

**Phylogeography of the Cape girdled lizard,
Cordylus cordylus: investigating biogeographic
patterning in the Cape floristic region (CFR)**

by

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Declaration

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Abstract

In the present study I examined the phylogeography of the rupicolous Cape girdled lizard, *Cordylus cordylus*. Samples were collected across the species distribution range from 63 localities in the Eastern and Western Cape and Free State provinces of South Africa, yielding a total sample size of 207 specimens. Four DNA loci, two nuclear (PRLR, PTPN12) and two mitochondrial (16S rRNA, ND2), were sequenced. Bayesian inference, maximum likelihood and maximum parsimony methods were employed to test evolutionary relationships among populations, followed by population structure analyses, divergence time estimations and niche modelling. My results confirm the species monophyly and revealed the presence of two distinct clades. Clade 1 comprised specimens from the western and southern portions of the Western Cape coast, while clade 2 comprised specimens from the southern and eastern Cape coast and adjacent interior of the Eastern and Western Cape and Free State provinces. An area of sympatry between the two clades was observed in the Breede river valley. The divergence time estimates revealed an Early Pliocene (4.31 Ma), Late Miocene (6.01 Ma) divergence for each of the two clades retrieved. Phylogeographic data suggest that clade 1 is younger (lower haplotypic and nucleotide diversity), in comparison to clade 2. Furthermore, the niche modelling shows that *C. cordylus* occupies a wide range of unfavourable habitats. The absence of marked phylogeographic patterning within clades is very uncharacteristic for a rupicolous vertebrate species. The ecological pliability and generalist nature of *C. cordylus* presumably contributed to the observed phylogeographic pattern and have facilitated the absence of within clade

differentiation. Moreover, I suggest that microclimatic variables, rather than geographic barriers influence the genetic structuring of *C. cordylus*.

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CHAPTER 1

Introduction

Phylogeography seeks to merge population genetics and biogeography by describing the interplay between historic abiotic (climate, environment and geology) and biotic (a species life history characteristics and ecology) factors in an attempt to understand a species contemporary distribution and genetic structure (Avice et al., 1987; Avice and Walker, 1998; Hewitt, 2001; Avice, 2009; Knowles, 2009). Historical climatic ameliorations are major drivers of cladogenesis that globally result in habitat shifts and contractions, inducing either extinction or divergence (Flagstad et al., 2001; Nielsen et al., 2011; Montgelard and Matthee, 2012). It is generally accepted that the earth's climate oscillated between warm and cool periods with frequent climate fluctuations being associated with the Quaternary (2.4 Mya until Holocene) (Hewitt, 2000, 2004). In the northern hemisphere, Quaternary climatic oscillations are thought to have been the key factor driving the phylogeographic patterning among various faunal and floral groups (Klicka and Zink, 1997; Avice and Walker, 1998; Flagstad et al., 2001; Bowie et al., 2006; Guillaumet et al., 2008; Zhang et al., 2010; Recuero and Garcia-Paris, 2011). However, in the southern hemisphere, particularly in Africa, less severe glaciation cycles were experienced (Boelhouwers and Meiklejohn, 2002; Linder, 2003; Chase and Meadows, 2007; Cowling et al., 2009). This was as a direct consequence of the distance from the southern polar ice sheets in relation to the continental land masses, coupled with the impact of larger oceanic basins that moderated the climate, resulting in older phylogeographic divergence in comparison to northern hemisphere taxa (Flagstad et al., 2001; Glor et al., 2001; Daniels et al., 2007; Cowling et al., 2009; Lorenzen et al., 2012). However, a limited number of

phylogeographic studies have been conducted on southern hemisphere taxa, thus hampering direct comparisons with those from the northern hemisphere (Beheregaray, 2008).

The fauna and flora of the biodiversity rich Cape Floristic Region (CFR), which straddles the western- and southern Cape regions of South Africa, has been significantly impacted by climatic shifts during the Miocene/ Pliocene/ Pleistocene (Linder et al., 2010). The diversity of the herpetofauna of the CFR is well documented and the group exhibits high levels of endemism (Matthee and Flemming, 2002; Daniels et al., 2004, 2006; Tolley et al., 2006, 2009; Measey and Tolley, 2011). In addition, the majority of phylogeographic studies conducted in the CFR demonstrated that the Cape Fold Mountains act as a barrier to gene flow (Branch and Bauer, 1995; Daniels et al., 2001, 2009; Swart et al., 2009; Spinks et al., 2010). In comparison to the CFR, a limited number of studies have been conducted in the southern and eastern portions of the CFR, and consequently phylogeographic breaks within this region have largely remained undocumented. In contrast to these studies, Lawes (1990), reported the first observed biogeographical break between Port Elizabeth and East London, which he coined the Bedford Gap. The Bedford Gap is characterised by the neighbouring Karroo sub-desert biome's significant effect on the region as well as the fact that this biogeographical break receives a maximum winter rainfall followed by a more prominent summer dry period than neighbouring biomes (Lawes, 1990; Lawes et al., 2007). Recently, various studies have attributed mammalian phylogeographic patterning to the Bedford Gap (Engelbrecht et al., 2011a; Willows-Munro and Matthee, 2011; du Toit et al., 2012), however, the importance of this barrier in non-mammalian taxa requires validation.

Despite the fact that the aforementioned barriers have been shown to play a role in the genetic diversification of different taxa, each species requires individual scrutiny as their life history characteristics, dispersal capability, habitat preference and ecology determine their spatial distribution and thus their phylogeographic patterning (Smit et al., 2007; Willows-Munro and Matthee, 2011; Batalha-Filho et al., 2012; Ozinga et al., 2013).

Ubiquitously distributed taxa such as the Cape girdled lizard, *Cordylus cordylus*, are ideal to test the impact of climatic oscillations on habitat shifts in the CFR. The Cape girdled lizard (snout vent length (SVL) 65 - 85 mm) is endemic to South Africa (Branch, 1998), and has an extensive distribution in the Western, southwestern and Eastern Cape and the southern portions of the Free State province (Brody et al., 1993; Branch, 1998). The occurrence of *C. cordylus* is primarily governed by the presence of suitable rocky crevices, resulting in the formation of dense colonies in the presence of favourable habitats (Branch, 1998). *Cordylus cordylus* occurs from the coast at sea level to as high as 2000 meters above sea level (Diedericks pers. obs.). The species is viviparous, bearing one to three large, fully developed young in summer (Branch 1998). Both adults and young have a generalistic diet and exhibit a wide thermal tolerance range (Clusella-Trullas and Botes, 2008; Clusella-Trullas et al., 2009). Collectively these factors suggest that *C. cordylus* is an ecological generalist.

It has been demonstrated that, among rupicolous taxa, the presence of suitable habitat govern the phylogeographic pattern of the species, leading to marked genetic structuring (Bauer, 1999; Matthee and Flemming, 2002; Swart et al., 2009). Nevertheless, numerous studies have demonstrated that generalist species

lack phylogeographic patterning compared to specialist species and show lower genetic divergence in comparison to habitat specialist taxa (Joseph and Moritz, 1994; Joseph et al., 1995; Matthee and Robinson, 1996; Stuart-Fox et al., 2001; Vandergast et al., 2004; Smit et al., 2007; Colgan et al., 2009; Daniels et al., 2010; du Toit et al., 2012). In comparison to specialist taxa, generalist species have been demonstrated to persist in multiple refugia during periods of climatic instability (Stuart-Fox et al., 2001; Moodley and Bruford, 2007; O'Neill et al., 2009; Sillero et al., 2009; Ozinga et al., 2013). Jansson and Dynesius (2002) postulated that generalists respond better to abiotic variables as they are able to track environmental change and effectively colonise new areas over large distances. Recently, Ozinga et al. (2013) confirmed the latter observation and concluded that generalists occupy multiple environmental niches, ultimately leaving more descendants in comparison to specialist. These observations led us to postulate that the Cape girdled lizard should exhibit limited phylogeographic subdivision throughout its distribution range.

A small scale study conducted among *C. cordylus* specimens in the Western Cape retrieved a coastal and mountainous clade (Daniels et al., 2004). The limited geographic sampling by the aforementioned authors precluded them from underpinning phylogeographic patterning in the Cape girdled lizard. In addition, the lack of nuDNA sequence data and divergence time estimations in the later study, provide limited insight into the spatial and temporal shifts the species may have historically undergone.

Aims

The aims of this study are twofold. Firstly, to examine the phylogeographic patterning of *C. cordylus* throughout the species distribution range. I hypothesize that its distribution and phylogeographic patterning is as a direct consequence of its generalist ecology. The second aim is to determine the dispersal and colonization route of *C. cordylus* and the impact that past climatic ameliorations may have had on the phylogeographic patterning. I hypothesize that the species dispersed from the south eastern portions of its historic distribution range towards the west, following the last major marine regression resulting in a recent Plio/Pleistocene colonization of the Western Cape.

CHAPTER 2

Materials and Methods

2.1 *Sample collection*

Cordylus cordylus specimens were collected from 55 localities ($N = 175$) throughout its distribution range in the Eastern and Western Cape as well as Free State provinces of South Africa (Permit numbers: Western Cape, AAA004-01051-0035; Eastern Cape, CRO 46/11CR). Free State tissue samples as well as additional samples were acquired from the National Museum Bloemfontein, Durban Natural Science museum, Bayworld and the South African National Biodiversity Institute (SANBI) collections. Newly collected specimens were combined with specimens from eight localities ($N = 32$) within the Western Cape collected by Daniels et al. (2004), yielding a total of 207 specimens from 63 localities (Fig. 1, Table 1). Where possible, sampling occurred at 50 km intervals, ideally collecting at least three to five specimens per sample locality to maximize the detection of genetic diversity (Morando et al., 2003). However, at a number of localities this was not possible, presumably due to low sample numbers. Specimens were identified using Branch (1998), and mtDNA sequences were blasted against reference sequences deposited in GenBank. Upon capture, a small tail biopsy was taken and the animals were released on site. Tissue samples were stored in 95% ethanol prior to use while a hand-held GPS was used to record the latitude and longitude at each sampling site. A single voucher specimen was retained per sample locality and euthanized using pentobarbital sodium solution (Ethical clearance reference number 11NP-DIE01, University of Stellenbosch). Voucher specimens were preserved in 4% buffered

formalin and will be deposited in the herpetological collection at the National Museum, Bloemfontein, South Africa.

2.2 Molecular techniques

Total genomic DNA was extracted from muscle, liver or tail tissue using a Qiagen DNEasy kit, following the manufacturer's protocol. All DNA samples were stored in a fridge at 4°C until required for PCR. Two partial mitochondrial (mtDNA) loci, 16S rRNA and nicotinamide dehydrogenase subunit 2 (ND2) were amplified and sequenced for all specimens, and combined with the sequences generated by Daniels et al. (2004) (GenBank accession numbers *C. cordylus*: AY519661-AY519718 and AY519777-AY519720 respectively). The two sister species *C. oelofseni* (AY519704 and AY519765) and *C. niger* (AY519690 and AY519750) were used as outgroups (Daniels et al., 2004; Stanley et al., 2011). I attempted to amplify several nuclear DNA (nuDNA) loci, including exophilin 5 (EXPH5; Portik et al., 2010), natural killer tumor recognition gene (NKTR; Heideman et al., 2011), ubinclin 1 (UBN1; Portik et al., 2012), prolactin receptor gene and protein tyrosine phosphatase, non-receptor type 12 (PRLR and PTPN12 respectively; Townsend et al., 2008). However, only two loci consistently amplified, PRLR and PTPN12 (Table 2). The prolactin receptor locus is a pituitary hormone (PRL) bound to a receptor and is responsible for the molting of the epidermis and tail regeneration in reptiles (Bole-Feysot et al., 1998). The PTPN12 gene forms part of the protein tyrosine phosphatase (PTP) family and creates a domain for protein-protein interaction, essential for cytoskeletal rearrangements involved in cell migration and cell division (Tiganis and Bennett, 2007). These two nuDNA loci were selected on the basis that they have successfully amplified across several reptile taxa (Breitman et al., 2011;

Gvoždík et al., 2010; Stanley et al., 2011; Edwards et al., 2012), with PRLR being the most variable nuclear DNA marker (Townsend et al., 2008; Portik et al., 2012).

Multiple nuclear loci are required when attempting to delineate and identify units for conservation and when determining phylogeographic structure (Hare, 2001; Camargo et al., 2010; Portik et al., 2012). However, phylogeographic studies among Squamata have generally revealed low levels of nuDNA sequence variation (Caccone et al., 1999, 2004; Spinks and Shaffer, 2005; McGaugh et al., 2008; Spinks et al., 2010). Hence, in the present study a single specimen was sequenced per sample locality for each of the two partial nuclear loci, since preliminary analyses of nuDNA sequence data revealed genetic invariance within sample localities (Diedericks unpublished). The PCR profile for both the mtDNA and the two nuDNA partial gene fragments were 95 °C for 2 min, 95 °C for 30 s, 54 or 55 °C for 40 s, 72 °C for 1 min. The last three steps were repeated for 32 - 40 cycles followed by a final extension of 10 min at 72 °C. PCR products were visualized on a 1% agarose gel containing ethidium bromide, and amplicons were excised and purified using a PCR gel purification kit (Bioflux, Bioer Technology Co., Ltd.). Sequencing was performed by Macrogen Inc. (www.macrogen.com) on an automated machine (ABI 3730 XL DNA analyzer, applied Biosystems).

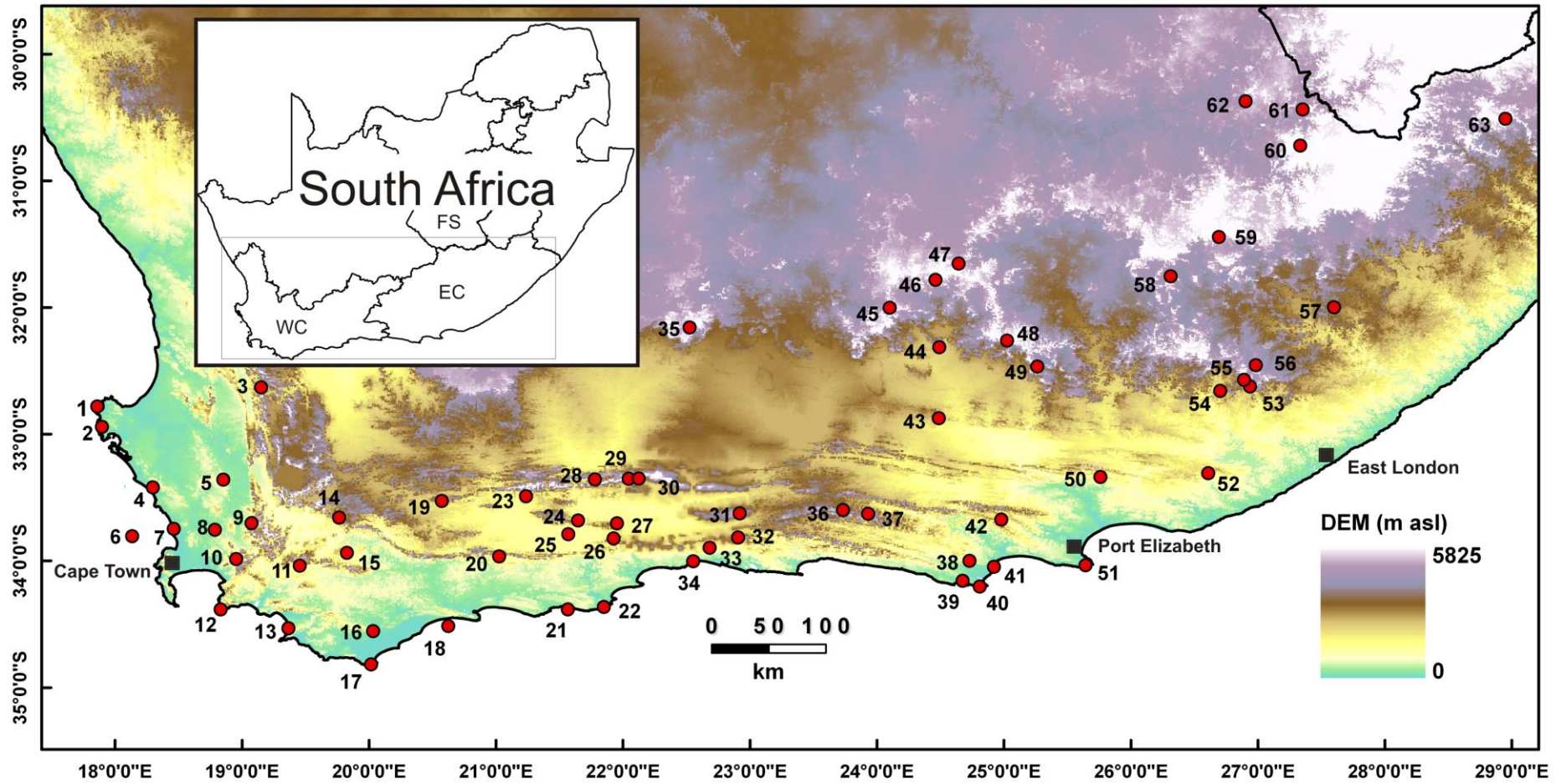


Figure 1. *Cordylus cordylus* localities sampled during the present study. The locality numbers correspond to those in Table 1. The abbreviations WC, EC and FS denotes the Western Cape, Eastern Cape and Free State provinces of South Africa.

Table 1. The locality sample number corresponding to Fig. 1. The sample size (*N*) at each locality, the province in which the locality is situated, the GPS coordinates, and the number of individuals sequenced for each gene. The data from Daniels et al. (2004) is indicated by a †, with additional specimens obtained from the following museums: South African National Biodiversity Institute (SANBI) (‡), National Museum Bloemfontein (#), Bayworld Museum, Port Elizabeth (γ).

Sample site	Locality	<i>N</i>	Province	Coordinates	16S rRNA	ND2	PRLR	PTPN12
1	Tieties Bay ‡	1	WC	32°50'21"S 17°51'43"E	1	1	1	1
2	Trekoskraal †	5	WC	32°53'13"S 17°53'56"E	5	5	1	0
3	Middelberg Pass †	4	WC	32°37'53"S 19°09'06"E	4	4	1	0
4	Rondeberg †	4	WC	33°25'18"S 18°17'56"E	4	4	1	1
5	Kasteelberg †	4	WC	33°21'39"S 18°51'16"E	4	4	1	0
6	Robben Island	3	WC	33°48'26"S 18°21'45"E	3	3	1	1
7	Blouberg †	4	WC	33°44'57"S 18°27'47"E	4	4	1	0
8	Stellenbosch	5	WC	33°46'23"S 18°47'12"E	5	5	1	1
9	Du Toitskloof Pass	3	WC	33°42'12"S 19°04'36"E	3	3	1	1
10	Jonkershoek N.R	4	WC	33°59'15"S 18°57'17"E	4	4	1	1
11	Amanzi Mnts. ‡	1	WC	34°02'22"S 19°27'23"E	1	1	1	1
12	Pringle Bay	5	WC	34°23'05"S 18°49'60"E	5	5	1	1
13	Gansbay	3	WC	34°32'00"S 19°22'00"E	3	3	1	1
14	Montagu ‡	5	WC	33°39'31"S 19°45'54"E	5	5	1	0

Table 1 (continued)

15	McGregor	4	WC	33°56'13"S	19°49'37"E	4	4	1	1
16	Bredasdorp †	4	WC	34°32'41"S	20°02'03"E	4	4	1	0
17	Cape Agulhas	3	WC	34°49'06"S	20°01'07"E	3	3	1	1
18	De Hoop N.R	5	WC	34°27'23"S	20°23'87"E	5	5	1	1
19	Anysberg ‡	1	WC	33°30'00"S	20°34'31"E	1	1	1	1
20	Heidelberg	3	WC	33°57'58"S	21°01'35"E	3	3	1	1
21	Stillbay	5	WC	34°21'37"S	21°25'34"E	5	5	1	1
22	Gouritsmond	5	WC	34°21'52"S	21°51'04"E	5	5	1	1
23	Ladismith ‡	3	WC	33°29'31"S	21°16'22"E	3	3	1	1
24	Rooiberg Pass	5	WC	33°40'55"S	21°38'49"E	5	5	1	1
25	Assegaay Bosch Pass	4	WC	33°44'32"S	21°34'17"E	4	4	1	1
26	Perdebond	1	WC	33°49'24"S	21°55'38"E	1	1	1	1
27	Gamka N.R	5	WC	33°42'19"S	21°57'13"E	5	5	1	1
28	Die Hel ‡	2	WC	33°21'30"S	21°46'49"E	2	2	1	1
29	Die Top ‡	4	WC	33°21'09"S	22°02'46"E	4	4	1	1
30	Swartberg Pass †	4	WC	33°21'05"S	22°03'03"E	4	4	1	0
31	Kammanassie Mnts. ‡	5	WC	33°37'37"S	22°55'11"E	5	5	1	1
32	Spitskop ‡	1	WC	33°49'4.0"S	22°54'29"E	1	1	1	0

Table 1 (continued)

33	Hoekwill	3	WC	33°53'54"S 22°41'03"E	3	3	1	1
34	Victoria Bay	4	WC	34°00'14"S 22°33'15"E	4	4	1	1
35	Beaufort West	5	WC	32°09'32"S 22°31'38"E	5	5	1	1
36	Baviaanskloof †	3	EC	33°38'17"S 23°48'01"E	3	3	1	0
37	Smutsberg ‡	4	WC	33°37'48"S 23°47'59"E	4	4	1	1
38	Humansdorp γ	3	EC	34°00'00"S 24°43'48"E	3	3	1	1
39	Thyspunt ‡	2	EC	34°10'41"S 24°43'59"E	2	2	1	1
40	Cape St. Francis γ	1	EC	34°11'24"S 24°50'58"E	1	1	1	1
41	Jeffrey's Bay ‡	3	EC	34°02'52"S 24°55'23"E	3	3	1	1
42	Elandsberg ‡	2	WC	33°41'47"S 24°58'50"E	2	2	1	0
43	Jansenville ‡	3	EC	32°52'28"S 24°29'22"E	3	3	1	1
44	Graaff Reinet	5	EC	32°15'59"S 24°29'34"E	5	5	1	1
45	Murraysburg	5	WC	32°00'14"S 24°06'05"E	5	5	1	1
46	Nieu Bethesda	3	EC	31°47'1.0"S 24°27'43"E	3	3	1	1
47	Sneeuberg ‡	2	EC	31°42'43"S 24°37'31"E	2	2	1	1
48	Zuurkloof γ	1	EC	32°15'44"S 25°01'32"E	1	1	1	1
49	Pearston	5	EC	32°29'24"S 25°15'53"E	5	5	1	1
50	Zuurberg Pass	3	EC	33°20'19"S 25°45'38"E	3	3	1	1

Table 1 (continued)

51	Port Elizabeth	5	EC	34°02'08"S	25°38'38"E	5	5	1	1
52	Grahamstown	1	EC	33°19'53"S	26°36'34"E	1	1	1	1
53	Amatole Mnts. #	3	EC	32°34'33"S	26°56'23"E	3	3	1	1
54	Hogsback γ	6	EC	32°34'23"S	26°53'28"E	6	6	1	1
55	Katberg γ	3	EC	32°35'26"S	26°47'32"E	3	3	1	1
56	Cathcart	5	EC	32°27'25"S	26°59'08"E	5	5	1	1
57	Cofimvaba #	1	EC	31°59'59"S	27°36'06"E	1	1	1	1
58	Sterkstroom ‡	1	EC	31°37'6.0"S	26°18'54"E	1	1	1	1
59	Wodehouse #	3	EC	31°26'41"S	26°41'37"E	3	3	1	1
60	Lady Grey ‡	2	EC	30°43'24"S	27°20'05"E	2	2	1	1
61	Zastron ‡	2	FS	30°26'22"S	27°21'13"E	2	2	1	1
62	Rouxville ‡	1	FS	30°22'23"S	26°54'18"E	1	1	1	1
63	Henwood ‡	2	EC	30°30'43"S	28°57'30"E	2	2	1	1
Total		207				207	207	63	53

The abbreviations WC, EC and FS denotes the Western Cape, Eastern Cape and Free State provinces respectively, while NR denotes a Nature Reserve and Mnts denotes mountains.

Table 2. The gene, primer pairs, references, and number of base pairs used in the current study. The '✓' represents loci that were able to amplify and that were used in this study.

Locus	Primer pair and sequence	Reference	Amplified	# of bp used
16S	16Sa: 5' CGCCTGTTTACTAAAAACAT 3' 16Sb: 5' CCGGTCTGAACTCAGATCACG 3'	Simon (et al.,) 1994 Simon (et al.,) 1994	✓	474
ND2	vMet3: 5' GTCCATACCCCGAAAATGTTG 3' vTrp3: 5' GCTCTTATTTAGGGCTTTGAA 3'	Daniels (et al.,) 2004 Daniels (et al.,) 2004	✓	535
PRLR	PRLRf1: 5' GACARYGARGACCAGCAACTR 3' PRLRr1: 5' GACYTTGTGRACTTCYACRTA 3'	Townsend (et al.,) 2008 Townsend (et al.,) 2008	✓	439
PTPN12	PTPN12F1: 5' AGTTGCCTTGTWGAAGGRGATGC 3' PTPN12R6: 5' CTRGCAATKGACATYGGYAATAC 3'	Townsend (et al.,) 2008 Townsend(et al.,) 2008	✓	494

2.3 Phylogenetic analysis

All DNA sequences were aligned and edited using BioEdit Sequence Alignment Editor 7.0.5.3 (Hall, 2005). The three protein coding loci (ND2, PRLR and PTPN12) were translated into amino acids using EMBOSS Transeq (<http://www.ebi.ac.uk/emboss/transeq>), to confirm the sequence framework and to check for the presence of pseudogenes. The two mitochondrial genes were analyzed independently, followed by a combined 16S rRNA and ND2 analysis. In addition, a single representative sample from each of the four loci (two mtDNA and two nuDNA) were combined and analyzed in a coalescent data set. Where I was unable to amplify the PTPN12 locus, the data was coded absent for the phylogenetic analyses. Each data set was subjected to a Bayesian Inference (BI) analysis, while only the concatenated data set was subjected to maximum parsimony (MP) and maximum likelihood (ML) analyses. The DNA substitution models were obtained from JModeltest (Posada, 2008), using the Akaike information criterion (AIC) (Akaike, 1973). For each of the three protein-coding gene fragments (ND2, PRLR and PTPN12) a substitution model was obtained for the partial gene fragment as well as for each of the three codon positions of that fragment (Table 3). This allowed partitioning by codon for the BI (Kornilios et al., 2012). Because 16S rRNA is a non-protein coding locus a single substitution model was calculated. In addition, a substitution model was obtained for each of the gene fragments prior to analysing the concatenated data set as each of the four gene fragments were represented by a single sequence per sample site.

Maximum parsimony as well as ML were executed in MEGA5 (Tamura et al., 2011). For the MP analyses, trees were generated using the heuristic close-neighbour interchange (CNI) search option. All sites were included and 2000 bootstrap iterations were performed. For the ML analysis, the substitution model of choice was determined by MEGA5 and was used to determine the rates. Again, the heuristic search option, CNI, was selected and all sites were included. Nodal support was assessed by 2000 bootstrap iterations and was taken to be significant when greater or equal to 75%. All gaps for 16S rRNA were treated as missing data. A Bayesian Inference was performed using MrBayes v.3.2.0 (Ronquist et al., 2012) with the command block being specified by the results obtained from JModeltest based on the AIC criteria (Posada, 2008). Five chains were run, sampling every 5000 generations for a total number fluctuating between 5 to 10 million generations. The first 25% was discarded as burn-in. Nodes were considered well supported if it had a posterior probability (pP) greater or equal to 0.95. All trees were visualized in FigTree v1.3.1 (Rambaut, 2009).

Table 3. The best-fit substitution model for each gene fragment selected by JModeltest using the AIC criterion.

	16S rRNA	ND2	PRLR	PTPN12
Model	HKY* + I + G	TIM3 + I + G	HKY* + G	HKY*
Nst	2	6	2	2
Gamma (G) value	0.607	0.782	0.016	Not applicable
Invariance (I) value	0.248	0.421	Not applicable	Not applicable

* (Hasegawa et al., 1985)

2.4 Divergence time estimations

To date the cladogenic events within *C. cordylus*, BEAST (v.1.6.1) (Drummond and Rambaut, 2007) was used on the combined mtDNA dataset, as a range of mutational rates are known for the two loci. A mutation range of 0.57 – 0.70% per million years was used for the ND2 fragment (Liggins et al., 2008; Macey et al., 1998, 1999; Kornilios et al., 2012). Estimation around 0.22% per million years was employed for the 16S rRNA gene fragment (Graybeal, 1997; Emerson et al., 2000; Honda et al., 2006; Engelbrecht et al., 2013). Due to the poor fossil record for the ingroup Cordylidae, no fossil calibration points were incorporated.

The models obtained for both mtDNA loci, together with their parameters, were used for specifying the site models in BEAUti, using a relaxed uncorrelated lognormal clock model for both genes, while the coalescent constant size was selected as a tree prior. The Markov Chain Monte Carlo (MCMC) ran for 200 million generations, sampling every 20 000 generations. Tracer v1.5 (Rambaut and Drummond, 2009) was used to assess the chain convergence and ensure that the effective sample size (ESS) values for each parameter were above 200. The first 1000 trees were discarded as burn-in using TreeAnnotator v1.6.1, part of the BEAST package, and the remainder was used to obtain the maximum clade credibility tree. A chronogram was constructed using FigTree v1.3.1 (Rambaut, 2009).

2.5 Population and demographic analyses

Haplotype networks were constructed for each locus under the 95% connection limit of parsimony, using TCS 1.21 (Clement et al., 2000). Since the ND2

locus was the most rapid evolving marker used during the present study, I conducted an analysis of molecular variance (AMOVA) on this locus using ARLEQUIN version 3 (Excoffier et al., 2005). A hierarchical AMOVA was performed on each of the two clades (detected in preliminary analysis) and over all sample localities. The population history for *C. cordylus* was examined using Fu's F_s test (Fu, 1997), with 10 000 permutations as implemented in ARLEQUIN version 3.0. DnaSP 5 (Librado and Rozas, 2009) was used to estimate the population expansions for each phylogroup by calculating the nucleotide and haplotype diversity. In addition, the population structure was explored using a Bayesian approach, as implemented in BAPS v. 5.3 (Bayesian Analysis of Population Structure) (Corander et al., 2008). The program treats both the nucleotide frequencies of the marker used as well as the number of genetically diverged groups as random variables. Because haploid DNA sequence data (ND2) was used for this analysis, I conducted the linked molecular data analysis (Corander and Tang, 2007; Corander et al., 2008) and partitioned the data set by codon. BAPS was run with the maximal number of groups (K) set to 1–63, repeating each run three times. To analyze the population size fluctuations through time, an extended Bayesian skyline plot (EBSP) (Heled and Drummond, 2008) was used for each derived clade, and implemented in BEAST v.1.6.1 (Drummond and Rambaut, 2007), using the combined mtDNA data set. All 'skyline plot' (Pybus et al., 2000) methods rely on coalescent theory, which uses sequence data to decipher the demographic history of a population as groups of lineages randomly coalesce till a single common ancestor is obtained (Ho and Shapiro, 2011). The EBSP was chosen above the conventional Bayesian skyline plot (BSP) because multiple unlinked loci improve the demographic estimates and are more likely to detect population bottlenecks (Ho and Shapiro, 2011; Mulcahy et al., 2012). The

input file was once again created in BEAUti, using the same parameters and mutation rates used in the BEAST analysis. In addition, the input file was constructed for each clade obtained in the phylogenetics analysis in order to detect the clade which had undergone the most range expansion.

In order to model the species geographic distribution, the popular MaxEnt (Maximum Entropy) algorithm (Phillips et al., 2006; Phillips and Dudik, 2008) was employed. This method uses presence only data, does well with small sample sizes and does not 'over-fit' the data (Elith et al., 2006, 2011). The sampled localities used in this study were used in conjunction with museum records to provide accurate presence data for the ecological niche models. Bioclimatic variables representing mean temperature, seasonality and rainfall (Bio 1, Bio 4, Bio 12 and Bio 15) for both the present and past (Last Glacial Maximum (LGM); ~ 21,000 BP) as well as altitude for the present conditions only, were downloaded from the WORLDCLIM website (<http://biogeو.berkeley.edu/worldclim/>; Hijmans et al., 2005). The environmental layers were projected and clipped according to a map of South Africa, with a spatial resolution of 5 X 5 km square grids using ArcGIS® software. The presence records, in conjunction with the environmental layers were then used to predict the potential distribution of *C. cordylus* using MaxEnt v3.3.3e (Phillips et al., 2006). Parameters were left as default, only changing the maximum iteration to 1000 and the convergence threshold to 1×10^{-5} . Models were evaluated based on the AUC (area under the curve) value, which reflects how accurately the model predicts the species presence.

CHAPTER 3

Results

3.1 16S rRNA

Sequencing of the 16S rRNA locus yielded a 474 bp fragment for 175 specimens. These sequences were combined with the 32 sequences generated by Daniels et al. (2004) to yield a total of 207 sequences. Novel sequences were deposited in GenBank (accession numbers KC700366 – KC700435). The HKY + I + G (Hasegawa et al., 1985) was selected as the best-fit substitution model for this locus (Table 3) using the AIC criteria ($-\ln L = 1934.49$; $AIC = 4736.99$). The A, C, G and T base pair frequencies were 31.96%, 24.05%, 21.56% and 22.44% respectively. The A - C bias observed in the present study has also been reported for cordylids (Daniels et al., 2004; Engelbrecht et al., 2011b; Stanley et al., 2011) and skinks (Daniels et al., 2009; Heideman et al., 2011; Engelbrecht et al., 2013). The Bayesian inference topology demonstrated that *C. cordylus* is monophyletic, (Fig. 2), with a single well-supported clade retrieved for the specimens sampled along the western and southern portions of the Western Cape coast (clade 1) (0.99 *pP*). Deeper nodal relationships were poorly resolved due to the slow evolutionary rate of the 16S rRNA locus, and is a pattern typical in reptiles (Bauer and Lamb, 2001; Matthee and Flemming, 2002).

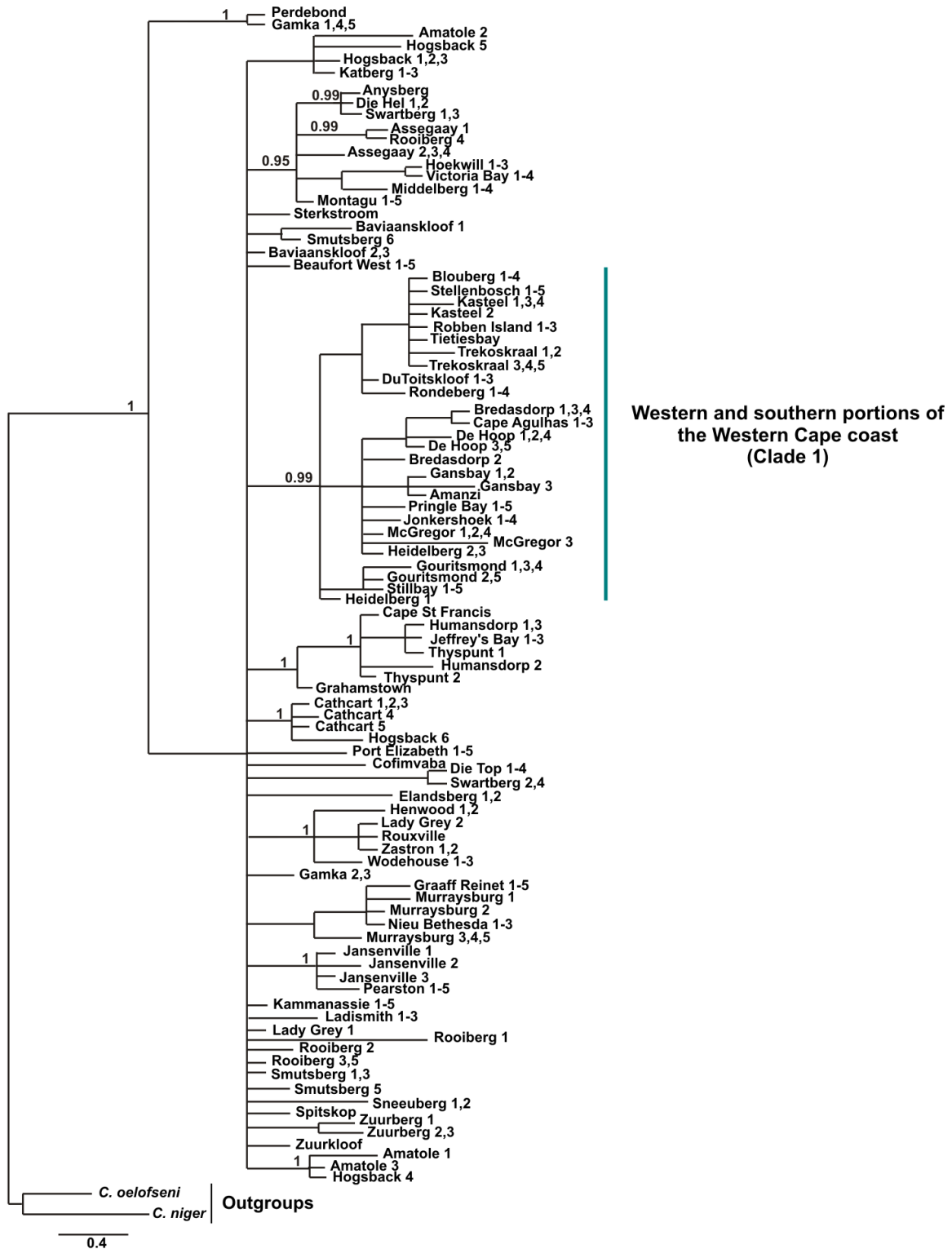


Figure 2. Bayesian inference phylogram derived from the 16S rRNA gene fragment for *C. cordylus*. Nodal support above each node depicts the posterior probability (≥ 0.95 pP).

3.2 ND2

For the partial ND2 locus a 535 bp fragment was sequenced for 175 *Cordylus* specimens, and combined with the 32 sequences from the study by Daniels et al. (2004) to yield a total of 207 sequences. Novel sequences were deposited in GenBank (accession numbers KC700436 – KC700531). JModeltest selected the TIM3 + I + G model for the gene fragment (Table 3), while TPM1uf + G was selected for each of the three codon positions (results not shown) using the AIC criteria (-lnL = 3752.56; AIC = 8345.12). The A, C, G and T base frequencies were 35.28%, 28.04%, 12.08% and 24.60% respectively. The high A - C bias is a common pattern for a protein coding marker and has been observed in other reptiles (Daniels et al., 2004, 2009; Engelbrecht et al., 2011b; Recuero and García-París, 2011).

Cordylus cordylus was retrieved as monophyletic (1.00 *pP*) (Fig. 3). In contrast to the 16S rRNA gene tree where a single clade was retrieved (Fig. 2), two monophyletic clades was evident. Clade 1 comprised samples from the western and southern portions of the Western Cape coast (0.96 *pP*) while clade 2 comprised samples from the southern and eastern Cape coast and adjacent interior of the Eastern and Western Cape and Free State provinces (0.95 *pP*) (Fig. 3). The maximum uncorrected sequence divergence between the two clades was 7.3%. Uncorrected sequence divergence values within *C. oelofseni* and *C. niger* was 10% and 5% respectively (Daniels et al., 2004). In addition, uncorrected sequence divergence values of 5% were recorded for *Karusasaurus polyzonus* specimens from the Cape west coast (Engelbrecht et al., 2011b).

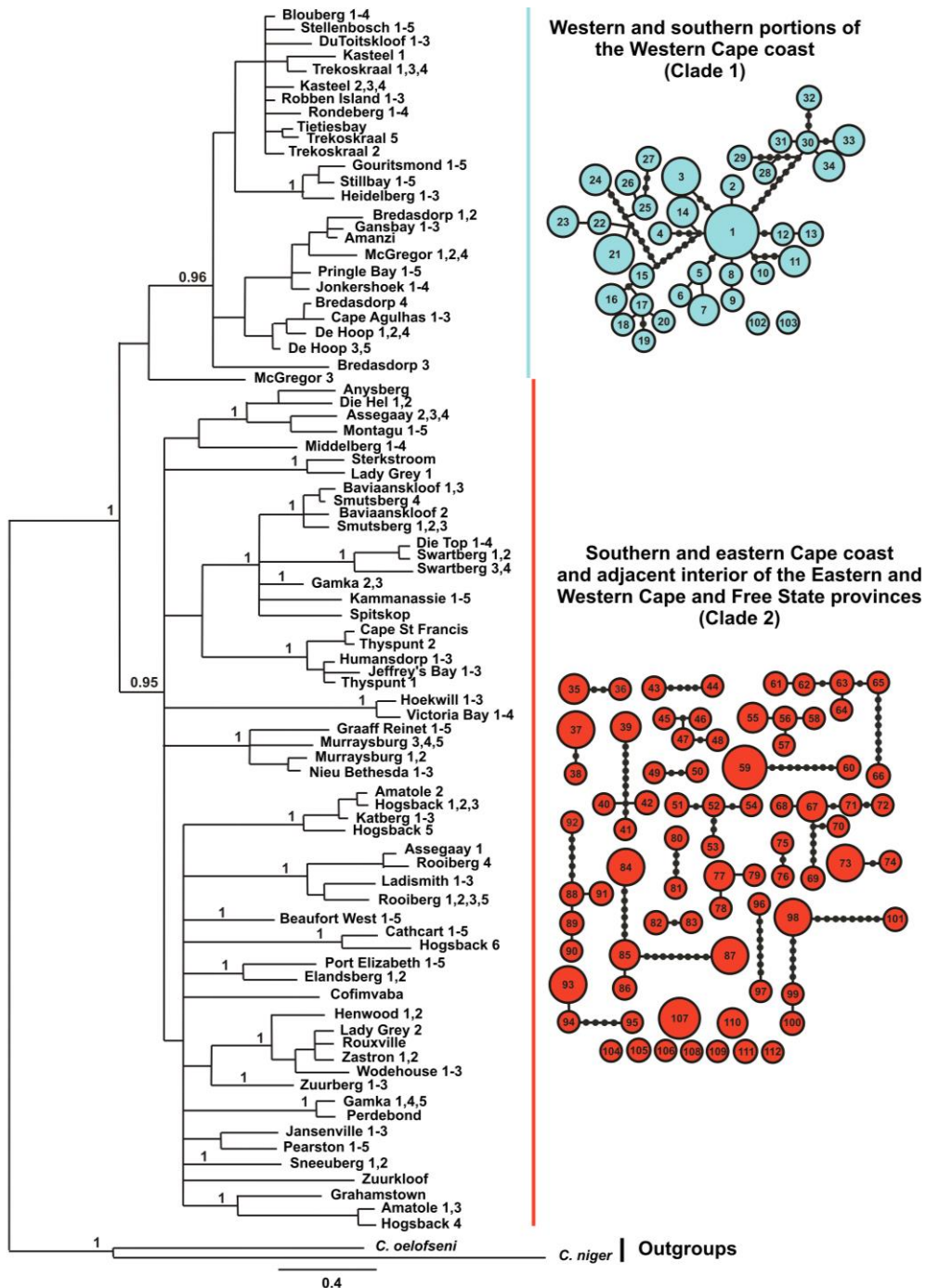


Figure 3. Bayesian inference phylogram derived from the protein-coding ND2 gene for *C. cordylus*. Nodal support above each node depicts the posterior probability (≥ 0.95 pP). The corresponding ND2 haplotype network for each clade is depicted to the right of each clade, with the size of each haplotype proportional to its frequency. Each black dot represents one mutational step, unsampled haplotype or missing haplotype, while the number denoted to each haplotype corresponds to the haplotype table (Appendix A).

3.3 Population structure

The 207 ND2 sequences yielded a total of 112 haplotypes (Fig. 3) with two unconnected haploclades being retrieved with 95% confidence. Haploclade 1 corresponded to the western and southern portions of the Western Cape coast (clade 1) while haploclade 2 corresponded to the southern and eastern Cape coast and adjacent interior of the Eastern and Western Cape and Free State provinces (clade 2). Haploclade 1 comprised 36 haplotypes while haploclade 2 consisted of 76 haplotypes. The nucleotide diversity and haplotype diversity was lower for haploclade 1 ($\pi = 0.0198 \pm 0.0101$, $h = 0.9751 \pm 0.0063$) in comparison to haploclade 2 ($\pi = 0.0446 \pm 0.0218$, $h = 0.9890 \pm 0.0023$) (Table 4). No haplotypes were shared between the two haploclades, suggesting no recent maternal dispersal (Appendix A).

The AMOVA over all sampled localities revealed that 31.28% ($df = 1$, $V_a = 4.56$, $p < 0.05$) of the total ND2 variation was explained by the differences between the two clades, with 56.07% of the variance partitioned among sampled localities within clades ($df = 62$, $V_b = 8.18$, $p < 0.05$), and 12.65% of the variance occurring within sample localities ($df = 149$, $V_c = 1.85$, $p < 0.05$). In clade 1 77.40% of the variation observed occurred among sampled localities and 22.60% of the variance occurring within sampled localities. In clade 2, 82.31% of the variation was partitioned among sampled localities within the clade, while 17.69% of the variation occurring within sampled localities. Fu's F_s was statistically significant and negative for both haploclades 1 and 2 (-9.01 ($p < 0.05$) and -20.38 ($p < 0.05$) respectively). These results provide evidence for an excess number of alleles and indicate selection or population expansion (Fu, 1997).

Table 4. Diversity indices retrieved from the ND2 data set for each sample locality. N, number of individuals; π , nucleotide diversity, h , haplotype diversity. Population numbers correspond to those of Fig. 1, with population numbers 1, 2, 4 – 13, 15 – 18, 20 – 22 representing the western and southern portions of the Western Cape coast (clade 1) and population numbers 3, 14, 19, 23 – 63 representing the southern and eastern Cape coast and adjacent interior of the Eastern and Western Cape and Free State provinces (clade 2).

Population #	Locality	N	# of haplotypes	Nucleotide diversity (π)	Haplotype diversity (h)
1	Tietiesbay	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
2	Trekoskraal	5	3	0.004887 +/- 0.003654	0.7000 +/- 0.2184
3	Middelberg	4	2	0.002846 +/- 0.002540	0.5000 +/- 0.2652
4	Rondeberg	4	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
5	Kasteelberg	4	3	0.007885 +/- 0.005992	0.8333 +/- 0.2224
6	Robben Island	3	2	0.001248 +/- 0.001557	0.6667 +/- 0.3143
7	Blouberg	4	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
8	Stellenbosch	5	3	0.001869 +/- 0.001740	0.7000 +/- 0.2184
9	Du Toitskloof	3	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000

Table 4 (continued)

10	Jonkershoek	4	2	0.000935 +/- 0.001159	0.5000 +/- 0.2652
11	Amanzi Mnts.	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
12	Pringle Bay	5	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
13	Gansbay	3	2	0.001246 +/- 0.001554	0.6667 +/- 0.3143
14	Montagu	5	2	0.001581 +/- 0.001574	0.4000 +/- 0.2373
15	McGregor	4	2	0.020561 +/- 0.014192	0.5000 +/- 0.2652
16	Bredasdorp	4	3	0.025954 +/- 0.017719	0.8333 +/- 0.2224
17	Cape Agulhas	3	3	0.004984 +/- 0.004474	1.0000 +/- 0.2722
18	De Hoop	5	2	0.001412 +/- 0.001547	0.6000 +/- 0.1753
19	Anysberg	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
20	Heidelberg	3	2	0.004984 +/- 0.004474	0.6667 +/- 0.3143
21	Stillbay	5	3	0.001869 +/- 0.001740	0.7000 +/- 0.2184
22	Gouritsmond	5	2	0.005607 +/- 0.004095	0.6000 +/- 0.1753

Table 4 (continued)

23	Ladismith	3	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
24	Rooiberg	5	4	0.014369 +/- 0.009469	0.9000 +/- 0.1610
25	Assegaay Bosch	4	3	0.031776 +/- 0.021519	0.8333 +/- 0.2224
26	Perdebond	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
27	Gamka	5	4	0.029533 +/- 0.018639	0.9000 +/- 0.1610
28	Die Hel	2	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
29	Die Top	4	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
30	Swartberg	4	2	0.011236 +/- 0.008091	0.6667 +/- 0.2041
31	Kammanassie	5	4	0.005981 +/- 0.004325	0.9000 +/- 0.1610
32	Spitskop	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
33	Hoekwill	3	2	0.001580 +/- 0.001970	0.6667 +/- 0.3143
34	Victoria Bay	4	2	0.001869 +/- 0.001852	0.5000 +/- 0.2652
35	Beaufort West	5	3	0.001495 +/- 0.001489	0.7000 +/- 0.2184

Table 4 (continued)

36	Baviaanskloof	3	2	0.006680 +/- 0.005806	0.6667 +/- 0.3143
37	Smutsberg	4	3	0.002820 +/- 0.002516	0.8333 +/- 0.2224
38	Humansdorp	3	2	0.003738 +/- 0.003525	0.6667 +/- 0.3143
39	Thyspunt	2	2	0.013109 +/- 0.014014	1.0000 +/- 0.5000
40	Cape St. Francis	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
41	Jeffrey's Bay	3	3	0.004444 +/- 0.004190	1.0000 +/- 0.2722
42	Elandsberg	2	2	0.003781 +/- 0.004630	1.0000 +/- 0.5000
43	Jansenville	3	2	0.002492 +/- 0.002561	0.6667 +/- 0.3143
44	Graaff Reinet	5	2	0.008972 +/- 0.006156	0.4000 +/- 0.2373
45	Murraysburg	5	2	0.008972 +/- 0.006156	0.6000 +/- 0.1753
46	Nieu Bethesda	3	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
47	Sneeuberg	2	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
48	Zuurkloof	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000

Table 4 (continued)

49	Pearston	5	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
50	Zuurberg	3	2	0.004984 +/- 0.004474	0.6667 +/- 0.3143
51	Port Elizabeth	5	2	0.001495 +/- 0.001489	0.4000 +/- 0.2373
52	Grahamstown	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
53	Amatole	3	2	0.033645 +/- 0.025900	0.6667 +/- 0.3143
54	Hogsback	6	5	0.035846 +/- 0.021687	0.9333 +/- 0.1217
55	Katberg	3	2	0.001246 +/- 0.001554	0.6667 +/- 0.3143
56	Cathcart	5	2	0.001375 +/- 0.001748	0.4000 +/- 0.2373
57	Cofimvaba	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
58	Sterkstroom	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
59	Wodehouse	3	2	0.001246 +/- 0.001554	0.6667 +/- 0.3143
60	Lady Grey	2	2	0.050467 +/- 0.051393	1.0000 +/- 0.5000
61	Zastron	2	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000

Table 4 (continued)

62	Rouxville	1	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000
63	Henwood	2	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000

The latter results was corroborated by the EBSPs (Fig. 4), though clade 1 appeared to have had undergone demographic stasis for the past 5 Mya, while clade 2 displayed rapid population expansion over the last 5.5 Mya. Bayesian analysis of molecular variance (BAPS) assigned all *C. cordylus* samples to 10 clusters (Fig. 5). Cluster 1 and 2 corresponded to the two subclades within the coastal clade (Fig. 3), with Bredasdorp and McGregor being shared between two clusters. The 3rd cluster is restricted to three localities, namely Ladismith, Rooiberg and Assegaay Bosch, with last mentioned also clustering with cluster 6. The Great Swartberge, Kammanassie, Baviaanskloof, Tsitsikamma and Kouga Mountains all form part of the 4th cluster. Victoria Bay and Hoekwill comprised cluster 5, while the 6th cluster contained specimens from across the sampled range, stretching from Montagu in the Western Cape to Lady Grey in the northern part of the Eastern Cape. The 7th cluster followed the escarpment and encompassed 13 localities from across the sampled range, with an additional six localities being shared between cluster 7 and clusters 2, 4, 6, 9 and 10. Cape St. Francis and adjacent localities defined the 8th cluster, while cluster 9 was restricted to the Amatole Mountains. Hogsback represented three Bayesian clusters whilst Amatole was comprised of two. Lastly, cluster 10 encompassed three localities in the Graaff Reinet district.

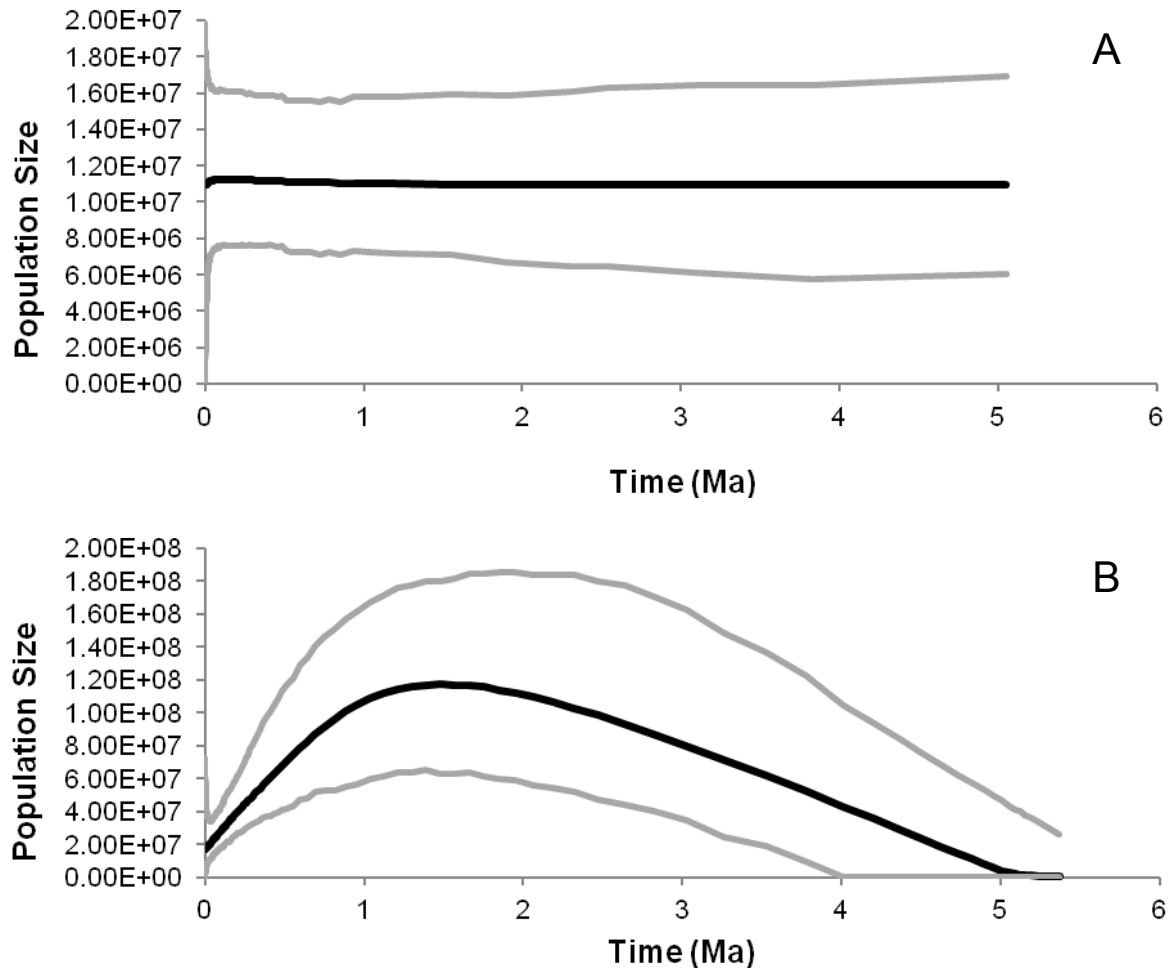


Figure 4. Extended Bayesian Skyline Plots (EBSP) inferred from the two mtDNA loci. The graphs represent population size fluctuations over time in clade 1 (A) and clade 2 (B) respectively. The black line represents the median while the grey lines above and below the median depict the hpd upper 95 and hpd lower 95 respectively.

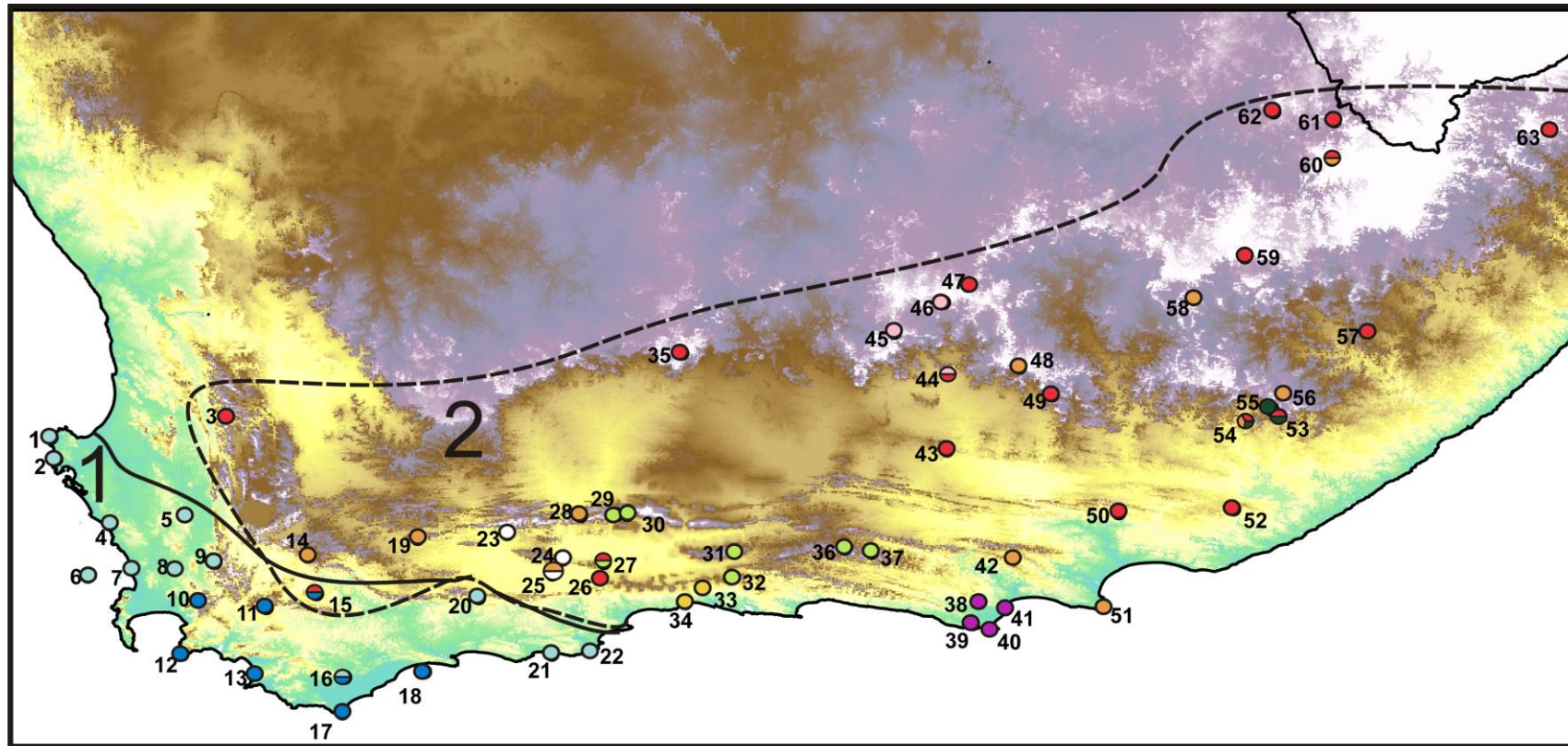


Figure 5. A graphic depiction of the BAPS clusters, with each colour indicating a genetic grouping and locality numbers corresponding to those in Table 1. Group 1 represents clade 1 and group 2 represents clade 2 as shown by Fig. 3. Localities that represent multiple clusters are shown as pie charts.

3.4 Combined mtDNA (16S rRNA and ND2) (topology not shown)

The combined mtDNA datasets (16S rRNA and ND2) yielded a total of 1011 bp for the 207 specimens. The tree topology retrieved *C. cordylus* as monophyletic (1.00 pP). Similar to the ND2 tree (Fig. 3), two monophyletic clades were retrieved, with clade 1 comprising samples from the western and southern portions of the Western Cape coast (1.00 pP) while clade 2 comprised samples from the southern and eastern Cape coast and adjacent interior of the Eastern and Western Cape and Free State provinces (1.00 pP). The localities associated with each clade were congruent with those reported for the ND2 tree topology. The majority of the internal groupings were well resolved with $pP > 0.95$.

3.5 Divergence time estimations

The divergence time estimates for the combined mtDNA dataset suggested a Late Miocene (6.84 Mya) origin for *C. cordylus* (95% HPD: 5.41 – 8.42 Mya; 1.00 pP), with clade 1 diversifying 4.31 Mya (95% HPD: 2.90 – 5.95 Mya; 1.00 pP) and clade 2 diversifying 6.01 Mya (95% HPD: 4.90 – 7.36 Mya; 0.79 pP) (Fig. 6).

3.6 nuDNA (PRLR and PTPN12)

For the prolactin receptor locus (PRLR) a 439 bp fragment was sequenced for 63 specimens. Sequences were deposited in GenBank (accession numbers KC700532 – KC700545). JModeltest selected the HKY + G (Hasegawa et al., 1985) substitution model for this locus (Table 3) using the AIC criteria ($-\ln L = 734.86$; AIC = 1735.73). The A, C, G and T base pair frequencies were 31.49%, 19.30%, 26.14%

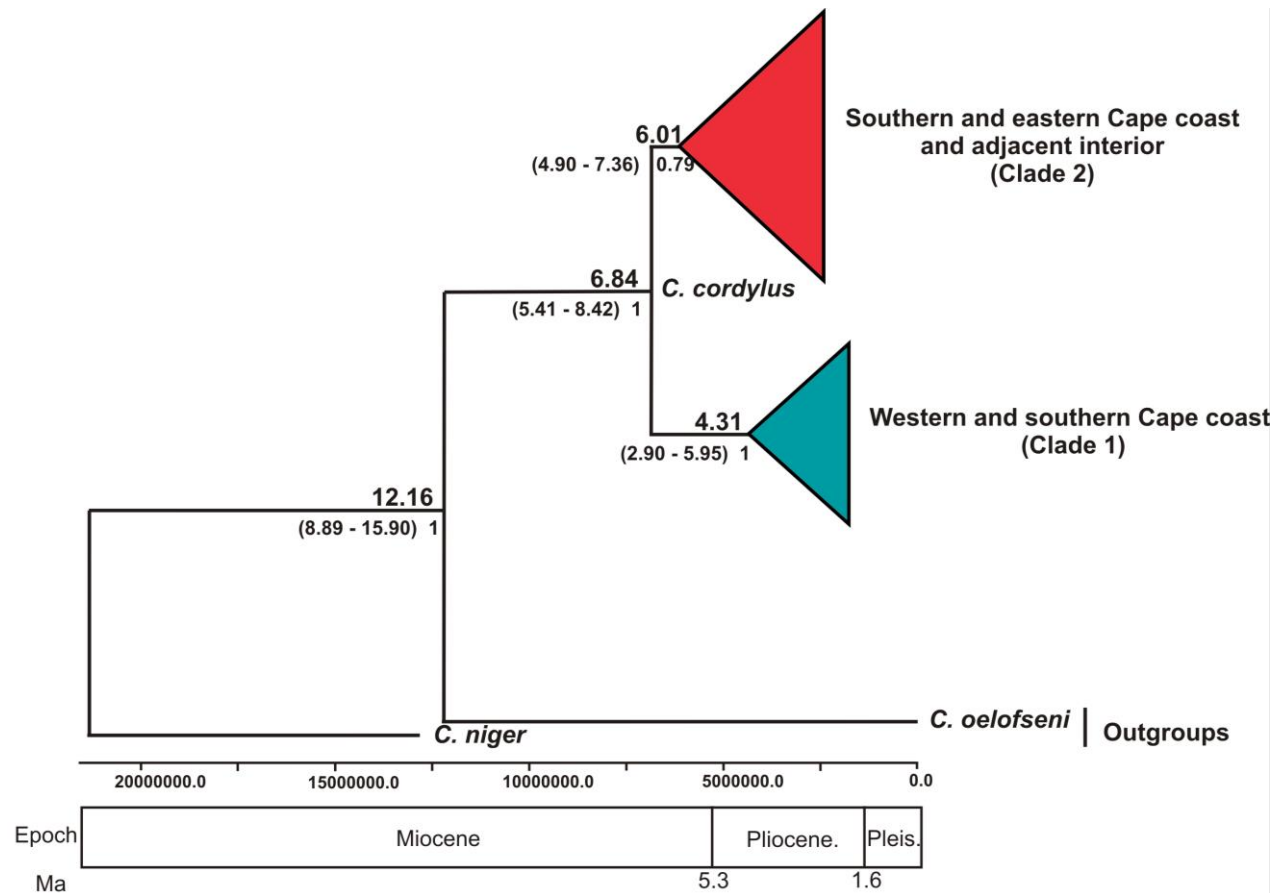


Figure 6. Maximum clade credibility tree attained for the BEAST analysis using the mutation rates for 16S rRNA (0.22% change per million years) and ND2 (0.57 – 0.70 % change per million years). The values above the nodes indicate the mean divergence date in millions of years before present, while the values below in brackets represent the 95 % HPD credibility intervals. The non-bracketed values below the nodes indicate the posterior probability.

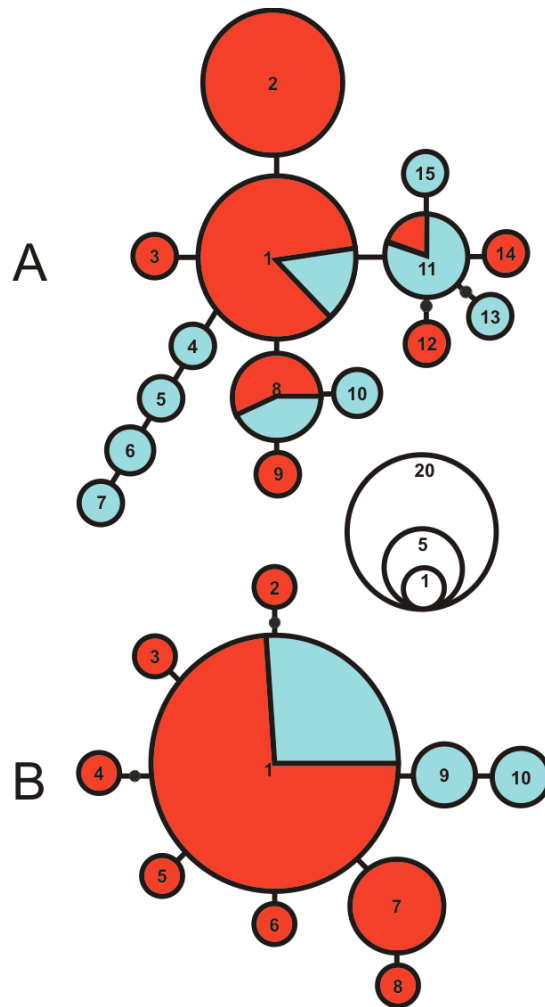


Figure 7. Haplotype networks depicting the relationship between *C. cordylus* localities for (A) the Prolactin receptor locus (PRLR) and (B) the protein tyrosine phosphatase, non-receptor 12 locus (PTPN12). Colours correspond to that of Fig. 3, with blue indicating the western and southern portions of the Western Cape coast (clade 1) and red representing the southern and eastern Cape coast and adjacent interior of the Eastern and Western Cape and Free State provinces (clade 2) (Appendix B.1 and B.2).

and 23.08% respectively. TCS retrieved 15 haplotypes (Fig. 7.a; Appendix B.1), with no differentiation observed between the two mtDNA clades retrieved earlier (Fig. 3).

A 494 bp fragment for 53 specimens was analysed for the PTPN12 locus. Sequences were deposited in GenBank (accession numbers KC700546 – KC700555). JModeltest selected the HKY (Hasegawa et al., 1985) substitution model for this locus (Table 3) using the AIC criteria ($-\ln L = 763.23$; $AIC = 1790.46$). The A, C, G and T base pair frequencies were 32.72%, 21.60%, 23.49% and 22.19% respectively. Ten haplotypes were retrieved (Fig. 7.b; Appendix B.2) and no differentiation was observed between the two mtDNA clades (Fig. 3).

3.7 Combined DNA analysis (16S rRNA, ND2, PRLR and PTPN12)

The total evidence data set comprised 1942 bp for 63 specimens. For the Bayesian analysis the models selected by JModeltest for each fragment is presented in Table 3, and the analyses was conducted using the partition for each locus (the 16S rRNA and ND2 models were recalculated since only a single sample was used per locality, the recalculated best-fit models are not shown). The HKY + I + G (Hasegawa et al., 1985) model was selected for the ML analysis. The gamma value (G) was 0.1168 while the invariable sites (I) had a value of 0.6970 and log likelihood ($-\ln L$) of -6925.6517. The BI, ML and MP topologies were congruent. *Cordylus cordylus* was retrieved as monophyletic (1.00 *pP* and 90% MP), with only clade 1 retrieved as statistically supported (1.00 *pP*, 94% MP and 88% ML) (Fig. 8). The samples from the southern and eastern Cape coast and adjacent interior of the Eastern and Western Cape and Free State provinces grouped together, however, no statistical support was retrieved for the group. These results suggest that the slowly

evolving nuDNA markers were of limited utility in supporting deeper nodal relationships.

3.8 Ecological niche modeling

Model performance was evaluated by calculating the area under the curve (AUC). An AUC value of 0.963 (SD = 0.016) was obtained, indicative of a model performing better than expected due to chance. In addition, Swets (1988) stated that AUC values > 0.9 are 'very good' with regards to discrimination abilities. The MaxEnt prediction for *C. cordylus*' current distribution closely mimicked that of the sampled distribution range (Fig. 9.a), although slightly wider in range as would be expected due to other factors such as biotic interactions and physical barriers influencing a species' range (Phillips et al., 2006). The mean annual temperature (BIO 1), altitude and precipitation of the driest month (BIO 14) were the most influential factors, accounting for 94% of the predicted range (Fig. 9.b). In addition, jack-knife tests showed these variables to have the greatest test gain when used in isolation, indicative of these variables being most informative when used in isolation. Similarly, these three variables were the variables to decrease the gain most when omitted from the model, showing that they possess more information that is not present in the other variables. With regards to the LGM (AUC = 0.957, SD = 0.019) (results not shown), it is evident that more areas with high suitability were available. Once again the mean annual temperature (BIO 1) was the most influential factor and together with annual precipitation (BIO 12) and precipitation seasonality (BIO 15) accounted for 79.4% of the variation within the model.

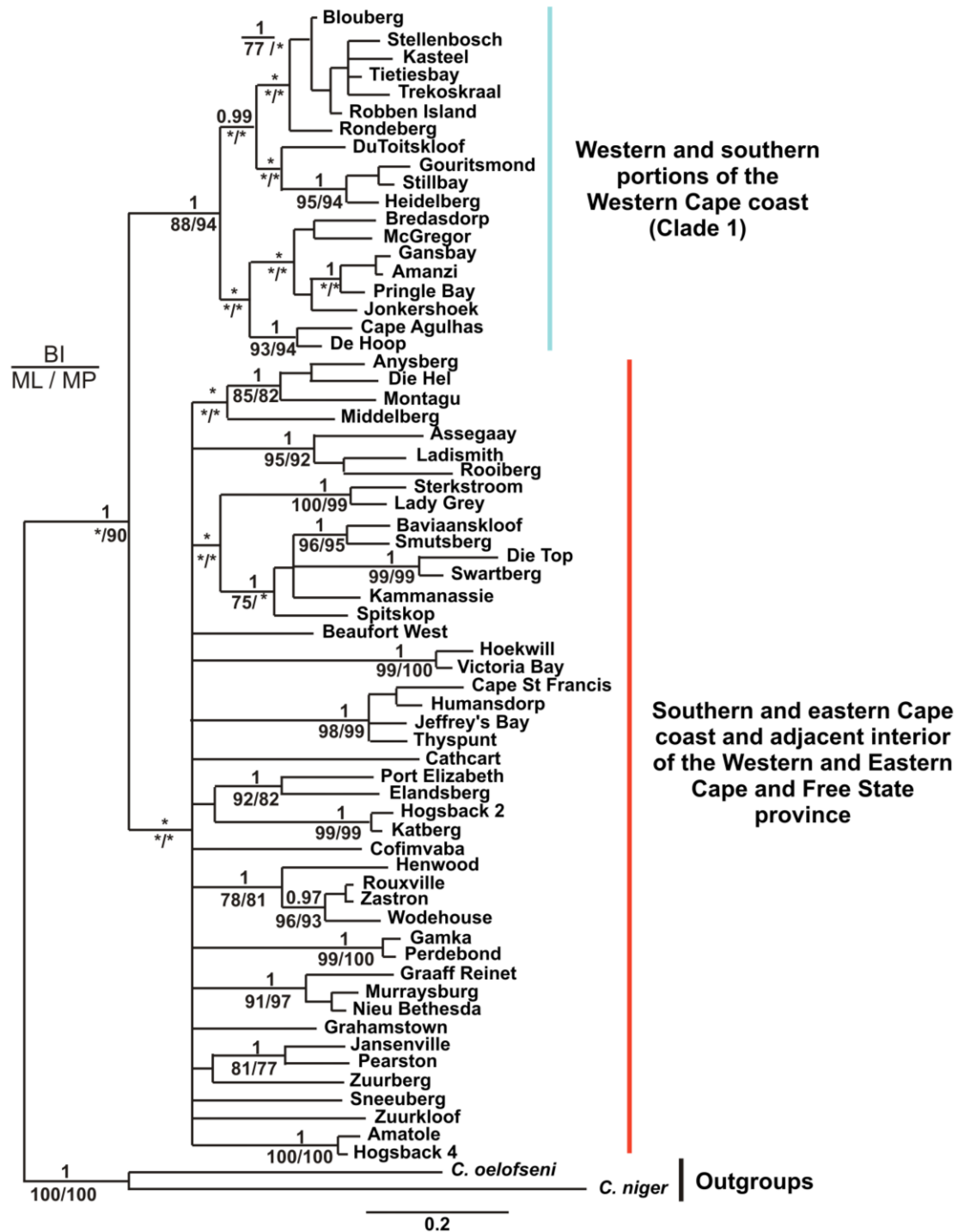


Figure 8. Bayesian inference phylogram derived from the total evidence data set (16S rRNA, ND2, PRLR, PTPN12) for *C. cordylus*. The values above each node represent the posterior probability (pP), while the values below each node represent the bootstrap values (%) for the maximum likelihood and maximum parsimony analyses respectively. Asterisks indicate statistically poorly supported nodes (< 75% or < 0.95 pP).

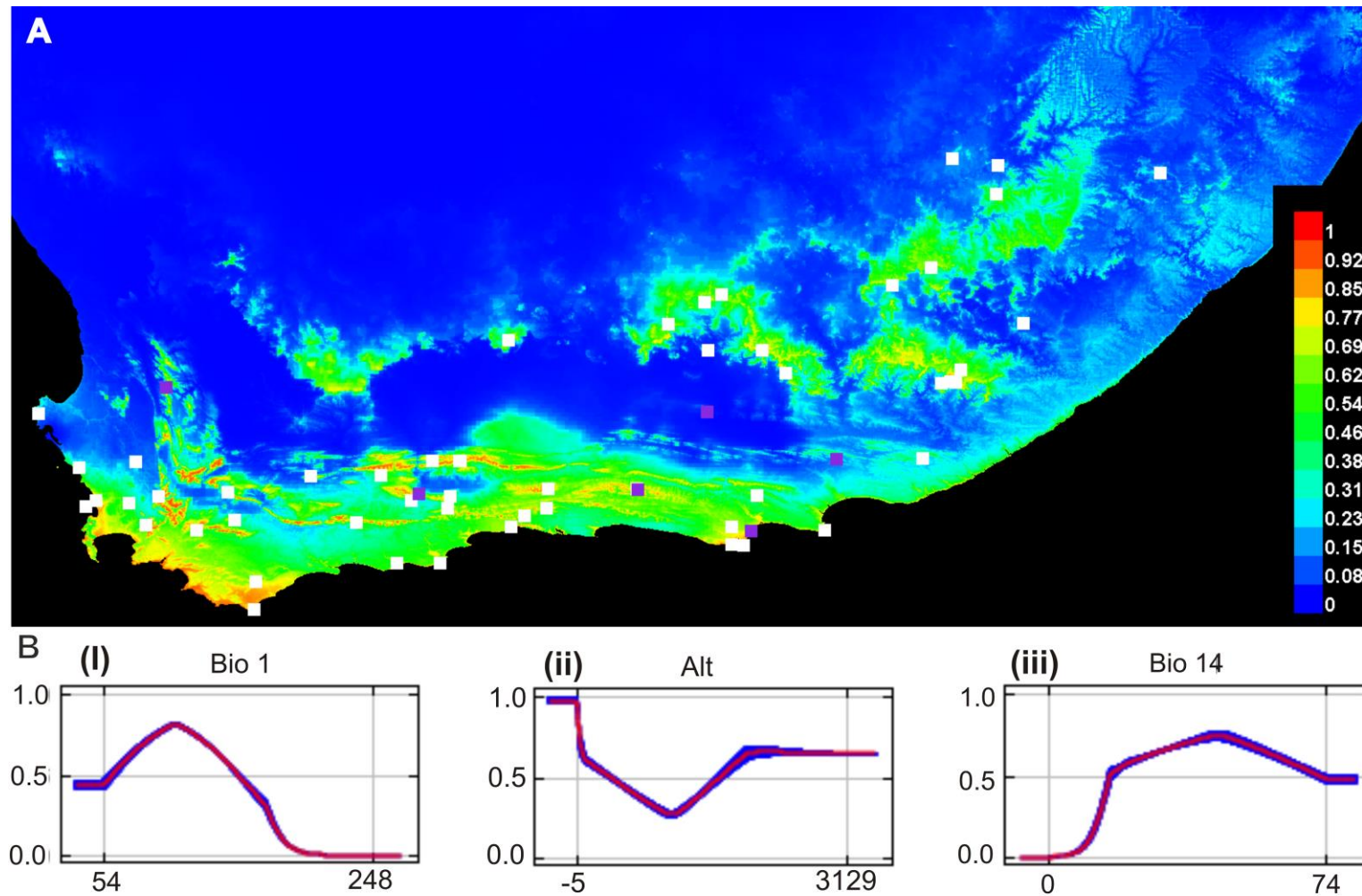


Figure 9. Ecological niche modelling predictions (A) for *C. cordylus* as obtained from MaxEnt during current climatic conditions. (B) Response curves for the three most important variables shaping *C. cordylus* distribution in the current climatic conditions.

CHAPTER 4

Discussion

Extensive geographic sampling and the inclusion of four partial DNA sequencing loci, incorporating both the mitochondrial and nuclear genomes, revealed a complex phylogeographic pattern for *Cordylus cordylus*. The present study retrieved the species as monophyletic and comprised of two clades. Clade 1 contained specimens from the western and southern portions of the Western Cape coast and adjacent interior, while clade 2 encompassed a broader geographic distribution and included specimens from the southern portions of the Western Cape, expanding along the Eastern Cape coastline and adjacent interior into the southern portions of the Free State province. The area adjacent to the Breede river drainage valley, extending from its origin in the Witzenberg Mountains towards the river mouth at Witsand in the southern parts of the Western Cape, is an area of near sympatry between the two clades and corresponds to a region of confluence in rainfall and biomes in the southern Cape of South Africa (Chase and Meadows, 2007). Divergence time estimates revealed an early Pliocene (5.95 – 2.90 Mya) / late Miocene (7.36 – 4.90 Mya) formation for clades 1 and 2 respectively. Despite the fact that both the mtDNA loci were incorporated into the divergence time estimations, caution should be applied when interpreting these results since the nuDNA loci used in this study are novel, their mutation rates unknown, and no fossils are known for the ingroup that could be used as additional calibration points. While the mutation rates of the two nuDNA loci can be estimated from the two mtDNA loci, such inferences are dubious and not optimal (Brown et al., 2012; Mulcahy et al., 2012).

Hence our divergence time estimations should be regarded as approximations of cladogenesis within *C. cordylus*.

The mtDNA haplotype network retrieved two major haploclades and revealed the absence of shared haplotypes between clades 1 and 2 (Fig. 3), negating any recent maternal dispersal between the two clades. Despite the marked phylogeographic pattern detected using the mtDNA loci, shared haplotypes were observed for both of the two nuDNA loci (Fig. 7). Similar results have been interpreted to reflect a sex biased dispersal pattern (Keogh et al., 2007; Zarza et al., 2011; Barlow et al., 2013). However, in the present study this cannot be validated. The discrepancy between the nuclear and mitochondrial DNA sequence data sets most likely reflect differences in the mutation rates of the two genomes (Townsend et al., 2008). Experimental validation of a sex biased dispersal is required and can be obtained by employing more sensitive nuDNA markers such as microsatellite markers or AFLP's (Escorza-Treviño and Dizon, 2000; Van der Wurff et al., 2003). A detailed long-term ecological study documenting the dispersal capabilities of the species, particularly different dispersal capabilities between the sexes, is required to corroborate or refute potential sex biased dispersal patterns (Keogh et al., 2007) in *C. cordylus*.

Both clades demonstrated expansions as evident from the EBSP's and Fu's F_s , while clade 1 had a lower haplotypic and nucleotide diversity in comparison to clade 2 (Table 4). Collectively, these results, along with the divergence time estimation, suggest that clade 1 represents a more recent colonization of the coastal low-lying plains and the adjacent interior along the southern and western portions of the Western Cape, while clade 2 represents an older clade. The latter clade likely

reflects the ancestral distribution of the species, furthermore validated by the divergence time estimation. Yet, despite the antiquity of the two clades, no additional subclustering or haplogroups were observed within either of the two clades, suggesting marked within clade dispersal.

The observed phylogeographic patterning in *C. cordylus* most likely reflects the interplay of climatic ameliorations impacting habitat suitability and availability during the Mio/ Plio/ Pleistocene epochs. The Miocene (23.8 – 5.3 Mya) was characterised by tectonic instability, with the first upliftment occurring during the Early Miocene followed by another at the onset of the Late Miocene (Cowling et al., 2009). The greatest upliftment (± 200 m) was experienced in the eastern part of the Cape, when compared to the western part (± 150 m) (Partridge and Maud, 2000). Sea levels were up to 150 m higher than present (Siesser and Dingle, 1981) resulting in the inundation of much of the Cape coastal plain (Cowling et al., 2009), with minimal coastal dunes being formed (Hesp et al., 1989). Furthermore, the formation of the Benguela Current along the west coast of the Western Cape (Siesser, 1980) coincided with this timeframe, facilitating the return of warm and wet climatic conditions to the Cape (Cowling et al., 2009). Collectively all the aforementioned factors significantly contributed to the increased heterogeneity within southern Africa and formed the template for cladogenesis in several groups (Cowling et al., 2009).

With the onset of the Pliocene epoch (5.3 – 2.6 Mya) rapid cooling was experienced due to the renewed glaciation of Antarctica (Partridge, 1997). This, in turn, led to a marine regression, sporadically interrupted by no less than three transgressions of up to 35 m above the present sea level (Grützner et al., 2005).

These regressions gave the inland *C. cordylus* populations the opportunity to recolonise the coastal low lying areas. Towards the end of the Pliocene regional cooler and drier climatic conditions were experienced, with present-day landscapes being established and floral radiations proliferating (Cowling et al., 2009, and references within), leading to the diversification of numerous taxa within the CFR (Smit et al., 2007; Swart et al., 2009; du Toit et al., 2012; Engelbrecht et al., 2013).

Extensive dune formation, warm-cold cycles and marine trans- and regressions, extending the coastline seaward from its current position, characterised the Pleistocene (2.6 - 0.1 Mya) (Cowling et al., 2009). A stable winter rainfall regime was established in the west, while a fluctuating rainfall pattern persisted in the east (Chase and Meadows, 2007). In addition, the west coast experienced a 60 km seaward shift, with the Agulhas Bank extending approximately 200 km, while the south-east coast moved seaward with 100 km. This regression, greatly enhanced the availability of suitable habitat for *C. cordylus*, and likely allowed the species to colonise the west and south-west coast, forming clade 1. With the onset of the transgressive phase, however, strong winds promoted dune formation, yet again erasing some of this newly created rocky habitat along the Agulhas Bank (Hesp et al., 1989), encouraging *C. cordylus* to move to the adjacent interior of the west coast while providing fossorial taxa with novel habitats (Engelbrecht et al., 2013). This hypothesis is corroborated by the localities comprising both grouping 1 and 2 within clade 1 (Fig. 5). Grouping 1 encompasses localities from either side of the Agulhas bank, while grouping 2 contains individuals from localities found within the bank (Figs. 3, 8). Following these last marine regressions around 2 Mya, the current coastline was established. The sea sand deposits were eroded, leaving the rocky coastal

areas stretching from Hangklip to De Hoop and adjacent interior, exposed. Subsequently *C. cordylus* from the localities within grouping 1 recolonised this area around 1.8 Mya. Both haplotype and nucleotide diversities support this observation (Table 4). Moreover, the BAPS analysis also corroborates this hypothesis as Bredasdorp falls within both grouping clusters (Fig. 5). The integrity of these two groupings were presumably maintained over time by the formation of the Breede River valley to the east and the Cape Fold Mountains to the west, with similar patterns being observed in skinks (Engelbrecht et al., 2013), toads (Tolley et al., 2010) and tortoises (Daniels et al., 2007). This is most likely due to the microclimate within the valley, as more xeric conditions prevail within the valley when compared to opposite sides of the valley (Schulze, 1997; Tolley et al., 2010). Similar results were obtained by Glor and Warren (2010), leading them to conclude that ribbons of unfavourable habitat and/ or sharp environmental gradients are causal of the observed phylogeographic structure. As indicated by the SDMs (Fig. 9), mean annual temperature, rainfall of the driest month and altitude are the most important factors governing the species' distribution, thus potentially corroborating why *C. cordylus* does not appear to transverse this area of near sympatry.

The phylogeographic distribution of *C. cordylus* is uncharacteristic for a rupicolous vertebrate species, since all the southern African rupicolous taxa that have been subjected to phylogeographic study to date have demonstrated marked genetic structuring (Prinsloo and Robinson, 1992; Matthee and Robinson, 1996; Bauer 1999; Matthee and Flemming, 2002; Daniels et al., 2010). The ecological pliability and non-specialist nature of *C. cordylus* as evident from its generalistic diet (comprising various Coleoptera and Hymenoptera species), the species ability to

utilise a variety of rocky substrate as habitat, coupled with its relatively large body size (SVL: 65 – 85 mm) and broad thermal tolerance range (CT_{min}: 7.5 °C, CT_{max}: 41.6 °C) presumably contributed to the observed phylogeographic pattern and have facilitated the absence of within clade differentiation (Branch, 1998; Clusella-Trullas and Botes, 2008; Clusella-Trullas et al., 2009; Diedericks pers. obs.). Flagstad et al. (2001), noted that generalist species may take advantage of several different habitat types, as this may facilitate dispersal events and ultimately, surviving as a metapopulation or multiple patchy populations (Driscoll and Hardy, 2005). It is therefore not surprising that *C. cordylus* distribution spans the Western and Eastern Cape and southern Free State provinces. The present study highlights the importance of a species' ecology in driving and maintaining phylogeographic continuity within clades.

Conclusions

Our results suggest that microclimatic variables together with the species life history characteristics, rather than physical geographic barriers have sculpted the observed phylogeographic pattern. Because reptile taxa are more reliant on climatic variables to govern their distribution, this pattern may be more prominent in squamate taxa when compared to small mammals or invertebrates. The question now arises as to whether the two clades represent distinct lineages. I am of the opinion that one species is present (considering the absence of nuDNA differences, as well as any fixed morphological differences between the two clades), however, the two clades should at least be considered as two distinct management units, considering the mtDNA differences (Crandal et al. 2004).

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