Fold Mountains which included the basal species *P. cederbergiensis* and the putative *Peripatopsis* species from Tulbagh. A one-way between subjects Anova was carried out to compare the average PD, ED, and SR for each geomorphic province. There was not a significant difference for average PD at the $p<0.05$ level [$F (4, 47) = 1.937$, $p = 0.119$] (Table. 2). Similarly there was no significant difference between means for ED at the $p<0.05$ level [$F (4, 49) = 0.969$, $p = 0.43$] (Table. 2), as well as for SR at the $p<0.05$ level [$M = 3.6$, $SD = 0.547$ conditions; $t (4) = 2.77$, $p = 0.05$].

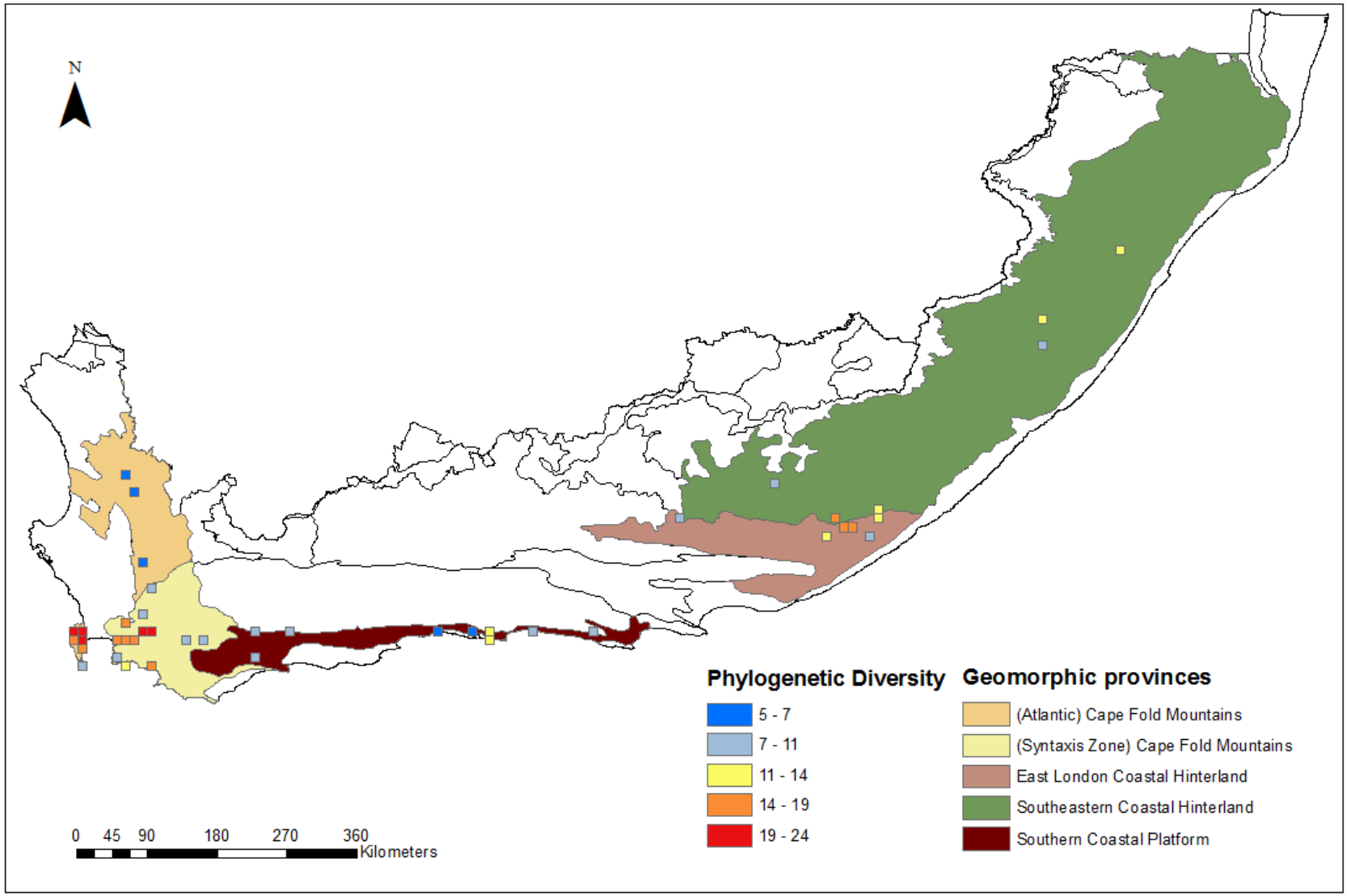


Figure 13: Distribution of phylogenetic diversity for *Peripatopsis* within the geomorphic provinces represented within its range

Table 6: Results for the ANOVAs for phylogenetic diversity and evolutionary distinctiveness for the five geomorphic provinces represented in the Peripatopsis distribution. When F<F crit (in bold), do not reject null that means are equal.

Phylogenetic Diversity

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	42.45517	4	10.61379	**1.937082**	0.119855	**2.56954**
Within Groups	257.5256	47	5.479268			
Total	299.9808	51				

Evolutionary distinctiveness

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	45.26132	4	11.31533	**0.969063**	0.432974	**2.561124**
Within Groups	572.152	49	11.67657			
Total	617.4133	53				

4.3.4 Extent of occurrence, area of occupancy, and extent of suitable habitat

A total of 70 occurrence records were used to calculate extent of occurrence, area of occupancy, and the extent of suitable habitat. Extent of occurrence for each species was calculated using the minimum convex polygon method. Applying the IUCN criteria to the extent of occurrence measure for *Peripatopsis* across South Africa resulted in 1 Extinct (EX), 1 Critically Endangered (CR), 8 Endangered (E), 1 Vulnerable (V), and 6 Data Deficient (DD) species (Table 3). Area of occupancy was calculated using a 4km² fishnet grid. Applying IUCN criteria to the latter measure resulted in 10 Endangered, 6 Critically Endangered and 1 Extinct species (Table 3). The area of occupancy takes into account that a taxon will not usually occur throughout its extent of occurrence, which may contain unsuitable or unoccupied habitats (IUCN, 2012). Hence the threat categories were reconciled according to which measure yielded the highest category to reflect the most appropriate status and included 1 Extinct, 7 Critically Endangered, 8 Endangered, and 1 Vulnerable. Forest areas for each species were calculated using the South African Vegetation Map. The extent of suitable habitat ranged from a minimum of 0.25km² to a maximum of 228.53km² for *Peripatopsis* species.

Table 7: IUCN Red List category for 16 *Peripatopsis* species using extent of occurrence, area of occupancy, and extent of suitable habitat

Species	EOO (Km^2)	AOO (Km^2)	Forest habitat size (Km^2)	IUCN criteria	Category
P. capensis	14.02	24.00	1.87	B1ab(iii)	CR
P. balfouri	2458.20	44.00	2.89	B1ab(iii)+B2ab(iii)	EN
P.bolandi	860.00	24.00	0.25	B1ab(iii)+B2ab(iii)	EN
P. purpureus	202.70	12.00	0.53	B1ab(iii)+B2ab(iii)	EN
P. lawrencei	5381.13	52.00	8.07	B1ab(iii)+B2ab(iii)	VU
P. overbergiensis	537.10	12.00	2.60	B1ab(iii)+B2ab(iii)	EN
P. clavigera	303.11	24.00	167.11	B1ab(iii)+B2ab(iii)	EN
P. sedgwicki	596.46	20.00	228.53	B1ab(iii)+B2ab(iii)	EN
P. moseleyi	1397.67	28.00	155.13	B1ab(iii)+B2ab(iii)	EN
P. birgiri	1342.62	12.00	36.84	B1ab(iii)+B2ab(iii)	EN
P. cederbergiensis	-	8.00	-	B2ab(iii)	CR
P. hamerae	-	4.00	-	B2ab(iii)	CR
P. storchi	-	4.00	-	B2ab(iii)	CR
P. alba	-	4.00	-	B2ab(iii)	CR
P. janni	-	8.00	-	B2ab(iii)	CR
P. sp. Tulbagh	-	4.00	-	B2ab(iii)	CR
P. leonina	-	-	-	-	EX

4.4 Discussion

4.4.1 Divergence time and biogeography

The ancestor of the *Peripatopsis* genus originated in the region which currently spans that Syntaxis Cape Fold and Southern Coastal Platform geomorphic provinces (Fig. 3), while cladogenesis within the group started in the early Miocene and coincided with progressive climatic and geotectonic amelioration in southern Africa (Linder, 2003; Cowling, *et al.* 2009; Coetzee, 1983; Axelrod & Raven, 1978; Tyson, 1986; King, 1978). Despite using a different dating technique (fossil calibration) Daniels *et al.* (2009) similarly retrieved cladogenesis within the group during this period. A significant expansion in the East Antarctic ice sheet was brought about by a period of Earth low orbital eccentricity between 13.87 and 13.84 Ma (Holbourn *et al.*, 2005). Antarctic glaciation increasingly locked marine water at the south polar ice cap resulting in marine regressions and falling sea levels (Hendey, 1983; Tyson & Partridge, 2000). A reduction in the surface area of ocean waters at the coast would have reduced onshore precipitation with a concomitant increase in aridity. Furthermore the strengthening of the anti-cyclonic system led to the development of southerly and south-easterly trade winds which initiated the Benguela upwelling system (12-3 Ma) on the west coast of southern Africa, a further driver of aridity from the south west (Deacon, 1983; Linder, 2003). By the early to mid-Miocene (~16 Ma) the troglobitic *P. alba* had assumed its caverniculous evolutionary trajectory in the moist and humid cave systems of the Cape Peninsula mountains (Sharratt et al., 2000), a time of divergence (~10 Ma) roughly congruent with Daniels *et al.* (2013). The divergence of the clade comprising *P. cederbergiensis*, *P. clavigera*, and the putative species from Tulbagh during the early to mid-Miocene strengthens the hypothesis for the distribution of widespread forest habitat across the southern Africa and specifically a link between the southern Cape and the Cederberg region for the latter period (Daniels *et al.* 2009; Daniels *et al.* 2013). Evidence for the latter is further supported by the phylogenetic relationship between sister taxa of the freshwater fish genus *Pseudobarbus* which indicates a link between river

systems of the southern Afrotemperate forests and the Olifants river system on the west coast (adjacent to the Cederberg region) (Swartz *et al.*, 2009). According to Hendey (1983) the picture of the early Pliocene (~5 Ma) emerges as one of relatively low temperatures and rainfall with forests confined to well-watered mountain slopes. The late Miocene-early Pliocene (~15-8 Ma) saw the diversification of *Peripatopsis* into the ancestral lineages of contemporary species (Daniels *et al.*, 2009) including *P. balfouri sensu latu, P. capensis sensu latu, P. sedgwicki sensu latu, and P. moseleyi sensu latu*. The latter diversification episode further coincides with a period of geotectonic uplift which lasted approximately two million years and amounted to uplift of 600-900m in eastern South Africa (Cowling *et al.*, 2009) and less intense uplift of 150-300m in the west (Partridge & Maud, 2000). According to Cowling *et al.* (2009) the erosion cycle which followed uplift had a profound impact on the Cape scenery leading to the fragmentation of the Cape Fold Mountains into several mountain blocks separated by low-lying valleys, sheltered ravines, and deeply incised gorges. From the Pliocene to Pleistocene (~5-0.1 Ma) *Peripatopsis* had undergone a final phase of diversification reflecting speciation in allopatry due to more pronounced xeric climatic conditions and increasingly isolated forest habitat (Daniels *et al.* 2009).

4.4.2 Systematics

The genus *Peripatopsis* forms a well-supported monophyletic grouping. While twelve described species within the genus are monophyletic and consistent with previous molecular systematic studies (McDonald *et al.*, 2012; Daniels *et al.*, 2013; Ruhberg & Daniels, 2013) three species, *P. moseleyi*, *P. birgiri* and *P. clavigera*, are non-monophyletic and/or not well supported. According to Ruhberg & Daniels (2013) *P. moseleyi* is monophyletic for the combined *COI* and *18S rDNA* loci, while for the topology of *COI* alone Daniels & Ruhberg (2010) retrieved a similar phylogenetic tree structure as the present study, whereby the specimens from Amathole Mountains and Pirie Forest do not form part of the main monophyletic grouping (see Daniels & Ruhberg (2010) fig. 2). Based

on morphology and scanning electron microscopy Ruhberg & Daniels (2013) classified the latter specimens as *P. moseleyi*. The use of different segments of the *18S rDNA* gene could explain the discordant phylo-groupings. The use of additional markers (e.g. *12 rRNA*) and a bigger sample size could further elucidate taxonomic status of *P. moseleyi* and could facilitate the approximation towards a species tree (Baum, 1992). Crandall (1998) posits an explanation for the non-monophyly of mtDNA haplotypes within a species as the problem of gene trees reflecting species (or population) trees. According to Crandall (1998) the results of theoretical population genetic work have indicated that for a certain period after the divergence of two or more populations, there is a high probability that populations may show non-monophyletic relationships for a specific gene. Hence the gene genealogy may not accurately reflect the population divergence. Similarly, the monophyly of *P. birgiri* (Ruhberg & Daniels, 2013) was not supported in the present study and was a reflection of the *COI* phylogeny in (Daniels & Ruhberg 2010) again suggesting that the choice of markers may play a role. A reappraisal of the chosen species concept may be beneficial as Mount Currie specimens and the clade comprising Amatole Mountain and Pirie Forest specimens could be deemed "separately evolving lineages" as per the criteria employed by McDonald *et al.* (2012) in the description of *P. lawrencei* and *P. overbergiensis*. Failing the latter, the designation of evolutionary significant units (ESU's) should be considered to aid conservation decision-making (Moritz, 1994). *P. lawrencei*, *P. overbergiensis*, and *P. capensis* formed well supported monophyletic clades which underpins the stable phylogeny of the species. Myburgh & Daniels (2015) undertook a fine-scale phylogenetic study to elucidate the relationships between the three main *P. overbergiensis* localities (Grootvadersbos, Marloth, and Potberg De Hoop). The latter authors found insufficient genetic differentiation to consider *P. overbergensis* a species complex comprising multiple lineages. *P. capensis* and *P. lawrencei* could also benefit from intra-specific taxonomic scrutiny, particularly the latter which has a wide, fragmented distribution across the heterogeneous central Cape Fold Mountains (Mcdonald & Daniels 2012). The three *P. sedgwicki* specimens formed a statistically well supported monophyletic grouping. The deep divergence

between the Diepwalle and Port Elizabeth/Lady Slipper specimens, the geographic separation between the localities in the Southern Cape and Eastern Cape respectively, and limited sampling raises the possibility that *P. sedgwicki* is a species complex comprising at least two genealogically distinct lineages. Daniels *et al.* (2009) included a bigger sample size employing three gene markers (*COI, 12S rRNA,* and *18S rDNA*) and found that *P. sedwicki* was a 'narrow-distribution' species complex comprising two clades divided into the aforementioned geographic regions. *P. bolandi, P. purpureus,* and *P. balfouri* formed moderate to well supported monophyletic groups, although a high level of phylogenetic structuring was revealed by the tree topology. Deep divergences and long terminal nodes demonstrate that *P. bolandi* and *P. purpureus* are subjected to the mechanism of allopatric speciation to a great degree. Daniels *et al.* (2013) reached a similar conclusion using a bigger sample size. The taxonomy of the latter species in addition to *P. balfouri* which displays a lower level of phylogenetic structure among its Cape Peninsula localities but shares a clade with geographically distant and isolated Simonsberg and Kogelberg localities, will benefit from fine-scale molecular studies and population genetic studies to ascertain the level of geneflow among localities. *P. cederbergiensis* (Boschkloof and Helskloof localities) formed a well supported clade and was sister to the Diepwalle (*P. clavigera*) specimen and the specimen representing a tentative new species from Secret Falls in Tulbagh. The recent discovery of the specimen at the latter locality suggests that several sampling gaps exist between the aforementioned mountainous regions (Daniels pers comm). *P. clavigera* specimens were non-monophyletic and not resolved. The polyphyletic nature of *P. clavigera* on the topology suggests that the taxon may harbor multiple species or evolutionary significant units.

4.4.3 Diversity patterns and conservation

The recommendation for IUCN threat status of species within *Peripatopsis* has changed dramatically in light of recent taxonomic endeavors on the group. Where the previous assessment

assigned an IUCN threat status to three species out of seven (Hamer, 2003a, 2003b, 2003c), the present study recommends a threat status for all sixteen described species within the genus namely 7 critically endangered, 8 endangered, and 1 vulnerable (Table 2). Phylogenetic diversity is concentrated on the Cape Fold Mountains in the Western Cape with a smaller centre of PD in the Amatole forests in the Eastern Cape at the interface of the East London Coastal Hinterland and the Southeastern Coastal Hinterland geologic provinces (Fig. 3).

Phylogenetic diversity is not uniformly distributed for *Peripatopsis* species across South Africa. Phylogenetic diversity is highest in the southwestern regions of the Cape Fold Mountains of the Western Cape implying a rich evolutionary history for this region (Fig. 3). Species diversification in this region occurred primarily during the Pliocene/Pleistocene epochs due to climatic ameliorations in the Western Cape. The latter coincided with the development of the pyrophytic Cape fynbos along fire pathways and led to high levels of allopatric speciation for lineages in the region (Bytebier *et al.*, 2011; Linder 2003). Areas with lower phylogenetic diversity including the Overberg region, Southern Cape, Amatole Mistbelt, and KwaZulu-Natal Mistbelt correspond with areas of stable ancient forest refugia (Lawes *et al.*, 2007). A similar pattern was found for tropical flora in Australia where areas with a higher than expected phylogenetic diversity corresponded to extant rainforest that was unstable during glacial periods, while lower than expected phylogenetic diversity was correlated with rain forest refugia that have remained stable throughout the last glacial cycle (Costion *et al.*, 2015). Conversely, the smaller hotspot of phylogenetic diversity in the Amatole forests may represent an exception to the latter pattern where a high level of phylogenetic diversification was observed for the *P. moseleyi sensu latu* species complex (Daniels & Ruhberg, 2010). A study on brevicipitid frogs from the Eastern Afromontane Biodiversity Region by Loader *et al.* (2014) found that persistent forests accumulate more diversity than regions with intermittent or less stable forests. The low levels of phylogenetic diversity retrieved for the basal species *P. clavigera*, *P. cederbergiensis*, and the putative species from Secret Falls in Tulbagh can be

explained by longer branch lengths and a lack of closely related sister taxa compared to species on the rest of the topology. Tolley *et al.* (2011) considers the latter a hallmark of palaeoendemism where long branch lengths reflect a distinct evolutionary history. Species richness mirrors hotspots of phylogenetic diversity across the *Peripatopsis* distribution, but provides a lower resolution than the latter measure. The major hotspot of phylogenetic diversity on the Cape Fold Mountains is reflected to a lesser extent by the species richness measure in the eastern part of its extent, while the smaller hotspot in the Amatole Forests is also less pronounced. Over a large series of samples Barker (2002) found phylogenetic diversity to be correlated with species richness in an approximately linear fashion. However despite the linear correlation phylogenetic diversity values can vary substantially between communities of similar richness and therefore provides a useful complement to richness in differentiating biodiversity value (Barker, 2002). We consider our findings for the spatial geographic analysis of *Peripatopsis* diversity a working hypothesis as we found no significant differences for phylogenetic diversity (PD), evolutionary distinctiveness (ED), and species richness (SR) for the group. A larger sample size is likely to reveal significant differences.

Molecular taxonomic scrutiny on *Peripatopsis* has led to tentatively higher IUCN threat status across the group. Former species complexes with wide distributions including *P. capensis sensu latu*, *P. balfouri sensu latu*, and *P. moseleyi sensu latu* were shown to comprise multiple cryptic lineages (Daniels *et al.*, 2009; McDonald & Daniels, 2012; Daniels & Ruhberg, 2010), leading to the description of several new species (McDonald *et al.*, 2012; Daniels *et al.*, 2013; Ruhberg & Daniels, 2013). These species are now characterised by significantly narrower distributions rendering them vulnerable to extinction in varying degrees. For example, *P. capensis* previously occupied a distribution ranging from the Cape Peninsula in the Western Cape to the Overberg region in the Southern Cape (Hamer *et al.*, 1997). The latter species is now limited to the biogeographically isolated Cape Peninsula with an extent of occurrence of 14km² and a threat

category of critically endangered if IUCN criteria is employed (Table. 2) (McDonald & Daniels, 2012; IUCN, 2000). Similarly, *P. moseleyi* was distributed from the Eastern Cape to KwaZulu-Natal (Hamer *et al.*, 1997). Following molecular studies and closer morphological scrutiny the latter species was found to be limited to the Eastern Cape and hence categorised as endangered. Samways (2002) notes the trend whereby species which decline in geographic range result in a raise in the Red List category. The taxonomic progress leading up to the IUCN recommendations suggested by the present study follows a similar trajectory to studies carried out on South African Odonata (dragonflies) albeit at a smaller scale (McGeoch, 2002). The number of scientifically described Odonata species in South Africa has increased progressively over the past 95 years and enabled thorough conservation assessments and new national records (Samways, 2002). However, the threats facing *Peripatopsis* may be underestimated considering that species within the group are habitat specialists which are largely confined to Afromontane forests across its distribution. An assessment of the extent of suitable habitat within the group has revealed that species occupy significantly smaller habitat pockets within the extent of occurrence and area of occupancy (Table 2). For example, *P. balfouri* has an extent of occurrence of 2458.2km² while the extent of suitable forest habitat within the latter area is 2.89km². Similarly, *P. lawrencei* has an area of occupancy of 52km² and an area of suitable habitat covering 8.07km². In addition to their fragmentary occurrence, forest habitats face several threats which could impact the habitat specialists they harbour. Pryke & Samways (2008) documented the impact of the invasive Argentine ant which uses waterways on the Cape Peninsula to invade forests and hence impact forest invertebrate diversity. According to Berliner (2005) forest are under threat from a number of land-use pressures including coastal development, mining, agriculture, and over-harvesting for subsistence or illegal commercial use. The Ngele mistbelt forest in KwaZulu-Natal harbouring the critically endangered *Opistopatus roseus* faces several threats which are characteristic of conservation threats facing *Peripatopsis* in general. Daniels (2011) cites anthropogenic activities including the logging of indigenous trees, construction of a national highway, commercial timber plantations, and the introduction of alien

plant species which result in the fragmentation of habitat and potential range contraction for the latter species. Habitat and range contraction is likely to be more pronounced due to anthropogenically induced climate change which has reached its highest levels in the last 1400 years (IPCC, 2014). According to Eeley *et al.* (1999) forest ecosystems are spatially and temporally dynamic and respond relatively rapidly to climatic change.

Considering the high levels of phylogenetic diversity and species richness obtained for the *Peripatopsis* species on the Cape Fold Mountains, this region should be prioritized for conservation efforts. The smaller centre of phylogenetic diversity in the Eastern Cape should be the second priority for conservation. Winter *et al.* (2013) argues that phylogenetic diversity can be used as a proxy for distinct functional traits and that the loss of evolutionary distinct species could result in an irreversible loss of functions for entire ecosystems. Several species geographic ranges have been reduced with a concomitant elevation in the Red List category. *P. capensis* and *P. alba* is of particular concern with both having a status of critically endangered. The latter species are restricted to the Cape Peninsula which is surrounded by the Cape Town urban area and is increasingly being subjected to human activities including hiking, caving, mountain biking, and religious rituals (Pers obs.). The cave-dwelling *P. alba* is a point endemic known only from the Wynberg cave system. According to Sharratt *et al.* (2000) human visitation is currently a major threat to the Table Mountain cave fauna and can disrupt the environmental conditions that are essential for the maintenance of troglobite populations. Currently species in the genus occur within areas under nature conservation protection or privately owned properties. However, indigenous forests are currently threatened by alien invasive flora, encroachment form timber plantations, and fire events. It is recommended that the latter factors be considered in future conservation plans.

Chapter 5

5. Conclusions

The present study shows that traditional taxonomy using gross morphological assessment has underestimated diversity within the genus *Peripatopsis* and specifically the *Peripatopsis capensis* species complex. Molecular analysis using DNA markers is an advanced approach which reveals higher levels of diversity than morphological methods. The former also enables the testing of biogeographical hypothesis and the application of phylogenetic diversity indexes to strengthen conservation assessments. Yet, differentiating characters based on gross morphological analysis still has an important role to play in species delineation.

The application of molecular techniques and scanning electron microscopy to the *Peripatopsis capensis* species complex reveals the presence of two novel species with a high sequence divergence, differentiating morphological characters, and geographical exclusivity. High levels of sequence divergence in comparison with findings for other genera (e.g. Australian and New Zealand taxa) support species recognition within the framework of the phylogenetic species concept. The latter was closely supported by geographical information which suggested barriers to genetic dispersal. The use of scanning electron microscopy provides limited differentiating morphological characters largely associated with dermal papillae, male genital pads, and pre-genital legs while traditional gross morphological characters like dorsal colour and number of leg pairs have low discriminatory power. The latter findings are in accordance with other species complexes in the genus namely *P. balfouri* and *P. moseleyi* where three and four novel species were described respectively. Further studies should focus on the identification of additional characters with taxonomic value. While the taxonomy of the genus will benefit from closer taxonomic scrutiny of minor complexes including *P. clavigera* and *P. sedgwicki*, it would also be worthwhile to examine population level genetic structure throughout the genus. For example, the high level of genetic

structure in the geographically widespread *P. lawrencei* which occurs on the heterogeneous Central Cape Fold Mountains warrants further study.

The present study adds to a growing body of literature which shows that species diversification within the South African montane faunal element was driven by Miocene/Pliocene climatic amelioration. The *P. capensis* species complex serves as a model for how the genus tracked expansion and contraction cycles of forest habitat across South Africa. Future studies should explore patterns for the whole genus to map the development of the genus through space and time. The latter endeavour should draw on the present study as a starting hypothesis which further suggests that the genus originated in the southern cape of South Africa with a series of colonization and recolonization routes along Afromontane belts in the region.

From the present study biogeographic histories has emerged as a significant driver of diversity patterns within *Peripatopsis*. Consequently different phylogenetic diversity indexes enable a nuanced assessment and strengthens biogeographic hypothesis. The employment in this study of the phylogenetic diversity index developed by Faith (1992) highlighted the Cape Peninsula and the Central Cape Fold mountains as regions with a rich evolutionary history and potentially high evolutionary potential. We consider the latter a working hypothesis as no significant differences were found for PD, ED, and SR for the geomorphologic provinces harbouring *Peripatopsis*. Further work should include sampling gaps and a larger sample size.

In contrast with earlier work on the conservation of the genus which assigned IUCN redlist categories to three species, the present study assigns a redlist status to all species including those newly described. The description of eight new species within the genus has led to range contractions and hence elevations of redlist status across the group. Two species, namely *P. capensis* and *P. alba*, require urgent conservation attention due to the threat of urban encroachment.

References

Acocks, J.P.H. (1953) Veld Types of South Africa. *Memoirs of the Botanical Survey of South Africa*. **28**, 1-192.

Adelana, S., Xu, Y. & Vrbka, P. (2010) A conceptual model for the development and management of the Cape Flats aquifer, South Africa. *Water SA*, **36**, 461-474.

Agapow P.M. & Crozier R. (1998) Conserve 3.2.1. Department of Human Genetics and Variation, LaTrobe University, Melbourne, Australia.

Akaike, H. (1973) Information theory as an extension of maximum likelihood principle. In: Petrov B.N. & Csake F. (eds). *Second international Symposium on Information theory. Akademiai Kaido. Budapest*: 267-281.

Allwood, J., Gleeson, D., Mayer, G., Daniels, S, Beggs, J.R. & Buckley, T.R. (2010) Support for vicariant origins of the New Zealand Onychophora. *Journal of Biogeography*, **37**, 669-681.

Alston, K. P. & Richardson, D. M. (2006). The roles of habitat features, disturbance, and distance from putative source populations in structuring alien plant invasions at the urban/wildland interface on the Cape Peninsula, South Africa. *Biological Conservation.* **132**: 183-198.

Álvarez-Presas, M., Carbayo, F., Rozas, J. & Riutort, M. (2011) Land planarians (Platyhelminthes) as a model organism for fine-scale phylogeographic studies: understanding patterns of biodiversity in the Brazilian Atlantic Forest hotspot. *Journal of Evolutionary Biology*, **24**, 887-896.

Alston. K.P. & Richardson, D.M. (2006) The roles of habitat features, disturbance, and distance from putative source populations in structuring alien plant invasions at the urban/wildland interface on the Cape Peninsula, South Africa. *Biological Conservation*, **132**, 183-198.

Avise, J.C. (2004) *Molecular Markers, Natural History, and Evolution* (Second Edition). Sinauer Associates, Sunderland.

Axelrod, D.I., Raven, P.H. (1978) Late Cretaceous and Tertiary vegetation history of Africa. In: Biogeography and ecology of southern Africa, Springer, Netherlands, pp.77-130.

Baillie, J. E. M., Hilton-Taylor, C., Stuart, S. N. (Editors) 2004. 2004 IUCN Red List of Threatened Species. A Global Species Assessment. IUCN, Gland, Switzerland and Cambridge, UK.

Ballard, J. W. O., Olsen, G. J., Faith, D. P., Odgers, W. A., Rowell, D. M., Atkinson, P. W. (1992) Evidence from 12S ribosomal RNA sequences that onychophorans are modified Arthropods. *Science*, **258**, 1345-1347.

Barker, G. M. (2002) Phylogenetic diversity: A quantitative framework for measurement of priority and achievement in biodiversity conservation. *Biological Journal of the Linnean Society*, **76**, 165-194.

Barnard K. H. (1916) Contributions to the crustacean fauna of South Africa. 5. *The Amphipoda. Annals of the South African Museum*, **15**, 105-302.

Baum, D. (1992) Phylogenetic species concepts. *Trends in Ecology & Evolution*, **7**, 1-2.

Beenaerts, N., Pethiyagoda, R., NG, P. K. L., Yeo, D. C. J, Bex, G. J., Bahir, M. M., Artois, T. (2009) Phylogenetic diversity of Sri Lankan freshwater crabs and its implications for conservation. *Molecular Ecology*, **19**, 183-196.

Berliner, D. (2005) Systematic conservation planning for the forest biome of South Africa. Department of Water Affairs and Forestry report.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., Das Indraneil (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, **22**, 148-155.

Bouvier, E. L. (1907) Monographie des Onychophores. *Annales de Sciences Naturelles.Zoologie*, **5**, 61-31.

Boyer, S.L., Baker, J.M. & Giribet, G. (2007) Deep genetic divergences in *Aoraki denticulate* (Arachnida, Opiliones, Cyphophthalmi) a widespread 'mite harvestman' defies DNA taxonomy. *Molecular Ecology*, **16**, 4999-5016.

Brain, C.K. & Sillen, A. (1988) Evidence from the Swartkrans cave for the earliest use of fire. *Nature*, **336**, 464-466.

Brinck, P. (1957) *Onychophora*. In: Hanstrom B, Brinck P, Rudebeck G, eds, South African Animal Life, Results of theLund University Expedition in 1950-1951. Vol 4 Uppsala, Almquist & Wiksell.

Briscoe, D. A., & Tait, N. N. (1995). Allozyme evidence for extensive and ancient radiations in Australian Onychphora. *Zoological Journal of the Linnean Society.* **114**: 91-102.

Brower, A.V.Z. (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences, USA*, **91**, 6491-6495.

Butchart, S. H. M. et al. (2010). Global biodiversity: indicators of recent declines. *Science*, **328**, 1164–1168.

Bytebier, B., Antonelli, A., Bellsted, D.U. & Linder, H.P. (2011) Estimating the age of fire in the Cape flora of South Africa from an orchid phylogeny. *Proceedings of the Royal Society B*, **278**, 188-195.

Cardoso, P., Erwin, T. L., Borges, P. A. V., New, T. R. (2011) The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, **144**, 2647–2655.

Carnaval, A.C., Hickerson, M.J., Haddad, C.F.B., Rodrigues, M.T. & Moritz, C. (2009) Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*, **323**, 785-789.

Castley, J.G. & Kerley, G.I.H. (1996) The paradox of forest conservation in South Africa. *Forest Ecology and Management*, **85**, 35-46.

Chase, B.M. & Meadows, M.E. (2007) Late Quaternary dynamics of southern Africa's winter rainfall zone. *Earth Science Reviews*, **84**, 103-138.

Clusella-Trullas, S. & Chown, S. L. (2008) Investigating onychophoran gas exchange and water balance as a means to inform current controversies in arthropod physiology. *Journal of experimental biology*, **211**, 3139–3146.

Coetzee, J. A. (1983) Intimations of the tertiary vegetation of southern Africa. *Bothalia*, **14**, 345-354.

Cook, L. G., Edwards, R.D., Crisp, M.D., & Hardy, N. B. (2010). Need morphology always be required for new species descriptions? *Invertebrate Systematics.* **24:** 322-326.

Costion, C. M., Edwards, W. F., Ford, A. J., Metcalfe, D. J., Cross, H. B., Harrington, M. G., Richardson, M. G., Hilbert, J. E., Lowe, D. W., Crayn, D. M. (2015) Using phylogenetic diversity to identify ancient rain forest refugia and diversification zones in a biodiversity hotspot. *Diversity and Distributions*, **21**, 279-289.

Cowling, R.M., Holmes, P.M. & Rebelo, A.G. (1992) *Plant diversity and endemism. Ecology of fynbos. Nutrients, fire and diversity* (ed. By R.M. Cowling), Oxford University Press, Cape Town.

Cowling, R.M. & Lombard, A.T. (2002) Heterogeneity, speciation/extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. *Diversity and Distributions*, **8**, 163-179.

Cowling, R.M., Pressey R.L., Rouget M. & Lombard, A.T. (2003) A conservation plan for a global biodiversity hotspot- the Cape Floristic Region, South Africa. *Biological Conservation*, **112**, 191-216.

Cowling, R.M., Procheş, S. & Partridge, T.C. (2009) Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. *Molecular Phylogenetics and Evolution*, **51**, 64-74.

Cowling, R.M., Richardson, D.M. & Pierce, S.M. (1997) *Vegetation of southern Africa*, Cambridge University Press, Cambridge.

Cowling, R.M., Richardson, D.M., Schulze, R.J., Hoffman, M.T., Midgley, J.J. & Hilton-Taylor, C. (1997) *Species diversity at the regional scale*. Vegetation of Southern Africa (ed. By R.M. Cowling, D.M. Richardson and S.M. Pierce), pp. 447-473. Cambridge University Press, Cambridge.

Cracraft, J. (1989) *Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation*: In: Otte D, Endler, JA, eds. Speciation and its consequences. Sunderland, MA: Sinauer Associates, 3-87.

Crandall, K. (1998) Conservation phylogenetics of Ozark crayfishes: Assigning priorities for aquatic habitat protection. *Biological Conservation*, **84**, 107-117.

Daniels, S. R., Stewart, B. A., Gibbons, M. J. (1998) *Potamonautes granularis* sp. nov. (Brachyura, Potamonautidae), a new cryptic species of river crab from the Olifants River systems, South Africa. *Crustaceana*, **71**, 885-903.

Daniels, S.R., Stewart, B.A. & Burmeister, L. (2001) Geographic patterns of genetic and morphological divergence amongst populations of a river crab (Decapoda, Potamonautidae) with the description of a new species from mountain streams in the Western Cape, South Africa. *Zoologica Scripta*, **30**, 181-197.

Daniels, S. R., Gouws, G., Stewart, B. A., Coke, M. (2003) Molecular and morphometric data demonstrate the presence of cryptic lineages among freshwater crabs (Decapoda: Potamonautidae:

Potamonautes) from the Drakensberg Mountains, South Africa. *Biological Journal of the Linnaen Society*, **78**: 129-147.

Daniels, S. R., Mouton, F. N., Du Toit, D. A. (2004) Molecular data suggest that melanistic ectotherms at the southwestern tip of Africa are products of Miocene climatic events: evidence from cordylid lizards. *Journal of Zooology*, **263**, 373-383.

Daniels, S.R., Gouws, G. & Crandall, K.A. (2006) Phylogeographic patterning in a freshwater crab species (Decapoda: Potamonautidae: *Potamonautes*) reveals the signature of historical climatic oscillations. *Journal of Biogeography*, **33**, 1538-1549.

Daniels, S. R., Hofmeyr, M. D., Henan, B., Crandall, K. A. (2007) Living with the genetic signature of Miocene induced change: evidence from the phylogeographic structure of the endemic angulate tortoise *Chersina angulata. Molecular Phylogenetics and Evolution*, **45**, 915-926.

Daniels, S.R., Picker, M.D., Cowlin, R.M. & Hamer, M.L. (2009) Unravelling evolutionary lineages among South African velvet worms (Onychophora: *Peripatopsis*) provides evidence for widespread cryptic speciation. *Biological Journal of the Linnean Society*, **97**, 200-216.

Daniels, S.R. & Ruhberg, H. (2010) Molecular and morphological variation in a South African velvet worm, *Peripatopsis moseleyi* (Onychophora, Peripatopsidae): evidence for cryptic speciation. *Journal of Zoology*, **282**, 171-179.

Daniels, S.R. 2011. Genetic variation in the Critically Endangered velvet worm *Opistopatus roseus* (Onychophora: Peripatopsidae). *African Zoology*. **46**: 419-424.

Daniels, S. R., McDonald, D. E., Picker, M. D. (2013) Evolutionary insight into the *Peripatopsis balfouri* sensu lato species complex (Onychophora: Peripatopsidae) reveals novel lineages and zoogeographic patterning. *Zoologica Scripta*, **42**, 656–674.

Daniels, S. R., Dambire, C., Klaus, S., Sharma, P. P. (2016) Unmasking alpha diversity, cladogenesis and biogeographical patterning in an ancient panarthropod lineage (Onychophora: Peripatopsidae: *Opistopatus cintipes*) with the description of five novel species. *Cladistics*.

Darriba, D., Taboada, G. L., Doallo, R., Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature methods*, **9**, 772-772.

Deacon, H.J. (1983) An introduction to Fynbos region, time scales and palaeoenvironments. *CSIR Report*, **75**, 1-99.

Deacon, H.J. (1983) Another look at Pleistocene climates of South Africa. *South African Journal of Science*, **79**, 325-328.

De Queiroz, K. (1998) The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. Pg 57-75 In: Endless forms: Species and speciation (DJ Howard and SH Berlocher, eds.). Oxford Univ. Press, Oxford, England.

De Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*. **56**: 879-886.

Dippenaar-Schoeman, A. S. (2002) Baboon and trapdoor spiders of Southern Africa: An identification manual, Plant Protection Research Institute Handbook 13, Agricultural Research

Council, Pretoria.

Dirzo R., Young H. S., Galetti M., Ceballos G., Isaac N. J. B., Collen B. (2014) Defaunation in the Anthropocene. *Science*, **385**, 401-406.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut A. (2006) Relaxed phylogenetics and dating with confidence. *Public Library of Science, Biology*, **4**:88.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**: 214.

Drummond A., Suchard M. A., Xie D., Rambaut A. (2012) Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969-1973.

Drummond A. J., Bouckaert R. R. (2015) Bayesian Evolutionary Analysis with BEAST. Cambridge University Press.

Dynesius, M. & Jansson, R. (2000) Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proceedings of the National Academy of Sciences, USA*. **97**, 9115-9120.

Eeley H. A. C., Lawes M. J. J., Piper S. E. E. (1999) The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *Journal of Biogeography*, **26**, 595–617.

Enrody-Younga, S. & Peck, S. B. (1983) Onychophora from the mesic grassveld in South Africa (Onychophora: Peripatopsidae). *Annals of the Transvaal Museum*, **33**, 347-352.

Evans, R. (1901) On two new species of Onychophora from the Siamese Malay States. *Quarterly Journal of Microscopical Science*, **44**, 473-538.

Excoffier, L. (2010) Arlequin: An integrated software package for population genetics data analysis. *Swiss Institute of Bioinformatics*, University of Bern.

Faith, D. P. (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation*, **61**, 1–10.

Faith, D. P. (2013) Biodiversity and evolutionary history: Useful extensions of the PD phylogenetic diversity assessment framework. *Annals of the New York Academy of Sciences*, **1289**, 69–89.

Faith, D. P., Reid, C. A. M., Hunter, J. (2004) Integrating Phylogenetic Diversity, Complementarity, and Endemism for Conservation Assessment. *Conservation Biology*, **18**, 255–261.

Farrell, B.D. (2001) Evolutionary assembly of the milkweed fauna: cytochrome oxidase I and the age of *Tetraopes* beetles. *Molecular Phylogenetics and Evolution*, **18**, 467-478.

Felsenstein, J.F. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783-791.

Fitzpatrick, S.W. (2009) Geographical variation in the genetic structure of an Atlantic coastal forest frog reveals regional differences in habitat stability. *Molecular Ecology*, **18**, 2877-2896.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vreijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biological Biotechnology*, **3**, 294-299.

Forest, F., Grenyer, R., Rouget, M., Davies, T. J., Cowling, R. M., Faith, D. P., Balmford, A., Manning, J. C., Proches, S., Van der Bank, M., Reeves, G., Hedderson, T. A. J., Savolainen, V. (2007) Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature*, **445**, 757–760.

Fu, Y. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915-925.

Garrick, R.C., Sands, C.J., Rowell, D.M., Hillis, D.M. & Sunnucks, P. (2007) Catchments catch all: long-term population history of a giant springtail from the southeast Australian highlands-a multigene approach. *Molecular Ecology*, **16**, 1865-1882.

Geldenhuys, C.J. (1997) Composition and biogeography of forest patches on the inland mountains of the southern Cape. *Bothalia*, **27**, 54-74.

Ghiselin, M. T. (1984) Peripatus as a living fossil. In: "Living Fossils" (N. Eldredge and S.M. Stanley, Eds.), pp. 214-217. Springer-Velag, New York.

Giribet, G., Carranza, S., Baguña, J., Riutort, M. & Ribera, C. (1996) First molecular evidence for the existence of a Tardigrada + Arthropoda Clade. *Molecular Biology and Evolution*, **13**, 76-84.

Giribet, G., Edgecombe, G. G., Wheeler, W. C. (2001) Arthropod phylogeny based on eight molecular loci and morphology. *Nature*, **413**, 157-161.

Gleeson, D. M. (1996) Onychophora of New Zealand: past, present, and future. *New Zealand Entomologist*, **19**, 51-55.

Gleeson, D.M., Rowell, D.M., Tait, N.N., Briscoe, D.A. & Higgins, A.V. (1998) Phylogenetic relationships among Onychophora from Australia Inferred from the mitochondrial cytochrome oxidase subunit I gene. *Molecular Phylogenetics and Evolution*, **10**, 237-248.

Goldblatt, P. & Manning J.C. (2002) Plant diversity of the Cape region of Southern Africa. *Annals of the Missouri Botanical Garden*, **18**, 281-302.

Goldstein, P. Z., Desalle R., Amato, G., Vogler, A. P. (2000) Conservation genetics at the species boundary. *Conservation Biology*, **14**, 120-131.

Gouws, G., Stewart, B. A., Coke, M. (2000) Evidence for a new species of river crab (Decapoda, Brachyura, Potamonautidae) from the Drakensberg, South Africa. *Journal of Crustacean Biology*, **20**, 743-758.

Gouws, G., Stewart, B. A., Reavell, P. E. (2001) A new species of freshwater crab (Decapoda, Potamonautidae) from the swamp forests of KwaZula-Natal, South Africa: Biochemical and Morphological evidence. *Crustaceana*, **74**, 137-160.

Gouws, G., Stewart, B.A. & Daniels, S.R. (2004) Cryptic species within the freshwater isopod *Mesamphisopus capensis* (Phreatoicidea: Amphisopodidae) in the Western Cape, South Africa:

allozyme and 12S rRNA sequence data and morphometric evidence. *Biological Journal of the Linnean Society*, **81**, 235-253.

Gouws, G. (2008) New species of *Mesamphisopus*, an endemic South African freshwater

isopod genus (Isopoda: Phreatoicidea: Mesamphisopidae). *Zootaxa*, **1690**, 1-62.

Gouws, G., Matthee, C.A. & Stewart, B.A. (2010) A multiple data set phylogeny for the endemic South African freshwater phreatoicidean isopod genus *Mesamphisopus*: Taxonomic and biogeographic implications. *Molecular Phylogenetics and Evolution*, **55**, 541-551.

Griffiths, C.L. (1981) The freshwater Amphipoda (Crustacea) of South and South West Africa. *Annals of the South African Museum*, **83**, 79-97.

Grube, E. (1866). *Peripatus capensis* n. sp. *Jber. Schl. Ges. Vaterl. Cult*., Breslau. **34**: 65-66.

Hamer, M.L., Samways, M.J. & Ruhberg, H. (1997) A review of the Onychophora of South Africa, with discussion of their conservation. *Annals of the Natal Museum*, **38**, 283-312.

Hamer, M. (2003a) *Peripatopsis clavigera*. The IUCN Red List of Threatened Species 2003.

Hamer, M. (2003b) *Peripatopsis leonina*. The IUCN Redlist of Threatened Species 2003.

Hamer, M. (2003c) *Peripatopsis alba*. The IUCN Redlist of Threatened Species 2003.

Harris, C., Oom, B.M. & Diamond, R.E. (1999) A preliminary investigation of the oxygen and hydrogen of the greater Cape Town area and an assessment of the potential for using stable isotopes as tracers. *Water SA*, **25**, 15-24.

Hebert, P.D.N., Billington, N., Finston, T.L., Boileau, M.G., Beaton, M.J. & Barrette, R.J. (1991) Genetic variation in the onychophoran *Plicatoperipatus jamaicensis*. *Heredity*, **67**, 221-229.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal society of London. Series B. Biological Sciences*. **270**: 313-321.

Heled, J. & Drummond, A. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570-580.

Henning, S.F. & Henning, G. A. (1989) South African Red Data Book: Butterflies. South African National Scientific Program Report No. 158, Foundation for Research Development, Pretoria.

Hickson R. E., Simon C., Cooper A., Spicer G. S., Sullivan J., Penny D. (1996) Conserved sequence motifs, alignments, and the secondary structure for the third domain of animal 12S rRNA. *Molecular Biology and Evolution*, **13**, 150-169.

Hidasi-Neto, J., Loyola, R. D., Cianciaruso, M. V. (2013) Conservation Actions Based on Red Lists Do Not Capture the Functional and Phylogenetic Diversity of Birds in Brazil. *PLoS ONE*, **8(9)**.

Holbourn, A., Kuhnt, W., Schulz, M., Erlenkeuser, H. (2005) Impacts of orbital forcing and atmospheric carbon dioxide on Miocene ice-sheet expansion. *Nature*, **438**, 483–487.

Hou, X., Bergstrom, J. (1995) Cambrian lobopodians- ancestors of extant onychophorans? *Zoological Journal of the Linnean Society*, **114**, 3-19.

Hurst, G. D. D., Jiggins, F. M. (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society of Biological Sciences*, **272**, 1525-1534.

IUCN (2012) IUCN Redlist Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.

IUCN Standards and Petitions Subcommittee (2014) Guidelines for using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee, Downloadable from http://www.iucnredlist.org/documents/RedListGuidelines.pdf

Keppel, G., Van Niel, K. P., Wardell-Johnson, G.W., Yates, C.J., Byrne, M., Mucina, L., Schut, A. G. T., Hopper, S.D., Franklin, S.E. (2012) Refugia: identifying and understanding safe havens for biodiversity under climate change. *Global Ecology and Biogeography*, **21**, 393-404.

Kidd, D. & Liu, X. (2007) GeoPhyloBuilder v1.0 for ArcGIS. *National Evolutionary Synthesis Center*, Durham.

King, L. C. (1978) The geomorphology of central and southern Africa. In: Werger MJA, ed. Biogeography and ecology of southern Africa. The Hague, Netherlands: W. Junk, pp. 3-17.

Kirchner, J., Moolman, J.H., du Plessis, H.M. & Reynders, A.G. (1997) Causes and management of salinity in the Breede River Valley, South Africa. *Hydrogeology Journal*, **5**, 98-108.

Knowlton, N., Weigt, L. A. (1998) New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 2257-2263.

Krajewski, C. (1994) Phylogenetic measures of biodiversity: A comparison and critique. *Biological Conservation*, **69**, 33–39.

Kumar, S., Stecher, G., Tamara, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*.

Lacorte, G. A., Oliveira, I. S. & Da Fonseca, C. G. (2011). Phylogenetic relationships among the *Epiperipatus* lineages (Onychophora: Peripatidae) from the Minas Gerais State, Brazil. *Zootaxa*. **2755**: 57-65.

Laffan, S. W., Lubarsky, E., Rosauer, D. F. (2010) Biodiverse, a tool for the spatial analysis of biological and related diversity. *Ecography*, **33**, 643–647.

Laity, T., Laffan, S. W., González-Orozco, C. E., Faith, D. P., Rosauer, D. F., Byrne, M., Miller, J. T., Crayn, D., Costion, C., Moritz, C. C., Newport, K. (2015) Phylodiversity to inform conservation policy: An Australian example. *Science of The Total Environment*, **534**, 131-143.

Lawes, M. J., Eeley, H. A. C., Findlay, N. J., Forbes, D. (2007) Resilient forest faunal communities in South Africa: A legacy of palaeoclimatic change and extinction filtering? *Journal of Biogeography*, **34**, 1246-1264.

Lewis, P. O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, **50**, 913-925.

Linder, H.P. (2003) The radiation of the Cape Flora. *Biological Reviews of the Cambridge Philosophical Society*, **78**, 597-638.

Loader, S. P., Ceccarelli, F. S., Menegon, M., Howell, K. M., Kassahun, R., Mengistu, A. A., Saber, S. A., Gebresenbet, F., de Sá R, Davenport, T. R. B., Larson, J. G., Müller, H., Wilkinson, M., Gower, D. J. (2014) Persistence and stability of Eastern Afromontane forests: Evidence from brevicipitid frogs. *Journal of Biogeography*, **41**, 1781-1792.

Maddison, W. P., Mooers, A. O. (2007) Tuatara: Conservation priority in a phylogenetic context. Version 1.0, http://mesquiteproject.org/packages/tuatara

Maddison, W. P., Maddison DR (2015) Mesquite: a modular system for evolutionary analysis. Version 3.04, http://mesquiteproject.org

Mallet, J. (2001) Species, concepts of. In Encyclopedia of Biodiversity (Vol. 5) (Levin, S.A, ed.), pp. 427-440, Academic press.

Manton, S. M. & Ramsay, J. A. (1937) Studies on the Onychophora III. The control of water loss in Peripatopsis. *Journal of experimental Biology*, 471–472.

Mayer, G., Harzsch, S. (2008) Distribution of serotonin in the trunk of *Metaperipatus blainvillei* (Onychophora, Peripatopsidae): implications for the evolution of the nervous system in Arthropoda. *Journal of Comparative Neurology*, **507**, 1196-1208.

Mayer, G., Tait, N. N. (2009) Position and development of oocytes in velvet worms shed light on the evolution of the ovary in Onychophora and Arthropoda. *Zoological Journal of the Linnean Society*, **157**, 17-33.

Mayr, E. (1963) Animal species and Evolution, Harvard University Press.

McDonald, D.J., Cowling, R.M. & Boucher, C. (1996) Vegetation-environment relationships on a species-rich coastal mountain range in the fynbos biome (South Africa). *Vegetatio*, **123**, 165-182.

McDonald, D.J. & Cowling, R. M. (1995) Towards a profile of an endemic mountain fynbos flora: implications for conservation. *Biological Conservation*, **72**, 1-12.

McDonald, D. E. & Daniels, S. R. (2012) Phylogeography of the Cape velvet worm (Onychophora: Peripatopsis capensis) reveals the impact of Pliocene/Pleistocene climatic oscillations on Afromontane forest in the Western Cape, South Africa. *Journal of Evolutionary Biology*, **25**, 824–835.

McDonald, D. E., Ruhberg, H. & Daniels, S. R. (2012). Two new Peripatosis species (Onychophora: Peripatopsidae) from the Western Cape province, South Africa. *Zootaxa*, **3380**, 55–68.

McGeoch, M. (2002) Insect conservation in South Africa: an overview. *African Entomology*, **10**, 1-10.

McGeoch, M. et al. (2011) Conservation and monitoring of invertebrates in terrestrial protected areas. *Koedoe*, **53**, 1–13.

Meadows, M.E. & Linder, H.P. (1993) A palaeoecological perspective on the origin of Afromontane grasslands. *Journal of Biogeography*, **20**, 345-55.

Monge-Najera, J. (1995) Phylogeny, biogeography and reproductive trends in the Onychpora. *Zoological Journal of the Linnean Society*, **114**, 21-60.

Moritz, C. (1994) Defining 'evolutionarily significant units' for conservation. *Trends in Ecology & Evolution*, 373-375.

Mosblech, N.A.S., Bush, M.B., & van Woesik, R. (2011) On metapopulations and microrefugia: palaeoecological insights. *Journal of Biogeography*, **38**, 419-429.

Mucina, L. & Rutherford, M.C. (eds) (2006) The vegetation of South Africa, Lesotho and Swaziland. Strelitzia, **19**. South African National Biodiversity Institute, Pretoria.

Murienne, J., Daniels, S. R., Buckley, T. R., Mayer, G., Giribet, G. (2014) A living fossil tale of Pangaean biogeography. *Proceedings of the Royal Society B*, **281,** 1775 (20132648)

Myburgh, A. M. & Daniels, S. R. (2015). Exploring the Impact of Habitat Size on Phylogeographic Patterning in the Overberg Velvet Worm Peripatopsis overbergiensis (Onychophora: Peripatopsidae), *Journal of Heredity*, **106**, 296-305.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca & G.A.B., Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853-858.

Nel JL, Roux DJ, Maree G, Kleynhans CJ, Moolman J, Reyers B, Rouget M, Cowling RM (2007) Rivers in peril inside and outside protected areas: a systematic approach to conservation assessment of river ecosystems. Diversity and Distributions, 13: 341–352.

New, T. R. (1995) Onychophora in invertebrate conservation: Priorities, practises, and prospects. *Zoological Journal of the Linnean Society*, **114**, 77-89.

Newlands, G. & Ruhberg, H. (1978) Onychophora. In: Werger MJA, ed., Biogeography and ecology of southern Africa. The Hague: Junk pp. 677-684.

Nixon, K.C., Wheeler, Q. D. (1992) Measures of phylogenetic diversity. In Novacek MJ, Wheleer QD (Eds.), Extinction and Phylogeny. Columbia University Press, New York, pp. 216-234

Nylander, J. A. A. A., Ronquist, F., Huelsenbeck, J. P., Nieves-Aldrey, J. L. (2004) Bayesian phylogenetic analyses of combined data. *Systematic Biology*, **53**, 47-67.

Oliveira, I., Wieloch, A.H., Mayer, G. (2010) Revised taxonomy and redescription of two species of the Peripatidae (Onychophora) from Brazil: a step towards consistent terminology of morphological characters. *Zootaxa*, **2492**, 16-34.

Oliveira, I.S., Lacorte, G.A., Fonseca, C.G., Wieloch, A.H., Mayer, G. (2011) Cryptic speciation in Brazilian *Epiperipatus* (Onychophora: Peripatidae) reveals an underestimated diversity among the Peripatid velvet worms. *Public Library of Science, Biology*, **6**, e19973.

Partridge, T.C. & Maud, R.R. (2000) *Macro-scale geomorphic evolution of southern Africa*. In: In: Partridge, T.C., Maud, R.M. (Eds.) Cenozoic of Southern Africa. Oxford University Press, New York.

Partridge, T. C., Dollar, E. S. J., Moolman, J., Dollar, L. H. (2010) The geomorphic provinces of South Africa, Lesotho, and Swaziland: A physiographic subdivision for earth and environmental scientists. *Transactions of the Royal Society of South Africa*, **65**, 1-47.

Perez-Losada, M., Jara, C. G., Bond-Buckup, G., Crandall, K. A. (2002) Conservation phylogenetics of Chilean freshwater crabs *Aegla* (Anomura, Aeglidae): assigning priorities for aquatic habitat protection. *Biological Conservation*, **105,** 345-353.

Pérez-Losada, M., Crandall, K. A. (2003) Can taxonomic richness be used as a surrogate for phylogenetic distinctness indices for ranking areas for conservation? *Animal Biodiversity and Conservation*, **26**, 77-84.

Posada, D, Buckley, T. R. (2004) Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio test. *Systematic Biology*, **53**, 793-808.

Polasky, S., Csuti, B., Vossler, C. A., Meyers, S. M. (2001). A comparison of taxonomic distinctness versus richness as criteria for setting conservation priorities for North American birds. *Biological Conservation*, **97**, 99–105.

Prain, D. (1913) The South African species of Cluytia. *Bulletin of Miscellaneous Information (Royal Gardens, Kew)*, **10**, 373-416.

Price, B.W., Barker, N.P. & Villet, M.H. (2007) Patterns and processes underlying evolutionary significant units in the *Platypleura stridula* L. Species complex (Hemiptera: Cicadidae) in the Cape Floristic Region, South Africa. *Molecular Ecology*, **16**, 2574-2588.

Projecto-Garcia, J., Cabral, H., Schubart, C. D. (2009) High regional differentiation in a North American crab species throughout its native range and invaded European waters: A phylogeographic analysis. *Biological Invasions*, **12**, 253-263.

Pryke, J. S., Samways, M. J. (2008) Conservation of invertebrate biodiversity on a mountain in a global biodiversity hotspot, Cape Floral Region. *Biodiversity and Conservation*, **17**, 3027-3043.

Purcell, W. F. (1899) On the South African species of Peripatidae in the collection of the South African Museum. *Annals of the South African Museum*, **1**, 331-351.

Rambaut, A., Suchard, M. A., Xie, D., Drummond, A. J. (2014) Tracer v1.6.

Reid, A. L., Tait, N. N., Briscoe, D. A., Rowell, D. M. (1995) Morphological, cytogenetic and allozymic variation within *Cephalofovea* (Onychophora: Peripatopsidae) with descriptions of three new species. *Zoological Journal of the Linnean Society*, **114**, 115-138.

Reid, A.L. (1996) Review of the Peripatopsidae (Onychophora) in Australia, with comments on the peripatopsid relationships. *Invertebrate Taxonomy*, **10**, 663-936.

Reid, A.L. (2000a). Eight new *Plannipapillus* (Onychophora: Peripatopsidae) from south-eastern Australia. *Proceedings of the Royal Society of Victoria.* **122**, 1-23.

Reid, A. L. (2000b). Description of *Lathropatus nemorum*, gen. et sp., and six new *Ooperipatus* Dendy (Onychophora: Peripatopsidae) from south-eastern Australia. *Proceedings of the Royal Society of Victoria.* **112**: 153-184.

Reinhard, J., Rowell, D. M. (2005) Social behaviour in an Australian velvet worm, *Euperipatoides rowelli* (Onychophora: Peripatopsidae). *Journal of Zoology*, **267**, 1-7.

Rockman, M.W., Rowell, D.M. & Tait, N.N. (2001) Phylogenetics of *Planipapillus*, lawn-headed Onychophorans of the Australian Alps, based on nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution*, **21**, 103-116.

Rodrigues, A. S. L., Gaston, K. J. (2002) Maximising phylogenetic diversity in the selection of networks of conservation areas. *Biological Conservation*, **105**, 103-111.

Rodrigues, A. S. L., Brooks, T. M., Gaston, K. J. (2005) Integrating phylogenetic diversity in the selection of priority areas for conservation: does it make a difference. *Phylogeny and conservation*, **8**, 101-119.

Roe, D., Elliott, J. (2005) Poverty-Conservation linkages: A conceptual framework. Available from http://www.povertyandconservation.info/docs/200511-Conceptual_Framework(Roe-Elliott).pdf [accessed 30 March 2010]

Ronquist, F., & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572-1574.

Rosauer, D., Laffan, S. W., Crisp, M. D., Donnellans, S. C., Cook, L. G. (2009) Phylogenetic endemism: A new approach for identifying geographical concentrations of evolutionary history. *Molecular Ecology*, **18**, 4061–4072.

Rowell, D.M., Higgins, A.V., Briscoe AV, & Tait, N. N. (1995). The use of chromosomal data in the systematic of viviparous Onychphoran from Australia (Onychphora: Peripatopsidae). *Zoological Journal of the Linnean Society*. **114**: 139-153.

Ruhberg, H. (1985). Die Peripatopsidae (Onychophora. Systematik, Ökologie, Chorologie und phylogenetische Aspekte. *Zoologica.* **137**: 1-184.

Ruhberg, H., Tait, N. N., Briscoe, D. A., Storch, V. (1988) *Cephalofovea tomahmontis*. gen., n. sp., an Australian peripatopsid (Onychophora) with a unique cephalic pit. *Zoologischer Anzeiger*, **221**, 117-133.

Ruhberg, H. (1992) "Peripatus"-an Approach towards a Modern Monograph. *Ber. Nat.-med Verein Innsbruck*, **10**, 441-458.

Ruhberg, H., Hamer, M. (2005) A new species of *Opisthopatus* (Onychophora: Peripatopsidae) from KwaZulu-Natal, South Africa. *Zootaxa*, **1039**, 27-38.

Ruhberg, H. & Daniels, S. R. (2013) Morphological assessment supports the recognition of four novel species in the widely distributed velvet worm *Peripatopsis moseleyi sensu lato* (Onychophora: Peripatopsidae). *Invertebrate Systematics*, **27**, 131–145.

Rutherford, M.C. & Westfall, R.H. (1986) Biomes of Southern Africa: and objective catergorization. *Memoirs of the Botanical Survey of South Africa*, **54**, 1-98.

Samways, M. J. (2002) A strategy for the national red-listing of invertebrates based on experiences with Odonata in South Africa. *African Entomology*, **10**, 43–52.

Samways, M. J. (2006) National Red List of South African Odonata. *Odonatologica*, **35,** 341-368.

Savolainen, V., Cowan, R. S., Vogler, A. P., Roderick, G. K., Lane, R. (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B*, **360,** 1805-1811.

Schalke, H.J.W.G. (1973) The upper Quaternary of the Cape Flats area (Cape Province, South Africa). *Scripta Geologica*, **15**, 1-57.

Schellenberg, A. (1926) Die Gammariden der Deutschen Südpolar-Expedition 1901-1903. *Deutsche Südpolar-Expedition 1901-1903*, **18**, 235-414.

Schloms, B.H.A., Ellis, F. & Lambrechts, J.J.N. (1983) Soils of the Cape coastal platform. *CSIR Report*, **75**, 70-85.

Schnitzler, J., Barraclough, T.G., Bootwright J.S., Goldblatt, P., Manning, J.C., Powell, M.P., Rebelo, T. & Savolainen, V. (2011) Causes of plant diversification in the Cape Biodiversity Hotspot of South Africa. *Systematic Biology*, **60**, 1-15.

Scholtz, C. H., Chown, S. L. (1995) Insects in southern Africa: How many species are there? *South African Journal of Science*, **91**, 124-126.

Schubart, C. D., Diesel, R., Hedges, S. B. (1998) Rapid evolution to terrestrial life in Jamaican crabs. *Nature*, **393**, 363-365.

Sedgwick, A. (1885). On the fertilized ovum and formation of the layers of the South African *Peripatus*. *Proceedings of the Royal Society, London.* **39**: 239-244.

Sharratt, N. J., Picker, M. D., Samways, M. J. (2000) The invertebrate fauna of the sandstone caves of the Cape Peninsula (South Africa): patterns of endemism and conservation priorities. *Biological Conservation*, **9**, 107-143.

Sherbon, B.J. & Walker, M.H. (2004) A new species of *Peripatopsis* from South Africa, *P. stelliporata*, with observations on embryonic development and sperm degradation (Onychophora: Peripatopsidae). *Journal of Zoology*, **264**, 295-305.

Shimodaira, H., Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inferences. *Molecular Biology and Evolution*, **16**, 114-116.

Siesser, W. G. (1980) Late Miocene origin of the Benguela upwelling system of northern Namibia. *Science*, **208**, 283-285.

Siesser, W.G. & Dingle, R.V. (1981) Tertiary Sea-Level Movements around Southern Africa. *Journal of Geology*, **89**, 523-536.

Sim, T.R. (1907) *The forests and forest flora of the Cape Colony*. Aberdeen.

Slotow, R. & Hamer, M. (2000) Biodiversity research in South Africa: comments on current trends and methods. *South African Journal of Science*, **96**, 222–224.

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology, 57: 758-771.

Stewart, B. A. (1992) Morphological and genetic differentiation between allopatric populations of a freshwater amphipod. *Journal of Zoology*, **228**, 287-305.

Stewart, B. A., Griffiths, C. L. (1992a) Four new species of the genus *Paramelita* (Amphipoda, Crangonyctoidea) from South Africa. *Annals of the South African Museum*, **101**, 139-158.

Stewart, B. A., Griffiths, C. L. (1992b) A taxonomic re-examination of freshwater amphipods in the *Paramelita auricularius-P. crassicornis* complex, with descriptions of three additional species. *Crustaceana*, **62**, 166-192.

Stewart, B. A., Griffiths, C. L. (1992c) Further new species within the freshwater amphipod genus *Paramelita* (Crangonyctoidea: Paramelitidae) from South Africa. *Journal of Natural History*, **26**, 489-506.

Stewart, B. A., Snaddon, C. D., Griffiths, C. L. (1994) Morphological and genetic differentiation among populations of the freshwater amphipod *Paramelita spinicornis* (Amphipoda: Crangonyctoidea), with description of new species. *Zoological Journal of the Linnean Society*, **111**, 179-195.

Stewart, B. A., Coke, M., Cook, P. A. (1995) *Potamonautes dentatus*, new species, a fresh-water crab (Brachyura: Potamoidea: Potamonautidae) from KwaZulu-Natal, South Africa. Journal of Crustacean Biology, 15: 558-568.

Stewart, B. A., Griffiths, C. L. (1995) Revision of the family Paramelitidae (Crustacea, Amphipoda) from South Africa fresh waters. *Annals of the South African Museum*, **104**, 181-247.

Stewart, B. A. (1997) Biochemical and morphological evidence for a new species of river crab *Potamonautes parvispina* sp. nov (Brachyura, Potamonautidae). *Crustaceana*, **70**, 737-753.

Stewart, B. A., Cook, P. A. (1998) Identification of a new species of river crab (Decapoda: Brachyura: Potamonautidae) from South Africa using morphological and genetic data. *Journal of Crustacean Biology*, **18**: 556-571.

Stuckenburg, R. (1962) The distribution of the montane palaeogenic elements in the South African invertebrate fauna. *Annals of the Cape Provincial Museum*, **2**, 190-205.

Swart, B.L., Tolley, K.A. & Matthee, C.A. (2009) Climate change drives speciation in the southern rock agama (*Agama atra*) in the Cape Floristic Region, South Africa. *Journal of Biogeography*, **36**, 78-87.

Swartz, E. R., Skelton, P. H., Bloomer, P. (2009) Phylogeny and biogeography of the genus Pseudobarbus (Cyprinidae): Shedding light on the drainage history of rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution*, **51**, 75-84.

Swofford, D.L. (2002) PAUP* Phylogenetic analysis using Parsimony (and other methods), Version 4.10. *Illinois Natural History Survey*, Champaign, Illinois.

Tait, N. N., Briscoe, D. A., & Rowell, D. M. (1995). Onychophora ancient and modern radiations. *Memoirs of the Association for Australian Palaentology*. **18**: 21-30.

Tait, N. N., & Norman, J. M. (2001). Novel mating behaviour in *Florelliceps stutchburyae* gen. nov., sp. nov (Onychophora: Peripatopsidae) from Australia. *Journal of Zoology, London*. **53**: 301-308.

Theron, J.N. (1983) Geological setting of the Fynbos. *CSIR report*, **75**, 21-34.

Thompson, I., Jones, D. S. (1980) A possible onychophoran from the Middle Pennsylvanian Mazon Creek Beds of northern Illinois. *Journal of Paleontology*, **54**, 588-596.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research*, **24**, 4876-4882.

Thorne, J.L. & Kishino, H. (2002) Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, **51**, 689-702.

Thurston, M. H. (1973) A new species of *Paramelita* (Crustacea: Amphipoda) from Africa. *Annals of the South African Museum*, **62**: 159-168.

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. *Molecular Ecology*, **15**, 781-793.

Tolley, K.A., De Villiers, A.L., Cherry, M.I. & Measey, G.J. (2010) Isolation and high genetic diversity in dwarf mountain toads (*Capensibufo*) from South Africa. *Biological Journal of the Linnean Society*, **100**, 822-834.

Tolley, K. A., Tilbury, C. R., Measey, J. G., Menegon, M., Branch, W. R., Matthee, C. A. (2011) Ancient forest fragmentation or recent radiation? Testing refugial speciation models in chameleons within an African biodiversity hotspot. *Journal of Biogeography*, **38,** 1748-1760.

Trewick, S. A. (1998). Sympatric cryptic species in New Zealand Onychophora. *Biological Journal of the Linnean Society.* **63**: 307-329.

Trewick, S. A. (1999) Molecular diversity of Dunedin peripatus (Onychophora: Peripatopsidae). *New Zealand Journal of Zoology*, **26**, 381-393.

Trewick, S.A. (2000) Mitochondrial DNA sequences support allozyme evidence for cryptic radiation of New Zealand Peripatoides (Onychophora). *Molecular Ecology*, **9**, 269-281.

Trewick, S.A. & Wallis, G.P. (2001) Bridging the 'beech-gap': New Zealand invertebrate phylogeography implicates Pleistocene glaciations and Pliocene isolation. *Evolution*, **55**, 2170-2180.

Tyson, P. D. (1986) Climate change and variability in southern Africa. Oxford University Press, USA.

Tyson, P.D. & Partridge, T.C. (2000) *Evolution of Cenozoic climates*. In: Partridge, T.C., Maud, R.M. (Eds.) Cenozoic of Southern Africa. Oxford University Press, New York.

Vane-Wright, R. I., Humphries, C. J., Williams, P. H. (1991) What to protect?—Systematics and the agony of choice. *Biological Conservation*, **55**, 235–254.

Van Wilgen, B. W., Higgins, K. B., Bellstedt, D. U. (1990) The role of vegetation structure and fuel chemistry in excluding fire from forest patches in the fire-prone fynbos shrublands of South Africa. *Journal of Ecology*. **78**: 210-222.

Verboom, G.A., Archibald, J.K., Bakker, F.T., Bellstedt, D.U., Conrad, F., Dreyer, L.L., Forest, F., Galley, C., Goldblatt, P., Henning, J.F., Mummenhoff, K., Linder, H.P., Muasya, A.M., Oberlander,

K.C., Savolainen, V., Snijman, D.A., Van der Niet, T. & Nowell, T.L. (2009) Origin and diversification of the Greater Cape flora: ancient species repository, hot-bed of recent radiation, or both? *Molecular Phylogenetics and Evolution*, **51**, 44-53.

Walker, F. (1952) *The geology*. In: The Cape Peninsula. Maskew Miller Ltd., Cape Town.

Walker, M. H. (1995) Relatively recent evolution of an unusual pattern of early embryonic development (long germ band) in a South African onychophoran, *Opisthopatus cintipes* Purcell (Onychophora: Peripatopsidae). *Biological Journal of the Linnean Society*, **114**, 61-75.

Wiens, J. J., Penkrot, T. A. (2002) Delimiting species DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic biology*, **51**: 69-91.

Will, K. W., Rubinoff, D. (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics*, **20,** 47-55.

Winter, M., Devictor, V., Schweiger, O. (2013) Phylogenetic diversity and nature conservation: Where are we? *Trends in Ecology and Evolution*, **28**, 199–204.

Wishart, M.J. & Hughes, J.M. (2001) Exploring patterns of population subdivision in the net-winged midge, *Elporia barnardi* (Diptera: Blephariceridae), in mountain streams of the south-western Cape, South Africa. *Freshwater Biology*, **46**, 479-490.

Wishart, M.J. & Hughes, J.M. (2003) Genetic population structure of the net-winged midge, *Elporia barnardi* (Diptera: Blephariceridae) in streams of the south-western Cape, South Africa: implications for dispersal. *Freshwater Biology*, **48**, 28-38.

Wood-Mason, J. (1879) Morphological notes bearing on the origin of insects. *Transactions of the entomological Society, London.* **2**: 155.

Wuyts, J., De Rijk, P., Van de Peer, Y., Pison, G., Rousseeuw, P., De Wachter, R. (2000) Comparative analyses of more than 3000 sequences reveals the existence of two pseudoknots in areas V4 of eukaryotic small subunit ribosomal RNA. *Nucleic Acids Research*, **28**, 4698-4708.

Zhang, D., & Hewitt, G. M. (2003). Nuclear DNA analyses in the genetic studies of populations: practise, problems, and prospects. *Molecular Ecology*. **12**: 563-584.