

Systematics of the Cape legless skink *Acontias meleagris* species complex

by
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Abstract

This study examined the biogeography and taxonomic status of the Cape legless skink, *Acontias meleagris* species complex using phylogenetic analyses, population genetics, demographic history aspects, time of lineage diversification estimation, environmental statistic analyses and a morphological evaluation. A total of 231 specimens from 55 localities were collected from the entire known distribution range of the *A. meleagris* complex throughout the Eastern, Northern and Western Cape, South Africa. Partial sequence data were collected from two mitochondrial DNA loci, *16S rRNA* and cytochrome oxidase subunit one (*COI*), and one protein-coding nuclear DNA locus, exophilin 5 (*EXPH 5*). DNA sequences were analyzed for phylogenetic methods and biogeographical dating, while population genetic analyses were conducted on the *COI* sequences. Geographical boundaries amongst cryptic lineages were determined and evolutionary drivers of cladogenesis within the species complex were inferred. Marked genetic structure was observed within the *A. meleagris* complex, and five clades were retrieved, most of which were statistically well supported. These five clades were also evident within the haplotypic analyses and were characterized by demographic stability.

Lineage diversification and the current biogeographical patterning observed for lineages within the *A. meleagris* species complex reflect the impact of sea level oscillations on historical coastal habitat availability. Additional historical evolutionary drivers within this subterranean species complex were inferred and discussed. The five clades within this species complex were considered discrete species, characterised by phylogenetic and biogeographic distinctiveness. While, morphological characters that could be used to identify the five species demonstrated widespread overlap for morphometric and meristic characters as well as colour patterning. Consequently, the phylogenetic species concept was employed for a taxonomical revision of *A. meleagris sensu lato*. Here, three of the previously recognised subspecies *A. m. meleagris*, *A. m. orientalis* and *A. m. orientalis*-‘lineicauda’ were elevated to full species, and two new species *A. caurinus* sp. nov. and *A. parilis* sp. nov. were described.

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Table of contents

Declaration	ii
Abstract	iii
Acknowledgements	iv
Table of contents	v
List of tables	viii
List of figures	ix
Chapter 1: General introduction	1
1.1 Preamble.....	1
1.2 Aim of study.....	4
1.3 Research questions	4
Chapter 2: Phylogenetic and biogeographical aspects: Tracking the impact of Pliocene / Pleistocene sea level and climatic oscillations on the cladogenesis of the Cape legless fossorial skink species complex, <i>Acontias meleagris</i> in South Africa	5
2.1 Introduction	5
2.2 Materials and Methods	8
2.2.1 <i>Sampling</i>	8
2.2.2 <i>DNA extraction, PCR and sequencing</i>	12
2.2.3 <i>Phylogenetic analyses</i>	12
2.2.4 <i>Population genetic analyses</i>	14
2.2.5 <i>Historical demography and molecular diversity</i>	14
2.2.6 <i>Divergence time estimation</i>	15
2.2.7 <i>Environmental statistical analyses</i>	16

2.3 Results	17
2.3.1 <i>Phylogenetic analyses</i>	17
2.3.2 <i>Population genetics</i>	20
2.3.3 <i>Historical demography and molecular diversity</i>	25
2.2.4 <i>Divergence time estimation</i>	25
2.3.5 <i>Environmental statistical analyses</i>	25
2.4 Discussion	32
2.4.1 <i>Historical biogeography</i>	32
2.4.2 <i>Historical demography</i>	35
2.4.3 <i>Environmental influences on biogeographical patterning</i>	36
2.4.4 <i>Conclusions and taxonomical implications</i>	36
Chapter 3: A taxonomic revision of the South African Cape legless skink <i>Acontias meleagris</i> species complex (Squamata: Scincidae): with the description of two new species	38
3.1 Introduction	38
3.2 Materials & Methods	42
3.2.1 <i>Collection and preparation of specimens</i>	42
3.2.1 <i>Morphological analysis and character counts</i>	42
3.3 Taxonomy	49
3.3.1 <i>Acontias meleagris</i> (Linnaeus 1758)	51
3.3.2 <i>Acontias meleagris orientalis</i> (Hewitt 1938)	58
3.3.3 <i>Acontias meleagris orientalis lineicauda</i> (Hewitt 1938)	63
3.3.4 <i>Acontias caurinus</i> sp. nov.	66

3.3.5 <i>Acontias parilis</i> sp. nov.	71
3.4 Discussion	76
Chapter 4: General conclusions	83
References	85
Appendix 1: Clade assignment, TCS haplotype allocation and corresponding GenBank numbers for newly generated COI sequences.....	97
Appendix 2: Time calibrated phylogeny.....	105

List of tables

Table 2.1: Geographic and molecular sampling information for specimens used in the study of the <i>Acontias meleagris</i> species complex. Locality numbers correspond to the numbers on figure 2.1 and are further assigned to respective provinces, i.e. Eastern Cape (EC), Northern Cape (NC) and Western Cape (WC) provinces. Asterisks (*) denote mitochondrial DNA sequences (16S rRNA and COI) included from Daniels <i>et al.</i> , (2005, 2009)	10
Table 2.2: Diversity measures for various clades of the <i>Acontias meleagris</i> species complex, where Nh indicate the number of haplotypes, Np the number of polymorphic sites, h the haplotype diversity and π_n the nucleotide diversity for the COI data set.....	24
Table 2.3: Demography statistics conducted on the COI data set for the various clades of the <i>Acontias meleagris</i> species complex, indicating the sum of squared deviation (SSD), Harpending's raggedness index (RI) and Fu's F_s value for each clade.....	28
Table 2.4: Comparison of means of parameters using one-way ANOVA and LSD. Values followed with different letters (^{a,b,c}) are significantly different between clades.	29
Table 2.5: Environmental factors showing significant distinction between clades for monthly measurements as indicated by VEPAC with clade*month as fixed effects.	31
Table 3.1: Sequence divergence (%) for the COI marker, between selected species of the <i>Acontias</i> ..	55
Table 3.2: Morphological measurements expressed as ratios of SVL for novel and revised species within <i>Acontias</i> . Snout-vent length (SVL), head length (HL), head width (HW), head height (HH), rostral scale length (RL), nasal-rostral line length (NRL) and mental scale length (ML).	56
Table 3.3: Variation in head and body scale counts of novel and revised species within <i>Acontias</i> . Supraciliaries (SC), supraoculars (SO), suboculars (SB), upper labials (UL), lower labials (LL), chin shields (CS), mid-body scale rows (MSR), subcaudals (SUC) and ventral scales (V).	57

List of figures

- Figure 2.1:** Sampling localities for specimens of the *Acontias meleagris* species complex used in this study, along the coastal regions and adjacent interior of the Eastern, Northern and Western Cape provinces of South Africa. 9
- Figure 2.2:** Maximum Likelihood topology for the total evidence DNA data (16S rRNA, COI and EXPH5) for specimens of the *Acontias meleagris* species complex, including eight outgroups; *A. breviceps*, *A. gracilicauda*, *A. lineatus*, *A. litoralis*, *A. occidentalis*, *A. percivali*, *A. plumbeus* and *A. tristis*. Bootstrap values for maximum likelihood and maximum parsimony (%) are shown above the nodes and posterior probability (*pP*) below the nodes. Asterisks indicate statistically poorly supported nodes (< 70% / < 0.95 *pP*). Geometric symbols on the tree topology indicate the distribution of ‘subspecies’ / ‘morphotypes’. 19
- Figure 2.3:** Illustration of the seven haplogroups retrieved by TCS. Various colours in the haplotype network indicate genetic groupings as retrieved by phylogenetic analyses, where circle size indicates relative frequencies. See appendix 1 for distribution of haplotypes across sampled localities..... 22
- Figure 2.4:** Tessellation illustration of Bayesian analysis of population structure, where each cell of the tessellation corresponds to the physical neighbourhood of an observed data point and is coloured according to genetic distinctiveness..... 23
- Figure 2.5:** Bayesian skyline plots (BSPs) and mismatch distribution (MMD) conducted on the single COI data set for various clades retrieved by phylogenetic analyses. Bayesian skyline plots illustrate the population size as a product of effective population size (N_e) and generation time (τ) through time (years). The black line represents the median estimate of population size, where the blue lines indicate the upper and lower 95% posterior intervals. On the MMD graphs, the columns denote the observed frequency of pairwise differences, where the trend line represents the expected distribution under the sudden expansion model..... 27
- Figure 2.6:** Map indicating the five clades of the *Acontias meleagris* species complex as retrieved by phylogenetic analyses. Oyster Bay and Port Elizabeth are not assigned to a clade..... 34
- Figure 3.1:** Maximum-likelihood topology for the total evidence DNA data (16S rRNA, COI and EXPH5) amongst 55 *Acontias meleagris sensu lato* sample sites across the Eastern, Northern and Western Cape provinces of South Africa demonstrating the presence of the five clades (Engelbrecht *et al.* 2012)..... 40

Figure 3.2: Measurements of the physical proportions of an *Acontias* skink, **a**, ventral view and illustration of Snout-vent length and tail length measurement, **b**, dorsal view and illustration of head width measurement, **c**, dorsal view and illustration of head length measurement from the tip of the snout to the edge of the parietal scales, **d**, lateral view and illustration of the head height measurement, from the edge of the parietals scales in a straight line downwards to the throat area, **e**, dorsal view of the snout and illustration of the rostral scale length measurement, **f**, lateral view and illustration of the nasal-rostral line length measurement and **g**, ventral view and illustration of the mental scale length measurement..... 44

Figure 3.3: Head shields of an *Acontias* skink, **a**, ventral view of the snout and chin area of indicating anterior chin shields (CS), mental scale (M) and the rostral scale (R), **b**, lateral view indicating the supraocular scales (SO), supraciliaries (SC), loreal scale (L), preocular scale (PR), subocular scales (SB), upper labial scales (UL) and lower labial scales (LL), **c**, dorsal view indicating the parietal scales (P), interparietal scale (IP), the frontal scale (F) and the prefrontal scale (PF)..... 47

Figure 3.4: Image of preserved *A. meleagris* specimens, **a**, demonstrating the colour variation and **b**, dorsal colour and scale pigmentation..... 54

Figure 3.5: Image of preserved *A. orientalis* specimens, **a**, demonstrating the colour variation and **b**, dorsal colour and scale pigmentation..... 62

Figure 3.6: Image of preserved *A. lineicauda* specimens, **a**, demonstrating the colour variation and **b**, dorsal colour and scale pigmentation..... 65

Figure 3.7: Image of preserved *A. caurinus* sp. nov. specimens, **a**, demonstrating the colour variation and **b**, dorsal colour and scale pigmentation..... 68

Figure 3.8: *Acontias caurinus* sp. nov. holotype, SAM ZR 52377, **a**, dorsal view of head, **b**, lateral view of head, **c**, dorsal view of specimen and **d**, ventral view of specimen..... 69

Figure 3.9: Image of preserved *A. parilis* sp. nov. specimens, **a**, demonstrating the colour variation and **b**, dorsal colour and scale pigmentation..... 73

Figure 3.10: *Acontias parilis* sp. nov. holotype, SAM ZR 52394, **a**, dorsal view of head, **b**, lateral view of head, **c**, dorsal view of specimen and **d**, ventral view of specimen..... 74

Figure 3.11: Images indicating the variation of tail shape within species of *Acontias meleagris sensu lato*. **a**, *A. meleagris*, **b**, *A. orientalis*, **c**, *A. lineicauda*, **d**, *A. caurinus* sp. nov. and **e**, *A. parilis* sp. nov.

..... 78

Figure 3.12: Images indicating head shape within species of *Acontias meleagris sensu lato*. **a**, *A. meleagris*, **b**, *A. orientalis*, **c**, *A. lineicauda*, **d**, *A. caurinus* sp. nov. and **e**, *A. parilis* sp. nov..... 80

Chapter 1

General introduction

1.1 Preamble

Climatic and geomorphic events are two of the major catalytic abiotic factors responsible for inducing cladogenesis (Abe & Lieberman, 2009). Lineage diversification in a number of southern Africa herpetofaunal taxa, are products of vicariant events induced through paleoclimatic alterations that occurred primarily during the Miocene and Pliocene / Pleistocene epochs (Matthee & Flemming, 2002; Daniels *et al.*, 2004; Daniels *et al.*, 2006; Lamb & Bauer, 2006; Tolley *et al.*, 2006; Daniels *et al.*, 2007; Smit *et al.*, 2007; Swart *et al.*, 2009). Throughout this period southern Africa was characterized by significant climatic changes and geomorphic events giving rise to landscapes of high heterogeneity (Cowling *et al.*, 2009), generating the ideal template for allopatric speciation.

Climatic oscillations during the Miocene severely impacted the extent of coastal plain habitat, as a result of prolonged marine transgressions and regressions, altering habitat availability and leading to the displacement, fragmentation or extinction of taxa. The development of the proto-Benguela and upwelling system resulted in increased aridification, which became more pronounced during the Miocene / Pliocene transition (Hendey, 1983). In contrast, resurfacing of the coastal plain and the development of finely sculptured landscapes during the Pliocene / Pleistocene transition would have resulted in recent fauna and flora distribution patterns. Furthermore, this period was characterized by marked tectonic uplifts, with more predominant effects in the east of southern Africa and leading to significant changes in drainage systems in the Cape Floristic Region (CFR), promoting allopatric isolation and inducing speciation. The majority of evolutionary studies of southern African herpetofauna have focused on supraterranean taxa, hampering our understanding of factors that influence the partitioning of genetic divergence among subterranean taxa. Phylogeographical patterns for co-distributed supraterranean herpetofaunal taxa in the Cape Floristic Region depict broadly congruent patterns. Genetic partitioning within a diverse array of herpetofauna (for e.g. *Bradypodion*, *Chersina angulata*, *Agama atra*, *Pedioplanis burchelli*) present three general clades; in the north-west to west, south to central and in the east of the southern Cape (Tolley *et al.*, 2006, Daniels *et al.*, 2007, Tolley *et al.*, 2009). The west of the Western Cape province is

characterised by high levels of genetic structure compared to shallow differentiation evident in the east and southern Cape (Swart *et al.*, 2009; Tolley *et al.*, 2009). Cladogenesis within the aforementioned taxa ranged from the Miocene to Pleistocene. The biotic factors that influence the tempos of lineage diversification are habitat specificity, dispersal capacity, reproductive rate and other life history strategies (Hairston & Bohonak, 1998; James & Shine, 2000; Tolley *et al.*, 2006).

Limited comparative phylogeographic research has been undertaken to investigate the congruence of patterns in co-distributed supraterranean and subterranean herpetofauna. It remains unclear on what basis the evolutionary processes responsible for inducing overriding phylogeographic patterns, differ between these two habitat types. Phylogeographic structure in the Californian subterranean lizard, *Anniella pulchra* conforms to the phylogeographic pattern observed in most wide-ranging supraterranean Californian reptiles (Parham & Papenfuss, 2009). The finer scale differences evident were attributed to the taxon's subterranean ecology. In contrast, phylogeographical patterns among subterranean taxa seem to be relatively congruent. For example, two amphisbaenid species exhibiting near identical phylogeographic patterns (Mulvaney *et al.*, 2005; Albert *et al.*, 2007), which are furthermore congruent to phylogeographic patterns of co-distributed surface dwelling taxa with low dispersal abilities (Jockusch & Wake, 2002; Parra-Olea, 2002). Albert *et al.* (2007) concluded that a subterranean life-style permits long-term accumulation of genetic diversity in a similar manner that surface barriers do in supraterranean taxa with low dispersal abilities.

Phylogeographical research on southern African subterranean herpetofauna is limited to the endemic subfamily Acontiinae (Daniels *et al.*, 2002; 2005; 2009). Preceding these studies, the last examination of *Acontias* was undertaken by Broadley & Greer (1969) and comprised a morphological based generic revision. These authors suggest widespread polymorphisms have limited reliable species diagnosis in the genus. In addition, morphological "intermediates" has been recorded between species, suggesting possible hybridization, casting doubt on the current operational taxonomical units within *Acontias* (Broadley & Greer, 1969). Historically, *Acontias* comprised ten evolutionary units; *A. plumbeus*, *A. poecilus*, *A. breviceps*, *A. gracilicauda gracilicauda*, *A. g. namaquensis*, *A. m. meleagris*, *A. m. orientalis*, *A. percivali percivali*, *A. p. occidentalis* and *A. p. tasmani* (Branch, 1998). Recent, genetic studies suggest that convergence in morphology is widespread among fossorial taxa, limiting its utility at delineating species (Townsend *et al.*, 2004; Schmitz *et al.*, 2005; Wiens *et al.*, 2006). Phylogenetic research undertaken by Daniels *et al.* (2005; 2009) revealed that the widely distributed Cape legless

skink, *A. meleagris* was comprised of five evolutionary units, contrasting the existing taxonomic designation. These results furthermore showed *Acontias p. tasmani* to be phylogenetically imbedded in the *A. meleagris* species complex.

The *Acontias meleagris* species complex is distributed along the coastal regions of the western, southern and eastern Cape, including a couple of offshore islands, where the species is predominantly present in sandy soils as well as the adjacent interior of the Klein Karoo (Branch, 1991; 1998; Daniels *et al.*, 2009). This species complex currently includes two subspecies, *Acontias meleagris meleagris* and *Acontias meleagris orientalis* (Branch, 1998), where the latter subspecies comprise two morphs in sympatry in the Eastern Cape, *A. m. orientalis* as the typical morph and *A. m. orientalis*–‘lineicauda’ (Hewitt, 1938). *Acontias percivali tasmani* (included in the *Acontias meleagris* species complex), occur in sympatry with *A. m. orientalis* and *A. m. orientalis*–‘lineicauda’ in the Eastern Cape.

In the *Acontias meleagris* species complex five clades were evident. Two Western Cape clades (for *A. m. meleagris*), a southern Cape clade (for *A. m. meleagris*, *A. m. orientalis* and *A. p. tasmani*) and two Eastern Cape clades (for *A. m. orientalis*–‘lineicauda’) which are genealogically and geographically discrete for sampled areas, diagnostic at both mtDNA and nuDNA level, with marked sequence divergence and no shared haplotypes between clades. From these results the following two observations are worth mentioning one; *A. m. orientalis*–‘lineicauda’ is genetically distinct from *A. m. orientalis* and two; *A. p. tasmani* fall within the same clade (the southern Cape and little Karoo clade) as *A. m. orientalis*, with less than 1 % sequence divergence between these two species for cytochrome oxidase subunit one (Daniels *et al.*, 2009).

In the above mentioned study, large areas of the *A. meleagris* species complex in the Western Cape were unsampled partially the Breede River valley basin and Overberg regions and the southern coastal strip from Struis Bay to Mossel Bay, leaving putative novel species boundaries unknown. Also, the two *A. m. orientalis*–‘lineicauda’ clades at Port Alfred and Port Elizabeth may represent two extremes of a genetic cline, however their non – sister relationship suggest this to be unlikely. The absence of biogeographic dating in Daniels *et al.* (2009) do not allow for any inference as to what extend paleoclimatic and geomorphic events influenced genetic differentiation and cladogenesis within this species complex. The latter is particularly important as it would aid in the understanding of how abiotic factors influence cladogenesis among fossorial taxa.

1.2. Aim of study: To assess the taxonomical status of the *Acontias meleagris* species complex by means of a detailed biogeographic study and morphological examination in an attempt to search for diagnostic morphological characters amongst the genetic lineages.

1.3 The research questions that were addressed in this study were threefold:

1.3.1 Where are the species boundaries between the various clades within the *A. meleagris* species complex?

1.3.2 Can we infer the evolutionary drivers that are responsible for lineage diversification and the current distribution pattern in the *A. meleagris* species complex?

1.3.3 Are the putative novel species morphologically distinct?

Chapter 2

Phylogenetic and biogeographical aspects: Tracking the impact of Pliocene / Pleistocene sea level and climatic oscillations on the cladogenesis of the Cape legless fossorial skink species complex, *Acontias meleagris* in South Africa

*This work formed the bases of a peer reviewed paper accepted for publication:

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2.1 Introduction

Climatic and geomorphic events are two of the major catalytic abiotic factors responsible for inducing lineage diversification in terrestrial biota (Douglas *et al.*, 2006; Ribas *et al.*, 2007; Abe & Lieberman, 2009; D'horta *et al.*, 2011). Evolutionary studies of several southern Africa herpetofaunal taxa, have revealed that allopatric speciation induced by palaeoclimatic changes since the Miocene and Pliocene / Pleistocene epochs are responsible for cladogenesis in these groups (Matthee & Flemming, 2002; Daniels *et al.*, 2004; 2006; 2007; Lamb & Bauer, 2006; Tolley *et al.*, 2006; Smit *et al.*, 2007, Swart *et al.*, 2009). Throughout this period southern Africa was characterized by marked climatic transformations and geomorphic changes (Cowling *et al.*, 2009), resulting in large-scale landscape heterogeneity causing habitat changes that provided the ideal template for lineage diversification.

Climatic oscillations during the Miocene severely impacted the extent of coastal plain habitat, as a result of prolonged marine transgressions and regressions, altering habitat availability of low lying taxa and leading to the displacement, fragmentation or extinction of species in these environments (Hendy, 1982). Furthermore, the development of the proto-Benguela upwelling system resulted in increased aridification, which became more pronounced during the Miocene / Pliocene transition (Siesser, 1981). Geomorphic events during this period involved marked tectonic uplifts which significantly impacted eastern parts of southern Africa. This led to orographic elevation, drainage and substrata rearrangements in the Cape Floristic Region, promoting speciation (Partridge & Maud, 1987; Partridge, 1997; 2000, Cowling *et al.*, 2009). In contrast, resurfacing of the coastal plain, aeolianite formation, dune construction and the development of the Pliocene / Pleistocene landscape resulted in more recent fauna and flora dispersal and

recolonization of low lying coastal areas (Klein, 1972; Hesp *et al.*, 1989; Bateman, 2004; Cowling, 2009). This supports the hypothesis that low lying areas harbour recent evolved species, while mountain areas harbour older lineages (Stuckenberg, 1962). The majority of evolutionary studies pertaining to southern African herpetofauna, have focused on supraterranean taxa (ripiculous or vegetation dependent, for e.g. Tolley *et al.*, 2006; Daniels *et al.*, 2007; Swart *et al.*, 2009). It has been observed from these studies that the west of the Western Cape Province is characterised by high levels of genetic structure compared to shallow differentiation evident in the east and southern Cape (Tolley *et al.*, 2009). Further fine scale variation in distribution patterns and tempos of lineage diversification amongst these reptile lineages can be ascribed to differing biotic factors such as habitat specificity, dispersal capacity, reproductive rate and a suite of life history characteristics (Hairston & Bohonak, 1998; James & Shine, 2000).

Globally, limited comparative phylogeographic research has been conducted to investigate the congruence of patterns between co-distributed supraterranean and subterranean herpetofauna, hampering our understanding of factors that influence the partitioning of genetic divergence among subterranean taxa. It remains unclear which evolutionary processes are responsible for inducing phylogeographic patterning and cladogenesis in subterranean habitats. Considering the assortment of adaptations to the subterranean life style (for e.g. limblessness and habitat specificity), the availability and location of suitable habitat, it is likely that subterranean taxa would incur a disadvantage over supraterranean taxa in the event of colonizing refugial areas during unfavoured environmental conditions. The morphological convergence and homogenous habitat of the subterranean system furthermore limit the possibilities of ecological divergence (López & Martin, 2009). This would render their phylogeographic structure closely linked to historical and contemporary habitat shifts. Interlinked, abiotic processes such as marine transgressions, regressions, aeolianite development and dune construction are potentially major abiotic factors driving lineage diversification within coastal subterranean taxa. The Cape legless skink, *Acontias meleagris* forms the ideal template taxon with which to explore phylogeographic patterns in the low lying coastal regions of the Eastern, Northern and Western Cape provinces of South Africa.

The Cape legless skink, *Acontias meleagris* species complex, is a monophyletic grouping, comprised of five statistically well supported lineages (Daniels *et al.*, 2009). Species and subspecies within the Cape legless skink species complex are widely distributed in sandy soils in the low lying coastal regions of the Eastern, Northern and Western Cape provinces and the adjacent interior of the Little Karoo, including a number of continental offshore islands (Branch, 1991, 1998; Daniels *et al.*, 2009). The *Acontias*

meleagris species complex currently comprise three subspecies *Acontias meleagris meleagris*, *Acontias meleagris orientalis* and *Acontias percivali tasmani*, with the latter two subspecies being sympatric in the Eastern Cape (Branch, 1998; Daniels *et al.*, 2002; 2005; 2009). Within *A. m. orientalis* two morphs occur in sympatry in the Eastern Cape, as the typical morph and *A. m. orientalis* – *lineicauda* morph (Hewitt, 1938).

In Daniels *et al.*, (2009), large geographic areas in the distribution range of the *A. meleagris* species complex were unsampled particularly in the Breede River valley basin, the Overberg regions and the southern coastal plains from Struis Bay to Mossel Bay, limiting our phylogeographic inference. The absence of biogeographic dating in Daniels *et al.* (2009) precluded inference about what palaeoclimatic and geomorphic events influenced genetic differentiation and cladogenesis within this species complex. The latter is particularly important, as it would aid our understanding of how abiotic and biotic factors promote speciation among subterranean taxa.

The aim of this study is twofold; firstly, to conduct a fine scale phylogeographical study in order to determine the geographical boundaries of evolutionary lineages within the *A. meleagris* species complex. Secondly, to investigate the evolutionary drivers responsible for lineage diversification and current distribution patterns using a dated phylogeny of the species complex. We therefore combine phylogeographic data, time of lineage diversification, aspects of demographic history and population analyses, in addition with spatial environmental data to explore the phylogeography of the taxon. This holistic approach should permit elucidation of the evolutionary drivers operating within this species complex and allow for a comparison of phylogeographic patterning between co-distributed taxa in twodistinct habitat types.

2.2 Materials and Methods

2.2.1 Sampling

In the present study, 133 specimens were collected from 38 sample localities along the coastal regions and adjacent interior of the Eastern, Northern and Western Cape provinces of South Africa. Newly collected data were combined with data from 98 specimens collected during two earlier studies (Daniels *et al.*, 2005, 2009). A total of 231 specimens from 55 localities were used (Fig. 2.1, Table 2.1). A handheld global positioning system (GPS) was used to record the coordinates of sample localities. Sampling included the known distributions of all subspecies and morphs. Specimens were identified to species and / or subspecies based on their distribution and morphological characteristics, using Branch (1998). Animals were euthanized using, sodium pentobarbitone (200mg, dose: 60mg / kg) under ethical clearance from the Stellenbosch University Research Ethics Committee (REF: 10NP – ENG01). The use of sodium pentobarbitone for euthanasia of vertebrates is recommended by several International Ethics Committees including both the American Society for Ichthyologists and Herpetologists (ASIH, 2004) and the American Veterinary Medical Association (AVMA, Euthanasia 2007). A lethal dose of sodium pentobarbitone was administered intraperitoneally. Animals were confirmed dead if no muscle contraction and no heartbeat were observed, following a minimum period of 60 minutes post injection. A biopsy of the liver or muscle tissue was undertaken from specimens and tissue samples were stored in absolute ethanol until required for DNA extraction. Carcasses were labeled and preserved in a 4% buffered formalin solution. Both the large and smaller bodied *Acontias* species were shown to form monophyletic groups, sister to the monophyletic *Acontias meleagris* species complex. The following species were employed as outgroups; *A. breviceps*, *A. gracilicauda*, *A. lineatus*, *A. litoralis*, *A. occidentalis*, *A. percivali*, *A. plumbeus*, and *A. tristis* (Daniels *et al.*, 2005; Lamb *et al.*, 2010).

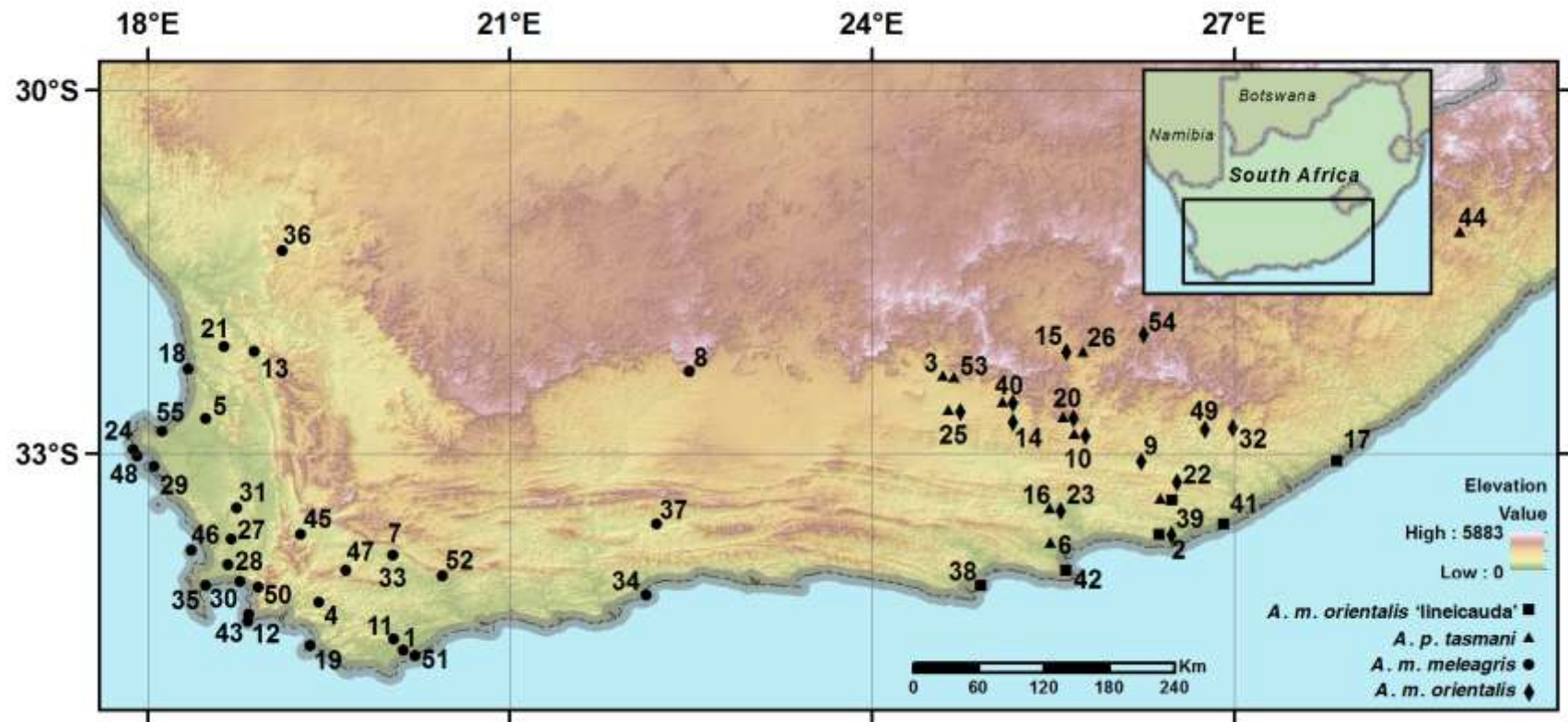


Figure 2.1: Sampling localities for specimens of the *Acontias meleagris* species complex used in this study, along the coastal regions and adjacent interior of the Eastern, Northern and Western Cape provinces of South Africa.

Table 2.1: Geographic and molecular sampling information for specimens used in the study of the *Acontias meleagris* species complex. Locality numbers correspond to the numbers on figure 2.1 and are further assigned to respective provinces, i.e. Eastern Cape (EC), Northern Cape (NC) and Western Cape (WC) provinces. Asterisks (*) denote mitochondrial DNA sequences (16S rRNA and COI) included from Daniels *et al.*, (2005, 2009).

Locality number on map	Sample locality	Province	N	GPS coordinates	16S rRNA	COI	EXPH5
1	Agulhas	WC	1	34.626730 20.120700	1	1	1
2	Alexandria	EC	3	33.671783 26.417313	3	3	3
3	Aberdeen	EC	6	32.352500 24.585666	6	6	3
4	Ashton	WC	4	34.226666 19.416666	4	4	3
5	Aurora	WC	1	32.708300 18.484154	1	1	1
6	Baakins Valley	EC	1	33.733477 25.477295	1	1	0
7	Barrydale	WC	6	33.838333 20.033333	6	6	3
8	Beaufort West *	WC	1	32.321111 22.486111	1	1	0
9	Bedford	EC	7	33.068666 26.230000	7	7	1
10	Bloemhof	EC	10	32.825000 25.725000	10	10	8
11	Bredasdorp	WC	7	34.533333 20.041667	7	7	3
12	Cape Hangklip *	WC	1	34.383333 18.833333	1	1	0
13	Clanwilliam *	WC	3	32.151944 18.887222	3	3	0
14	Cookhouse	EC	4	32.590000 25.135833	4	4	3
15	Cradock	EC	3	32.158756 25.613251	3	3	1
16	Dunbrody	EC	4	33.471666 25.541666	4	4	1
17	East London	EC	4	33.058333 27.852166	4	4	2
18	Elands Bay *	WC	2	32.302500 18.336666	2	2	0
19	Gansbay	WC	1	34.583917 19.346881	1	1	1
20	Graaff Reinet	EC	2	32.701833 25.635166	2	2	1
21	Graafwater *	EC	2	32.117500 18.635555	2	2	0
22	Grahamstown *	EC	10	33.270277 26.536111	10	10	0
23	Hope Fountain	EC	3	33.471666 25.541666	3	3	2
24	Jacobs Bay *	WC	2	32.966666 17.883333	2	2	0
25	Jansenville	EC	5	32.638333 24.685000	5	5	4
26	Katberg	EC	4	32.151833 25.750000	4	4	1
27	Klipheuwel	WC	6	33.703611 18.695277	6	1	1
28	Kuilsriver *	WC	1	33.916666 18.666666	1	1	0
29	Langebaan *	WC	5	33.104444 18.051666	5	5	0
30	Macassar	WC	1	34.053886 18.765593	1	1	1
31	Malmesbury *	WC	1	33.450000 18.737777	1	1	0
32	Middeldrift	EC	1	32.791892 26.993408	1	1	1
33	Montagu	WC	4	33.838333 20.033333	4	4	3

Table 2.1 (continued)

Locality number on map	Sample locality	Province	<i>N</i>	GPS coordinates		16S rRNA	COI	EXPH5
34	Mossel Bay *	WC	5	34.166666	22.133333	5	5	0
35	Muizenberg	WC	1	34.091336	18.480549	1	1	1
36	Nieuwoudtville	NC	3	31.324100	19.114820	2	2	3
37	Oudtshoorn *	WC	6	33.583333	22.216666	6	6	0
38	Oyster Bay	EC	1	34.090277	24.902222	1	1	0
39	Paterson	EC	4	33.365000	26.502166	4	4	2
40	Pearston	EC	5	32.586666	25.135833	4	4	5
41	Port Alfred *	EC	8	33.583333	26.916666	8	8	0
42	Port Elizabeth *	EC	6	33.961444	25.612392	6	5	0
43	Pringle Bay	WC	2	34.347617	18.831511	2	2	2
44	Qumbu	EC	2	31.162106	28.869554	2	2	1
45	Rawsonville	WC	3	33.666666	19.268333	3	3	3
46	Robben Island *	WC	18	33.800000	18.366666	18	18	0
47	Robertson	WC	1	33.966600	19.644000	1	1	1
48	Saldanha Bay *	WC	1	33.016666	17.916666	1	1	0
49	Salem	EC	7	32.801833	26.758333	7	7	1
50	Sir Lowry's Pass *	WC	1	34.100000	18.916666	1	1	1
51	Struis Bay	WC	7	34.666666	20.216666	7	7	2
52	Swellendam	WC	1	34.008843	20.443153	1	1	1
53	Tandjiesberg	EC	1	32.369523	24.682846	1	1	0
54	Tarkastad	EC	4	32.016666	26.250000	4	4	1
55	Velddrif *	WC	28	32.816933	18.117222	28	28	0
Total			231			229	223	71

2.2.2 DNA extraction, PCR and sequencing

Total genomic DNA was isolated from liver or muscle tissue. DNA was extracted from ethanol preserved tissue using a Qiagen DNEasy kit (USA), following the manufacturer's protocol. Prior to use 1 µl of extracted DNA was diluted with 19 µl of distilled water. Two mitochondrial (mtDNA) markers 16S ribosomal RNA (16S rRNA) and the cytochrome oxidase subunit one (COI), in combination with the protein coding nuclear marker (nuDNA) exophilin 5 (EXPH5) were used to examine evolutionary affinities within the *Acontias meleagris* species complex. The 16S rRNA locus has a slow mutation rate and has been used to examine relationships among both closely and distantly related taxa, whilst the COI locus has a faster mutation rate and has been used at a number of taxonomic levels (Daniels *et al.*, 2002; Podnar *et al.*, 2005; Albert *et al.*, 2007; Recuero *et al.*, 2007; Passoni *et al.*, 2008; Paulo *et al.*, 2008; Fonseca *et al.*, 2009; Musilova *et al.*, 2010). The use of the two mtDNA markers further allow the combination of data generated in this study with that of Daniels *et al.*, (2005; 2009). The functional constraints of exophilin 5 (EXPH5) is poorly understood, but the locus has recently been used in phylogeographic studies of reptiles (Portik *et al.*, 2010, 2011). The three loci (16S, COI and EXPH5) were amplified using the primer pairs, 16Sa and 16Sb (Cunningham *et al.*, 1992) for the 16S rRNA partial gene fragment, COIA and COIF (Palumbi *et al.*, 1991) for the COI partial gene fragment (COI) and the primer pairs EXPH F1 and EXPH R1 (Portik *et al.*, 2010) were used to amplify EXPH5.

The PCR temperature regime for both mitochondrial gene fragments were similar to Daniels *et al.*, 2009. While the PCR regime for the nuclear marker was 94°C for 4 min; 94°C for 30 s; 60°C for 40 s; 72°C for 40 s cycles for the last three steps, followed by a final extension of 10 min at 72°C for the nuclear gene fragment. PCR products were electrophoresed in 1% regular agarose gel containing ethidium bromide, for about 30 min at 70 V and visualized under a UV light and purified using a PCR purification kit (Qiagen). Purified PCR products were cycle sequenced using standard protocols (3 µl of the purified PCR product, 4 µl of the fluorescent-dye terminators with an ABI PRISM Dye Terminator Cycle Sequencing Reaction Kit (Perkin-Elmer), and 3 µl of a 10 µM primer solution for each primer pair). Unincorporated dideoxynucleotides were removed using Sephadex G-25 (Sigma). Sequencing was performed on an ABI 3700 automated machine.

2.2.3 Phylogenetic analyses

Forward and reverse sequences were checked for base ambiguity in Sequence Navigator (Applied Biosystems) and a consensus sequence was created. The 16S rRNA sequences were aligned in

CLUSTAL X (Thompson *et al.*, 1997), using the default parameters of the program. Mismatches made by computational alignment, were adjusted by eye. The protein coding mitochondrial COI and nuclear EXPH5 data sets were adjusted manually. Phylogenetic analyses were executed in PAUP *(4 beta 10), RAxML (version 7.0.4) and MrBayes (version 3.1.2) for maximum parsimony (MP), maximum likelihood (ML) and an analysis of Bayesian inference (BI) respectively (Swofford, 2002; Stamatakis, 2006; Ronquist & Heulsenbeck, 2003).

All three phylogenetic analyses were conducted on a reduced (using a single representative sample per locality), concatenated mtDNA and nuDNA data set (16S rRNA, COI and EXPH5) while BI was further conducted on the complete concatenated mtDNA data set (16S rRNA and COI), the complete single COI data set as well as the complete single EXPH5 data set. Phylogenetic patterns and tree topologies were established on independent data sets for all three markers prior to analyses on reduced data sets. The EXPH5 sequence data were only generated for newly collected (71) specimens collected during this study, due to amplification difficulties of degraded DNA samples from the two earlier studies by Daniels *et al.* (2005, 2009, Table 2.1). For Maximum Likelihood and Bayesian Inference analysis, the appropriate substitution models were calculated using MODELTEST (version 3.06, Posada & Crandall, 1998). The best fit maximum likelihood score was chosen using the Akaike Information Criterion (AIC, Akaike, 1973). For the MP analysis, trees were generated using a heuristic search option with tree bisection, reconnection (TBR) and branch swapping with 100 random taxon additions. Gaps and missing data were excluded as characters. Phylogenetic confidence in the nodes recovered from parsimony was estimated by bootstrapping 1000 pseudo replicates of data sets. Uncorrected sequence divergence values (“p”) were calculated in PAUP* for each of the three loci sequenced. Maximum Likelihood was performed according to the GTR model with the addition of Γ -distribution rate heterogeneity (GTRGAMMA) as implemented in RAxML, where support was assessed by analyzing 1000 replicates of the topology. Bootstrap values < 70% were considered as weakly supported and values > 70% as statistically well supported (Hollis & Bull, 1993). For ML and BI the combined data sets were partitioned by gene and run according to the substitution models inferred by MODELTEST; 16S rRNA (TrN + I), COI (GTR + I + G) and EXPH5 (K81uf + I). For each analysis of BI four Markov chains was run, with each chain starting from random trees and run for five million generations; sampling each tree every 10,000 generations. A 50% majority rule consensus tree was generated from the trees retained (after burn in trees were discarded using likelihood plots); with posterior probabilities (pP) for each node estimated by the percentage of time the node was recovered. A posterior probability of > 0.95 was considered statistically well

supported (Wilcox *et al.*, 2002). Maximum Parsimony and ML were conducted on the Smithsonian cluster (Harvard – Smithsonian cluster, Centre of Astrophysics).

2.2.4 Population genetic analyses

Since the COI tree topology was identical to that of the total evidence DNA data set and was thoroughly sampled geographically; population genetic analyses, historical demography and molecular diversity indices were conducted exclusively on the COI data set. While the Extended Bayesian Skyline Plots (EBSPs) were conducted on the multilocus data set (16S rRNA, COI and EXPH5). To examine fine scale evolutionary relationships between sample localities, haplotype networks were constructed in TCS (version 1.21, Clement *et al.*, 2000), using statistical parsimony with 95% confidence. As a complementary approach, spatial clustering analysis was conducted using Bayesian Analysis of Population Structure (BAPS version 5.2, Coriander *et al.*, 2003). Spatial clustering was carried out on individuals, i.e. phylogroups were not prespecified. Hierarchical analysis of molecular variance (AMOVA) and evaluation of molecular diversity indices were performed on the mtDNA COI data for the phylogroups retrieved (evident from the phylogeny), using ARLEQUIN (version 3.0, Excoffier *et al.*, 2005). In addition pairwise fixation indices (Φ_{ST}), nucleotide diversity (π_n), number of haplotypes (Nh), number of polymorphic sites (Np) and haplotype diversity (h) were calculated, using 10 000 randomizations.

2.2.5 Historical demography and molecular diversity

To test for deviations from the neutral theory model of equilibrium, i.e. sudden changes in effective population size due to bottlenecks and population expansions, Fu's F_s (Fu, 1997) test was conducted for each phylogroup separately, using ARLEQUIN. This neutrality test is proven to be a more powerful test than Tajima's D in detecting population growth for large sample sizes (Ramos-Onsins & Rozas, 2002). Values near zero indicate stable populations, i.e. adhering to the neutral theory model of equilibrium, where negative values could be an indication of either expansion or positive selection (Fu, 1997). Therefore, the McDonald- Kreitman test (MK; McDonald & Kreitman, 1991) for selection (comparing synonymous and non-synonymous substitution rates) was conducted across all localities using DnaSP (version 5.1, Librado & Rozas *et al.*, 2009). Furthermore, mismatch distribution analysis (MMD) was conducted on the COI data set for each phylogroup, where the goodness of fit between observed and expected MMD were further assessed with sum of square deviations (SSD) and Harpending's raggedness

index (RI) as implemented in ARLEQUIN. Significant (p) values indicate the rejection of the expansion hypothesis model (Rogers & Harpending, 1992). Time since the beginning of the expansion was estimated using $\tau = 2ut$, where “ t ” is the time elapsed between initial and current population size, and $u = 2\mu k$, where “ μ ” is the mutation rate and “ k ” is the length of the sequence (Rogers & Harpending, 1992).

To further explore differences of demographic history among the clades retrieved, both Bayesian and Extended Bayesian Skyline Plots (BSPs and EBSPs) were constructed. Bayesian Skyline Plots were conducted on the reduced COI data set, while EBSPs were conducted on the reduced data sets for all three markers (16S rRNA, COI and EXPH5) for all phylogroups retrieved, using BEAST (version 1.6, Drummond *et al.*, 2005; Drummond & Rambaut, 2007; Heled & Drummond, 2008). This was to test for conflicting patterns between methods applied to examine demographic history patterns and should therefore allow for a comparison of results obtained from MMD and BSPs, both conducted on the COI data set. In contrast to Bayesian skyline plots, EBSPs allow the incorporation of multiple loci to estimate effective population size through time, which are thus more accurately estimated. Bayesian and Extended Bayesian Skyline Plots generate a posterior distribution of effective population size through time using a Markov Chain Monte Carlo (MCMC) sampling method. For EBSPs, all models were unlinked for all analyses, linear models were applied and run for 10 million generations, sampled every 1,000 generations and further examined for stationarity using Tracer (version 1.5.0, Rambaut and Drummond, 2009). Since the fossil record for lizards (specifically Scincidae) are generally poor, fixed mutation rates for each of the mitochondrial gene fragments (16S and COI) were used and further used to estimate the mutation rate of the nuclear marker (EXPH5).

2.2.6 Divergence time estimation

Divergence time assessment between the phylogroups retrieved was estimated in BEAST, using the total evidence DNA data. A multilocus approach should result in more accurate estimations of divergence times especially when the methods applied to estimate divergence times, permit different evolutionary rates for the different loci used (Thorne & Kishino, 2002; Yoder & Yang, 2004; Noonan & Chippendale, 2006; Drummond & Rambaut, 2007). Divergence time estimation was conducted using a Bayesian framework thereby employing a probabilistic model to define rates of molecular sequence evolution of lineages over time, using the MCMC method to derive clade ages as implemented in BEAST. A relaxed molecular clock was employed (Drummond *et al.*, 2006), using a fixed mutation rate of 0.22% per million years for 16S rRNA (Graybeal, 1997; Emerson *et al.*, 2000; Honda *et al.*, 2006) and minimum and

maximum of 0.61% and 0.7% per million years for COI (Macey *et al.*, 1998a,b; Weisrock *et al.*, 2001; Honda *et al.*, 2006). The mutation rate for EXPH5 was estimated using a non-informative (1/x) prior since the mutation rate for this locus is unknown. A multiple coalescent model was used for *A. meleagris* (Heled & Drummond, 2010). Twelve independent MCMC chains were run for 10 million generations and sampled every 10,000 generations. The convergence of the 12 combined chains was determined by EES for each parameter in Tracer after appropriate burnin cut-off. Trees in the 12 chains were combined using LogCombiner and were assessed using TreeAnnotater. A chronogram was constructed using FigTree (version 1.3.1, Rambaut, 2009).

2.2.7 Environmental statistical analyses

A total of 103 environmental variables were extracted for each sample locality using a geographical information system (GIS). The variables included climatic parameters (e.g. monthly mean precipitation, monthly mean temperature, monthly minimum temperature and monthly maximum temperature, Van Niekerk & Joubert, 2011); soil properties (e.g. depth and clay content, ARC, 2006); and terrain information (e.g. elevation, slope gradient, and slope aspect, Van Niekerk, 2001). All datasets were projected to a common coordinate system (based on the Hartebeesthoek 94 datum) to enable spatial comparison. Due to the large number of variables, the extraction process was automated in ArcView GIS 3.3 software using the Avenue programming language. Environmental variables were subjected to hierarchical tree clustering, by means of Ward's method, using squared Euclidean distances as a measure to uncover the intrinsic structure of the data set and to reduce the number of environmental variables. Unlike principal component analysis, cluster analysis uses all the variances or information contained in the original data set (Razmkhah *et al.*, 2010). Interpretation of the resulting hierarchical structure was context dependent and 26 variables were subjected to further statistical analyses. The reduced data set was divided into individual parameters and those that were measured over twelve months. Clade 5 was excluded from all environmental statistical analyses due to small sample sizes. Individual parameters were soil depth, soil clay content, altitude, number of fog days, number of cloudy days, summer aridity index, precipitation seasonality, topographic wetness index, distance to sea, continentality index, latitude, number of frost days, mean annual potential evaporation, mean soil water stress percent days under stress, mean annual precipitation, coefficient of variation annual precipitation, mean annual minimum temperature, mean annual maximum temperature and mean annual temperature. Measurements from January to December were potential evaporation, daily mean relative humidity, median precipitation,

solar radiation per month, mean daily mean temperature, mean daily minimum temperature and mean daily maximum temperature.

The means of individual parameters were compared using analysis of variance (ANOVA) and least significance difference (LSD) was used for post-hoc testing. The homogeneity of the variances was tested with Levene's test. A mixed model repeated measures ANOVA was conducted to assess variation due to monthly effects (i.e. on repeated measurements of environmental variables). The variance estimation and precision package (VEPAC) was used in ANOVA analysis of monthly measurements utilizing the restricted maximum likelihood as a method of parameter estimation (REML). Least square means from mixed models were used to assess interaction factors and fixed effects were "month" (for the seven monthly measurements), "clade" (clades as retrieved by phylogenetic analyses) and their interaction term. Least significance difference (LSD) was conducted as a post-hoc test. Analyses were conducted using the computer software programme STATISTICA (version 10, StatSoft, Inc. 1984 – 2011)

2.3 Results

2.3.1 Phylogenetic analyses

The BI topology of the combined mtDNA data set (16S rRNA and COI) revealed similar clades and were near identical to the total evidence DNA data set (16S rRNA, COI and EXPH5) for all phylogenetic analytical methods (MP, ML). Hence, only the topology derived from the total evidence DNA data set is shown and discussed (Fig. 2.2). The reduced concatenated mtDNA and nuDNA data set yielded a total of 1655 bp (461 bp for 16S rRNA locus, 552 bp for the COI locus and 642 bp for EXPH5). Newly generated sequences from the present study have been deposited in GenBank (16 rRNA; JQ692450-JQ692571; COI; JQ692328-JQ692449, and EXPH5; JQ278035-JQ278112).

The monophyly of the *Acontias meleagris* species complex were supported by both MP and ML for the total evidence DNA data set. For MP, 1000 trees were retrieved, with tree length of 623 steps, CI = 0.47 and RI = 0.77 from 176 parsimony informative characters. Five clades were consistently retrieved for the species complex of which most were statistically well supported ($> 70\%$ / > 0.95 pP, Fig. 2.2). Similar to Daniels *et al.*, (2009) two distinct, distantly related Western Cape clades were evident for the species complex (Clade 2 and 4). The basal Western Cape clade (Clade 4) in the northern parts of the province occurs predominantly in the interior from Aurora to Robertson and encompassed several coastal localities

(> 70% / > 0.95 pP). The second Western Cape clade (Clade 2) in the southern parts of the province has a coastal distribution from Jacobs Bay to Sir Lowry's Pass and includes Robben Island (> 70% / > 0.95 pP). Fine scale geographical sampling during the present study, revealed an additional clade (Clade 3) spanning the Breede River Valley from Nieuwoudtville in the Northern Cape Province to the Agulhas plain in the southern parts of the Western Cape Province (> 70% / > 0.95 pP). The geographically widely distributed clade (Clade 1) spanning the coastal and interior sections of both the Western and Eastern Cape provinces previously retrieved by Daniels *et al.*, (2009), now have its geographical boundaries from Ashton in the west (Breede River Valley), Mossel Bay to Qumbu in the far north eastern part of the Eastern Cape. Clade 1 was statistically poorly supported except for the BI analysis. The eastern *A. m. orientalis* – lineicauda morph (Clade 5, includes Port Alfred, Alexandria, Paterson occurring inland and East London and the surrounding areas (Clade 5, > 70% for ML, < 70% for MP and > 0.95 pP for BI). Oyster Bay and Port Elizabeth were unresolved on the MP and BI topology, however, Oyster Bay grouped with Clade 5 on the ML topology, with weak statistical support (Fig. 2.2).

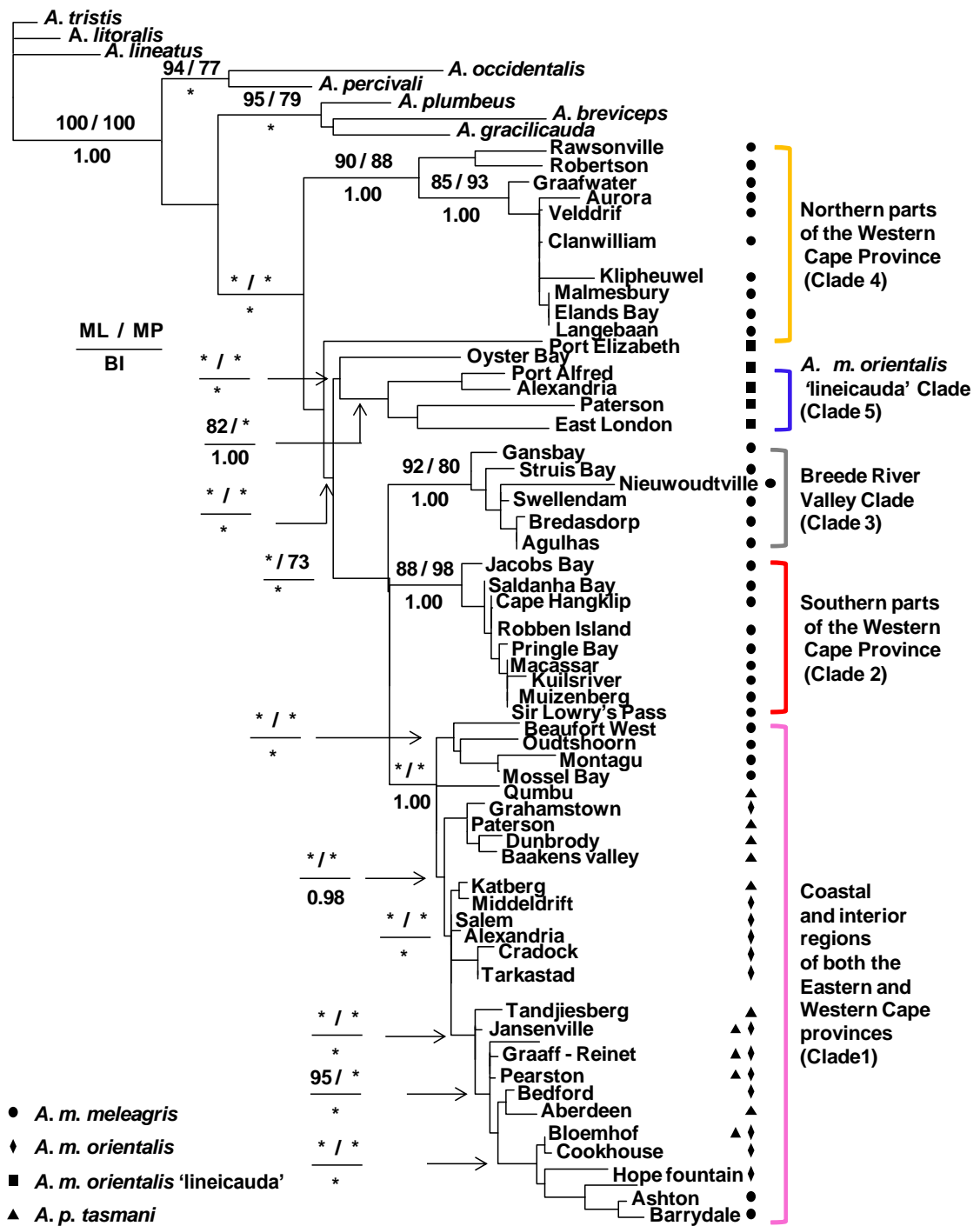


Figure 2.2: Maximum Likelihood topology for the total evidence DNA data (16S rRNA, COI and EXPH5) for specimens of the *Acontias meleagris* species complex, including eight outgroups; *A. breviceps*, *A. gracilicauda*, *A. lineatus*, *A. litoralis*, *A. occidentalis*, *A. percivali*, *A. plumbeus* and *A. tristis*. Bootstrap values for maximum likelihood and maximum parsimony (%) are shown above the nodes and posterior probability (pP) below the nodes. Asterisks indicate statistically poorly supported nodes (< 70% / < 0.95 pP). Geometric symbols on the tree topology indicate the distribution of 'subspecies' / 'morphotypes'.

Uncorrected sequence divergence for the COI locus was 3.4% between the clade spanning the coastal and interior regions of both Eastern and Western Cape provinces (Clade 1) and the Breede River Valley (Clade 3) and 3.8% between Clade 1 and the clade spanning the southern parts of the Western Cape Province (Clade 2), while the sequence divergence between the two Western Cape clades (Clades 2 and 4) were 6.7%.

2.3.2 Population genetics

TCS collapsed the 223 COI sequences into 82 haplotypes at 95% confidence and retrieved seven distinct haplogroups (Fig. 2.3). The largest haplogroup (haplogroup 1) comprised individuals from both the Breede River Valley (Clade 3) and the clade spanning the coastal and interior regions of both the Eastern and Western Cape provinces (Clade 1). The sharing of haplotypes only occurred between Clades 1 and 3 and is evident at Bredasdorp, Nieuwoudtville, Struis Bay and Tarkastad (haplotype 47). Haplogroup 2 corresponds to the Clade 2. Both haplogroups 3 and 4 corresponds to the clade spanning the northern regions of the Western Cape (Clade 4), where Rawsonville and Robertson formed a distinct haplogroup in the Breede River Valley (haplogroup 4). The *A. m. orientalis* – lineicauda morph was subdivided into haplogroup 5 (Port Alfred and Alexandria) and haplogroup 6 (East London and surrounding area). Port Elizabeth was retrieved as a distinct group; haplogroup 7. Refer to appendix 1 for the distribution of haplotypes across sampled localities.

Five genetically distinct groups were retrieved, using BAPS (Fig. 2.4). Similar to the TCS results, the Breede River Valley (Clade 3) and the clade spanning both the Eastern and Western Cape provinces (Clade 1) are regarded as a single genetic unit and Port Elizabeth is considered to be genetically distinct as a second group. Furthermore, the two Western Cape clades (Clades 2 and 4) are genetically distinct as evident from the phylogenetic analyses, forming a third and fourth group. The fifth group contained specimens from East London and surrounding areas, Port Alfred, Oyster Bay and the *A. m. orientalis* – lineicauda specimen from Paterson, Rawsonville and Robertson in the Western Cape. Specimens from Alexandria clustered with both the Breede River Valley group and the East London genetic unit.

Marked genetic structure was observed using AMOVA indicating that 67.28% of the variation occurred between phylogroups of the *A. meleagris* species complex ($df = 4$, $V_a = 67.28$, $p < 0.05$) with 23.31% variation occurring among sample localities within phylogroups ($df = 54$, $V_b = 23.31$, $p < 0.05$), while 9.41% variation occurring within sample localities ($df = 174$, $V_c = 9.41$, $p < 0.05$). All comparisons between sample localities yielded highly significant F_{ST} values (results not shown). Nucleotide diversity

decreases from the eastern parts of the *A. meleagris* species complex distribution range (i.e. the *A. m. orientalis* – lineicauda morph, Clade 5) towards the interior (Clade 1) followed by the Breede River Valley (Clade 3), the clade spanning the northern regions of the Western Cape Province (Clade 4) and the clade in the southern regions of the Western Cape Province (Clade 2) having the lowest nucleotide diversity. In general haplotype diversity decreased from east to west. Molecular diversity indices for the various clades are summarized in table 2.2.

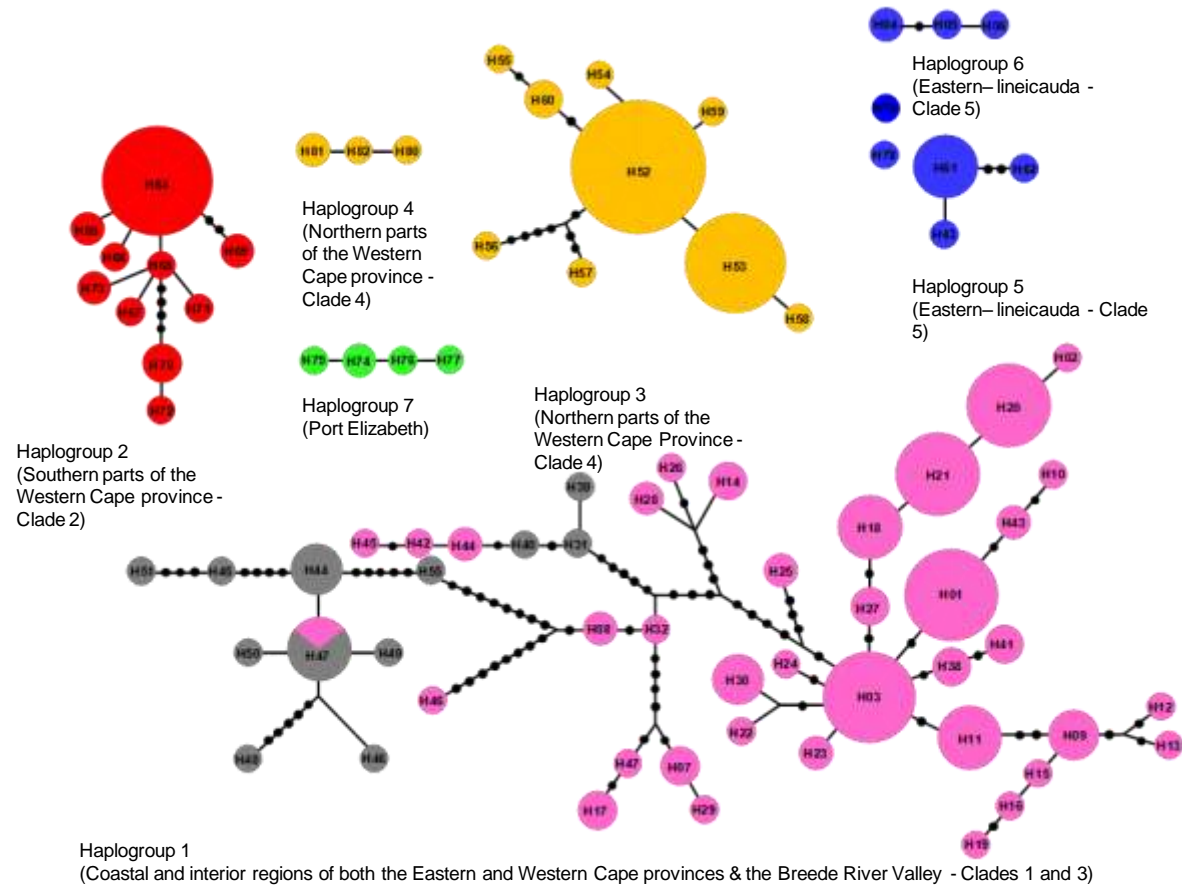


Figure 2.3: Illustration of the seven haplogroups retrieved by TCS. Various colours in the haplotype network indicate genetic groupings as retrieved by phylogenetic analyses, where circle size indicates relative frequencies. See appendices S2 – S5 in Supporting Information for distribution of haplotypes across sampled localities.

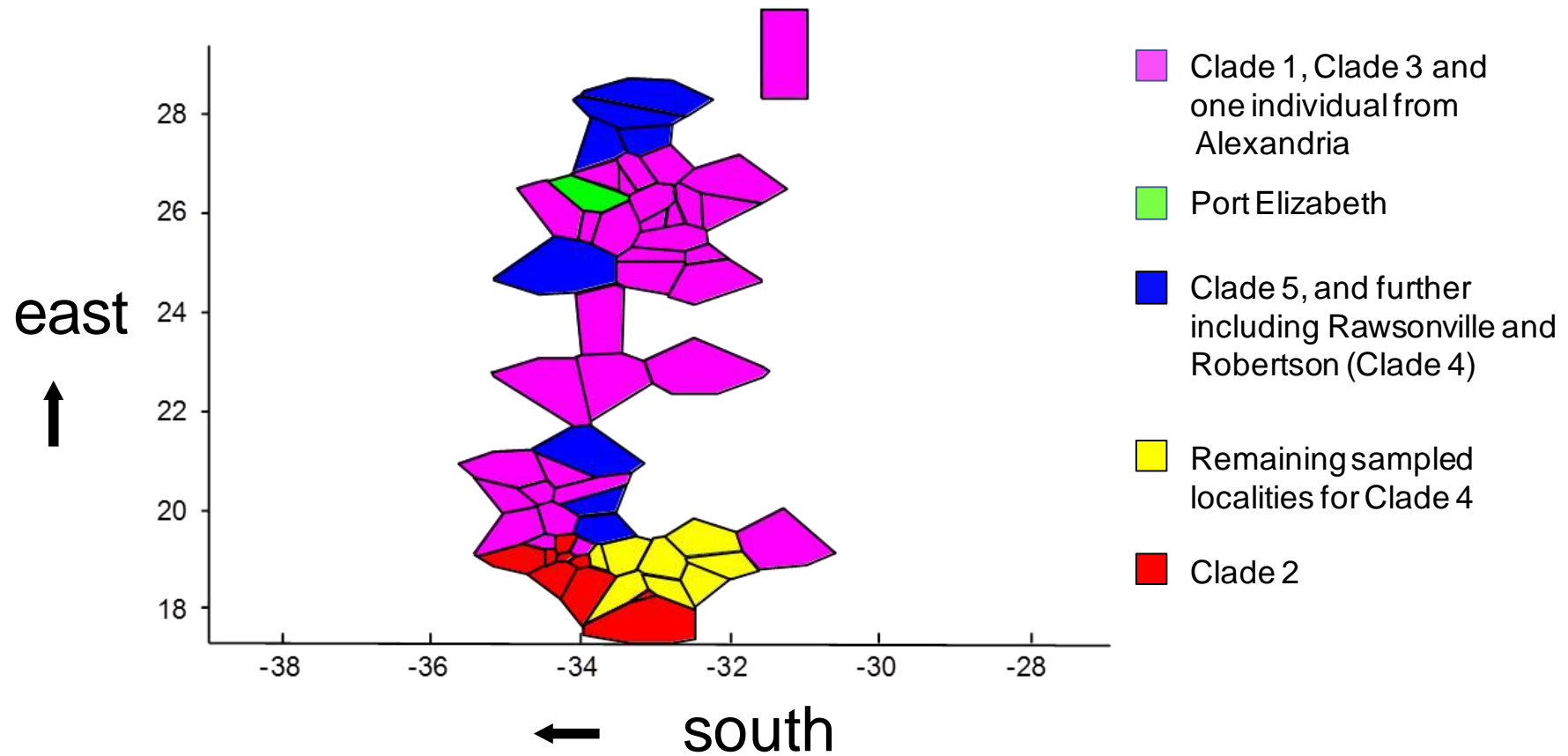


Figure 2.4: Tessellation illustration of Bayesian analysis of population structure, where each cell of the tessellation corresponds to the physical neighbourhood of an observed data point and is coloured according to genetic distinctiveness.

Table 2.2: Diversity measures for various clades of the *Acontias meleagris* species complex, where Nh indicate the number of haplotypes, Np the number of polymorphic sites, h the haplotype diversity and π_n the nucleotide diversity for the COI data set.

	N	Nh	Np	h	π_n
Clade 1 – Coastal and interior of the Eastern and Western Cape provinces	118	43	79	0.9631 ± 0.0061	0.016208 ± 0.008332
Clade 2 – Southern parts of the Western Cape Province	28	10	27	0.7434 ± 0.0840	0.005981 ± 0.003528
Clade 3 – Breede River Valley	19	11	31	0.9006 ± 0.0489	0.016036 ± 0.008656
Clade 4 – Northern parts of the Western Cape Province	47	12	32	0.7216 ± 0.0544	0.008322 ± 0.004618
Clade 5 – Eastern <i>A. m. orientalis</i> - lineicauda	15	9	40	0.8476 ± 0.0878	0.023047 ± 0.012359

2.3.3 Historical demography and molecular diversity

Mismatch analyses and BSPs of the COI data revealed similar results to that of EBSPs of the total evidence DNA set, therefore only BSPs and MMD for the COI data are shown (Fig. 2.5). Four of the five clades retrieved by phylogenetic analyses, showed demographic stability (statistically well supported, Table 2.3). The clade spanning the coastal and interior regions of both the Eastern and Western Cape provinces (Clade 1) displays a unimodal distribution of observed frequencies of pairwise differences and does not reject deviation from the expansion model (goodness of fit, $SSD = 0.00386$, $p > 0.05$; raggedness index = 0.004, $p > 0.05$). The negative and statistically significant Fu's F_s value for Clade 1 further corroborate an expansion event (Fu's $F_s = -12.01$, $p < 0.02$). The MK test did not reveal significant differences in synonymous and nonsynonymous changes between clades ($G = 0.586$; $p = 0.4439$), suggesting that the COI marker is not deviating from neutrality. Therefore the pattern observed might be resultant of recent expansion. However, both BSPs and EBSPs indicate demographic stasis for this clade for the last 12 500 years. Fu's F_s for clades 2 to 5 were not statistically significant limiting inference regarding demographic expansions and contracting events.

2.3.4 Divergence time estimation

Lineage diversification within the *Acontias meleagris* species complex occurred approximately 2.24 Mya (95% confidence interval = 7.2–0.84 Mya). Within this species complex most clades diverged simultaneously, where the eastern *A. m. orientalis* – *lineicauda* Clade (Clade 5) diverged approximately 1.26 Mya (confidence interval = 3.77–0.41 Mya), the clade in the northern parts of the Western Cape Province (Clade 4), approximately 1.24 Mya (confidence interval = 3.4–0.35 Mya) and the clade spanning both the Eastern and Western Cape provinces (Clade 1), approximately 1.13 Mya (confidence interval = 3.23–0.43 Mya). The diversification between the southern parts of the Western Cape Province (Clade 2) and the Breede River Valley clade (Clade 3), occurred approximately 1.12 Mya (confidence interval = 3.01–0.36 Mya), where Clade 3 age approximately 0.63 Mya (1.8–0.14 Mya) and Clade 2 approximately 0.53 Mya (confidence interval = 1.60–0.10 Mya).

2.3.5 Environmental statistical analyses

Most of the significant differences occurred between Clade 1 and the rest of the clades (clades 2, 3 and 4). Daily relative humidity measured over a monthly period is the only environmental factor that distinguishes all clades from each other, especially during the months of May to October. Environmental

factors that show a significant distinction between specific clades for both independent and monthly measurements are summarized in tables 2.4 and 2.5 respectively.

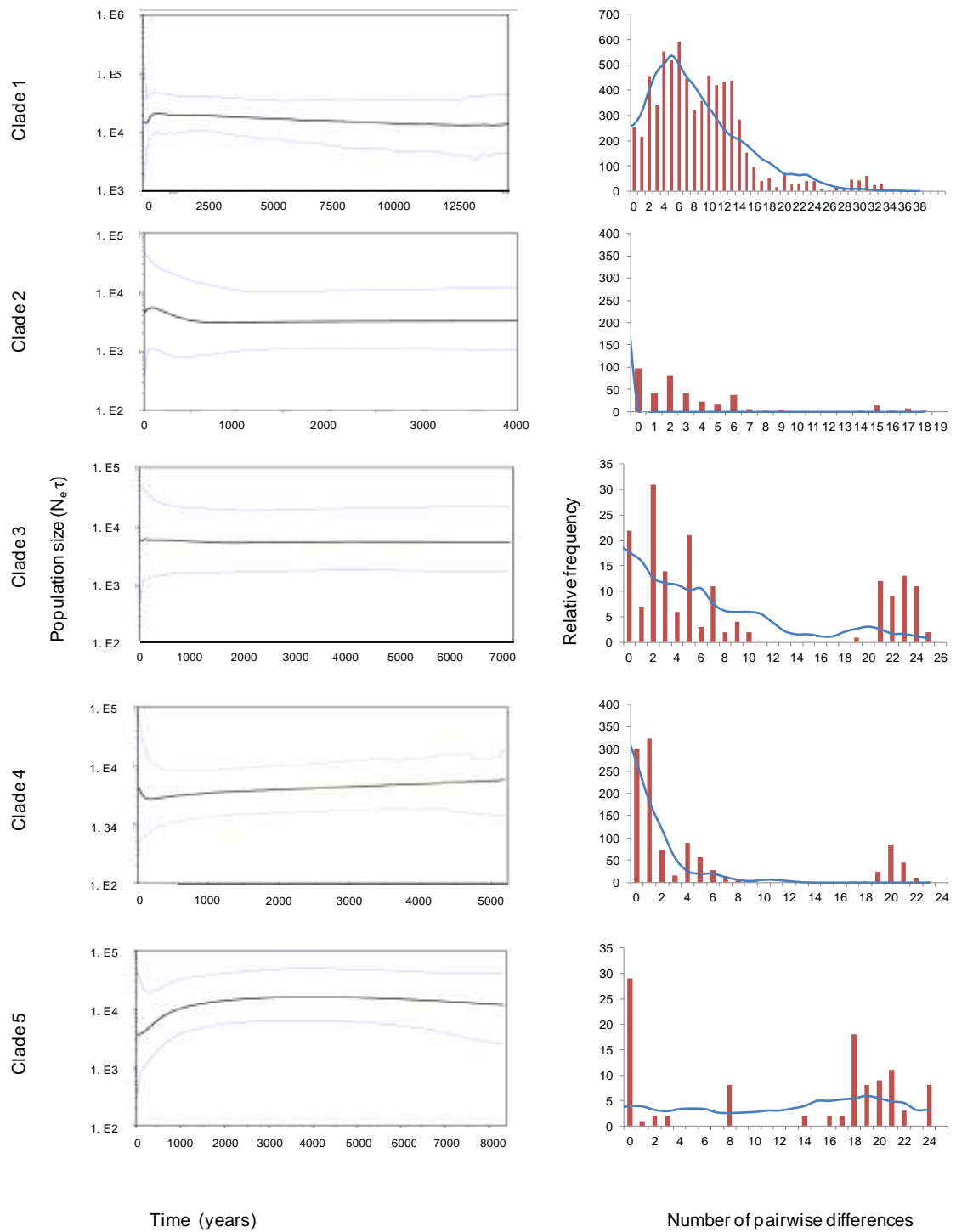


Figure 2.5: Bayesian skyline plots (BSPs) and mismatch distribution (MMD) conducted on the single COI data set for various clades retrieved by phylogenetic analyses. Bayesian skyline plots illustrate the population size as a product of effective population size (N_e) and generation time (τ) through time (years). The black line represents the median estimate of population size, where the blue lines indicate the upper and lower 95% posterior intervals. On the MMD graphs, the columns denote the observed frequency of pairwise differences, where the trend line represents the expected distribution under the sudden expansion model.

Table 2.3: Demography statistics conducted on the COI data set for the various clades of the *Acontias meleagris* species complex, indicating the sum of squared deviation (SSD), Harpending's raggedness index (RI) and Fu's F_s value for each clade.

Clade	N	SSD	p	RI	p	Fu's F_s	p
Clade 1 – Coastal and interior of the Eastern and Western Cape provinces	118	0.0039	> 0.05	0.004	> 0.05	-12.011	< 0.02
Clade 2 – Southern parts of the Western Cape Province	28	0.64	< 0.05	0.06	> 0.05	-1.28	> 0.02
Clade 3 – Breede River Valley	19	0.037	> 0.05	0.073	> 0.05	-0.05	> 0.02
Clade 4 – Northern parts of the Western Cape Province	47	0.029	> 0.05	0.069	> 0.05	0.005	> 0.02
Clade 5 – Eastern <i>A. m. orientalis</i> – lineicauda	15	0.1	< 0.05	0.14	< 0.05	1.6	> 0.02

Table 2.4: Comparison of means of parameters using one-way ANOVA and LSD. Values followed with different letters (^{a,b,c}) are significantly different between clades.

Clade	Soil depth (mm)		Soil clay content (%)		Altitude (m)	
	Mean ± SD	<i>p</i>	Mean ± SD	<i>p</i>	Mean ± SD	<i>p</i>
Clade 1	422.79 ± 205.062 ^b	< 0.01	13.59 ± 4.76 ^a	< 0.01	547.52 ± 361.24 ^a	< 0.01
Clade 2	763.51 ± 283.50 ^a		5.79 ± 5.19 ^b		70.11 ± 103.67 ^b	
Clade 3	534.18 ± 240.42 ^{ab}		8.57 ± 6.73 ^b		167.33 ± 260.48 ^b	
Clade 4	711.31 ± 319.82 ^a		7.62 ± 6.33 ^a		131.5 ± 105.87 ^b	
	Number of cloudy days (days)		Summer aridity index (na)		Precipitation seasonality (%)	
	Mean ± SD	<i>p</i>	Mean ± SD	<i>p</i>	Mean ± SD	<i>p</i>
Clade 1	3.34 ± 0.44 ^a	< 0.01	4.31 ± 0.57 ^c	< 0.01	28.96 ± 13.97 ^c	< 0.01
Clade 2	3.00 ± 0.22 ^b		5.21 ± 0.79 ^b		69.00 ± 2.83 ^a	
Clade 3	3.47 ± 0.73 ^a		4.92 ± 0.89 ^b		53.5 ± 7.40 ^b	
Clade 4	2.76 ± 0.35 ^b		5.99 ± 0.66 ^a		67.4 ± 5.32 ^a	
	Distance to sea (km)		Continental index (na)		Latitude (degrees)	
	Mean ± SD	<i>p</i>	Mean ± SD	<i>p</i>	Mean ± SD	<i>p</i>
Clade 1	90.75 ± 62.52 ^a	< 0.01	20.88 ± 5.99 ^a	= 0.02	33.063 ± 0.78	< 0.01
Clade 2	3.83 ± 5.36 ^b		15.18 ± 1.56 ^b		33.87 ± 0.53	
Clade 3	29.054 ± 37.64 ^b		16.93 ± 6.27 ^{ab}		34.025 ± 1.33	
Clade 4	29.14 ± 23.97 ^b		19.052 ± 3.68 ^{ab}		33.025 ± 0.69	

Table 2.4 (continued)

Clade	Number of frost days (days)		mean annual maximum temperature (°C)	Mean soil water stress percent days under stress (%)
	Mean ± SD	<i>p</i>		
Clade 1	11.85 ± 11.97 ^a	< 0.01		
Clade 2	3.00 ± 0.00 ^b			
Clade 3	4.83 ± 4.49 ^{ab}			
Clade 4	3.3 ± 0.68 ^b			
	temperature (°C)			
	Mean ± SD			
Clade 1	16.82 ± 1.11	= 0.02		
Clade 2	15.89 ± 0.60			
Clade 3	16.33 ± 0.52			
Clade 4	17.00 ± 0.94			

Table 2.5: Environmental factors showing significant distinction between clades for monthly measurements as indicated by VEPAC with clade*month as fixed effects.

	Clade 1 – Coastal and interior of the Eastern and Western Cape provinces	Clade 2 – Southern parts of the Western Cape Province	Clade 3 – Breede River Valley	Clade 4 – Northern parts of the Western Cape Province
Clade 1 – Coastal and interior of the Eastern and Western Cape provinces		<ul style="list-style-type: none"> • Daily mean relative humidity • Mean daily minimum temperature • Median precipitation • Potential evaporation • Solar radiation per month 	<ul style="list-style-type: none"> • Daily mean relative humidity • Mean daily minimum temperature • Solar radiation per month 	<ul style="list-style-type: none"> • Mean daily maximum temperature • Mean daily mean temperature • Mean daily minimum temperature • Daily mean relative humidity • Solar radiation per month
Clade 2 – Southern parts of the Western Cape Province			<ul style="list-style-type: none"> • Daily mean relative humidity 	<ul style="list-style-type: none"> • Daily mean relative humidity
Clade 3 – Breede River Valley				<ul style="list-style-type: none"> • Daily mean relative humidity

2.4 Discussion

The *Acontias meleagris* species complex is differentiated into five clades of which most of them were statistically well supported, and each clade is characterized by marked genetic structure. Exophilin 5 has been proven useful for diagnosing intraspecific phylogenetic relationships in supraterranean skinks (Portik *et al.*, 2011; 2012). However, considering the recent divergence and rapid radiation, the *A. meleagris* species complex may not have acquired enough genetic differentiation for a comparable level of genetic distinctiveness, which may explain the low resolution retrieved for the BI topology of the EXPH5 data set. Furthermore, the EXPH5 data set may lack dense taxon sampling, resulting in a low resolution nuclear topology.

Fine scale sampling revealed the Breede River Valley acting as an area of convergence between clades 1, 3 and 4 (Fig. 2.6). Divergence time estimates revealed Pliocene / Pleistocene cladogenesis for this species complex with overall demographic stability within clades. The divergence time estimation suggests that lineage diversification is the result of recent niche exploitation, following the stabilization of sea levels along the South African coastline. The subterranean nature of the *A. meleagris* species complex and habitat preference for low lying coastal dune regions, lends itself to genetic partitioning induced by oscillations in sea levels caused by historical cycles of glacial and interglacial periods. The extensive geographic distribution of this species complex in the interior regions of the Eastern, Northern and Western Cape provinces are likely the result of dispersal events when coastal conditions were unsuitable due to habitat inundation by rising sea levels. Subsequent marine regressions during the early Pleistocene would have facilitated the occupation of the coastal plain while late Pleistocene transgressions would have reinforced genetic differentiation.

2.4.1 Historical biogeography

While the clade spanning the northern parts of the Western Cape Province (Clade 4) is basal, it diverged approximately at the same time as the *A. m. orientalis* – *lineicauda* morph (Clade 5) during the early Pleistocene. A marine regression during the mid-Pliocene (Hendy, 1982) coincides with the intensification of the Benguela Current upwelling system, which was associated with arid and more open habitats along the western coastal plains (deMenocal, 2004). This arid, open habitat would have allowed the ancestral *A. meleagris* to disperse into the north-western parts of the Western Cape Province. However, the subsequent transgression during the late Pliocene (Hendy, 1982), could have impacted *A. meleagris* distribution along the west coast, causing this taxon to retreat into refugia in the Breede River

Valley. Following the last major marine regression during the late Pleistocene (Hendy, 1982), *A. meleagris* presumably recolonized the west coast and became isolated.

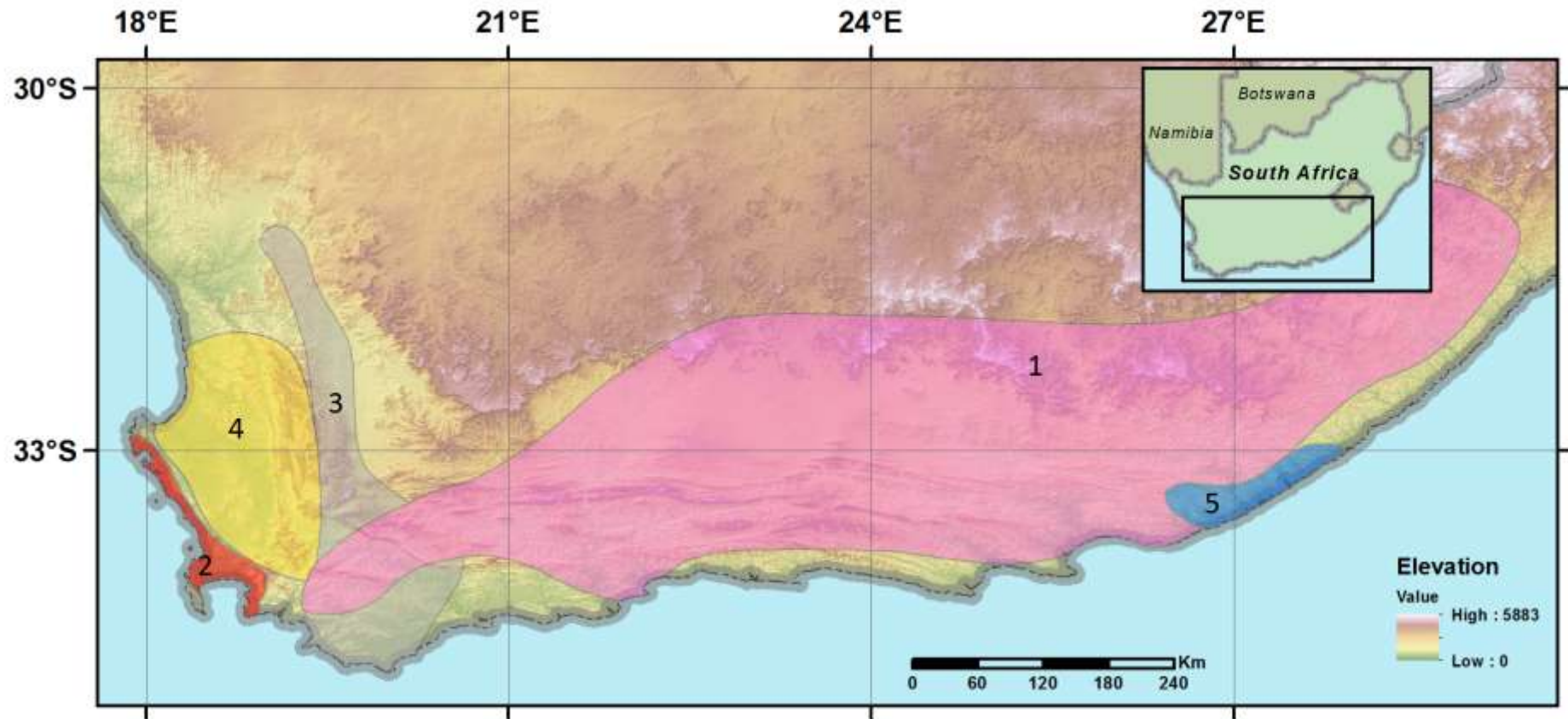


Figure 2.6: Map indicating the five clades of the *Acontias meleagris* species complex as retrieved by phylogenetic analyses. Oyster Bay and Port Elizabeth are not assigned to a clade.

The starlike haplotype network and relative low haplotype diversity for Clade 4 corroborates a possible recent population expansion. In Clade 5, further geographical expansion towards the west, during the early Pleistocene was most likely curbed by existing and interlinked Neogene rejuvenation of the Zuurberg fault along the northern margin of the Algoa basin and the episodic eastward migrations of the lower Sundays River (Hattingh *et al.*, 1996). These geomorphic phenomena potentially resulted in the historical and contemporary biogeographical isolation of the *A. m. orientalis* – *lineicauda* lineage to the immediate east of the lower Sundays River.

The remaining *A. meleagris* clades, 2 and 3 currently occupying both the interior and coastal regions would have occupied vacant coastal areas due to a receding coast line during the early Pleistocene (Hendy, 1982). The presence of the Nieuwoudtville specimens in the Breede River Valley (Clade 3) is most likely due to *A. meleagris* dispersing into the coastal Agulhas plain and the Cape Fold Mountains in the Western Cape Province along a north-south axis. Subsequently, *A. meleagris* would have expanded its range along the low lying coastal areas and colonized offshore continental islands (Robben and Dassen Islands) that were part of the mainland during the Last Glacial Maximum 16 000 BP (Tankard, 1976). Thus, the clade spanning the southern regions of the Western Cape Province (Clade 2) occupied the west coast recently and as a result occurs in near sympatry with specimens in the northern regions of the Western Cape (Clade 4). These latter two clades are genetically discrete, are characterized by the absence of shared haplotypes, both possess high Φ_{ST} values and marked uncorrected sequence divergences, corroborating their genetic distinctiveness.

2.4.2 Historical demography

The intermontane Breede River Valley appears to be an area where clades converge geographically (clades 1, 3 and 4). Higher nucleotide diversity indices for representative sample localities of these three clades indicate that the Breede River Valley is refugial. Similarly, the San Joaquin Valley in California harbours deep genetic lineages of *Aniella pulchra* (Parham & Papenfuss, 2009), suggesting that intermontane valleys may serve the same role as mountains do in supraterranean taxa, providing favorable conditions for refugia during adverse environmental conditions. Bayesian Analyses of Population Structure recognizes the Breede River Valley Clade (Clade 3) and the clade spanning the coastal and interior regions of both the Eastern and Western Cape provinces (Clade 1) as genetically closely related. Furthermore, MMD suggests a population expansion between 3000 and 4000 years ago for Clade 1 that could be indicative of introgression between Clade 1 and 3. However, the implied introgression event is not supported by BSPs and EBSPs suggesting demographic stability

for Clade 1 for the last 12 500 years. Thus, possible hybridization between clades of the *A. meleagris* species complex warrants examination by exploring potential contact zones in the Breede River Valley (clades 1 and 3) and along the west coast of the Western Cape coast (clades 2 and 4).

2.4.3 Environmental influences on biogeographical patterning

The four clades used for the environmental analyses (clades 1 to 4) occur in different climatic regions. This is evident from the comparison of the environmental variables between the western and eastern clades. Less differentiation exists between clades 1 and 3 and are most likely due to the Breede River Valley acting as a transition zone between differing rainfall patterns and other linked environmental factors. Genetic partitioning within the *A. meleagris* species complex may be partially influenced by the differing climatic regions in which the various clades occur. However, this needs to be verified with ecological investigations.

2.4.4 Conclusions and taxonomical implications

Acontias meleagris showed phylogeographic similarities with ectotherms in the Cape Floristic Region (CFR) that are characterized by low dispersal capabilities and habitat fastidiousness (Price *et al.*, 2007; Gouws *et al.*, 2010). Accordingly, Albert *et al.*, (2007) inferred that the subterranean lifestyle impact genetic partitioning in a similar manner that surface barriers do in taxa with low dispersal abilities. Factors maintaining the geographic boundaries between the observed clades of the *A. meleagris* species complex are possibly reproductive / genetic isolation or ecological divergence, and require closer scrutiny.

Similar to the phylogeographical pattern of other herpetofaunal taxa occurring in the Cape Floristic Region (Daniels *et al.*, 2004; Swart *et al.*, 2009; Tolley *et al.*, 2006), the *A. meleagris* species complex shows stronger genetic partitioning in the Western parts of its distribution, while shallow structure is observed in the central and eastern clades. While there is broad biogeographic congruence between the subterranean *Acontias meleagris* species complex and co-distributed supraterranean herpetofauna, fine scale differences can be attributable to, life history characteristics habitat specificity and colonization patterns associated with the fossorial lifestyle of the *Acontias meleagris* species complex (Daniels, *et al.*, 2007; Tolley *et al.*, 2004, 2006, 2009; Swart *et al.*, 2009).

Lamb *et al.*, (2010) recognized the various subspecies and the *A. m. orientalis* – *lineicauda* morph within the *A. meleagris* species complex as “genetically distinct, morphologically diagnosable units” while *A. p. tasmani* was

synonymized with *A. m. orientalis*. While we agree that *A. p. tasmani* is synonymous with *A. m. orientalis* and that strong genetic partitioning exists amongst lineages of the *A. meleagris* species complex, two lineages for *A. m. meleagris* and one lineage for *A. m. orientalis* – lineicauda were retrieved, leaving the previously *A. m. orientalis* – lineicauda clade in Port Elizabeth (Daniels *et al.*, 2009) unresolved and rendering the informal designations by Lamb *et al.*, (2010) doubtful. The observation that these lineages are morphologically diagnosable is questionable based on preliminary morphological character evaluation for the five evolutionary lineages retrieved during this study (Engelbrecht unpublished data). The taxonomic designations within the *A. meleagris* species complex remain dubious and will form the focal aspect of a forthcoming revision (Engelbrecht, *in prep.*).

Chapter 3

A taxonomic revision of the South African Cape legless skink *Acontias meleagris* species complex (Squamata: Scincidae): with the description of two new species

3.1 Introduction

The fossorial subfamily Acontiinae (Greer 1970), has recently received considerable systematic attention (Daniels *et al.* 2002; 2006; Lamb *et al.* 2010). However, these taxonomic studies primarily focussed on phylogenetic relationships between the genera, while the alpha taxonomy remains unresolved and dubious, particularly among species complexes and subspecies (Daniels *et al.* 2005, 2009). Currently, two genera containing 32 species (27 *Acontias* and five *Typhlosaurus*) are recognised (Lamb *et al.* 2010). Within *Acontias* (Cuvier 1817), Broadley and Greer (1969) reported that widespread polymorphisms have hampered reliable species diagnosis. In addition, recent molecular systematic studies suggest that convergence in morphology is prevalent among fossorial reptile taxa including *Acontias*, limiting its utility at delineating species boundaries (Kearney & Stuart 2004; Crottini *et al.* 2009; Mott & Vieites 2009; Heideman *et al.* 2011).

In an attempt to resolve the phylogenetic relationships within *Acontias*, systematic investigations were initiated with specific focus on widely distributed species such as the Cape legless skink, *Acontias meleagris* (Daniels *et al.* 2002; 2005; 2009) (Linnaeus 1758). The latter species occurs from the northern region of the Western Cape province along the coastal belt and adjacent interior into north-eastern portions of the Eastern Cape province and is known to exhibit variable dorsal coloration. Phylogeographic studies have demonstrated that *A. meleagris* is a species complex comprised of five evolutionary lineages (Daniels *et al.* 2009). Two lineages were retrieved for *A. m. meleagris* (Hewitt 1938) distributed along the west coast of the Western Cape province, while a third lineage comprising *A. m. meleagris*, *A. m. orientalis* (Hewitt 1938) and *A. p. tasmani* (Hewitt 1938) was present in the interior and coastal regions of both the Western and Eastern Cape provinces (Daniels *et al.* 2009). Lastly, two lineages were retrieved for *A. m. orientalis*—‘lineicauda’ (Hewitt 1938), one in the Port Elizabeth region

and the second in the Port Alfred region along the coast of the Eastern Cape province. Following recent extensive geographic sampling Engelbrecht *et al.* (2012) uncovered congruent patterns with larger distribution ranges for most of the lineages retrieved by in Daniels *et al.* (2009), while an additional lineage was retrieved in the Breede River Valley, Western Cape province (Fig. 3.1).

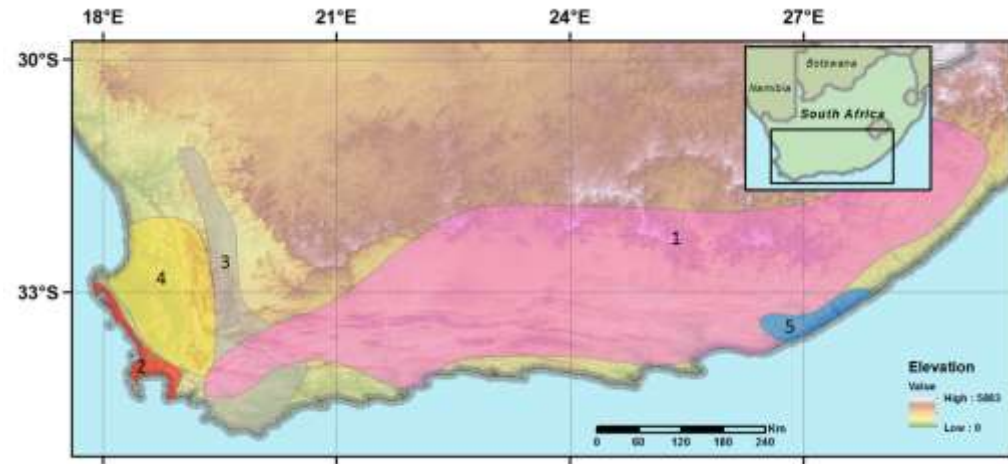
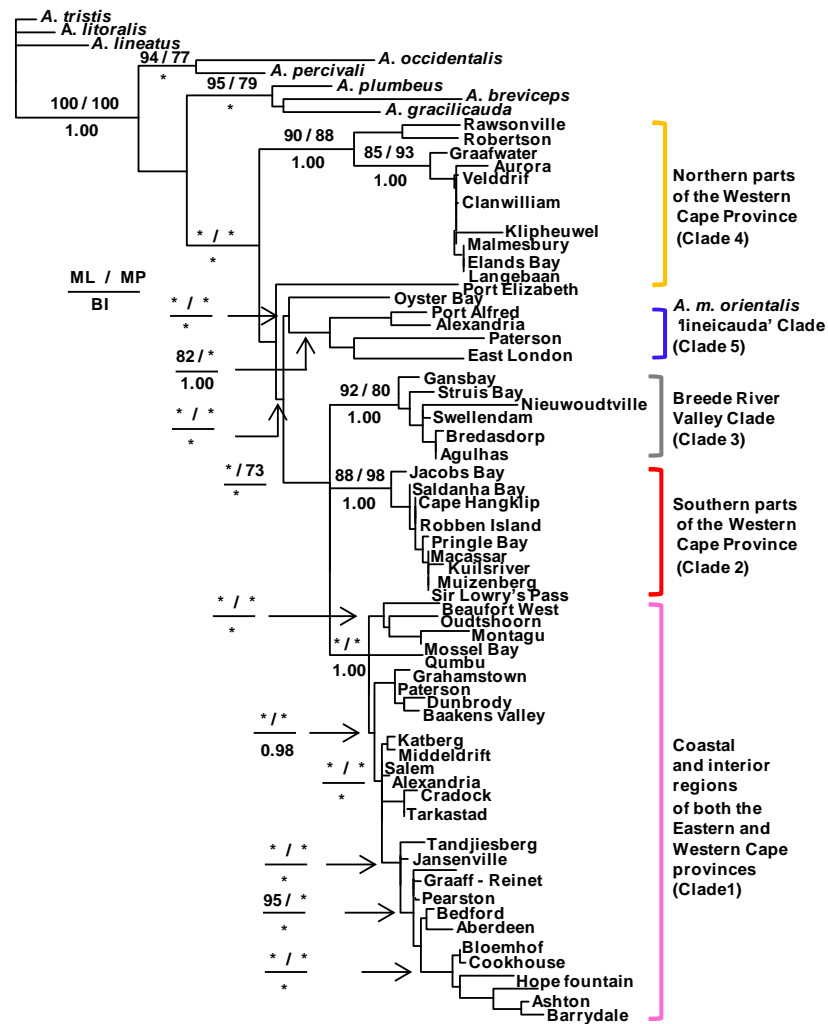


Figure 3.1: Maximum-likelihood topology for the total evidence DNA data (16S rRNA, COI and EXPH5) amongst 55 *Acontias meleagris sensu lato* sample sites across the Eastern, Northern and Western Cape provinces of South Africa demonstrating the presence of the five clades (Engelbrecht *et al.* 2012).

However, in contrast to Daniels *et al.* (2009), Engelbrecht *et al.* (2012) retrieved a single lineage for *A. m. orientalis*–‘*lineicauda*’. The five *Acontias meleagris* lineages were characterised by marked sequence divergence values, lacked shared haplotypes between lineages, were statically well supported in the phylogenetic analyses and show geographic exclusivity. These results suggest that five evolutionary lineages are present within the *A. meleagris* species complex. Lamb *et al.* (2010) elevated both *A. m. orientalis* and the ‘*lineicauda*’ morph to full species, without a formal taxonomic description. Limited geographic coverage by Lamb *et al.* (2010) prevented the authors from making a formal morphological diagnosis of the lineages within the *A. meleagris* species complex or provided a description of the distribution of the novel species. Noticeably, the primary objective of the latter study was not the description of species but to provide a generic revision of the Acontiinae. The aim of the present study was to conduct a morphological examination of the five genetic lineages within the *A. meleagris* species complex, in an attempt to search for diagnostic morphological characters amongst the lineages recognized by Engelbrecht *et al.* (2012). We hypothesize that the five lineages within the *A. meleagris* species complex will exhibit limited diagnostic morphological characters considering the presence of widespread morphological convergence among fossorial reptiles. Given the hypothesis, the phylogenetic species concept will be employed as point of reference for species delimitations (Cracraft 1989). This is furthermore in agreement with the notion of the unified species concept which distinguishes the theoretical concept of a species (separately evolving metapopulation lineages) from operational criteria (lines of evidence) to delimit lineages (de Queiroz 2007). Two of the lineages are taxonomic formally described, and a modern taxonomic revision of *Acontias meleagris*, *A. orientalis* and *A. lineicauda* is provided based on both molecular and morphological data.

3.2 Materials & Methods

3.2.1 Collection and preparation of specimens

Specimens used for morphological examination includes a total of 116 newly collected specimens, 26 from Daniels *et al.* (2005; 2009) and 20 additional specimens from the South African Museum of Natural History (SAM, IZIKO Museums of Cape Town), seven from the National Museum, Bloemfontein (NMB) and type specimens of *A. m. orientalis*, *A. m. orientalis* 'lineicauda' morph and *A. p. tasmani* from the Port Elizabeth Museum (PEM). Museum specimens were carefully selected based on its geographic inclusion within the five clades recognized by Engelbrecht *et al.* (2012). Animals were collected by active searching under rocks, logs, building rubble, corrugated iron sheets and leaf litter of *Acacia*, *Rhus* and *Eucalyptis* trees in sandy areas. Specimens were euthanized using, sodium pentobarbitone (200mg, dose: 60mg / kg) under ethical clearance from the Stellenbosch University Research Ethics Committee (REF: 10NP—ENG01). The use of sodium pentobarbitone for euthanasia of vertebrates is recommended by several International Ethics Committees including both the American society for ichthyologists and herpetologists (ASIH, 2004) and the American veterinary medical association (AVMA, Euthanasia 2007). A lethal dose of sodium pentobarbitone was administered intraperitoneally. Animals were confirmed dead if no muscle contraction and no heartbeat were observed, following a minimum period of 60 minutes post injection. All newly collected specimens were labeled and preserved in a 4% buffered formalin solution. Voucher specimens were deposited in the South African Museum of Natural History, IZIKO Museums of Cape Town (SAM).

3.2.1 Morphological analysis and character count

All specimens were examined for morphometric and meristic characters following FitzSimons (1943) and Broadley and Greer (1969). In order to discriminate between morphological characters that are sexually influenced (Heideman 2008), the sex of specimens was determined through ventral incisions and observation of the gonads and efferent ducts in the posterior part of the body wall. To furthermore remove the effect of physical proportions when comparing measurements among specimens of varying sizes, measurements were standardized by expressing it as ratios of log SVL. Snout-vent length (SVL) was measured by placing cotton string at the tip of the snout to the posterior edge of the cloacal scale (Fig. 3.2a). The cotton string was measured to the nearest 0.5 mm by placing it on a ruler. Remaining head and body measurements were done to the nearest 0.05 mm by using a digital calliper and included tail length (TL), head width (HW), head length (HL), head height (HH), rostral scale length (RL), nasal-rostral line length (NRL) and mental scale length (ML) (Figs. 3.2a—g).

Scale counts were conducted for mid-body scale rows, including ventral rows (MSR), ventral scales (V), subcaudal scales (SBC), anterior chin shields bordering the mental (CS), preocular scales (PR), loreal scales (L), supraciliaries (SC), supraocular scales (SO), number of subocular scales (SB) and the number of SB reaching the lip, number of upper labial scales (UL) in addition to how many UL enters the eye and edge the mental, lower labial scales (LL), shape of the prefrontal scale (PF), shape of the frontal scale (F), presence and shape of interparietal scale (IP) and separation from supraoculars, shape of the parietal scales (P) and whether the parietals contact posterior to the IP (Figs. 3.3a—c). Variable scale counts on individuals are reported as; left/right. Photos were taken with a Nikon D300S digital camera. The low sample sizes for certain clades (clade 2 *A. meleagris*, clade 3 *A. parilis* sp. nov, clade 4 *A. caurinus* sp. nov., clade 5 *A. lineicauda*) precluded us from undertaking a statistical analyses on the present data.

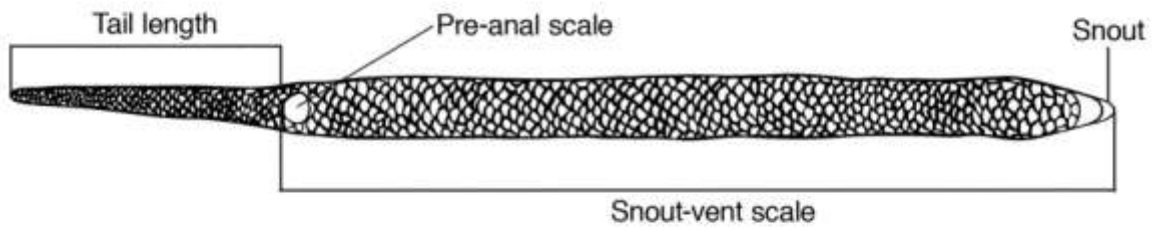


Figure 3.2a: Measurements of the physical proportions of an *Acontias* skink; ventral view and Illustration of snout-vent length and tail length measurement.

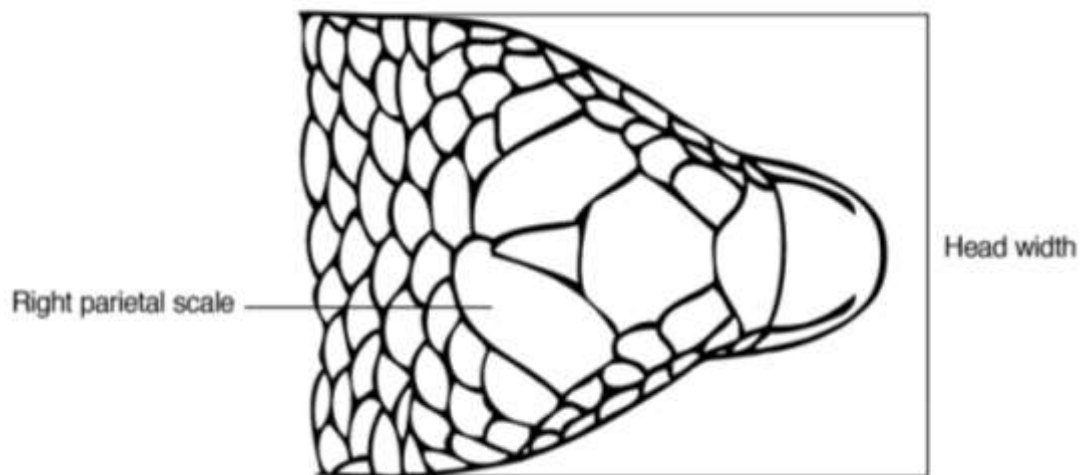


Figure 3.2b: Dorsal view and illustration of head width measurement.

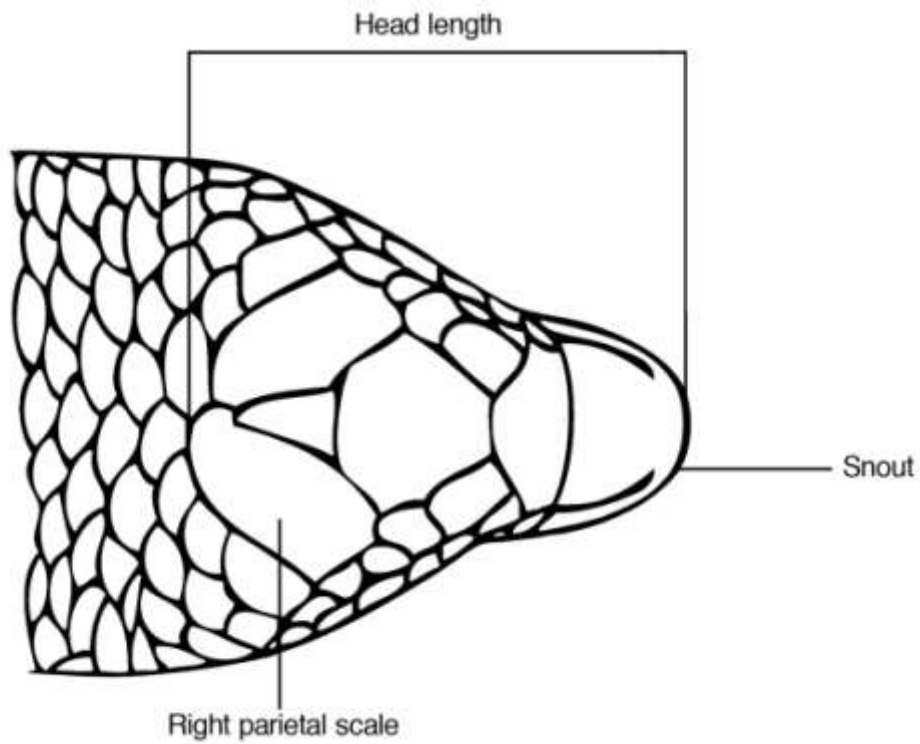


Figure 3.2c: Dorsal view and illustration of head length measurement from the tip of the snout to the edge of the parietal scales.

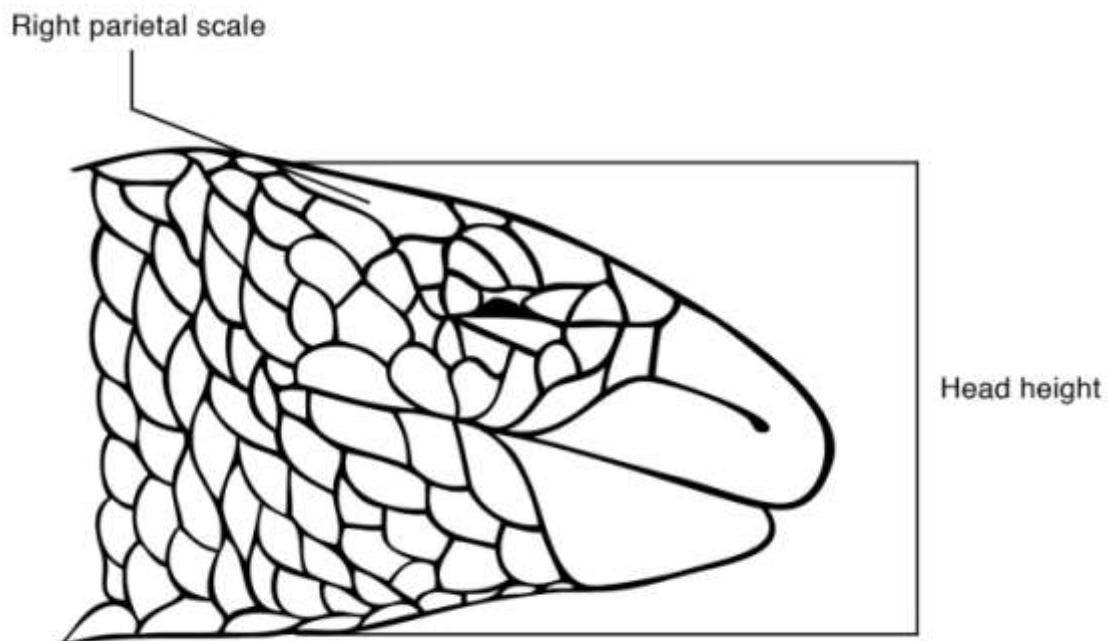


Figure 3.2d: Lateral view and illustration of the head height measurement, from the edge of the parietals scales in a straight line downwards to the throat area.

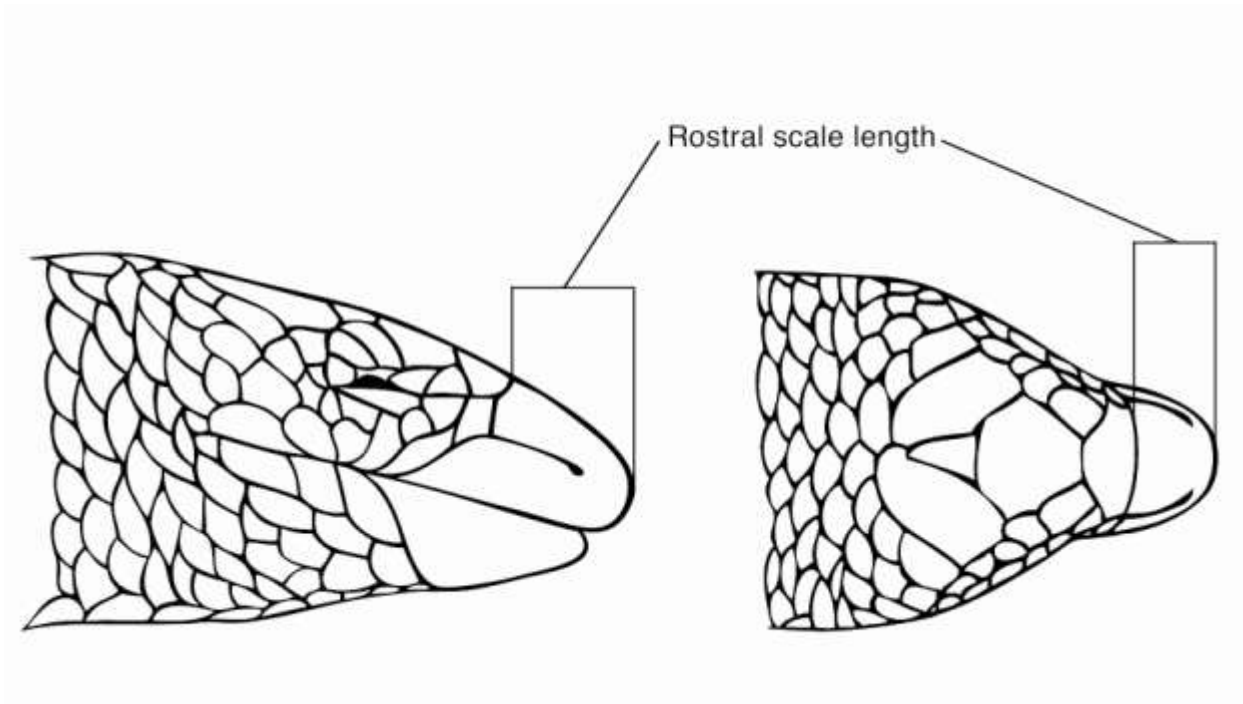


Figure 3.2e: Dorsal view of the snout and illustration of the rostral scale length measurement.

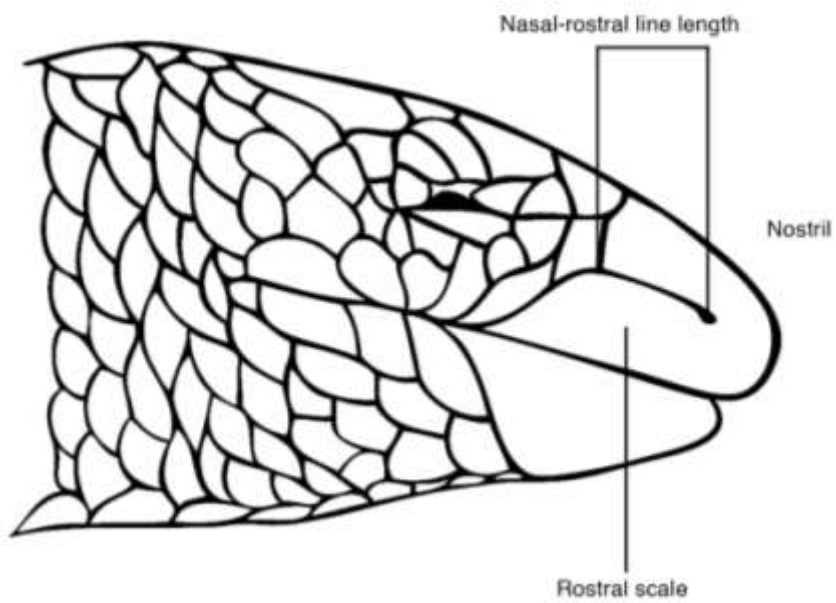


Figure 3.2f: Lateral view and illustration of the nasal-rostral line length measurement.

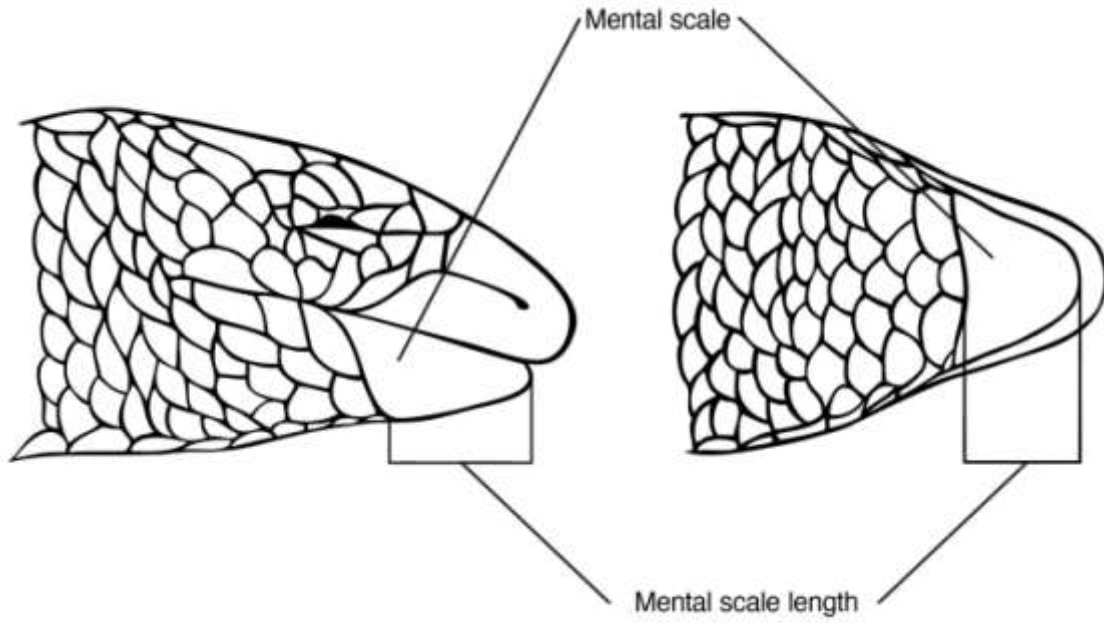


Figure 3.2g: Ventral view and illustration of the mental scale length measurement.

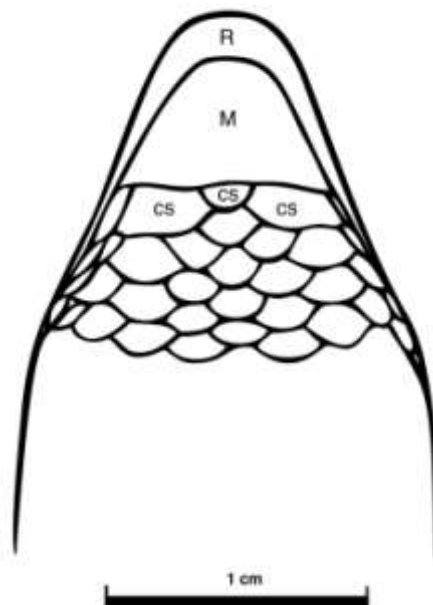


Figure 3.3a: Head shields of an *Acontias* skink, a, ventral view of the snout and chin area of indicating anterior chin shields (CS), mental scale (M) and the rostral scale (R).

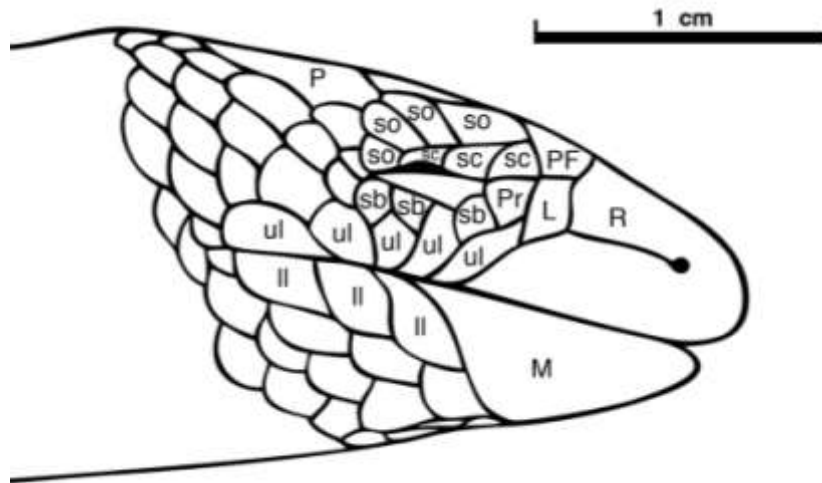


Figure 3.3b: Lateral view indicating the supraocular scales (SO), supraciliaries (SC), loreal scale (L), preocular scale (PR), subocular scales (SB), upper labial scales (UL) and lower labial scales (LL).

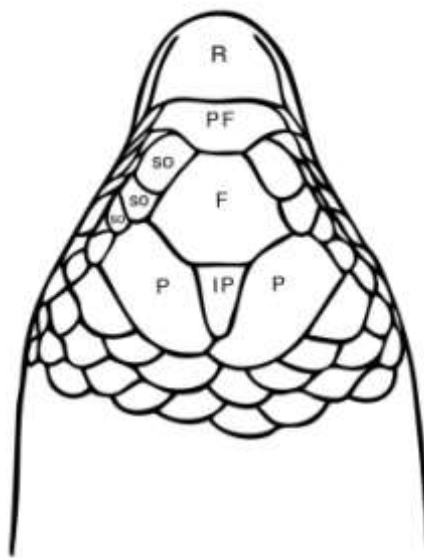


Figure 3.3c: Dorsal view indicating the parietal scales (P), interparietal scale (IP), the frontal scale (F) and the prefrontal scale (PF).

DNA sequence data

GENBANK accession numbers for the five clades detected using the cytochrome oxidase subunit one (*COI*), *16S rRNA* and exophilin 5 (*EXPH5*) are listed as corroborative evidence for species diagnosis (Daniels *et al.* 2005; 2009; Engelbrecht *et al.* 2012).

Acronyms

NMB—National Museum, Bloemfontein

PEMR—Port Elizabeth Museum Reptile Collection

SAM ZR—South African Museum of Natural History, Cape Town Herpetological collection (IZIKO Museums of Cape Town)

F—female

M—male

n—number of specimens

3.3 Taxonomy

Anguis meleagris Linnaeus, 1758, *Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Tomus I. Editio decima, reformata. Laurentii Salvii, Holmiæ. 10th Edition: pp 227.

Eryx meleagris Daudin, 1803, *Histoire naturelle, générale et particulière des Reptiles* 7, Paris.

Acontias meleagris Merrem, 1820, *Versuch eines Systems der Amphibien—Testamen Systematics Amphibiorum* p. 89.

Acontias meleagris **part.** Boulenger, 1887, *Catalogue of the lizards in the British Museum (Natural History)*. p. 427

Acontias meleagris Lamb, T., Biswas, S. & Bauer, A.M. (2010) A phylogenetic reassessment of African fossorial skinks in the subfamily Acontinae (Squamata: Scincidae): evidence for parallelism and polyphyly. *Zootaxa* **2657**, 33–46.

Description: Head conical, somewhat elongate, though often (in old specimens) very broad across parietal region. Snout obtusely rounded projecting beyond labial margin. Ear hidden. Rostral very large, about as long as frontal+frontonasal, indented on either side at terminus of nasal groove, equal in length to or a little longer than distance from anterior corner of eye to nostril. Frontonasal 2 to 2.25 times as broad as long. Frontal subhexagonal, broader than long, slightly narrower but much longer (1.5 to 2 times) than frontonasal. Interparietal, small, triangular, more or less equilateral. Parietals usually in contact with one another behind interparietal, sides not parallel but converging towards one another. An elongate band-like nuchal shield on each side adjoining parietals behind. Three supraoculars, 1st about as large as other two together but not elongate, 3rd smallest. Four supraciliaries, 1st largest and 3rd smallest. Lower eyelid narrow and elongate, translucent. A preocular and three suboculars. Five upper labials, 1st highest and 5th longest. Mental very large, extending to below middle of eye, posterior border slightly notched in the middle. Three lower labials, last elongate. Scales on body hexagonal above and subquadrangular below, those of the two median dorsal rows being broader than others; usually 18 (exceptionally 20) scales around body anteriorly and 16 (exceptionally 14) posteriorly. A single large semicircular preanal shield with breadth greater than distance from frontal to tip of snout. Tail short, cylindrical rounded at end, not or feebly tapering, from a fifth to sixth length of head and body. Ventrals from chin-shield 160—170 to preanal plate; 30—40 subcaudals, median dorsal series not very strongly broadened.

Colour: Above, olive brown, greyish-brown to dark liver-brown, more or less uniform with scales paler edged, or with a darker concentration or spot on distal half of each scale, or yellow above with longitudinal series of dark purplish brown transversally elongate spots; the dark area above (from 6 or 8 scale rows broad) is usually sharply demarcated from immaculate pale lower surfaces, or sometimes breaks up into series of spots. Below, yellowish, usually immaculate, sometimes with series of small dark spots on chin and throat and a few scattered dark spots over belly; under side tail usually with series of somewhat transversely elongate spots. Sometimes specimens are found which are yellow above, with 6—8 series (rarely 10) longitudinal series of dark spots, which may fuse in varying degree to form stripes.

Dimensions: Head and body maximum length $250 + 49 = 299$ mm, breadth head 7.3 mm, diameter of body 8.8 mm.

Biology & habitat: Usually found in sandy soil and often exposed on turning over stones, logs, etc. When placed on hard surface, they move forward slowly, by vigorous wriggling from side to side in serpentine fashion. They live largely on small worms, insect larvae and other small creatures such as are to be found below ground. The young, usually ranging from 3—4 in number are born in late summer or early autumn and measure from 70—80mm in total length.

Distribution: Widely distributed in sandy soils of continental offshore islands along the west coast of the Western Cape and low-lying coastal regions of the Eastern, Northern and Western Cape provinces, the Breede River Valley and the adjacent interior of the Little Karoo. The five clades genetically distinct clades were identified by Engelbrecht *et al.* (2012) and are here recognized as distinct taxa; Clade 1 is distributed from Ashton in the Western Cape to Qumbu in the north-eastern part of the Eastern Cape province, Clade 2 occurs along the southern coastal parts of the Western Cape province encompassing sampled localities from Jacobs Bay to Pringle Bay and includes Robben Island, Clade 3 spans the Breede River Valley and includes Nieuwoudtville in the Northern Cape province and sampled localities in the Agulhas Plain region along the southern parts of the Western Cape, Clade 4 occurs in the northern parts of the Western Cape encompassing coastal regions, but occurring predominantly in the interior from Aurora to Robertson, lastly Clade 5, the *A. m. orientalis* ‘lineicauda’ morph occurs along the coast of the Eastern Cape and includes Alexandria, Paterson occurring inland, Port Alfred, Port Elizabeth, and East London and surrounding areas.

3.3.1 *Acontias meleagris* (Linnaeus 1758)

(Figs. 3.4a, b)

(“Clade 2”, Engelbrecht *et al.* 2012)

The holotype of the species complex appears to be lost. Exhaustive attempts to locate the holotype were fruitless. The need for a neotype is warranted by the fact that similar taxa cannot be ruled out from the Linnaean description therefore, a neotype is designated in the present study. The type locality for the lost holotype is ‘In Indiis’ and is believed to be most likely the Cape of Good Hope, hence the species is here restricted to Clade 2 (Engelbrecht *et al.* 2012).

Neotype. An adult female; **SAM ZR 52432**, Saldanha Bay, 33° 00′ 60″ S, 17° 54′ 60″ E, Western Cape province, South Africa; collected 5 August 2012 by H.M. Engelbrecht, X. Human and F. Van Zyl.

Additional material examined. **SAM ZR 52433, 52434, 52428, 52435—52437**; Saldanha Bay, 33° 00′ 60″ S, 17° 54′ 60″ E, Western Cape province, South Africa, 2 F and 4 M collected 5 August 2012 by H.M. Engelbrecht, X. Human and F. Van Zyl. **PEMR 1264**, Goodwood, 33° 54′ 37″ S, 18° 33′ 02″ E, Western Cape province, South Africa, sex not determined, collected 24 July 1979 by A. de Cock. **PEMR 9088**, Wynberg district, 34° 01′ 28″ S, 18° 27′ 58″ E, Western Cape province, South Africa, sex not determined, collected 17 May 1904 by S. Schönland. **PEMR 9077—9081**, Robben Island, 33° 47′ 30″ S, 18° 34′ 06″ E, Western Cape province, South Africa, sex not determined, collected 10 March 1927. **SAM ZR 52429**, Macassar, 34° 03′ 13″ S, 18° 45′ 56″ E, Western Cape province, South Africa, 1 M collected February 2012 by H.M. Engelbrecht and R. D. Engelbrecht. **SAM ZR 52440**, Sir Lowry’s Pass, 34° 06′ 00″

S, 18° 54' 60" E, Western Cape, South Africa, 1 M collected 2 August 2012 by H.M. Engelbrecht, X. Human and F. Van Zyl. **SAM ZR 52425, 52438**, Kuilsriver, 33° 54' 60" S, 18° 39' 60" E, Western Cape province, South Africa, 2 F collected 2 August 2012 by H.M. Engelbrecht and F. Van Zyl. **SAM ZR 43939, 2041, 43938, 43940—43943**; Robben Island, 33° 48' 00" S, 18° 22' 36" E, Western Cape province, South Africa, 1 M and 6 F collected 6 October 1976 by McLachlan and van den Heever. **NMB 9029, 9030**; Robben Island, 33° 48' 21" S, 18° 22' 36" E, Western Cape province, South Africa, 1M and 1F collected 23 October 2001 by M. Bates. **SAM ZR 44559**; Jacobs Bay, 32° 58' 37" S, 17° 52' 54" E, Western Cape province, South Africa, 1 F collected 25 August 1978 by André and Prins. **SAM ZR 52420**; Jacobs Bay, 32° 57' 60" S, 17° 52' 60" E, Western Cape province, South Africa, 1 M collected 4 August 2012 by H.M. Engelbrecht, X. Human and F. Van Zyl. **SAM ZR 43235**; Claremont, 33° 52' 30" S, 18° 22' 30" E, Western Cape province, South Africa, 1 M collected 17 July 1958 by de Villiers. **SAM ZR 47041**; Fish Hoek, 33° 07' 30" S, 18° 22' 30" E, Western Cape province, South Africa, 1 M collected 7 November 1985. **SAM ZR 43558**, Newlands, 33° 52' 30" S, 18° 22' 30" E, Western Cape province, South Africa, 1 M collected 29 August 1965 by Gov.

Genbank numbers. *COI*: Cape Hangklip: **EU855734**, Jacobs Bay: **EU855736**, Kuilsriver **EU855677**, Macassar: **JQ692406**, Muizenberg: **JQ692412**, Pringle Bay: **JQ692424**, Robben Island: **EU855720—855724, 855726, 855728, 855730, 855732**, Saldanha Bay: **EU855718**, Sir Lowry's Pass: **EU855717**. Sequence divergence amongst selected species of *Acontias* are summarised in table 3.1.

16S rRNA: Cape Hangklip: **EU855638**, Jacobs Bay: **EU855640**, Kuilsriver: **EU855579**, Macassar: **JQ692528**, Muizenberg: **JQ692534**, Pringle Bay: **JQ692546**, Robben Island: **AY683700—683704, EU855624—855632, 855634, 855636**, Saldanha Bay: **EU855622**, Sir Lowry's Pass: **EU855621**.

EXPH 5: Macassar: **JQ278086**, Pringle Bay: **JQ278101—278102**.

Description. Dimensions (Neotype with the variation shown within species in parantheses): SVL 208 mm (68—226, mean 165.8); TL 42.97 mm (13—43.23, mean 33.9); HL 8.64 (4.76—8.96, mean 7.39); HW 6.34 mm (3.28—8.15, mean 5.77); HD 5.56 (2.53—7.07, mean 4.94); RL 3.37mm (1.98—3.78); NRL 1.97 mm (1.3—2.7, mean 1.86); ML 3.35 mm (1.78—3.35, mean 2.72). Ratios for morphological measurements are summarised in table 3.2.

Lepidosis. Supraciliaries 4/4, supraoculars 3/3, suboculars 3/3, upper labials 5/5, lower labials 3/3, chin shields 3, midbody scale rows 14—18, subcaudals 33—41, ventrals 161—190. However, see table 3.3 for variation within species.

Colouration (in life). More often than not this species is uniformly coloured, where scales are either pale edged or have a partially darker pigmentation concentration on one half of the scale (not necessarily the distal half). Often the scales are just mottled resulting in a generally spotty appearance, where spots can fuse to form stripes. In uniformly coloured animals the colour variation includes light-brown, medium golden-brown to dark liver-brown. Bright yellow animals are also observed with very dark to nearly black pigmentation on the dorsum. In very rare cases only the two mid-dorsal rows are pigmented resulting in an overall pale appearance with a band of pigmentation on the dorsum. In all morphotypes the tail is slightly tapered with a blunt rounded end. Ventral markings on the tail are frequently present and are either light-brown mottling or form three stripes that end towards cloacal scale, leaving a pale spot posterior to the cloaca. The chin and throat area are frequently mottled in all morphotypes. Colour and scale pigmentation of recently preserved specimens of *A. meleagris* resemble the colouration of life animals (Figs. 3.4a, b)

Habitat. Subterranean in habits, usually found in sandy soils in low lying coastal regions. Animals were exposed by active digging through loose sand and turning over building rubble at derelict buildings and rock on beachfronts

Distribution. Southern coastal region of the Western Cape province encompassing sampled localities from Jacobs Bay to Pringle Bay and include continental offshore Robben Island (Clade 2). Occur in sympatry with *Scelotes bipes*, *S. gronovii* and *Typosaurus caecus*.



Figure 3.4a: Image of preserved specimens demonstrating the colour variation within *Acontias meleagris sensu stricto*.



Figure 3.4b: Image of preserved specimens demonstrating the dorsal colour and scale pigmentation variation within *A. meleagris sensu stricto*.

Table 3.1: Sequence divergence (%) for the COI marker, between selected species of the *Acontias* genus.

	<i>A. orientalis</i>	<i>A. meleagris</i>	<i>A. parilis</i> sp.nov.	<i>A. caurinus</i> sp. nov.	<i>A. lineicauda</i>	<i>A. percivali</i>	<i>A. breviceps</i>	<i>A. plumbeus</i>	<i>A. occidentalis</i>	<i>A. gracilicauda</i>	<i>A. lineatus</i>
<i>A.orientalis</i>											
<i>A.meleagris</i>	4.1										
<i>A.parilis</i> sp. nov.	3.4	3.8									
<i>A.caurinus</i> sp. nov.	6.1	6.7	6								
<i>A.lineicauda</i>	4.8	5.6	4.9	7.6							
<i>A.percivali</i>	6.8	7.0	6.7	8.1	7.0						
<i>A.breviceps</i>	6.4	5.9	6.3	7.7	6.5	5.2					
<i>A.plumbeus</i>	6.9	5.8	6.5	7.4	5.4	5.3	1.5				
<i>A.occidentalis</i>	6.6	6.1	6.1	7.3	6.4	2.7	4.9	4.9			
<i>A.gracilicauda</i>	6.4	6.1	6.6	6.6	6.3	5.5	5.3	1.6	4.7		
<i>A.lineatus</i>	12.3	12.0	12.4	11.0	11.9	13.3	11.8	12.3	12.3	11.7	
<i>A.tristis</i>	10.0	10.1	10.2	9.7	9.9	10.9	9.6	10.1	10.3	9.5	3.8

Table 3.2: Morphological measurements expressed as ratios of SVL for novel and revised species within *Acontias*. Snout-vent length (SVL), head length (HL), head width (HW), head height (HH), rostral scale length (RL), nasal-rostral line length (NRL) and mental scale length (ML).

Taxa	Sex	<i>n</i>	<u>TL/SVL</u>		<u>HL/SVL</u>		<u>HW/SVL</u>		<u>HD/SVL</u>		<u>RL/SVL</u>		<u>NRL/SVL</u>		<u>ML/SVL</u>	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>A. orientalis</i>	F	28	0.21	0.01	0.04	0.00	0.03	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00
	M	36	0.21	0.02	0.05	0.01	0.04	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00
<i>A. meleagris</i>	F	13	0.20	0.01	0.04	0.01	0.03	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00
	M	12	0.21	0.02	0.05	0.01	0.04	0.01	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00
<i>A. parilis</i> sp. nov.	F	9	0.21	0.02	0.04	0.00	0.03	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00
	M	6	0.2	0.02	0.04	0.01	0.04	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00
<i>A. caurinus</i> sp. nov.	F	16	0.21	0.02	0.04	0.00	0.03	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00
	M	14	0.2	0.02	0.04	0.01	0.03	0.01	0.03	0.00	0.02	0.00	0.01	0.00	0.01	0.00
<i>A. lineicauda</i>	F	5	0.18	0.02	0.04	0.01	0.03	0.01	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00
	M	2	0.16	0.02	0.04	0.00	0.03	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.02	0.00

Table 3.3: Variation in head and body scale counts of novel and revised species within *Acontias*. Supraciliaries (SC), supraoculars (SO), suboculars (SB), upper labials (UL), lower labials (LL), chin shields (CS), mid-body scale rows (MSR), subcaudals (SUC) and ventral scales (V).

Taxa	<i>n</i>	<u>SC</u>			<u>SO</u>				<u>SB</u>				<u>UL</u>			<u>LL</u>			<u>CS</u>				<u>MSR</u>	<u>SUC</u>	<u>V</u>
		3/3	3/4	4/4	2/2	3/2	2/3	3/3	2/2	3/2	2/3	3/3	5/4	4/5	5/5	3/3	4/3	3/4	3	4	5	6			
<i>A. orientalis</i>	64	—	1	63	3	2	2	57	6	5	4	49	1	—	63	63	—	1	62	2	—	—	14-17	33-44	154-187
<i>A. meleagris</i>	25	—	—	25	—	—	—	25	3	2	1	19	—	—	25	24	1	—	23	2	—	—	14-18	33-41	161-190
<i>A. parilis</i> sp. nov.	15	—	—	15	—	—	1	14	1	3	1	10	—	—	15	15	—	—	15	—	—	—	14-18	31-44	153-207
<i>A. caurinus</i> sp. nov.	30	—	—	30	—	—	—	30	4	1	3	22	—	1	—	30	—	—	28	1	—	1	14-17	32-46	174-196
<i>A. lineicauda</i>	7	3	—	4	4	—	—	3	7	—	—	—	—	1	6	7	—	—	7	—	—	—	14-16	28-37	159-184

3.3.2 *Acontias meleagris orientalis* (Hewitt, 1938)

Acontias orientalis (Lamb *et al.* 2010)

(Figs. 3.5, b)

(“Clade 1”, Engelbrecht *et al.* 2012)

Acontias percivali tasmani is genetically nearly indistinguishable from *A. orientalis* (Daniels *et al.* 2002; 2005; 2009; Lamb *et al.* 2010; Engelbrecht *et al.* 2012) and therefore synonymised with *A. orientalis* by Lamb *et al.* (2010).

Lectotype. An adult (sex not determined), **PEMR 5116**, Grahamstown, 33° 18' 14" S, 26° 31' 27" E, Eastern Cape province, South Africa, designated as a type specimen for *A. meleagris orientalis* on 16 March 1968 by D.G. Broadley.

Paralectotypes. Two specimens (sex not determined), **PEMR 5117**, **PEMR 5118**, Grahamstown, 33° 18' 14" S, 26° 31' 27" E, Eastern Cape province, South Africa, collection details not specified.

Additional material examined. **PEMR 10190**, Grahamstown, 33° 18' 14" S, 26° 31' 27" E, Eastern Cape province, South Africa, sex not determined, collected 17 October 1973 by R. Ochtman. **PEMR 5146**, Grahamstown, 33° 18' 14" S, 26° 31' 27" E, Eastern Cape province, South Africa, sex not determined, collected by B. Alan, collection date not specified. **PEMR 5123—5129**, Dunbrody, 33° 28' 11" S, 25° 32' 27" E, Eastern Cape province, South Africa, sex not determined, collected by K. Tasman, collection date not specified. **PEMR 5152—5160**, Dunbrody, 33° 28' 11" S, 25° 32' 27" E, Eastern Cape province, South Africa, sex not determined, collected by K. Tasman, collection date not specified. **PEMR 5162—5180**, **5192**, Dunbrody, 33° 28' 11" S, 25° 32' 27" E, Eastern Cape province, South Africa, sex not determined, collected by K. Tasman, collection date not specified.

PEMR 5190, Redhouse, Port Elizabeth district, 33° 49' 36" S, 25° 33' 59" E, Eastern Cape province, South Africa, sex not determined, collection details not specified. **PEMR 2289**, Kirkwood Rifle, 33° 23' 42" S, 25° 28' 56" E, Eastern Cape province, South Africa, sex not determined, collection details not specified. **PEMR 4140**, King Williams Town, 32° 52' 36" S, 27° 23' 30" E, Eastern Cape province, South Africa, sex not determined, collected 20 May 1995 by Bowen. **PEMR 4131**, King Williams Town, 32° 52' 36" S, 27° 23' 30" E, Eastern Cape province, South Africa, sex not determined, collected 30 August 1984 by L. Wingate. **PEMR 4137**, King Williams Town, 32° 52' 36" S, 27° 23' 30" E, Eastern Cape province, South Africa, sex not determined, collected by D.G. Broadley, collection date not specified. **PEMR 4155**, King Williams Town, 32° 52' 36" S, 27° 23' 30" E, Eastern Cape province, South Africa, sex not

determined, collected 15 February 1964 by L. Gibson. **PEMR 6590, 6591**, King Williams Town, 32° 52' 36" S, 27° 23' 30" E, Eastern Cape province, South Africa, sex not determined, collection details not specified. **PEMR 10024**, King Williams Town, 32° 52' 36" S, 27° 23' 30" E, Eastern Cape province, South Africa, sex not determined, collected 18 December 1978 by L. Wingate. **PEMR 8118**, Hartenbos, Mossel Bay, 34° 07' 16" S, 22° 06' 52" E, Eastern Cape province, South Africa, sex not determined, collected 09 March 1993 by M. Snyman. **SAM ZR, 52524, 52529, 52530; 52525—52527, 52531**, Salem, 32° 48' 06" S, 26° 45' 29" E, Eastern Cape province, South Africa, 3 F and 4 M. **SAM ZR 52528, 52474—52475**; on route to Hope Fountain, 33° 21' 33" S, 26° 30' 49" E, Eastern Cape province, South Africa, 2 F and 1 M. **SAM ZR 52486**, on route to Kirkwood, 33° 26' 40" S, 25° 45' 05" E, Eastern Cape province, South Africa, 1 F. **SAM ZR 5250—52509, 52510**; on route to Dunbrody, 33° 28' 11" S, 25° 32' 19" E, Eastern Cape province, South Africa, 3 F. **SAM ZR 52512, 52513**, outside Katberg, 32° 09' 42" S, 25° 45' 00" E, Eastern Cape province, South Africa, 2 F collected 24-28 April 2010 by S.R. Daniels, H.M. Engelbrecht, H. Ruhberg and N. Solomons. **SAM ZR 52501, 52503**; on route to Bedford, 33° 04' 43" S, 26° 13' 29" E, Eastern Cape province, South Africa, 1 F and 1 M. **SAM ZR 52514, 52518, 52519**; Pearston, 32° 46' 09" S, 25° 39' 49" E, Eastern Cape province, South Africa, 2 M and 1 F. **SAM ZR 52468**; Cookhouse, 32° 35' 15" S, 25° 08' 54" E, Eastern Cape province, South Africa, 1 M. **SAM ZR 52452, 52454, 52462, 52453, 52455—52460, 52463**; Bloemhof, 32° 49' 19" S, 25° 43' 20" E, Eastern Cape province, South Africa, 3 F and 8 M. **SAM ZR 52469—52472**; Kruidfontein, 32° 42' 40" S, 25° 38' 41" E, Graaff-Reinet, Eastern Cape province, South Africa, 2 F and 2 M. **SAM ZR 52442, 52445, 52443, 52441, 52444, 52446**; Aberdeen, 32° 21' 55" S, 24° 35' 52" E, Eastern Cape province, South Africa, 2 M and 3 F. **SAM ZR 52533, 52534**; on route to Tarkastad, 32° 24' 16" S, 25° 44' 14" E, Eastern Cape province, South Africa, 1 M and 1 F. **SAM ZR 52504, 52505, 52506, 52507**; on route to Cradock, 32° 18' 35" S, 25° 32' 24" E, Eastern Cape province, South Africa, 3 F and 1 M. **SAM ZR 52478—52480, 52485**; on route to Jansenville, 32° 38' 20" S, 26° 00' 54" E, Eastern Cape province, South Africa, 4 M collected 4-10 September 2010 by S.R. Daniels, H.M. Engelbrecht, N. Solomons and P. Strauss. **SAM ZR 52522, 52521, 52520, 52523**; Qumbu, 31° 09' 44" S, 28° 52' 10" E, Eastern Cape province, South Africa, 2 M and 2 F collected by S.R. Daniels, N. Solomons and F. Van Zyl (date not specified). **SAM ZR 52495, 52499; 52497, 52494, 52498**, Mossel Bay, 34° 09' 59" S, 22° 07' 59" E, Western Cape province, South Africa, 2 M and 3 F collected 22 May 2001, 15 October 2001 and 14 January 2002 by N.J.L. Heideman and S.R. Daniels.

Diagnosis. COI: Aberdeen: **JQ692328—692333**, Alexandria: **JQ692335**, Ashton: **JQ692338—692341**, Baakens Valley: **JQ692343**, Barrydale: **JQ692344—692349**, Beaufort West: **EU855676**, Bedford: **JQ692350—692356**, Bloemhof: **JQ692358—692367**, Cookhouse: **JQ692375—692378**, Cradock: **JQ692379-692381**, Dunbrody:

JQ692382, 692383, 692385, Graaff-Reinet: JQ692391-692392, Hope Fountain: JQ692393—692395, Jansenville: JQ692396—692400, Katberg: JQ692401-692404, Middeldrift: JQ692407, Montagu: JQ692408—692411, Oudtshoorn: EU855678-855683, Paterson: JQ692415—692416, 692418, Pearston: JQ692419-692422, Qumbu: JQ692426-692427, Salem: JQ692431—692437, Tandjiesberg: JQ692445, Tarkastad: JQ692446-692449. Sequence divergence amongst selected species of *Acontias* are summarised in table 3.1.

16S rRNA: Aberdeen: JQ692450-692455, Alexandria: JQ692457, Ashton: JQ692460—692463, Baakens Valley: JQ692465, Barrydale: JQ692466—692471, Beaufort West: EU855578, Bedford: JQ692472—692478, Bloemhof: JQ692480—692489, Cookhouse: JQ692497—692500, Cradock: JQ692501—692503, Dunbrody: JQ692504—692507, Graaff-Reinet: JQ692513—692514, Hope Fountain: JQ692515—692517, Jansenville: JQ692518—692522, Katberg: JQ692523—692526, Middeldrift: JQ692529, Montagu: JQ692530—692533, Mossel Bay: AY683721—683725, 683727, Oudtshoorn: EU855580—EU855585, Paterson: JQ692537-692538, 692540, Pearston: JQ692541—692544, Qumbu: JQ692548—692549, Salem: JQ692554—692560, Tandjiesberg: JQ692568, Tarkastad: JQ692569—692571.

EXPH 5: Aberdeen: JQ278040—278042, Alexandria: JQ278044, Ashton: JQ278047—278049, Barrydale: JQ278051—278053, Bedford: JQ278054, Bloemhof: JQ278056—278063, Cookhouse: JQ278067—JQ8069, Cradock: JQ278070, Dunbrody: JQ278071, Graaff-Reinet: JQ278075, Hope Fountain: JQ278077—278078, Jansenville: JQ278079—278082, Katberg: JQ278083, Middeldrift: JQ278087, Montagu: JQ278088—278090, Paterson: JQ278095, Pearston: JQ278096—278100, Qumbu: JQ278103, Salem: JQ278108, Tarkastad: JQ278112.

Description. Dimensions (lectotype with the variation shown within species in parantheses): SVL 179 mm (116—212, mean 170.6); TL 41.31 mm (23.69—43.81, mean 35.84); HL 8.28 mm (5.6—9.27, mean 7.66); HW 6.16 mm (4.44—7.63, mean 5.97); HD 4.75 mm (3.57—6.51, mean 4.95); RL 3.05 mm (2.48—3.57, mean 3.04); NRL 1.76 mm (1.43—2.36, mean 1.94); ML 3.06 (2.23—3.8, mean 2.83). Ratios for morphological measurements are summarised in table 3.2.

Lepidosis. Supraciliaries 4/4, supraoculars 3/3, suboculars 3/3, upper labials 5/5, lower labials 3/3, chin shields 3, midbody scale rows 14, subcaudals 41, ventrals 176. However, see table 3.3 for variation within species.

Colouration (in life). Characterised by two main morphotypes, i.e. uniform colouration or striped, but intermediate colouration patterns are also observed. The stripe form is generally pale or bright yellow with four to six solid dark-brown to black broad stripes, broader than pale interspaces. In addition, there is one continuous row of spots and in some cases a second discontinuous row of spots. Animals that are uniformly coloured resembles that of *A. meleagris*

(clade 2), *A. caurinus* sp. nov. (clade 4) and *A. parilis* sp. nov. (clade 3) in that the dorsal pigmentation patterns shows similar variation, where it can be anything from grey-brown, medium-brown to dark-brown. Animals from Qumbu, Eastern Cape are nearly black. Scales are either pale edged; or mottled on the upper half with a pale distal half; or uniform in colour with darker pigmentation on the distal half. In some animals the dorsum is medium to dark-brown and scales are characterized by pigmentation concentration on the upper edge with a line running from it to the distal edge of the scale, resulting in ragged stripes on the dorsum, giving it an intermediate appearance of a dark dorsum with stripes. In all morphotypes the tail is slightly tapered with a blunt rounded end. Ventral markings on the tail are frequently present and are either light-brown mottling or form three stripes that end towards cloacal scale, leaving a pale spot posterior to the cloaca. The chin and throat area are frequently mottled in all morphotypes. Colour and scale pigmentation of *A. orientalis* resemble colouration of life animals, except striped morphotypes were characterized by a brighter yellow dorsum (Figs. 3.5, b).

Habitat. Subterranean in habits, usually found in sandy soils in both low lying coastal regions and sandy areas in the interior Klein Karoo and mostly rural settlements in the Eastern Cape. Animals were exposed by over turning logs, rocks, raking leaf litter and building rubble at derelict buildings sites and under corrugated iron sheets.

Distribution. Spanning both the Western and Eastern Cape provinces, occurring predominantly in the interior regions of both provinces from Ashton in the west to Qumbu in the far north-east. Found in sympatry with the Algoa Bay dwarf burrowing skink, *Scelotes anguineus* and the thin-tailed legless skink, *Acontias gracilicauda*.



Figure 3.5a: Image of preserved specimens demonstrating the colour variation within *A. orientalis*.



Figure 3.5b: Image of preserved specimens demonstrating the dorsal colour and scale pigmentation variation within *A. orientalis*.

3.3.3 *Acontias meleagris orientalis*–‘*lineicauda*’ (Hewitt 1938)

Acontias lineicauda (Lamb *et al.* 2010)

(Figs. 3.6a, b)

(“Clade 5”, Engelbrecht *et al.* 2012)

Lectotype. **PEMR 5128**, Dunbrody, 33° 28' 11" S, 25° 32' 27" E, Eastern Cape province, South Africa, sex not determined, collected by K. Tasman, collection date not specified.

Paralectotypes. **PEMR 5120—5127, 5129**, Dunbrody, 33° 28' 11" S, 25° 32' 27" E, Eastern Cape province, South Africa, sex not determined, collected by K. Tasman, collection date not specified.

Additional material examined. **PEMR 1268**, Port Elizabeth, 33° 49' 36" S, 25° 33' 59" E, Eastern Cape province, South Africa, sex not determined, collection details not specified. **PEMR 1275**, Port Elizabeth, 33° 49' 36" S, 25° 33' 59" E, Eastern Cape province, South Africa, sex not determined, collection details not specified. **PEMR 12921**, Schelm Hoek, west bank of Sundays River, 33° 43' 00" S, 25° 48' 00" E, Eastern Cape province, South Africa, sex not determined, collected 01 November 1996. **SAM ZR 52419**, Paterson, 33° 21' 33" S, 26° 30' 49" E, Eastern Cape province, South Africa, 1 M collected 27 April 2010 by S.R. Daniels, H.M. Engelbrecht, H. Ruhberg and N. Solomons. **SAM ZR 52416; 52418, 52415**, outside East London, 33° 03' 20" S, 27° 51' 48" E, Eastern Cape province, South Africa, 2 F collected 22 April 2010 and 3 September 2010 by S.R. Daniels, H.M. Engelbrecht, N. Solomons and P. Strauss. **NMB 9058, 9060**; Wells estate, 33° 49' 42" S, 25° 37' 59" E, Port Elizabeth, Eastern Cape province, South Africa, 2 F collected 4 June 2001. **NMB 9061**; Alexandria, 33° 39' 06" S, 26° 24' 30" E, Eastern Cape province, South Africa, 1 F collected 7 June 2001.

Diagnosis. *COI*: Alexandria: **JQ692336—692337**, East London: **JQ692386—692389**, Oyster Bay: **JQ692423**, Paterson: **JQ692417**. Sequence divergence amongst selected species of *Acontias* are summarised in table 3.1.

16S rRNA: Alexandria: **JQ692458—692459**, East London: **JQ692508—692511**, Oyster Bay: **JQ692545**, Paterson: **JQ692539**, Port Alfred: **AY683713—AY683720**, Port Elizabeth: **AY683705—AY683710**.

EXPH 5: Alexandria: **JQ278045—278046**, East London: **JQ278072—278073**, Paterson: **JQ278094**.

Description. Dimensions (Lectotype with the variation shown within species in parantheses): SVL 142 mm (120-164, mean 136.25); TL 29.97 mm (18.04-29.39, mean 23.16); HL 5.74 mm (5.01-7.09, mean 5.49); HW 3.82 mm (3.65-5.67, mean 4.25); HD 3.45 mm (3.21-5.09, mean 3.9); RL 2.27 mm (2.12-3.08, mean 2.46); NRL 1.41 mm (1.41-2.3, mean 1.74); ML 2.02 mm (1.61-2.96, mean 2.22). Ratios for morphological measurements are summarised in table 3.2.

Lepidosis. Supraciliaries 4/4, supraoculars 2/2, suboculars 2/2, upper labials 5/5, lower labials 3/3, chin shields 3, midbody scale rows 14-16, subcaudals 28-37, ventrals 159-184. However, see table 3.3 for variation within species.

Colouration (in life). Generally pale to bright yellow with four to six solid stripes which are in many cases thinner than the pale interspaces, but forms having stripes equally broad as pale interspaces are also observed. The solid stripes are followed by one continuous row of spots and often a second discontinuous row of spots. Frequently, this is a more slender form (with thin dark stripes), but stout forms resembling *A. orientalis* are also observed. In all morphotypes the tail is tapered and in some animals only slightly tapering, with a blunt rounded end. In all morphotypes the tail is slightly tapered with a blunt rounded end. Ventral markings on the tail are frequently present and are either light-brown mottling or form three stripes that end towards cloacal scale, leaving a pale spot posterior to the cloaca. The chin and throat area are frequently mottled in all morphotypes. Colour and scale pigmentation of preserved specimens of *A. lineicauda* resemble the colouration of life animals (Figs. 3.6a, b).

Habitat. Subterranean in habits, usually found in sandy soils in low-lying coastal regions. Animals were exposed by turning over logs, rocks and leaf litter on beachfronts. They were also found under building rubble at dumpsites.

Distribution. Occurs along the narrow coastal strip of the Eastern Cape province from East London towards Port Elizabeth, including the adjacent interior at Paterson. Found sympatric with the Algoa dwarf burrowing skink, *S. anguineus*. The phylogenetic status of the Port Elizabeth locality, pertaining to its inclusion with the *A. lineicauda* taxon requires further investigation. However, here, Port Elizabeth was included in the distribution range of *A. lineicauda* until further investigation.



Figure 3.6a: Image of preserved specimens demonstrating the colour variation within *A. lineicauda*.



Figure 3.6b: Image of preserved specimens demonstrating the dorsal colour and scale pigmentation variation within *A. lineicauda*.

3.3.4 *Acontias caurinus* sp. nov.

(Figs. 3.7a, b and 3.8a—d)

(“Clade 4”, Engelbrecht *et al.* 2012)

Holotype. An adult female; **SAM ZR 52377**, Robertson, 33° 48' 07" S, 19° 59' 19" E, Western Cape province, South Africa, collected 13 July 2010 by H.M. Engelbrecht, D. Erasmus, P. Strauss and F. Van Zyl.

Paratypes. Two specimens (1 M: **SAM ZR 5041**; 1 F: **SAM ZR 1969**), Robertson, 33° 52' 30" S, 19° 52' 30" E, Western Cape province, South Africa, collected by Melle (date not specified).

Additional material examined. **PEMR 2008, 2013, 2014**Langebaan, 33° 05' 08" S, 18° 02' 00" E, Western Cape province, South Africa, sex not determined, collected 18 March 1965 by J. Spence. **PEMR 2010**, Schaapen Island, 33° 05' 27" S, 18° 01' 16" E, Western Cape province, South Africa, sex not determined, collected collected 18 March 1965 by J. Spence. **SAM ZR 52359**, Aurora, 32° 42' 29" S, 18° 29' 02" E, Western Cape province, South Africa, 1 M collected by J.H. Visser (date not specified). **SAM ZR 2485, 2487**, Clanwilliam, 32° 09' 24" S, 18° 53' 51" E, Western Cape province, South Africa, 2 F collected October 1897 by Leipoldt. **SAM ZR 52360, 52361**; Clanwilliam, 32° 09' 24" S, 18° 53' 51" E, Western Cape province, South Africa, 2 M, collected 2 July 2006 by N.J.L. Heideman. **SAM ZR 52365, SAM ZR 52366, 52367, 52468**; Klipheuwel, 33° 42' 12" S, 18° 41' 42" E, Western Cape province, South Africa, 2 F and 2 M, collected by J. Van Der Vyver (date not specified). **SAM ZR 1527, 1532, 1535**; Malmesbury, 33° 22' 30" S, 19° 52' 30" E, Western Cape province, South Africa, 3 F collected by Gird (date not specified). **SAM ZR 52369—2371**; Langebaan, 33° 06' 57" S, 18° 03' 21" E, Western Cape province, South Africa, 1 F and 2 M. **SAM ZR 52379, 52393, 52383, 52386, 52388, 52389, 52380—52382, 52387, 52390**; Velddrif, 32° 49' 04" S, 18° 07' 03" E, Western Cape province, South Africa, 6 F and 5 M collected 29-30 June 2006 by N.J.L. Heideman. **SAM ZR 52364**, Graafwater, 32° 07' 09" S, 18° 38' 27" E, Western Cape province, South Africa, 1 M collected 27 June 2006 by S.R. Daniels and N.J.L. Heideman.

Diagnosis. *COI*: Aurora: **JQ692342**, Clanwilliam: **EU855684—EU855686**, Elands Bay: **EU855715**, Graafwater: **EU855687—EU855688**, Klipheuwel: **JQ692405**, Langebaan: **EU855690—EU855694**, Malmesbury: **EU855689**, Rawsonville: **JQ692428—692430**, Velddrif: **EU855695—EU855714**. Sequence divergence amongst selected species of *Acontias* are summarised in table 3.1.

16S rRNA: Aurora: **JQ692464**, Clanwilliam: **EU855586—EU855587** , Elands Bay: **EU855619**, Graafwater: **EU855589—EU855590**, Klipheuwel: **JQ692527**, Langebaan: **EU855593—EU855597**, Malmesbury: **EU855592**, Rawsonville: **JQ692550—692552**, Robertson: **JQ692553**, Velddrif: **EU855599—EU855617**.

EXPH 5: Aurora: **JQ278050**, Klipheuwel: **JQ278084—278085**, Rawsonville: **JQ278104—278106**, Robertson: **JQ278107**.

Description. Dimensions (Holotype with the variation shown within species in parantheses): SVL 213 mm (147—261, mean 198.28); TL 50.36 mm (28.71—50.36, mean 39.74); HL 8.66 mm (6.27—10.50, mean 8.43); HW 6.77 mm (4.47—8.7, mean 6.66); HD 5.35 (3.38—7.94, mean 5.53); 3.67 (2.47—3.95, mean 3.58); NRL 1.88 mm (1.6—2.86, mean 2.03); ML 2.74 mm (2.35—3.77, mean 2.96).

Lepidosis. Supraciliaries 4/4, supraoculars 3/3, suboculars 3/3, upper labials 5/5, lower labials 3/3, chin shields 3, midbody scale rows 14, subcaudals 46, ventrals 177. However, see table 3.3 for variation within species.

Colouration (in life). Resemble the exact colour variation and patterns of *A. meleagris*. However, low frequencies of striped forms are observed. The tail is seldom slightly tapered with a blunt rounded end. Colour and scale pigmentation of preserved specimens of *A. meleagris* resemble colouration of life animals (Figs. 3.7a, b).

Habitat. Subterranean in habits, usually found in sandy soils in low lying coastal regions and sandy patches inland spanning into the Breede River Valley.

Distribution. Northern region of the Western Cape province encompassing coastal regions, including the interior of the Western Cape from Aurora to Robertson. Inland animals were found by over turning building rubble. Found sympatric with *T. caecus*, *S. gronovii*, *S. kasneri*, *S. sexlineatus*, *A. litoralis*, *A. lineatus lineatus*, *A. l. tristis* and *A. l. grayi*.

Etymology. The specific epithet refers to the biogeographic region where the species occurs, i.e. the north-western parts of the Western Cape province.



Figure 3.7a: Image of preserved specimens demonstrating the colour variation within *A. caurinus* sp. nov.



Figure 3.7b: Images of preserved specimens demonstrating the dorsal colour and scale pigmentation variation within *A. caurinus* sp. nov.



Figure 3.8a: *Acontias caurinus* sp. nov. holotype, SAM ZR 52377, dorsal view of head.



Figure 3.8b: *Acontias caurinus* sp. nov. holotype, SAM ZR 52377, lateral view of head.



Figure 3.8c: *Acontias caurinus* sp. nov. holotype, SAM ZR 52377, dorsal view of specimen.



Figure 3.8d: *Acontias caurinus* sp. nov. holotype, SAM ZR 52377, ventral view of specimen.

3.3.5 *Acontias parilis* sp. nov.

(Figs. 3.9a, b and 3.10a—d)

(“Clade 3”, Engelbrecht *et al.* 2012)

Holotype. An adult female; **SAM ZR 52394**, Bredasdorp district, 34° 29' 04" S, 20° 05' 29" E, Eastern Cape province, South Africa, collected 25 September 2010 by H.M. Engelbrecht, S.R. Daniels, F. Gordon and D.E. McDonald.

Paratypes. Three specimens (2 F: **SAM ZR 52398, 2399**; 1 M: **SAM ZR 52400**), Bredasdorp, 34° 29' 04" S, 20° 05' 29" E, Western Cape province, South Africa, collected 24 September 2010 by H.M. Engelbrecht, S.R. Daniels, F. Gordon and D.E. McDonald

Additional material examined. **SAM ZR 52395, 52397**; Agulhas, 34° 37' 36" S, 20° 07' 14" E, Western Cape province, South Africa, 1 M and 1 F. **SAM ZR 52407, 52396, 52406, 52410, 52412**; Struis Bay, 34° 44' 11" S, 20° 01' 41" E, Western Cape, province, South Africa, 5 M, collected 24—25 September 2010 by S.R. Daniels, H.M. Engelbrecht, F. Gordon and D.E. McDonald. **SAM ZR 52402—52403**; Nieuwoudtville, 31° 19' 10" S, 19° 07' 02" E, Northern Cape province, South Africa collected 8 October 2010 by H.M. Engelbrecht, D.E. McDonald, K. Van Zyl and F. Van Zyl. **SAM ZR 52401**; Gans Bay, 34° 35' 02" S, 19° 20' 48" E, Western Cape Province, South Africa, 1 F collected 5 February 2011 by G. Beukman, G. Diedericks and H.M. Engelbrecht. **NMB 9551**; Bredasdorp, 34° 39' 26" S, 19° 31' 37" E, Western Cape province, South Africa, 1 M, collected 7 October 2008 by J. Marais.

Diagnosis. *COI*: Agulhas: **JQ692334**, Bredasdorp: **JQ692368—692374**, Gans Bay: **JQ692390**, Nieuwoudtville: **JQ692413—692414**, Struis Bay: **JQ692438—692443**, Swellendam: **JQ692444**. Sequence divergence amongst selected species of *Acontias* are summarised in table 3.1.

16S rRNA: Agulhas: **JQ692456**, Bredasdorp: **JQ692490—692496**, Gans Bay: **JQ692512**, Nieuwoudtville: **JQ692535—692536**, Struis Bay: **JQ692561—692566**, Swellendam: **JQ692567**.

EXPH 5: Bredasdorp: **JQ278064—278066**, Gans Bay: **JQ278074**, Nieuwoudtville: **JQ278091—278093**, Struis Bay: **JQ278109—278110**, Swellendam: **JQ278111**.

Description. Dimensions (Holotype with the variation shown within species in parantheses): SVL 162 mm (123—218, mean 174.09); TL 32.49 mm (19.89—46.62, mean 35.26); HL 7.8 mm (2.88—9.20, mean 7.48); HW 5.8 mm

(4.25—7.8, mean 6.22); HD 4.95 mm (3.45—6.41, mean 6.25); RL 2.86 mm (2.13—3.75, mean 2.99); NRL 2.05 mm (1.48—2.48); ML 2.78 mm (2.14—3.41, mean 2.89).

Lepidosis. Supraciliaries 4/4, supraoculars 3/3, suboculars 3/3, upper labials 5/5, lower labials 3/3, chin shields 3, midbody scale rows 15, subcaudals 31, and ventrals 170. However, see table 3.3 for variation within species.

Colouration (in life). Resemble *A. meleagris* and the slender striped form of *A. lineicauda* with thin black stripes. Animals occurring in the Agulhas Plain region are generally pale yellow with dark uniform pigmentation on dorsum (at least the two mid-dorsal rows). Animals from the Northern Cape are generally lighter in colour. Within this species animals may have ventral markings on mostly the anterior ventral region, including the ventral tail. Colour and scale pigmentation of recently preserved specimens of *A. parilis* resemble colouration of life animals (Figs. 3.9a, b).

Habitat. Subterranean in habits, usually found in sandy soils in low lying coastal regions, leaf litter, and often exposed on turning over logs, rocks and any debris that suffice as a basking retreat.

Distribution. Spans the Agulhas Plain region in the Western Cape province and includes Nieuwoudtville in the Northern Cape province. Found sympatric with the dwarf burrowing skink, *Scelotes bipes* in the Agulhas Plain region.

Etymology. The specific epithet was chosen from the Latin *parilis*, meaning equivalent, like or similar in reference to the colouration similarities between the new and genetically distinct species *A. meleagris* and *A. orientalis*.



Figure 3.9a: Image of preserved specimens demonstrating the colour variation within *A. parilis* sp. nov.



Figure 3.9b: Images of preserved specimens demonstrating the dorsal colour and scale pigmentation variation within *A. parilis* sp. nov.



Figure 3.10a: *Acontias parilis* sp. nov. holotype, SAM ZR 52394, dorsal view of head.



Figure 3.10b: *Acontias parilis* sp. nov. holotype, SAM ZR 52394, lateral view of head.



Figure 3.10c: *Acontias parilis* sp. nov. holotype, SAM ZR 52394, dorsal view of specimen.



Figure 3.10d: *Acontias parilis* sp. nov. holotype, SAM ZR 52394, ventral view of specimen.

3.4 Discussion

The five genetic clades defined by Engelbrecht *et al.* (2012) formed the basis of the diagnosis for the present study. Considerable morphological overlap was observed among the five lineages based on meristic, morphometric characters and the colouration patterns examined (Tables 3.2 and 3.3). The lack of diagnostic morphological differences among the five clades are not surprising and have hampered the reliable diagnosis of the novel lineages in the species complex. Similar observations have been documented among fossorial taxa. For example, overlap in scalation characters has masked the genetic diversity among species in the southern African fossorial skink genus, *Scelotes* (Heideman *et al.* 2011). In addition, in the Philippine's slender skinks *Brachymeles* it has been observed that traditional morphological based taxonomic characters have obscured genetic lineages among currently described species (Siler *et al.* 2011). While in a phylogeographic study of the Greek legless skink *Ophiomorus punctatissimum* it was documented that two genetically discrete lineages was evident that could not be linked to morphological differences (Poulakakis *et al.* 2008). Collectively, these studies suggest that evolutionary studies based on molecular (DNA sequence) data is required to unravel evolutionary lineages among fossorial skink taxa and that these taxa, may harbor considerable species diversity. Character incongruence between DNA sequence and morphology are widespread amongst subterranean reptiles and is often attributed to morphological convergence induced by the ecomorphological demands of the subterranean life-style (Gans 1960; 1978; Norris & Kavanau 1966; Lee, 1998; Kearney & Stuart 2004; Crottini *et al.* 2009; Mott & Vieites 2009; Heideman *et al.* 2011). The fossorial habitat is generally thought to have induced widespread convergence amongst genetically distinct lineages. Subsequently, the absence of diagnostic meristic, morphometric and colouration characters, within *A. meleagris* was anticipated.

In a pre-cladistic framework, dorsal colour and colouration patterns formed the basis of species diagnosis amongst previously recognized taxa of the *A. meleagris* species complex (Broadley and Greer 1969). However, the present study in conjunction with data from Engelbrecht *et al.* (2012) verify that three of the newly described and revised species, with the exception of *A. lineicauda*, comprise both uniformly coloured and striped morphotypes within a species (Figs. 3.4a, b; 3.5a, b; 3.9a, b). For example at Agulhas, Western Cape province we collected both the striped morphotype of *A. parilis* sp. nov. together with a uniformly coloured form of the same species. Similarly, at Sir Lowry's Pass, Western Cape province we collected both morphotypes of *A. meleagris* that were genetically identical. *Acontias percivali tasmani* was synonymized with *A. orientalis* by Lamb *et al.* (2010) and regarded as a dorsal uniformly coloured morphotype of *A. orientalis* during the present study, while striped morphotypes were collected at for example Salem, Hope Fountain, Grahamstown, Cradock, Tarkastad and intermediate morphotypes representing a variety of striped patterns were present at Bloemhof, Jansenville and Pearston.

Furthermore, *A. lineicauda* are characterized by variable dorsal stripe patterning including broad dark stripes resembling that of previously recognized *A. m. orientalis* (Figs. 3.6a, b). The striped morphotype of *A. orientalis* and *A. lineicauda* share the same variation for the number of continuous versus discontinuous stripes. No striped morphotypes of *A. caurinus* sp. nov., were sampled or observed during the course of this investigation (Figs. 3.7a, b).

Polymorphic colour patterns observed within *A. meleagris sensu lato* appears to be indiscriminate among the five clades. Comparable polymorphic colour states within species of *Scelotes* and previously recognized *Acontias* species, particularly *A. lineatus lineatus*, *A. l. tristis*, *A. l. grayi* and *A. litoralis* suggests that colour polymorphism may reflect local ecological adaptations to soil and vegetation types (Janse van Vuuren, 2009). In subterranean caecilians, Wollenberg and Measey (2009) found that colour patterns can be ascribed to limited above-ground movement and convergent selection. However, the evolutionary advantage of polymorphic colour patterns within *Acontias* requires formal investigation to validate its adaptive nature.

In addition, former descriptions pertaining to the shape of the tail, i.e. tapered versus non-tapered tails within *A. meleagris sensu lato* is phylogenetically uninformative (Figs. 3.11a—e). The head shape amongst these species pose the same scenario, however this needs to be formally tested through the application of geomorphometric methods (Figs. 3.12a—e). In part, the lack of diagnostic morphological characters could be resultant of the relative recent Pliocene / Pleistocene cladogenesis among the five lineages (Engelbrecht *et al.* 2012). Moreover, the average sequence divergence amongst species of *A. meleagris sensu lato* for cytochrome oxidase subunit one (*COI*) of 5.3% falls well within the sequence divergence range of currently recognized *Acontias* species (Table 3.1). The robustness of the *COI* locus for species delineation has been extensively validated and proven to be useful for all animal taxa (Herbert *et al.* 2003). The latter authors demonstrated that sequence divergence for *COI* within Chordata average $9.6\% \pm 3.8$ sd, while sequence divergence for *COI* amongst closely related skink species average 6.0%. For example, amongst species of the *Plestiodon latiscutatus* group in mainland Japan and several adjacent islets, Okamoto & Hikida (2012) found the lowest interspecific sequence divergence to be 6.1%. Correspondingly, a large-scale DNA barcoding assessment of Malagasy reptiles demonstrate that the sequence divergence between sister taxa of scincid lizards have a threshold of 6.5%—6.1% (Nagy *et al.* 2012). In addition, the latter authors demonstrate that sequence divergence for *COI* is comparatively lower between species of the Scincidae family.



Figure 11a: Image indicating the variation of tail shape within species of *Acontias meleagris sensu lato*, *A. meleagris sensu stricto*.



Figure 11b: Image indicating the variation of tail shape within species of *Acontias meleagris sensu lato*, *A. orientalis*.



Figure 11c: Image indicating the variation of tail shape within species of *Acontias meleagris sensu lato*, *A. lineicauda*.



Figure 11d: Image indicating the variation of tail shape within species of *Acontias meleagris sensu lato*, *A. caurinus* sp. nov.



Figure 11e: Image indicating the variation of tail shape within species of *Acontias meleagris sensu lato*, *A. parilis* sp. nov.



Figure 12a: Image indicating head shape within species of *Acontias meleagris sensu lato*, *A. meleagris sensu stricto*.



Figure 12b: Image indicating head shape within species of *Acontias meleagris sensu lato*, *A. orientalis*.



Figure 12c: Image indicating head shape within species of *Acontias meleagris sensu lato*, *A. lineicauda*.



Figure 12d: Image indicating head shape within species of *Acontias meleagris sensu lato*, *A. caurinus sp. nov.*



Figure 12e: Image indicating head shape within species of *Acontias meleagris sensu lato*, *A. parilis sp. nov.*

Conversely, putative evolutionary species of the *Sphenomorphus jagori* species complex have *COI* sequence divergence of as low as 3.47 %, but are morphologically diagnosable in parapatric areas with no evidence of geneflow between allopatric populations (Linkem *et al.* 2010). Notably, the five lineages detected in the *Acontias meleagris* species can also be differentiated based on nuDNA sequence data.

In the present study we subscribe to the phylogenetic species concept to diagnose the novel species (Cracraft 1989; de Queiroz 2007). The results from the present study demonstrate that DNA sequence data might render a superior tool in the diagnosis of fossorial lineages. In addition, the present study further demonstrates that some species are narrowly distributed (clades 2, 3 and 5 on Fig. 3.1) while other species (such as clades 1 and 4) are more widely distributed. Conservation concern should be directed towards the taxa with the narrow distributions for example *A. parilis* sp. nov., *A. lineicauda* and *A. meleagris* distributed in low-lying coastal regions since these areas are rapidly being transformed through residential estate development. The formal description of these narrow endemic lineages followed by a formal conservation status listing will likely highlight the need for proper conservation of the herpetofaunal group.

Chapter 4

General conclusions

From the present study it is evident that morphological taxonomic assessments of fossorial taxa remain unreliable in the absence of molecular techniques. For that reason, DNA based phylogeny construction is a superior tool and will most likely reveal larger genetic diversity and species richness than anticipated by morphological diversity. Additionally, molecular systematic methods provide significant insight into the evolutionary histories of these species. However, molecular systematics needs to be corroborated by ecological studies to provide a more holistic perspective into the processes of cladogenesis among fossorial taxa.

In comparison with supraterranean reptile taxa, a limited number of studies have examined reproductive isolation amongst closely related fossorial reptile taxa. It is possible that reproductive isolation by means of mate recognition through chemosensory discrimination (pheromones) is maintaining genetic structure amongst subterranean reptile taxa. Since, the chemosensory system of reptiles is generally well developed it is likely that chemoreception is enhanced amongst subterranean herpetofauna considering their reduced vision. In addition, scent characteristics vary with genetic diversification therefore, reproductive isolation by means of chemoreception discrimination warrants investigation amongst recently diverged fossorial reptile taxa. This is an area that requires future scrutiny.

In contrast, numerous fossorial reptile taxa are characterised by polymorphic morphological traits for example and more widespread; polymorphic states for colouration, limb loss, digit loss, head and tail shapes such as for example in *Scelotes*. However, these polymorphic morphological characters amongst closely related fossorial reptile species represent local ecological adaption. In general, the ecological divergence amongst reptiles in the subterranean habitat remains poorly studied, for example body proportion trade-offs (for e.g. burrowing speed versus feeding aspects) and resource partitioning, especially where species occur in sympatry and in close geographical contact (*Acontias*, *Typhlosauris* and *Scelotes*). An additional field that requires formal investigation amongst fossorial reptiles is the presence and adaptive nature of sexual dimorphism in the subterranean habitat. Given the limitations of the subterranean habitat and highly convergent morphology, the presence of sexual dimorphic characters within a single fossorial reptile taxon is unlikely. However, several studies report on the presence of sexual dimorphism within these taxa, yet the adaptive significance whether related to for example mate selection, or competition during reproduction remains unexplored.

The limited knowledge regarding the general biology and ecology of fossorial reptile taxa could benefit from an integrative research approach since these factors are interlinked and of little value if viewed in isolation. For example, investigations concerning pigmentation variation are most likely linked to partial above ground movement. In turn, knowledge regarding above ground movement could uncover general ecological strategies for subterranean reptiles such as behaviour ecology, interlinked with mating strategies and resource partitioning. Evidently, future research pertaining to subterranean reptiles should focus on the contemporary evolutionary drivers maintaining genetic diversity and species boundaries. In addition, a comparative research approach, comparing supraterranean reptile taxa and applying it to the subterranean system could be a point of departure in an attempt to bridge the gap between speciation processes and local ecological adaptation amongst fossorial reptiles. A comparative research approach may well reveal differing selection pressures between the terrestrial and fossorial habitat moreover, disclose traits that are under natural selection versus those that are not.

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Appendix 1

Clade membership, TCS haplotype allocation and corresponding GenBank numbers for newly generated *COI* sequences. Asterisks (*) denote *COI* sequences included from Daniels *et al.* (2002, 2005, 2009).

Clade membership	Sampling locality	Haplotype number	GenBank accession number
Clade 1	Aberdeen_HME133	H15	JQ692328
Clade 1	Aberdeen_HME135	H03	JQ692329
Clade 1	Aberdeen_HME136	H07	JQ692330
Clade 1	Aberdeen_HME137	H03	JQ692331
Clade 1	Aberdeen_HME140	H07	JQ692332
Clade 1	Aberdeen_HME144	H07	JQ692333
Clade 3	Agulhas_HME228	H44	JQ692334
Clade 1	Alexandria	H03	JQ692335
Clade 5	Alexandria_HME182	H63	JQ692336
Clade 1	Alexandria_HME184	H43	JQ692337
Clade 1	Ashton_02	H40	JQ692338
Clade 1	Ashton_03	H39	JQ692339
Clade 1	Ashton_04	H40	JQ692340
Clade 1	Ashton_05	H39	JQ692341
Clade 4	Aurora	H57	JQ692342
Clade 1	Baakens Valley	H14	JQ692343
Clade 1	Barrydale	H32	JQ692344
Clade 1	Barrydale_01	H36	JQ692345
Clade 1	Barrydale_02	H36	JQ692346
Clade 1	Barrydale_03	H33	JQ692347
Clade 1	Barrydale_04	H33	JQ692348
Clade 1	Barrydale_05	H33	JQ692349
Clade 1	Beaufort West *	H42	EU855676
Clade 1	Bedford_HME79	H20	JQ692350
Clade 1	Bedford_HME80	H20	JQ692351

Appendix 1 (continued)

Clade membership	Sampling locality	Haplotype number	GenBank accession number
Clade 1	Bedford_HME81	H20	JQ692352
Clade 1	Bedford_HME82	H20	JQ692353
Clade 1	Bedford_HME83	H20	JQ692354
Clade 1	Bedford_HMS84	H20	JQ692355
Clade 1	Bedford_HME85	H20	JQ692356
Clade 1	Bloemhof_HME104	H21	JQ692358
Clade 1	Bloemhof_HME105	H21	JQ692359
Clade 1	Bloemhof_HME106	H21	JQ692360
Clade 1	Bloemhof_HME108	H21	JQ692361
Clade 1	Bloemhof_HME110	H21	JQ692362
Clade 1	Bloemhof_HME111	H21	JQ692363
Clade 1	Bloemhof_HME112	H08	JQ692364
Clade 1	Bloemhof_HME114	H21	JQ692365
Clade 1	Bloemhof_HME119	H20	JQ692366
Clade 1	Bloemhof_HME120	H20	JQ692367
Clade 3	Bredasdorp_HME191	H47	JQ692368
Clade 3	Bredasdorp_HME192	H44	JQ692369
Clade 3	Bredasdorp_HME193	H47	JQ692370
Clade 3	Bredasdorp_HME197	H50	JQ692371
Clade 3	Bredasdorp_HME201	H31	JQ692372
Clade 3	Bredasdorp_HME214	H34	JQ692373
Clade 3	Bredasdorp_HME218	H35	JQ692374
Clade 2	Cape Hangklip *	H67	EU855734
Clade 4	Clanwilliam_01 *	H52	EU855684
Clade 4	Clanwilliam_02 *	H52	EU855685
Clade 4	Clanwilliam_03 *	H52	EU855686
Clade 1	Cradock_HME155	H23	JQ692379
Clade 1	Cradock_HME156	H23	JQ692380
Clade 1	Cradock_HME158	H24	JQ692381
Clade 1	Cookhouse_HME98	H21	JQ692375

Appendix 1 (continued)

Clade membership	Sampling locality	Haplotype number	GenBank accession number
Clade 1	Cookhouse_HME100	H22	JQ692376
Clade 1	Cookhouse_HME101	H21	JQ692377
Clade 1	Cookhouse_HME102	H08	JQ692378
Clade 1	Cradock_HME155	H23	JQ692379
Clade 1	Cradock_HME156	H23	JQ692380
Clade 1	Cradock_HME158	H24	JQ692381
Clade 1	Dunbrody_01	H11	JQ692382
Clade 1	Dunbrody_02	H11	JQ692383
Clade 1	Dunbrody_05	H11	JQ692385
Clade 5	East London_01	H04	JQ692386
Clade 5	East London_02	H04	JQ692387
Clade 5	East London_HME70	H05	JQ692388
Clade 5	East London_HME74	H06	JQ692389
Clade 4	Elands Bay_02 *	H52	EU855715
Clade 4	Elands Bay_01 *	H53	
Clade 3	Gansbaai	H51	JQ692390
Clade 1	Graaff Reinet_HME127	H18	JQ692391
Clade 1	Graaff Reinet_HME128	H18	JQ692392
Clade 4	Graafwater_01 *	H56	EU855687
Clade 4	Graafwater_02 *	H54	EU855688
Clade 1	Grahamstown_01 *	H01	AY683743
Clade 1	Grahamstown_02 *	H01	AY683742
Clade 1	Grahamstown_03 *	H02	AY683741
Clade 1	Grahamstown_04 *	H01	AY683740
Clade 1	Grahamstown_05 *	H01	AY683739
Clade 1	Grahamstown_06 *	H01	AY683738
Clade 1	Grahamstown_08 *	H01	AY683764
Clade 1	Grahamstown_07 *	H01	AY683737
Clade 1	Grahamstown_09 *	H01	AY683765
Clade 1	Grahamstown_10 *	H01	AY683773

Appendix 1 (continued)

Clade membership	Sampling locality	Haplotype number	GenBank accession number
Clade 1	Hope Fountain_01	H03	JQ692393
Clade 1	Hope Fountain_02	H03	JQ692394
Clade 1	Hope Fountain_03	H16	JQ692395
Clade 2	Jacobs Bay_01 *	H73	EU855735
Clade 2	Jacobs Bay_02 *	H73	EU855736
Clade 1	Jansenville_HME168	H17	JQ692396
Clade 1	Jansenville_HME169	H17	JQ692397
Clade 1	Jansenville_HME170	H18	JQ692398
Clade 1	Jansenville_HME171	H18	JQ692399
Clade 1	Jansenville_HME180	H03	JQ692400
Clade 1	Katberg_HME160	H09	JQ692401
Clade 1	Katberg_HME161	H09	JQ692402
Clade 1	Katberg_HME162	H09	JQ692403
Clade 1	Katberg_HME163	H09	JQ692404
Clade 4	Klipheuwel_2	H55	JQ692405
Clade 2	Kuilsriver *	H72	EU855677
Clade 4	Langebaan_01 *	H53	EU855690
Clade 4	Langebaan_02 *	H53	EU855691
Clade 4	Langebaan_03 *	H53	EU855692
Clade 4	Langebaan_04 *	H53	EU855693
Clade 4	Langebaan_05 *	H58	EU855694
Clade 2	Macassar	H70	JQ692406
Clade 4	Malmesbury *	H53	EU855689
Clade 1	Middeldrift	H10	JQ692407
Clade 1	Montagu_01	H38	JQ692408
Clade 1	Montagu_02	H38	JQ692409
Clade 1	Montagu_03	H38	JQ692410
Clade 1	Montagu_04	H37	JQ692411
Clade 1	Mossel Bay_01 *	H29	AY683781
Clade 1	Mossel Bay_03 *	H30	AY683779

Appendix 1 (continued)

Clade membership	Sampling locality	Haplotype number	GenBank accession number
Clade 1	Mossel Bay_05 *	H30	AY683778
Clade 1	Mossel Bay_07 *	H30	AY683777
Clade 2	Muizenberg	H70	JQ692412
Clade 3	Niewoudtville_HME253	H49	JQ692413
Clade 3	Niewoudtville_HME254	H47	JQ692414
Clade 1	Oudtshoorn_01 *	H27	EU855678
Clade 1	Oudtshoorn_02 *	H28	EU855679
Clade 1	Oudtshoorn_03 *	H28	EU855680
Clade 1	Oudtshoorn_04 *	H26	EU855681
Clade 1	Oudtshoorn_05 *	H27	EU855682
Clade 1	Oudtshoorn_06 *	H27	EU855683
Clade 1	Paterson_01	H11	JQ692415
Clade 1	Paterson_02	H11	JQ692416
Clade 5	Paterson_03	H78	JQ692417
Clade 1	Paterson_04	H11	JQ692418
Clade 1	Pearson_HME89	H18	JQ692419
Clade 1	Pearston_HME91	H19	JQ692420
Clade 1	Pearston_HME92	H18	JQ692421
Clade 1	Pearston_HME94	H18	JQ692422
Clade 5	Port Alfred_01 *	H61	AY683766
Clade 5	Port Alfred_02 *	H61	AY683767
Clade 5	Port Alfred_03 *	H61	AY683768
Clade 5	Port Alfred_04 *	H61	AY683769
Clade 5	Port Alfred_05 *	H62	AY683770
Clade 5	Port Alfred_06 *	H61	AY683771
Clade 5	Port Alfred_07 *	H61	AY683772
Clade 5	Port Alfred_08 *	H61	
N / A	Port Elizabeth_01 *	H74	AY683759
N / A	Port Elizabeth_02 *	H76	AY683760
N / A	Port Elizabeth_03 *	H77	AY683761

Appendix 1 (continued)

Clade membership	Sampling locality	Haplotype number	GenBank accession number
N / A	Port Elizabeth_04 *	H74	AY683762
N / A	Port Elizabeth_05 *	H75	AY683763
N / A	Oyster Bay	H79	JQ692423
Clade 2	Pringle Bay	H68	JQ692424
Clade 2	Pringle Bay_2	H71	JQ692425
Clade 1	Qumbu_2	H25	JQ692426
Clade 1	Qumbu_3	H25	JQ692427
Clade 4	Rawsonville_1	H80	JQ692428
Clade 4	Rawsonville_2	H82	JQ692429
Clade 4	Rawsonville_3	H81	JQ692430
Clade 2	Robben Island_01 *	H69	AY683754
Clade 2	Robben Island_02 *	H69	AY683755
Clade 2	Robben Island_03 *	H64	AY683756
Clade 2	Robben Island_04 *	H64	AY683757
Clade 2	Robben Island_05 *	H64	AY683758
Clade 2	Robben Island_06 *	H64	EU855720
Clade 2	Robben Island_07 *	H64	EU855721
Clade 2	Robben Island_08 *	H64	EU855722
Clade 2	Robben Island_09 *	H64	EU855723
Clade 2	Robben Island_10 *	H64	EU855724
Clade 2	Robben Island_11 *	H64	EU855725
Clade 2	Robben Island_12 *	H64	EU855726
Clade 2	Robben Island_13 *	H64	EU855727
Clade 2	Robben Island_14 *	H64	EU855728
Clade 2	Robben Island_15 *	H64	EU855729
Clade 2	Robben Island_16 *	H64	EU855730
Clade 2	Robben Island_17 *	H65	EU855731
Clade 2	Robben Island_18 *	H65	EU855732
Clade 2	Saldanha Bay *	H66	EU855718
Clade 1	Salem_1	H03	JQ692431

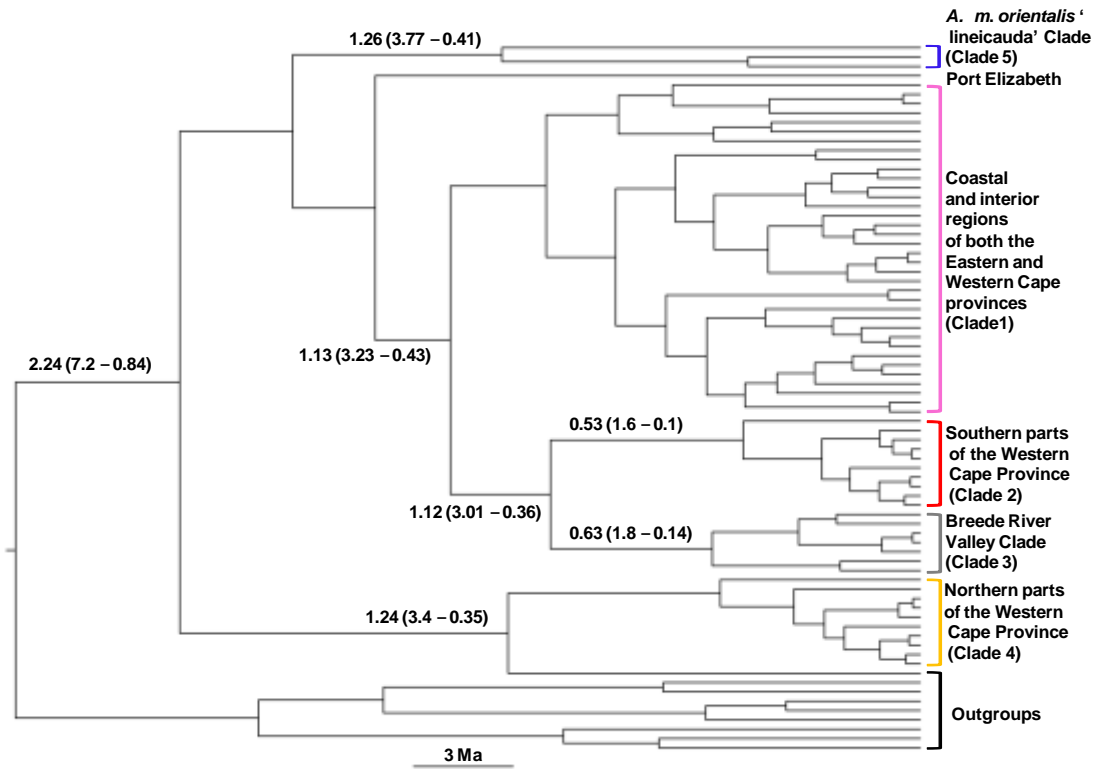
Appendix 1 (continued)

Clade membership	Sampling locality	Haplotype number	GenBank accession number
Clade 1	Salem_2	H03	JQ692432
Clade 1	Salem_3	H03	JQ692433
Clade 1	Salem_4	H03	JQ692434
Clade 1	Salem_5	H03	JQ692435
Clade 1	Salem_6	H03	JQ692436
Clade 1	Salem_7	H03	JQ692437
Clade 2	Sir Lowry's Pass *	H70	EU855717
Clade 3	Struis Bay *	H46	EU855719
Clade 3	Struis Bay_HME230	H44	JQ692438
Clade 3	Struisbaai_HME231	H41	JQ692439
Clade 3	Struisbaai_HME232	H47	JQ692440
Clade 3	Struisbaai_HME237	H45	JQ692441
Clade 3	Struisbaai_HME238	H47	JQ692442
Clade 3	Struisbaai_HME239	H45	JQ692443
Clade 3	Swellendam	H48	JQ692444
Clade 1	Tandjies	H17	JQ692445
Clade 1	Tarkastad	H01	JQ692446
Clade 1	Tarkastad_HME147	H01	JQ692447
Clade 1	Tarkastad_HME149	H47	JQ692448
Clade 1	Tarkastad_HME150	H47	JQ692449
Clade 4	Velldrif_01 *	H52	AY683789
Clade 4	Velldrif_02 *	H52	AY683788
Clade 4	Velldrif_03 *	H52	AY683787
Clade 4	Velldrif_04 *	H52	AY683786
Clade 4	Velldrif_05 *	H52	AY683785
Clade 4	Velldrif_06 *	H52	AY683784
Clade 4	Velldrif_07 *	H52	AY683783
Clade 4	Velldrif_08 *	H52	AY683782
Clade 4	Velldrif_09 *	H52	EU855714
Clade 4	Velldrif_10 *	H52	EU855713

Appendix 1 (continued)

Clade membership	Sampling locality	Haplotype number	GenBank accession number
Clade 4	Velddrif_11 *	H52	EU855712
Clade 4	Velddrif_12 *	H52	EU855711
Clade 4	Velddrif_13 *	H52	EU855710
Clade 4	Velddrif_14 *	H52	EU855709
Clade 4	Velddrif_15 *	H52	EU855708
Clade 4	Velddrif_16 *	H52	EU855707
Clade 4	Velddrif_17 *	H52	EU855706
Clade 4	Velddrif_18 *	H52	EU855705
Clade 4	Velddrif_19 *	H53	EU855704
Clade 4	Velddrif_20 *	H53	EU855703
Clade 4	Velddrif_21 *	H53	EU855702
Clade 4	Velddrif_22 *	H53	EU855701
Clade 4	Velddrif_23 *	H53	EU855700
Clade 4	Velddrif_24 *	H53	EU855699
Clade 4	Velddrif_25 *	H59	EU855698
Clade 4	Velddrif_26 *	H60	EU855697
Clade 4	Velddrif_27 *	H60	EU855696
Clade 4	Velddrif_28 *	H60	EU855695

Appendix 2



Appendix 2: Time-calibrated phylogeny of the *Acontias meleagris* species complex derived from BEAST (95% confidence intervals are provided in parentheses).