Molecular Phylogenetics and Phylogeography of sand lizards, *Pedioplanis* (Sauria: Lacertidae) in southern Africa

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Declaration

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

Signature: ……

Date: ……

Abstract

The present study aims to determine the phylogenetic relationships among the sand lizards**,** *Pedioplanis*. In addition, a single mitochondrial gene is used to investigate the geographic genetic structure of the widey distributed *P. burchelli*. With 11 species, *Pedioplanis* is the most speciose genus among the southern African genera of the family Lacertidae. All the species are restricted to the subcontinent with the exception of three (*P. namaquensis*, *P. undata* and *P. benguellensis)*, which extend their range northwards into Angola. A total of 2200 nucleotide positions derived from two mitochondrial markers (ND2 and 16S rRNA) and one nuclear gene (RAG-1) are used to determine the phylogenetic relationships among ten of the eleven *Pedioplanis* species. The first well resolved gene tree for the genus, drawn from 100 individuals, is presented and this is largely congruent with a phylogeny derived from morphology. Contrary to some previous suggestions, *Pedioplanis* forms a monophyletic assemblage with *Heliobolus* and *Nucras*. The genus *Pedioplanis* is monophyletic with *P. burchelli*/*P. laticeps* forming a sister clade to all the remaining congeners. Two distinct geographic lineages can be identified within the widespread *P. namaquensis;* one occurs in Namibia, while the other occurs in South Africa. The "P. undata" species complex is monophyletic, but one of its constituent species, *P. inornata*, is paraphyletic. Relationships among the subspecies of *P. lineoocellata* are much more complex than previously documented. An isolated population previously assigned to *P. l. pulchella* is paraphyletic and sister to the three named subspecies. The phylogeny identifies two biogeographical groupings that probably diverged during the mid-Miocene. The development of the Benguella Current could have

initiated isolation mechanisms associated with changes in habitat that could have generated barriers and played a role in the evolution of this group.

At the lower taxonomic level, the mtDNA phylogeographic structure of the wide spread *P. burchelli* in South Africa reveal at least six distinct clades that are geographically partitioned. The first one is restricted to the eastern mountains along the Great Escarpment (GE). The next three are found along the Cape Fold Mountains (CFM): the north-west CFM, central CFM and eastern CFM. The fifth one shares samples from central CFM and GE. The last clade is restricted to the eastern central mountains of the GE. These six geographic groupings are genetically divergent from each other and they started separating in the early Pliocene period. Phylogeographic studies on other taxa in the region have found different levels of genetic structuring among or within taxa. The fact that *P. burchelli* is restricted to high altitude areas could have resulted in limited dispersal and consequently contributed to its geographic structure. However, the exact cause of the pattern obtained is not readily apparent. Habitat fragmentation in the past is probably one of the most influential factors shaping the genetic distribution of the species across South Africa. The inclusion of nuclear markers will shed more light on the evolutionary history of *P. burchelli* in South Africa.

Opsomming

Die huidige studie stel ten doel om ‘n filogenie daar te stel vir die Sand akkedisse**,** *Pedioplanis*. ‘n Enkele mitochondriale geen is ook gebruik om die geografiese genetiese struktuur van die wydverspreide *P. burchelli vas te stel*. Met 11 spesies is *Pedioplanis* die mees spesieryke genus onder die suidelike Afrika genera wat aan die Lacertidae familie behoort. Al die spesies is beperk tot die subkontinent met die uitsondering van drie (*P. namaquensis*, *P. undata* en *P. benguellensis)*, wat ‘n uitgebreide verspreiding het noordwaarts tot in Angola. ‘n Totaal van 2200 nukleotied posisies wat afkomstig is van twee mitochondriale merkers (ND2 en 16S rRNA) en een nukluêre geen (RAG-1) is gebruik om die filogenetiese verwantskappe tussen 10 van die 11 *Pedioplanis* spesies vas te stel. Die eerste goed geondersteunde geen boom vir die genus, gebasseer op 100 individue, is verkry en dit is meestal ooreenstemmend met ‘n filogenie gebasseer op morfologie. In teenstelling met sekere voorstelle van die verlede vorm *Pedioplanis* ‘n monofiletiese groep tesame met *Heliobolus* en *Nucras*. Die genus *Pedioplanis* is monofileties met *P. burchelli*/*P. laticeps* wat ‘n suster groep vorm van al die oorblywende lede van die genus. Twee herkenbare geografiese lyne kan geidentifiseer word in die wydverspreide *P. namaquensis;* een kom in Namibia voor, terwyl die ander een in Suid Afrika voorkom. Die “P. undata” spesies kompleks is monofileties, maar een van die spesies wat deel uitmaak van die groep, *P. inornata*, is parafileties. Verwantskappe tussen die subspesies van *P. lineoocellata* is meer kompleks as wat aanvanklik aanvaar is. ‘n Geisoleerde bevolkimg wat voorheen toegesê is aan *P. l. pulchella* is parafileties en verteenwoordig ‘n suster groep van die benaamde subspesies. Die filogenie identifiseer twee biogeografiese groeperings wat moontlik gedivergeer het

gedurende die middel-Miocene. Die ontwikkeling van die Benguella stroom het dalk versperrings geinisiëer as gevolg van die gesamentlike veranderinge in habitat wat dalk ook ‘n rol gespeel het in die evolusie van die groep.

Op die laer taksonomiese vlak het die mtDNA filogeografiese struktuur van die wydverspreide *P. burchelli* in Suid Afrika ten minste ses groepe aangetoon wat geografies van mekaar geskei is. Die eerste een is beperk tot die oostelike berge wat aan die Groot Eskarpement (GE) behoort. Die volgende drie word gevind in die Kaapse Vouberge (KVB): die noord-westelike KVB, sentrale KVB en oostelike KVB. Die vyfde een deel eksemplare van beide die GE en die KVB. Die laaste groep is beperk tot die oostelike en sentrale berge van die GE. Hierdie ses geografiese groepe is geneties geskei van mekaar en hulle het begin om apart te ontwikkel gedurende die vroë Pliocene periode. Ander filogeografiese studies in die area het verskillende vlakke van genetiese struktuur vertoon tussen en binne taksa. Die feit dat *P. burchelli* beperk is tot hoogliggende dele kon moontlik bygedrae het tot die geografiese struktuur. Die presiese oorsaak van die patroon wat verkry is, is nie ooglopend nie. Habitat fragmentasie in die verlede is moontlik een van die mees invloedrykste faktore wat die genetiese verspreiding van die spesie in Suid Afrika beinvloed het. Die insluiting van nukluêre merkers sal meer lig warp op die evolusionêre geskiedenis van *P. burchelli* in Suid Afrika.

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Chapter 1: General introduction to lacertid lizards

1. 1. Biology of lacertid lizards

Lacertid lizards, family Lacertidae, are small-bodied lizards typically less than 120 mm from snout to vent, and with a tail longer than the body in most cases. Members of the family are all diurnal and mostly heliothermic. Lacertids occur from the tundra on high mountain habitats through heath, scrub and Mediterranean associates (*Gallotia*), to tropical forest (*Holaspis*), semi-desert and desert (*Meroles*) (FitzSimons, 1943; Arnold, 1989; Branch, 1998). Some species are habitat specific; for example within the genus *Pedioplanis*, *P. rubens* and *P. husabensis* are strictly rock dwelling (Branch, 1998). Lacertid lizards feed mainly on insects, although some species of the genus *Gallotia* are herbivorous (Arnold, 1989). They actively hunt to feed; although in some cases, for instance *Pedioplanis lineoocellata* and *Meroles suborbitalis* sit and wait for prey to come near (Pianka *et al.,* 1979; Branch, 1998). Most of the species in the family lay eggs, with a clutch size of between 1-25. Exceptions occur in *Lacerta vivipara* and some species in the genus *Eremias*, which bear fully formed young (Arnold, 1989; Branch, 1998).

1. 2. Lacertid dispersal and current distribution

Lacertid lizards are distributed throughout the Old World and are found mainly in Europe and Africa although some genera, *i.e. Takydromus* extend to the Far East (Arnold, 1989). Two competing biogeographical hypotheses exist regarding the origin of the modern lacertids and suggest that these lizards arose in Eurasia or alternatively in Africa. The Eurasian hypothesis has received much support recently from both morphological and

molecular data (Arnold, 1989; Harris *et al.,* 1998a; Fu, 1998, 2000). Deductions from the phylogeny imply that primitive groups of lacertid lizards are found in relatively mesic conditions in the Palearctic and Oriental regions, while the advanced forms occur in the Afrotropical region (Africa south of the Sahara). The intermediate groups are found in the deserts of north Africa and Eurasia, and also on the Indian subcontinent. If this holds, it would suggest that the group probably dispersed into Africa during the mid-Miocene when Africa was briefly connected with Eurasia not during the Cretaceous, as was proposed earlier (Estes, 1983a). Later on some of the lineages may have dispersed back to Eurasia from Africa and *vice versa* (Arnold, 1998). Fu (1998) suggests that this occurred about ten million years ago. The fossil record further supports the ancestral Eurasian hypothesis, as fossils are very rare for this group in Africa, and better represented from the Cenozoic of Europe (the latter are also much older). A single record is known from the Miocene of Morocco (Estes, 1983b). There is evidence of a drastic climatic change during the Miocene, when north Africa progressively became more arid. It is thought that the common ancestor of African lacertids may have adapted to xeric habitats during this time, penetrating the arid regions of Africa southwards and westwards (Arnold, 1989; Harris *et al.,* 1998a; Fu, 1998, 2000). The African origin hypothesis is mainly based on the fact that some of the morphologically primitive forms of lacertid lizards are found in Africa but some are also from Eurasia (Estes, 1984a; Arnold, 1989).

1. 3. Systematic relationships within Lacertidae

The family Lacertidae has long been regarded as a part of the Scleroglossa, a putatively monophyletic group of lizards supported by a diversity of morphological features (Estes *et al.,* 1988). Although a recent nuclear and mitochondrial sequence study (Townsend *et al.,* 2004) revealed a polyphyletic Scleroglossa, the Lacertiformes, (including teiids, gymnophthalmids, amphisbaenians, as well as lacertids) received strong support for monophyly. In addition, the monophyly of the Lacertidae has been uniformly accepted and is supported by both molecular studies (Harris *et al.,* 1998a; Fu, 1998, 2000) and a number of morphological synapomorphies. The latter include sexually dimorphic presacral vertebrae counts, hemipenial and jaw muscle characters, and the closure of the temporal fenestra by the postfrontal bone (Estes, 1988; Arnold, 1989).

Various workers (Arnold, 1989; Harris *et al.,* 1998a; Fu 1998, 2000) have tried to unravel phylogenetic relationships within the family using either morphological and/or molecular techniques. The most recent phylogenetic hypothesis for the family, based on mtDNA data (12S rRNA, 16S rRNA, Cyt-b, CO1, $tRNA^{Val}$ and $tRNA^{Thr}$), recognises two subfamilies: the Gallotiinae, consisting of two genera (*Gallotia* endemic to Canary Islands and *Psammodromus* occurring in Eurasia) and the Lacertiinae which is much more species rich (Fu, 2000). The latter subfamily is divided into two groups. One consists of African (*Tropidosaura*, *Meroles*, *Nucras*, *Heliobolus*, *Acanthodactylus*, *Adolfus* and *Pedioplanis*), Arabian (*Mesalina*, and *Latastia*) and Eurasian taxa (*Eremias* and *Ophisops*), while the second group consists of only Eurasian lacertids (*Lacerta*, *Algroides*, *Podacris* and *Takydromus*). Although the deep divergence of the lacertids into

the two subfamilies is retrieved with bootstrap support, the relationships among taxa within these subfamilies remains largely unresolved and controversial. This has been attributed to rapid speciation in the group as they adapted to changing climatic conditions during the Miocene, leaving no or few synapomorphic characters at the internodes (Fu, 1998, 2000; Harris *et al.,* 1998a). It has been suggested by Fu (2000) that increased sampling at the lower taxonomic level will improve resolution among members of this group.

1. 4. Lacertid lizards in southern Africa

Lacertid diversity is greatest in the Palearctic region; however, southern Africa is characterized by a diverse assemblage of lacertids encompassing eight genera. Two of these genera (Southern Rock Lizards - *Australolacerta*; Mountain Lizards - *Tropidosaura*) are strictly endemic to South Africa, whereas the remaining six genera occur more widely, with their ranges extending northwards into mesic and semi-arid environments and reaching as far north as central and east Africa (Sandveld Lizards - *Nucras*; Bushveld Lizards - *Heliobolus;* Rough-Scaled Lizards - *Ichnotropis*; Tree Lizards - *Holaspis*) or into the semi-arid and, arid regions of Namibia and Botswana (Desert Lizards - *Meroles*; Sand Lizards - *Pedioplanis*). These eight genera presently comprise 45 species, of which 28 are endemic to the southern African region (Arnold, 1989; Branch, 1998; Spawls *et al.,* 2002). Together the species richness represents a diversity hotspot for this group within sub-Saharan Africa. Despite this diversity, only one of the eight genera, *Meroles* (Harris *et al.,* 1998b; Lamb & Bauer, 2003), has been investigated from a phylogenetic perspective using molecular sequence data. As a

consequence, phylogenetic relationships between and within most genera remain uncertain. Although *Meroles* is a typical desert lizard group and *Pedioplanis* is more widely distributed, the two taxa are presumably closely related and in some instances their distributions overlap (Branch, 1998). Since there was phylogenetic congruence within *Meroles* when morphological (Arnold, 1991; Harris *et al.,* 1998) and molecular data (Harris *et al.,* 1998; Lamb & Bauer, 2003) were employed, there is high likelihood that the phylogenetic relationships within *Pedioplanis* will largely reflect the morphological hypothesis by Arnold (1991).

In second chapter of this study, molecular markers were employed to reconstruct the phylogenetic relationship among species currently assigned to the genus *Pedioplanis* and the position of genus among other southern African lacertids. At a lower taxonomic level in chapter three, mtDNA was analzed to determine the phylogeographic structure of *Pedioplanis burchelli,* which is one of the widespread endemic species in South Africa. The outcome of this study should significantly enhance the current undertanding of lizard evolution and may provide further insight into the processes responsible for the rich biodiversity in southern African lizards.

Chapter 2: Nuclear and mtDNA phylogenetic inferences among southern African sand lizards, *Pedioplanis* (Sauria: Lacertidae)

2. 1. Introduction

Lizards of the genus *Pedioplanis*, represent the most species rich lacertid genus in southern Africa (11 species). They occupy diverse habitats including montane grassland, coastal fynbos, succulent Karoo, Nama Karoo, arid and moist savannah, and the Namib desert (FitzSimons, 1943; Branch, 1998). All species are endemic to southern Africa except *P. namaquensis*, *P. undata* and *P. benguellenis*, which range into southern Angola. Boulenger (1921) and FitzSimons (1943) assigned most of the species now placed in *Pedioplanis* to the subgenus *Mesalina* within the large genus *Eremias*. Szczerbak (1975) recognized that *Eremias* was polyphyletic and divided the African sand lizards into five genera, including *Mesalina.* Balletto (1968) suggested that the subgeneric name *Pedioplanis* Fitzinger, 1843 was applicable to southern African *Mesalina* and this name has been used almost exclusively for these lizards since the 1980s, whereas *Mesalina* sensu stricto is currently restricted to North Africa and Asia.

Pedioplanis species share a number of morphological characters including the presence of a posterior projection and posterolateral process of the septomaxilla and fused frontal bones, with other derived lacertid genera (Arnold, 1991). Among these forms, however, Arnold (1991) found no support for the collective monophyly of the southern African genera, although *Pedioplanis* + *Meroles* shared numerous putative synapomorphies with

Sahara-Eurasian clade (Arnold, 1989). Allozyme (Mayer & Berger-Dell'mour, 1988) and some mtDNA data (12S rRNA, 16S rRNA and Cyt-b; Harris *et al.,* 1998b) have inferred a sister taxon relationship between *Pedioplanis* and *Meroles*, but Fu (2000), also using mtDNA data (12S rRNA, 16S rRNA, Cyt-b, CO1, $tRNA^{Val}$ and $tRNA^{Thr}$), placed *Meroles* as sister to a monophyletic *Pedioplanis* + *Tropidosaura*. Arnold (1991) considered the large number of features shared by both *Pedioplanis* and *Meroles* as parallelisms and identified 13 putative synapomorphies of *Pedioplanis*, one of which, the outer connectors of the hemipenis armature running close together dorsally or fused, is uniquely derived.

Within *Pedioplanis* the status of the members of two species or species complexes have remained inadequately resolved. Although now treated as separate species, *P. undata*, *P. inornata*, *P. gaerdesi* and *P. rubens* were collectively referred to as the "P. undata" species complex. Mertens (1954, 1955) recognized three subspecies in the complex, whereas Mayer and Berger-Dell'mour (1987) recognized up to seven forms of the "P. undata" species complex based on morphology and protein electromorphs. Recent studies (*e.g.,* Arnold, 1989, 1991; Branch, 1998) have recognized five of these forms as valid at the specific level. However, the suggestion that *P. undata* and *P. inornata* could each be divided into northern and southern forms (Mayer and Berger-Dell'mour, 1987) has not been subsequently corroborated. In the spotted sand lizard, *P. lineoocellata*, two subspecies are widely recognized, *P. l. lineoocellata*, and *P. l. pulchella*. Bauer and Branch (2001) suggested that these two subspecies should be elevated to full species given that they are allopatric and exhibit substantial morphological, color pattern, and

ecological differences. The nominate race has slightly overlapping, keeled scales on the back that are smaller than those on the forelimbs, whereas *P. l. pulchella* has smooth, juxtaposed scales on the dorsum that are comparable in size to those of the forelimb, which are not overlapping and smooth on the back. Specimens from Lüderitz Bay in Namibia are sometimes treated as a third subspecies, *P. l. inocellata* (Mertens, 1955), named for its dull, dark gray body (occasionally with four faint dorsal stripes) and lack of large flank spots (Branch, 1998).

Mayer and Berger-Dell'mour (1988) made the first attempt to elucidate phylogenetic relationships within the sand lizards, on the basis of electrophoretic data. Their results were preliminary due to incomplete sampling and a lack of support values for the inferred relationships. Subsequently, Arnold (1991) conducted a phylogenetic analysis of all *Pedioplanis* taxa based on morphological data. Relationships were weakly supported and most information was derived from genital characters (Arnold, 1986). The morphology, nevertheless, suggested that *P. lineoocellata* was sister to all other species. Arnold (1991) also found that the geographically proximal *P. burchelli* and *P. laticeps* are sister species, closely related to *P. breviceps*, and that these three taxa are the sister group to (*P. inornata* (*P. husabensis* (*P. namaquensis* (*P. benguellensis* (*P. rubens* (*P. undata*, *P. gaerdesi*)))))). Arnold's (1991) phylogeny thus excluded *P. inornata* from the "P. undata" species complex (*P. undata*, *P. inornata*, *P. gaerdesi*, and *P. rubens*).

2. 2. Aims

Analysis of DNA sequence data derived from both conserved nuclear and more variable mitochondrial genes stand to significantly enhance the current understanding of the phylogenetic relationships and evolution within *Pedioplanis*. This study specifically aims to:

(i) provide a gene tree addressing the sister taxon relationship of *Pedioplanis* relative to other southern African lacertids;

(ii) determine the phylogenetic relationships among sand lizards, *Pedioplanis*;

(iii) establish the status of named subspecies and unnamed forms in *P. lineoocellata* and the "P. undata" species group, respectively;

(iv) couple the *Pedioplanis* phylogeny to a molecular clock in an attempt to identify factors driving speciation in this group.

2. 3. Materials and methods

2. 3. 1. Sampling

Ten of the eleven recognized species in the genus were sampled; tissues from *P. benguellensis* were not available for inclusion. Where possible sampling was done to examine geographic variation within each species and also to address the validity of some of the recognized subspecies (Fig. 1; Table 1). Identifications of the specimens was done by the respective collectors and in case of any potential doubt, they were re-examined by Aaron Bauer, or Le Fras Mouton and Bill Branch. Due to the uncertain sister taxon relationship of *Pedioplanis,* several lacertid outgroup taxa were incorporated in the study. They included representatives of the genera *Meroles* (*M. knoxii*, *M. suborbitalis*, and *M. reticulatus*), *Ichnotropis* (*I. capensis*), *Nucras* (*N. tessellata*), *Heliobolus* (*H. lugubris*) and *Australolacerta* (*A. australis*). In total, 100 individual were incuded in this study.

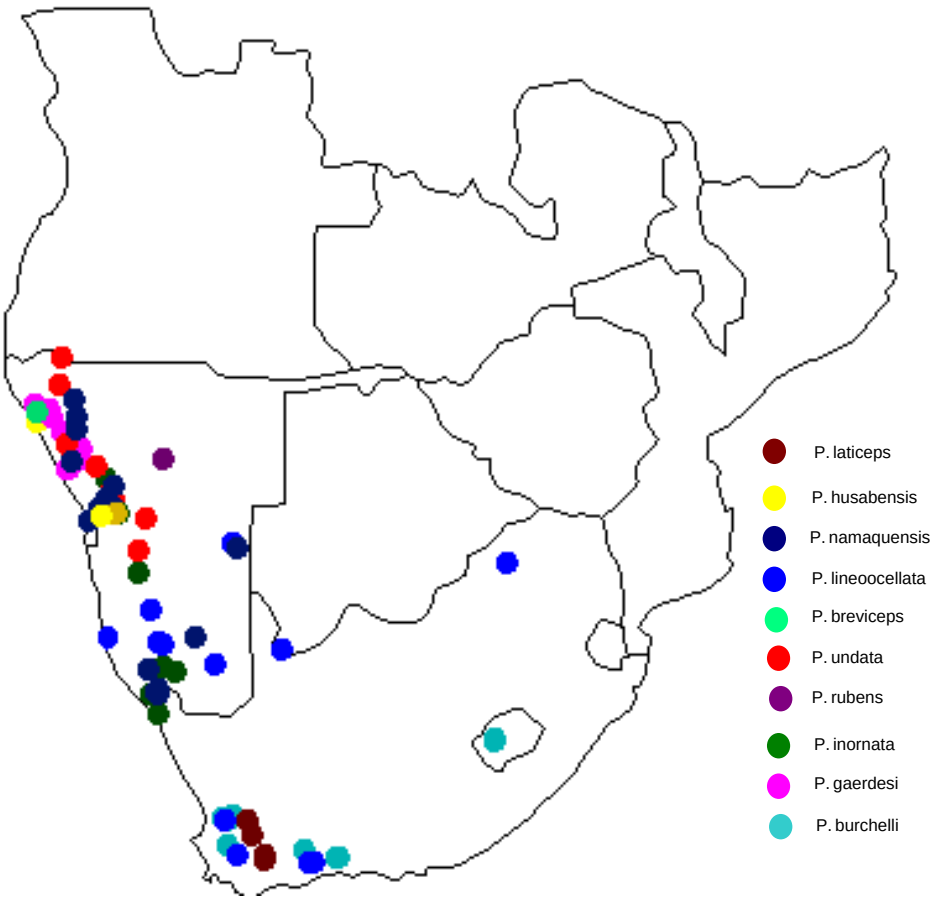


Fig. 1. Sampling localities of the species included in the present investigation. Ten of the eleven *Pedioplanis* species across the distribution range of the genus were included.

Table 1. Species locality information and GenBank accession numbers of the specimens (identical sequences in each taxa were excluded and the final was based on 58 specimens) used in this study. Collection codes: AMB = Aaron M. Bauer tissue collection (corresponding voucher specimens pending accession in the National Museum of Namibia); ABE and ABD = Molecular Systematics Section of the Naturhistorisches Museum in Wien (voucher and/or tissue sample only); CAS = California Academy of Sciences, KTH = Krystal Tolley (tissue accessioned at the South African National Biodiversity Institute), CF & MH = Cape Fold Herp project (tissue only, no voucher specimens); JSM = Jane S. Makokha (voucher specimens pending accession in Port Elizabeth Museum, South Africa); DDT = Dahne Du Toit (tissue only, no voucher); MCZ = Museum of Comparative Zoology, Harvard University, MCZ FS = Museum of Comparative Zoology, Harvard University field series (corresponding voucher specimens pending accession in the National Museum of Namibia); NHMW = Naturhistorisches Museum in Wien.

Collection Code	**Museum Numbers**	**Taxon Name**	**Locality**	**Gene Bank Accession Numbers**		
				ND2	**16S rRNA**	**RAG-1**
Outgroup						
KTH499	-	*Australolacerta australis*	Naudesberg-Langeberg, W. Cape South Africa	**DQ871092**	**DQ871150**	-
KTH569	-	*Australolacerta australis*	Goedemoed-Langeberg, W. Cape South Africa	**DQ871093**	**DQ871151**	-
MH0531	-	*Australolacerta australis*	Zuurberg Private Nature Reserve, W. Cape South Africa	**DQ871094**	**DQ871152**	**DQ871208**
AMB6001	NMNW…	*Ichnotropis capensis*	Road to Tsumkwe, Namibia	**DQ871090**	**DQ871148**	**DQ871206**
AMB6067	CAS 209602	*Ichnotropis capensis*	Kosi Bay, KwaZulu-Natal, South Africa	**DQ871091**	**DQ871149**	**DQ871207**
AMB5589	CAS 206735	*Meroles suborbitalis*	Groenriviermond, N. Cape, South Africa	**DQ871089**	**DQ871147**	**DQ871205**
AMB5629	CAS 206782	*Meroles knoxii*	Port Nolloth, Northern Cape, South Africa	**DQ871088**	**DQ871146**	**DQ871204**
AMB5921	NMNW…	*Meroles reticulatus*	11.3 Km south of Cape Cross, Namibia	**DQ871086**	**DQ871144**	**DQ871202**
MCZFS38343	NMNW…	*Meroles suborbitalis*	Near Grünau	**DQ871087**	**DQ871145**	**DQ871203**
AMB5582	CAS 206723	*Nucras tessellata*	Groenriviermond, N. Cape, South Africa	**DQ871085**	**DQ871143**	**DQ871201**
MCZFS37894	FS…	*Heliobolus lugubris*	Kamanjab, Namibia	**DQ871084**	**DQ871142**	**DQ871200**
MCZFS37870	MCZ R184277	*Heliobolus lugubris*	Kamanjab, Namibia	**DQ871083**	**DQ871141**	**DQ871199**

Ingroup						
MCZFS38393	MCZ R 184524	*P. l. pulchella*	Kgama, Limpopo, South Africa	**DQ871050**	**DQ871108**	**DQ871166**
ABA21	NHMW 35385:2	*P. l. inocellata*	Lüderitz, Namibia	**DQ871045**	**DQ871103**	**DQ871161**
ABA20	NHMW 35360:1	*P. l. lineoocellata*	Aranos, Namibia	**DQ871048**	**DQ871106**	**DQ871164**
AMB6862	CAS 223974	*P. l. lineoocellata*	45 Km North of Helmeringhausen, Namibia	**DQ871046**	**DQ871104**	**DQ871162**
MCZFS37656	MCZ R183775	*P. l. lineoocellata*	76.2 Km East of Ugab Crossing, Namibia	**DQ871047**	**DQ871105**	**DQ871163**
DDT09	-	*P. l. pulchella*	Matjiesrivier Nat. Res., W. Cape, S. Africa	**DQ871051**	**DQ871109**	**DQ871167**
MH0336	-	*P. l. pulchella*	Die Trap, Cederberg, W. Cape, South Africa	**DQ871049**	**DQ871107**	**DQ871165**
KTH222	-	*P. laticeps*	Tankwa Karoo, Western Cape, South Africa	**DQ871069**	**DQ871127**	**DQ871185**
JSM021	PEM…	*P. laticeps*	Anysberg Nature Reserve, W. Cape, S. Africa	**DQ871068**	**DQ871126**	**DQ871184**
JSM018	PEMR17212	*P. laticeps*	Anysberg Nature Reserve, W. Cape, S. Africa	**DQ871067**	**DQ871125**	**DQ871183**
JSM015	PEMR17214	*P. laticeps*	Anysberg Nature Reserve, W. Cape, S. Africa	**DQ871066**	**DQ871124**	**DQ871182**
KTH346	-	*P. burchelli*	Qwa Qwa, Free State, S. Africa	**DQ871065**	**DQ871123**	**DQ871181**
KTH137	-	*P. burchelli*	Wamboomberg nr. Ceres, W. Cape, S.Africa	**DQ871064**	**DQ871122**	**DQ871180**
CF169	-	*P. burchelli*	Sneeukop, Kouebokkeveld, W. Cape, S.Africa	**DQ871063**	**DQ871121**	**DQ871179**
MH0334	-	*P. burchelli*	Tafelberg, Cederberg, W. Cape, South Africa	**DQ871062**	**DQ871120**	**DQ871178**
MCZFS37819	NMNW…	*P. breviceps*	Gai-As, Namibia	**DQ871060**	**DQ871118**	**DQ871176**
MCZFS37818	NMNW…	*P. breviceps*	Gai-As, Namibia	**DQ871059**	**DQ871117**	**DQ871175**
AMB8473	NMNW…	*P. breviceps*	near Gai-As, Namibia	**DQ871061**	**DQ871119**	**DQ871177**
ABF16	NHMW 35356:1	*P. breviceps*	Hoanib, Namibia	**DQ871058**	**DQ871116**	**DQ871174**
MCZFS37127	R 184164	*P. husabensis*	Northern Bank of Swakop River, Namibia	**DQ871081**	**DQ871139**	**DQ871197**
ABE473	-	*P. husabensis*	Ukub-West, Namibia	**DQ871080**	**DQ871138**	**DQ871196**
ABE451	-	*P. undata*	Palmwag, Namibia	**DQ871053**	**DQ871114**	**DQ871172**
ABE385	NHMW 35339:13	*P. undata*	Kunene, Namibia	**DQ871054**	**DQ871112**	**DQ871170**
ABE423	NHMW 35339:25	*P. undata*	Nauchas, Namibia	**DQ871057**	**DQ871115**	**DQ871173**
ABE415	NHMW 35339:5	*P. undata*	Uis, Namibia	**DQ871053**	**DQ871111**	**DQ871169**
AMB6406	CAS 214643	*P. undata*	59 km west of Kamanjab, Namibia	**DQ871055**	**DQ871113**	**DQ871171**
KTH595	-	*P. inornata*	Farm Kuthula, 35 Km E. Upington South Africa	**DQ871081**	**DQ871140**	**DQ871198**

AMB4736	NMNW…	*P. inornata*	Richtersveld, Northern Cape, South Africa	**DQ871078**	**DQ871136**	**DQ871194**
ABE393	NHMW 35340:9	*P. inornata*	Fish River Canyon, Namibia	**DQ871079**	**DQ871137**	**DQ871195**
ABE472	-	*P. inornata*	Tsaobis Leopard Park in Swakop, Namibia	**DQ871073**	**DQ871131**	**DQ871189**
ABE428	NHMW 35340:5	*P. inornata*	Rössing, Namibia	**DQ871072**	**DQ871130**	**DQ871188**
ABE458	-	*P. inornata*	Usakos, Namibia	**DQ871071**	**DQ871129**	**DQ871187**
AMB6552	CAS 214789	*P. inornata*	South of Karibib, Namibia	**DQ871070**	**DQ871128**	**DQ871186**
ABE407	NHMW 35371:12	*P. gaerdesi*	Purros, Namibia	**DQ871077**	**DQ871135**	**DQ871193**
AMB6507	CAS 214745	*P. gaerdesi*	29 Km west of Sesfontein, Namibia	**DQ871075**	**DQ871133**	**DQ871191**
ABE448	-	*P. gaerdesi*	Palmwag, Namibia	**DQ871076**	**DQ871134**	**DQ871192**
AMB7584	NMNW…	*P. gaerdesi*	33.2Km E. of Ugab Crossing, Namibia	**DQ871074**	**DQ871132**	**DQ871190**
ABE384	NHMW 35341:8	*P. rubens*	Waterberg, Namibia	**DQ871052**	**DQ871110**	**DQ871168**
AMB4558	CAS 200033	*P. namaquensis*	Richtersveld, Northern Cape, South Africa	**DQ871043**	**DQ871101**	**DQ871159**
AMB4775	CAS 200105	*P. namaquensis*	Richtersveld, Northern Cape, South Africa	**DQ871042**	**DQ871100**	**DQ871158**
AMB4541	NMNW	*P. namaquensis*	Richtersveld, Northern Cape, South Africa	**DQ871041**	**DQ871099**	**DQ871157**
ABD54	-	*P. namaquensis*	Otjondeka, Namibia	**DQ871044**	**DQ871102**	**DQ871160**
AMB7577	NMNW…	*P. namaquensis*	17 Km east of Ugab crossing, Namibia	**DQ871040**	**DQ871098**	**DQ871156**
AMB7121	NMNW…	*P. namaquensis*	Road to Uis, Namibia	**DQ871039**	**DQ871097**	**DQ871155**
ABD47	NHMW 35351:20	*P. namaquensis*	Trekkopje, Namibia	**DQ871038**	**DQ871096**	**DQ871154**
AMB6549	CAS 214784	*P. namaquensis*	South of Karibib, Namibia	**DQ871037**	**DQ871095**	**DQ871153**

2. 3. 2. DNA extraction, amplification and sequencing

A piece of the tail, or the entire liver of voucher specimens, was preserved in 95% ethanol or saturated salt-DMSO buffer. Upon DNA extraction, tissue was homogenized in 250µl extraction buffer and 10µl of a 10mg/ml proteinase K solution. Total genomic DNA was extracted using the phenol/chloroform iso-amyl alcohol procedure as described by Palumbi *et al.* (1991). Two mitochondrial (ND2 and 16S rRNA) and one nuclear gene regions (RAG-1) were selected for sequencing. Lacertid specific primers for RAG-1 (F211 - 5'-ATTACTTCAGTGCCACAAGA-3' and R1392 - 5'-CCTGCATCATAGCTTCCAAC-3') were designed using *Eremias* sp. sequence from GenBank (AY662615) and Primer3 software (Rozen & Skaletsky, 2000). The published vMet2 and vTrp ND2 primers (Cunningham & Cherry, 2004) and L2510 and H3080 16S rRNA primers (Palumbi, 1996) were used for mtDNA amplification and sequencing. The PCR cycle profile was as follows: an initial 1 min denaturation at 94°C, followed by 35 cycles of 35 sec at 94°C, 30 sec at 50°C - 55°C (annealing) and 45 sec at 72°C; with a final extension of 5 min at 72°C using the Gene Amp PCR system 2700 (Applied Biosystems). The annealing temperature was set at 54°C, 50°C and 55°C for ND2, 16S rRNA and RAG-1 genes, respectively.

The PCR reaction mixture was separated on 0.8% agarose gels and amplified products were purified using Qiagen purification columns (Qiagen). Cycle sequencing was done with the BigDye terminator v. 3.1 cycle sequencing kit (Applied Biosystems). Excess terminator dye was removed by gel filtration through Centri-Sep 96 multi-well filter

plates (Princeton Separation). The cycle sequencing products were then analysed on an ABI Prism 3100 or 3130 XL 16-capillary genetic analyzer (Applied Biosystems).

2. 3. 3. Phylogenetic analysis

The sequences were visually inspected in Sequence Navigator v. 1.01 (Applied Biosystems) and alignment was done with Clustal X (Thompson *et al.,* 1997) using default parameters. Adjustments were then made by eye using MacClade v. 4.0 (Maddison & Maddison, 2002). All the sequences have been deposited in GenBank (Table 1). Two methods of phylogenetic analysis were used: parsimony and Bayesian inference. Congruence between the three gene partitions was tested using 100 replicates of the partition-homogeneity test (PHT) (Farris *et al.,* 1994, 1995) in PAUP* 4.0b10 (Swofford, 2002). Maximum parsimony tree construction was done in PAUP* 4.0b10 with all characters unordered and equally weighted. Tree searches were conducted using heuristic tree bisection and reconnection branch-swapping (TBR) with 100 random addition replicates. The support of the recovered nodes was calculated using 1000 non-parametric bootstrap replicates (Felsenstein, 1985). Modeltest 3.06 (Posada & Crandall, 1998) and Alkaike Information Criterion (AIC) was used to estimate the most likely model that best fits different data sets and these models were used to guide priors in a Bayesian Inference analyses performed with MrBayes v. 3.1.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). For the combined analysis, the data from the three genes were partitioned and parameters were un-linked allowing the assignment of different optimizations for each data set. The GTR + I + G model was selected for ND2 and 16S rRNA data, and the TIM + I + G model was chosen as the best-fit model for the

nucleotide substitutions in the RAG-1 gene. Priors in MrBayes were set to nst = 6 and rates = invgamma. Two runs each with four Markov chains were run simultaneously for five million generations. Trees were sampled every hundred generations, and the first 10% (5000 trees) of 50000 trees were discarded as the burn-in. The support for each clade was determined by calculating a 50% majority rule consensus tree in PAUP* 4.0b10 (Swofford, 2002). The GTR corrected pairwise differences between individual gene sequences was calculated to determine the genetic distance among and with species species in PAUP* 4.0b10 (Swofford, 2002). In all the phylogenetic analyses the genus *Australolacerta* was used to root the trees (Arnold, 1989).

2. 3. 4. Estimation of time of divergence

A constant molecular clock was rejected by the likelihood ratio test (without clock -LnL 18808.24570, with clock -LnL 18863.16074; $P < 0.05$; $X^2 = 74.4683241$). The relaxed Bayesian clock implemented in Estbranches and Multidivtime was used to generate an ultrametric tree (Thorne & Kishino, 2002; Kishino *et al.,* 2001). The maximum likelihood estimation of transition/transversion ratios, rate heterogeneity among sites and nucleotide frequencies were determined using PAML v.3.15 (Yang, 1997). We used a concensus tree identical to the tree resulting from the combined parsimony and Bayesian analyses. Due to time constraints the dataset was trimmed. The final tree included 29 taxa representing one or two individual of each species, all four *P. lineoocellata* taxa and both *P. namaquensis* and *P. inornata* lineages. The only fossil record for African lacertids from Morocco, has been dated to the middle Miocene, approximately 15 MYA (Estes, 1983b). On this basis, the node of the ancestor of all southern African genera was

arbitrarily constrained to lower and upper limits of 12 MYA and 18 MYA respectively. A somewhat earlier scenario was proposed by Busack & Maxson (1987), who estimated the divergence of *Heliobolus*/*Pedioplanis* from *Ichnotropis* to be early Miocene (17-24 MYA) based on immunological data (serum albumin). However, their inferred pattern of relationships was in conflict with the findings in this study and their dating estimates were possibly overestimated due to an exceptionally long branch associated with *Ichnotropis* (Mayer & Benyr, 1994).

2. 4. Results

2. 4. 1. Mitochondrial ND2 and 16S rRNA genes

The aligned ND2 matrix of 100 individuals had a total of 602 characters of which 234 (38.9%) were constant, 334 (55.5%) parsimony informative and 34 (5.6%) variable but not parsimony informative. All samples of *P. lineoocellata* shared a three base pair (bp) deletion (position 437 - 439 in the alignment) in the ND2 region and this did not interrupt the reading frame. The corrected genetic distance comparisons among ingroup taxa ranged between 7% - 34%. A parsimony analysis resulted in 16 equally parsimonious trees (L = 1702, CI = 0.3486, RI = 0.7509) from which a strict consensus was generated (not presented). The ND2 gene resolved most of the interspecific relationships among *Pedioplanis* species. Sixty-five percent of the nodes received over 75% bootstrap support and these also had significant posterior probabilities (≥ 0.95). Although the monophyly of *Pedioplanis* was not well supported by parsimony bootstrap (BS) (60%) it was recovered with a posterior probability (PP) of 1.0.

Exclusion of the highly variable and difficult to align sections of the 16S rRNA gene for 100 individuals (positions 225 - 230, 280 - 292, 309 - 312 in the alignment – 42 characters in total); resulted in a matrix with 498 characters of which 220 (44.2%) were constant, 28 (5.6%) variable but not parsimony informative and 250 (50.2%) parsimony informative. The corrected genetic distance within the ingroup taxa ranged from 2% to 12%. Parsimony analysis of the 16S rRNA data retrieved 721 equally parsimonious trees (L = 971; CI = 0.4602 and RI = 0.7631). The bootstrap tree of the 16S rRNA gene does not support any of the deeper nodes in the tree. However, 33% of the nodes on the

parsimony tree are supported with over 75% BS, and these are mainly restricted to those supporting the monophyly of species. The Bayesian inference tree was once again better supported, with 46% of the nodes receiving significant (≥ 0.95) PP support.

The PHT test performed to determine the congruence between the two mitochondrial data partitions (ND2 and 16S rRNA) did not reject the null hypothesis (P = 0.16), and because these two genes are linked, the two datasets were combined. Only 58 unique sequences were identified and used for the combined mtDNA genes, which is also presented for combined mtDNA and nDNA. A parsimony analysis of the mitochondrial genes resulted in 56 equally parsimonious trees (L = 2439, CI = 0.4121 and RI = 0.740). The mitochondrial gene parsimony tree supports the monophyly of *Pedioplanis* (64% BS and 1.0 PP). However, unlike the individual analysis of the mitochondrial genes, the combined data also resulted in a *P. burchelli* and *P. laticeps* clade being sister to all the other species, but without support (53% BS; see below), whereas the Bayesian analysis supported this association with 1.0 PP (Fig. 2).

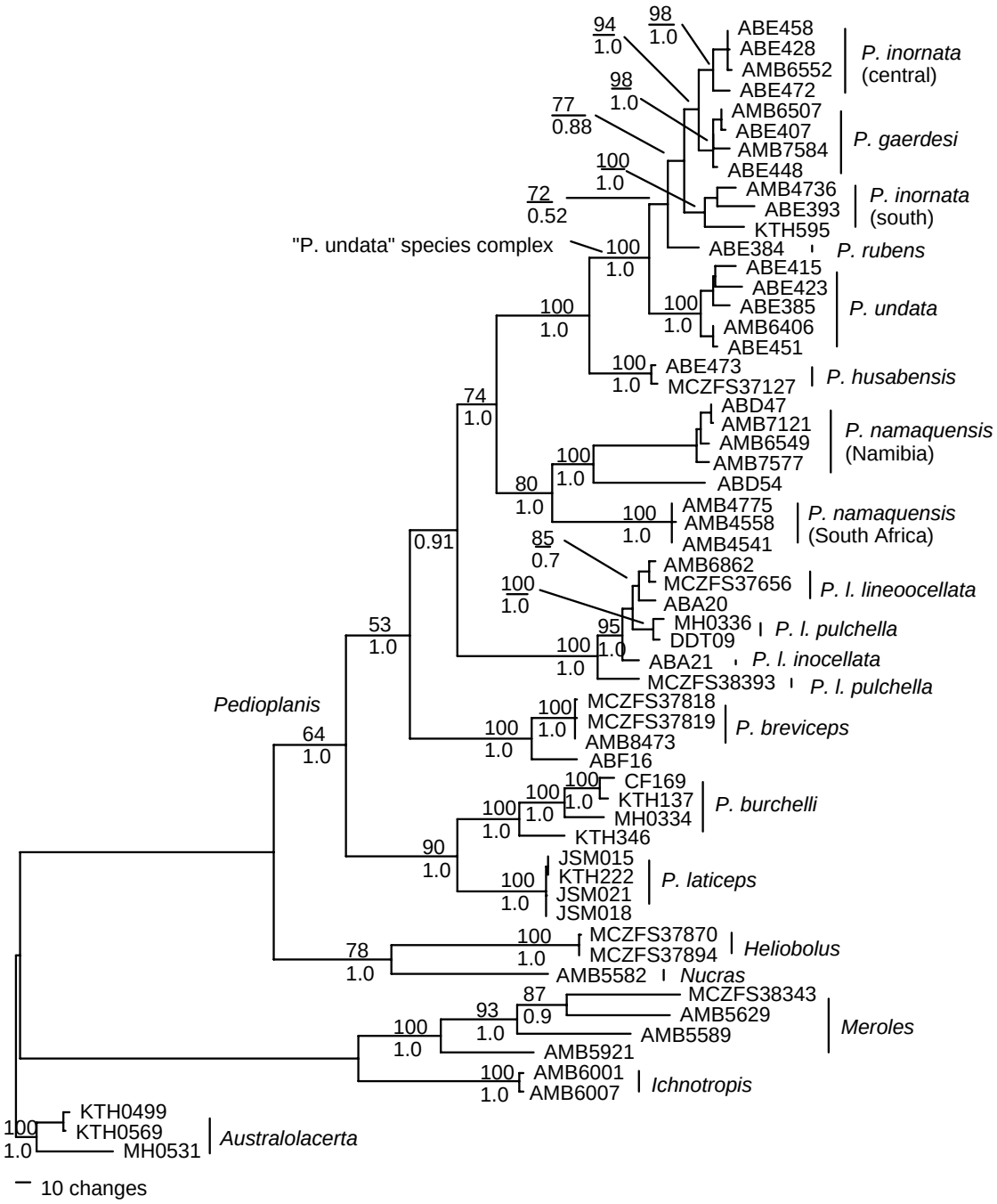


Fig. 2. A Bayesian inference analysis phylogram of the combined mtDNA data (ND2 & 16S rRNA) for the genus *Pedioplanis*. Parsimony analysis retrieved a total 56 most parsimonious trees (TL = 2439, CI = 0.4121 and RI = 0.740). Bootstrap support values are indicated above and Bayesian posterior probability below the nodes.

2. 4. 2. Nuclear RAG-1 gene

One hundred samples for the mtDNA genes were sequenced, and of these, 58 individuals with unique mtDNA haplotypes were selected for nDNA sequencing. The aligned RAG-1 matrix had 1100 characters of which 679 (61.7%) were constant, 250 (22.7%) variable and 171 (15.6%) parsimony informative. All samples of *P. breviceps* had a 12bp deletion (position 474 - 485) whereas *Meroles knoxii* and *M. suborbitalis* had a 15bp deletion (position 115 - 129) both at the same positions in the alignment, but these did not affect the reading frame. The corrected nDNA genetic distance values among taxa were low and ranged between 1% and 6%. In both parsimony and Bayesian analysis, the basal nodes of the topology (those defining relationships among genera) were well resolved but interspecific relations had little support (topology not shown).

2. 4. 3. Combined mitochondrial and nuclear genes

The results of the PHT test between mitochondrial and nuclear genes used in this study indicated incongruence (P = 0.02). However, the three genes were combined because there were no strongly supported nodes that were in conflict between the trees generated by the mitochondrial and the nuclear data sets. The significant PHT results could be attributed to the conservative nature of the test (Yoder *et al.*, 2001; Barker & Lutzoni, 2002). In addition, the combination of data frequently increases phylogenetic resolution. The concatenated dataset of 58 taxa consisted of 2200 characters of which 1303 (59.2%) were constant, 140 (6.4%) variable but uninformative and 757 (34.4%) were parsimony informative. I was unable to amplify the RAG-1 gene for two samples (KTH499 & KTH569), resulting in missing data for this marker in the combined data set (see Table

1). A parsimony analysis of the combined data resulted in 72 equally parsimonious trees (L = 2887, CI = 0.4465, RI = 0.7725). The inclusion of the nuclear data set decreases the support for recently divergent taxa (for example among the "P. undata" species complex) whereas it generally increased the support for the associations among species and genera (Figs. 2 and 3). The two genes clearly provide phylogenetic signal at different levels in the phylogeny and it is likely that for closely related taxa, lineage sorting at the nuclear DNA level is not yet complete (Maddison & Knowles, 2006). The associations among the closely related "P. undata" members are thus discussed mainly on the mtDNA findings.

In the Bayesian inference analysis, the average standard deviation for the split frequencies after five million generations was 0.007525. With the genus *Australolacerta* defined as the outgroup, *Meroles* forms a poorly supported clade with *Ichnotropis* (53% BS and 0.55 PP) (Fig. 3). This clusters as a basal sister clade to another fairly well supported clade of *Heliobolus* and *Nucras* (99% BS and 1.0 PP). The monophyletic relationship of *Heliobolus*/*Nucras* with *Pedioplanis* is well supported (91% BS and 1.0 PP). *Pedioplanis* is monophyletic with 74% BS and 1.0 PP and the *P. burchelli* / *P. laticeps* clade is the sister clade to the rest of the species in the genus (70% BS and 1.0 PP). Among the remaining taxa, *P. breviceps* and *P. lineoocellata* clustered as sister taxa without significant support (< 50% BS and 0.90 PP) and together are the sister group to the remaining species of *Pedioplanis*. Collectively, the subspecies of *P. lineoocellata* constitute a strongly supported clade (100% BS and 1.0 PP). Within *P. lineoocellata,* the samples from Kgama in the Waterberg District, Limpopo Province, considered to be an

isolated population of *P. l. pulchella* (Jacobsen, 1989), is basal to all the other three recognized subspecies (*P. l. lineoocallata*, *P. l. pulchella*, *P. l. inocellata*). Within the current concept of *P. namaquensis*, there seem to be two geographically distinct lineages; one occurs in Namibia, while the other occurs in South Africa. The two lineages form a monophyletic group (96% BS and 1.0 PP). *Pedioplanis husabensis* and the "P. undata" species complex form a strongly supported clade (100 % BS and 1.0 PP). Within this species complex, *P. undata* is sister to all other forms. *Pedioplanis rubens* is the sister to the *P. inornata*/*P. gaerdesi* clade, although this pattern does not receive significant support. *Pedioplanis inornata* is paraphyletic and consists of two separate clades, a strongly supported central Namibian clade that is sister to *P. gaerdesi* and a more southern Namibian and Northern Cape *P. inornata* clade.

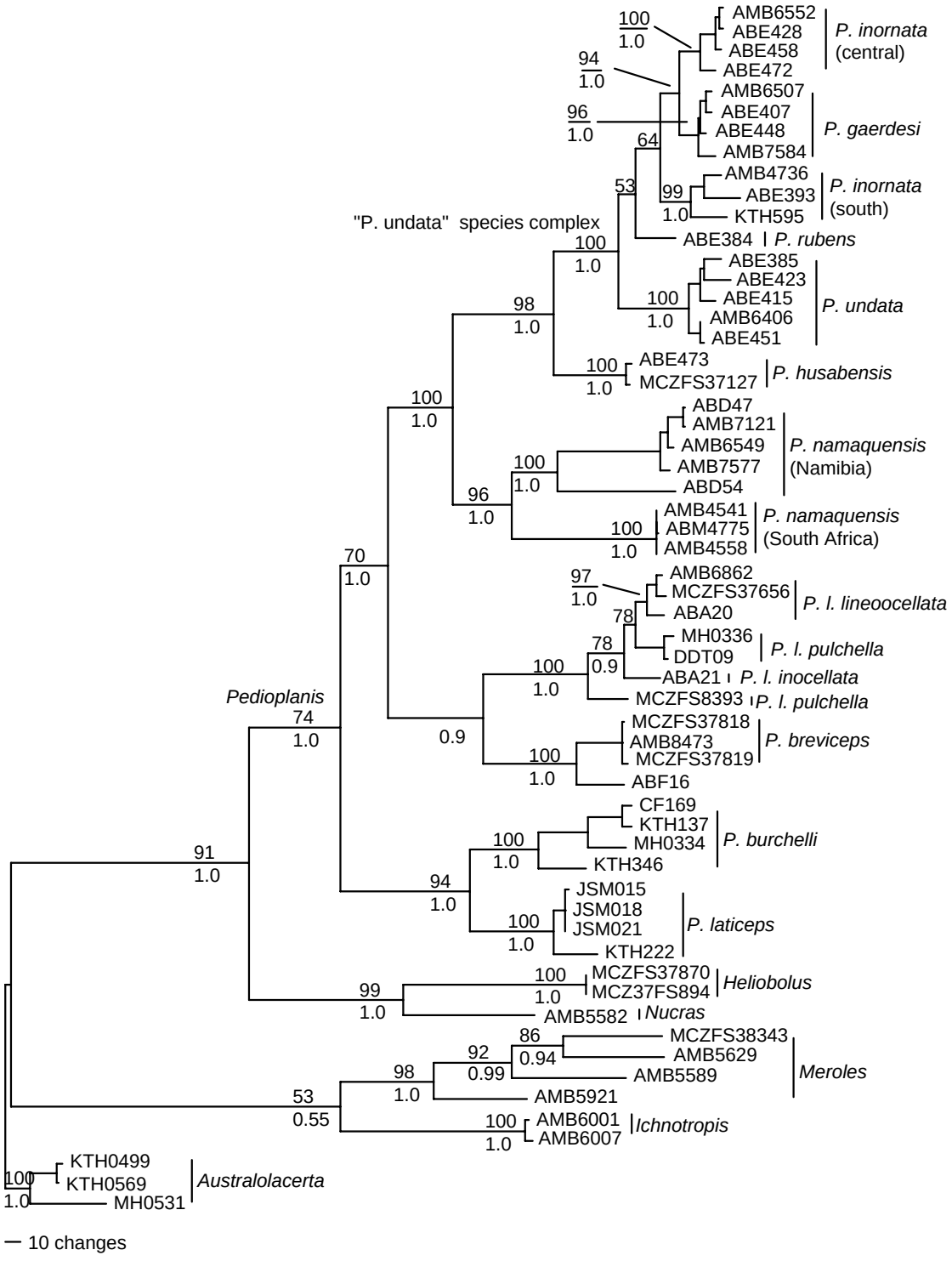


Fig. 3. A parsimony analysis phylogram for the genus *Pedioplanis* based on the combined data (mitochondrial and nuclear fragments) of the 72 most parsimonious trees (L = 2887, CI = 0.4465, RI = 0.7725) with bootstrap support values above and Bayesian posterior probabilities below the nodes.

2. 4. 4. Estimated time of divergence within *Pedioplanis*

The Relaxed Bayesian clock using the combined data resulted in posterior molecular divergence dates with relatively narrow standard errors (Fig. 4) and confidence interval (not shown). The divergence of the two clades (Fig. 4) within *Pedioplanis* is estimated to have occurred during the mid-Miocene (13.5 -/+ 1.8 MYA). Speciation within the "P. undata" species complex could have commenced in the late Miocene to the Pliocene period (5.3 -/+ 1.6 MYA) (Fig. 4). These values should be taken as rough estimates assuming that the calibration point is correct.

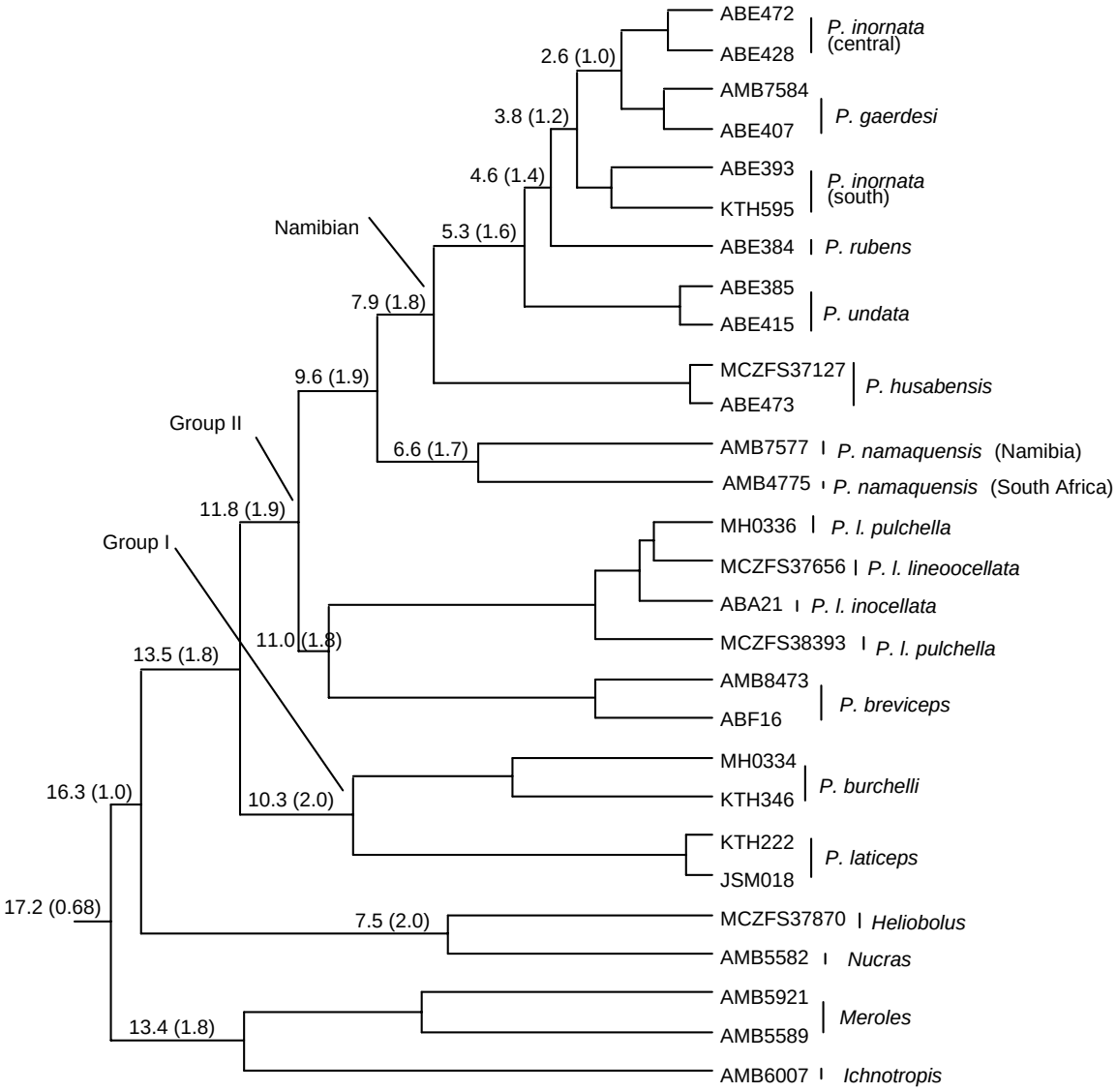


Fig. 4. Utrametric tree showing the estimated time of divergence and the standard error in parantheses in millions of years using a Miocene fossil as a calibration point (12 - 18 MYA, Estes, 1983b) and the programme Multidivtime.

2. 5. Discussion

2. 5. 1. Higher level taxonomy of *Pedioplanis*

Species belonging to the five potential outgroup genera (*Nucras*, *Heliobolus*, *Ichnotropis*, *Meroles* and *Australolacerta*) included in this study were all found to be monophyletic. When the root was placed at *Australolacerta,* there was good support for the sister relationship between *Nucras* and *Heliobolus,* and this clade was retrieved as sister to a monophyletic *Pedioplanis.* The monophyly of the three genera was well supported but conflicts with previous topologies based on morphology and mtDNA (Arnold, 1989; Harris *et al.,* 1998a; Fu, 1998, 2000). The close relationship between *Nucras* and *Heliobolus* (but not *Pedioplanis*) is consistent with Fu (2000). In this study *Meroles* is retrieved as sister to *Ichnotropis* although with poor support. This is in agreement with other molecularly derived patterns of relationship (Harris *et al.,* 1998a) but is contrary to the morphological as well as combined data inferences made by the same study. Nonetheless, for a few characters, such as tongue color, *Meroles* and *Ichnotropis* exhibit alternative states to *Nucras*, *Heliobolus* and *Pedioplanis* (Arnold, 1989).

2. 5. 2. Phylogenetic relationships within *Pedioplanis*

The monophyly of the genus *Pedioplanis* is well supported, corroborating previous studies based on morphology (Arnold, 1989, 1991) and protein electrophoresis data (Mayer & Berger-Dell'mour, 1988). This study strongly supports *P. burchelli* and *P. laticeps* as sister to all other species in the genus, in contrast to the morphologically derived phylogeny of Arnold (1991). He suggested that *P. lineoocellata* was sister to all the species in the genus but admitted that the evidence for this was not satisfactory as the

characters on which this inference was based, such as axillary mite pockets, loss of pterygoid teeth and the position of outer connectors of the hemipenes, were unreliable, of uncertain polarity, or not scored for all taxa. However, the close relationship between *P. burchelli* and *P. laticeps* based on hemipenial structure and general morphology has never been in doubt (Arnold, 1986, 1991). Indeed, these taxa have often been confused and a clear delimitation of species boundaries and ranges, especially for *P. laticeps*, is at present problematic (B. Branch, pers. comm.).

The combined gene tree suggests that *P. breviceps / P. lineoocellata* share a close evolutionary relationship but this is not well supported in parsimony or Bayesian analysis. Arnold (1991) suggested that *P. lineoocellata*, *P. burchelli*, *P. laticeps*, and *P. breviceps* are all closely related since they share the derived features of exposure of the ectopyrgoid as a lateral facet below the jugal bone and 25 presacral vertebrae in males. The molecular data, however, suggest that these "derived features" represent the symplesiomorphic state, which is more parsimonious.

Pedioplanis namaquensis with a wide distribution throughout Namibia and South Africa is strongly supported as sister to a clade consisting of *P. husabensis* and the "P. undata" species complex. According to Arnold (1991) this group (*P. namaquensis* + *P. husabensis* + "P. undata") shares derived genital features. *Pedioplanis namaquensis* itself consists of two geographically distinct clades, one in the Northern Cape Province of South Africa and the other in Namibia. These two lineages are separated by genetic distances of between 18%-20%, 5%-6%, 1%-3% for the ND2, 16S rRNA and RAG-1

genes, respectively. Samples from southern Namibia were not available, and with the present sampling it is difficult to determine where the boundary between the two forms lies. Alternatively, geographically intermediate populations could reveal these two clades to be an artifact of isolation by distance in a widespread species. Although these two lineages are genetically distinct, they show no obvious pattern of morphological differences. Specimens described from Kalkfontein, southern Namibia by Hewitt (1926) were initially assigned subspecific status (*P. n. quadrangularis*), but FitzSimons (1943) found no morphological characters to distinguish it from the nominate race and thus did not recognize the subspecies. Bauer *et al.* (1993) suggested that specimens from Hoanib River in Namibia might differ from the typical form, although they did not elaborate of the specific nature of the morphological differences and called for further investigation. Although it is difficult to delineate species based on sequence divergence only, the level of divergence found between these two lineages is significantly higher than between some species in this group, i.e, within the "P. unadata" species complex. A population genetic revision of *P. namaquensis* is required to investigate the possible validity of *P. n. quadrangularis* and to assess the morphological variation across the range of the species in light of its significant intraspecific genetic divergence. Range extension within this species has been recently recorded from Buffelsklip in the Little Karoo (Branch & Bauer, 1995). Specimens from this locality lack the regular barring associated with the Namibian and Namaqualand individuals although their colourations are similar to those from the central Karoo populations.

A tissue sample of *P. benguellensis* was not included in the current study. However, it is morphologically similar and thought to be to closely related to *P. namaquensis* (Bill Branch pers. com.). Indeed, because of a lack of clear morphological differences, Mertens (1955) considered it a synonym of *P. namaquensis*. *Pedioplanis benguellensis* is restricted to northern Namibia extending northwards into south of Angola (Branch, 1998). It is thus important to realize that specimens from northern Namibia, for instance ABD54 from Otjendeka (Figs. 2, 3 and Table 1) could potentially have been misidentified as *P. namaquensis* and in effect represent *P. benguellensi*. From the analyses, this specimen is sister to the Namibian lineage of *P. namaquensis*. More specimens from this region need to be examined, both morphologically and molecularly to asses the validity and distinctness of *P. benguellensis*.

2. 5. 3. The "P. undata" species complex

Contrary to the phylogeny proposed by Arnold (1991), which placed *P. inornata* as sister to *P. namaquensis* and *P. husabensis*, this study shows that the "P. undata" complex group is monophyletic and sister to *P. husabensis*. All the currently recognized taxa within the "P. undata" species complex were found to be monophyletic except *P. inornata*, which is made up of two distinct lineages, one from central Namibia and the other from southern Namibia and Northern Cape Province, South Africa. Due to low levels of genetic support, the relationships amongst members of the "P. undata" species group remain unclear; only the sister relationship between the *P. gaerdesi* and the central Namibian lineage of *P. inornata* is well supported. The phylogenetic relationships presented here for this species complex should therefore be considered tentative. The two

lineages of *P. inornata* are moderately divergent, with genetic distances between the two lineages varying from 7%-8%, 3%-4%, 1%-2% for the ND2, 16S rRNA and RAG-1 genes, respectively. These are, however, well within the ranges of between-species divergence for other recognized *Pedioplanis,* and given the strong support for paraphyly, it is suggested that the two forms be elevated to species level. This is consistent with Mayer & Berger-Dell'mour's (1987) suggestion that two forms of *P. inornata* occur parapatrically in Namibia, one with a limited distribution in west-central Namibia and the other widespread in southern Namibia and extending into northern South Africa. The southern form, characterized by brownish or reddish coloration and greenish spots, is correctly associated with the name *P. inornata*, which was described from the Orange River (Roux, 1907). The northern form, with a distinctive grayish fore body reddish hind body and yellow spots, may be specifically distinct and will be the subject of further investigation. On the other hand, the data does not support the recognition of two genetically distinct forms of *P. undata* and is thus consistent with Mayer & Berger-Dell'mour (1987), who considered these "forms" as probable color morphs rather than distinct evolutionary lineages.

2. 5. 4. Subspecific relationships within *P. lineoocellata*

The relationships amongst the currently recognized subspecies of *P. lineoocellata* appear more complicated than previously thought. The specimens from the Waterberg District, Limpopo Province, South Africa, previously assigned to *P. l. pulchella* (Jacobsen, 1989; Branch, 1998), are basal to all other *P. lineoocellata* specimens. In addition, the sample from Lüderitz Bay (*P. l. inocellata*) is sister to the samples assigned to *P. l. lineoocellata*

and the remainder of *P. l. pulchella*. This renders the subspecies *P. l. pulchella* paraphyletic. *Pedioplanis l. lineoocellata* and *P. l. pulchella* are morphologically and ecologically distinct (FitzSimons, 1943; Branch, 1998). Based on this, Bauer & Branch (2001) proposed that the two subspecies should be raised to specific status. *Pedioplanis l. inocellata* from Lüderitz Bay is also morphologically distinct (Mertens, 1955; Haacke, 1965; Branch, 1998). The high level of divergence between the Waterberg specimen and all other specimens belonging to the species for ND2 (9%-10%), for 16S and RAG-1 (1%-2%) genes indicates that they are an independently evolving lineage; it is therefore suggested that they too should be elevated to specific status. As no previously proposed names are available for this form, a full species description will be presented elsewhere. In addition, *P. lineoocellata* from Roodeplaat in South Africa, another population will be interesting to include in future studies (Jacobsen, 1989), was not considered here.

2. 5. 5. Biogeography of *Pedioplanis* in southern Africa

There are two well define, strongly supported biogeographic groups within *Pedioplanis* (Fig. 4, Group I and II). Group I consists of *P. burchelli* and *P. laticeps*, which are endemic to South Africa. Group II consists of *P. lineoocellata, P. namaquensis,* which are wide spread, and the remaining species. The relaxed Bayesian clock estimate suggest that the two geographical groups diverged during the mid-Miocene, which was characterized by unstable climate and is thought to be a period of major habitat change in the region (Linder, 2003, 2005). Within group II, the rest of the species form a coherent, chiefly Namibian assemblege (*P. husabensis*, *P. rubens*, *P. gaerdesi*, *P. undata* and *P. inornata*) group, with only *P. undata* extending its range in to southern Angola. The only

misplaced element is *P. breviceps,* which although endemic to Namibia, does not group within the Namibian group.

Speciation in the Namibian species occurred between the mid-Miocene and Pliocene. This is after the development of the Benguela Current along the west coast of southern Africa (Siesser, 1980), which is thought to have increased the aridity in the Namib Desert and may have been responsible for the rapid radiation (indicated by short branch lengths) in the Namibian group, particularly in the "P. undata" species complex (Figs. 2 and 3). These events have also been associated with speciation in other lizard groups such as *Meroles* (Arnold, 1991; Lamb & Bauer, 2003), *Pachydactylus* group geckos (Bauer & Lamb, 2005), and the desert plated lizard, *Gerrhosaurus skoogi* (Lamb *et al.,* 2003). The changes in aridity in the region could also have played an important role in habitat changes and especially the extensive sand deposition during the Miocene (Lancaster, 1990). This could have been reinforced with major habitat changes, which have been ascribed to climate change during the Quaternary (Vrba, 1995; Partridge *et al.,* 1987, 1995; Van Zinderen Bakker & Mercer, 1986). Together it is hypothesized that the climate changes caused isolation and fragmentation of the habitats and, in the process, shifted populations.

The phylogenetic relationships indicate a steady shift from the more mesic habitats occupied by species in Group I, i.e., from *P. burchelli* found in Cape fynbos and montane grassland, to the extremely habitats of the Namib Desert inhabited by *P. gaerdesi* of the Namibian group. This is in agreement with Arnold's (1981) model of speciation in which

competition, displacement and adaptation are important processes leading to speciation as populations colonize more extreme habitats. *Meroles*, which is a typical desert lizard group with almost all species having part of their range in the Namib Desert (Branch, 1998; Harris *et al.*, 1998b; Lamb & Bauer, 2003), clearly depicts this model. *Pedioplanis* shows similar patterns with the more recently divergences (within group II) associated with the dry habitats of the Namib Desert or surrounding dry savannah habitats. There has been a higher rate of speciation in the drier habitats, particularly in the Namibian species, with over 50% of the taxa in this group.

In Namibia, the Western Escarpment forms a zoogeographical transition zone with savannah in the east and Namib Desert in the west. Mayer & Berger-DellMour (1987) classified the various forms of "P. undata" species complex as either being Pro-Namib or Namib and/or having southern or northern transition distributions in Namibia. Although the Western Escarpment seems to be a barrier between *P. gaerdesi* and *P. undata*, the two lineages of *P. inornata* are divided into northern and southern populations separated roughly along the Swakop River. These west-east and north-south transition zones have also been demonstrated to have played a role in the evolution of the *Pachydactylus punctatus* group (Bauer & Branch, 1995; Bauer, 1999) and possibly in *Trachylepis sulcata sulcata* versus *T. s. ansorgii* (Bauer *et al.*, 1993).

Most of the basal species in the groups have very wide distributions, for instance *P. burchelli*, *P. lineoocellata* and *P. namaquensis*. However, isolation due to restriction to particular habitat types seems to have also played a role in the divergence between sister

species in this group. The separation between, *P. burchelli versus P. laticeps*, *P. lineoocellata versus P. breviceps*, and *P. undata versus P. rubens* is mainly due to habitat preference. For instance, *P. rubens* is restricted to red sandstone bedrocks in the Waterberg Plateau in Namibia, and *P. husabensis* is only found in the rocky desert between the Khan and Swakop rivers in the Husab Mountains (Branch, 1998). These are the only two members of Group II that are rock dwelling (Branch, 1998; Mayer & Richter, 1990). It has been suggested that they may have diverged from their relatives as a result of adaptation to a rupicolus lifestyle. Substrate specificity due to competition has been suggested to play an important role in evolution of other regional reptiles groups, for instance geckos (Bauer, 1999; Bauer & Lamb, 2005).

Chapter 3: Phylogeographic patterns in Burchell's sand lizard, *P. burchelli,* in South Africa

3. 1. Introduction

Pedioplanis burchelli is one of two species in the genus endemic to South Africa. Widely distributed, it ranges from Cape Agulhas, through the Cape Fold Mountains and inland along the escarpment to the eastern Free State, Lesotho and south-eastern Mpumalanga (Fig. 5). The species mainly inhabits montane fynbos in the south-western Cape and grassland in the central and eastern part of its range. In the southwestern part of its distribution, however, it is thought to extend into succulent Karoo and coastal fynbos (Branch, 1998). Because of its wide distribution across South Africa, *P. burchelli* could serve as a model to determine broad scale phylogeographic patterns within the region. The phylogeographic structure is dependent on various biotic and abiotic factors (Avise, 1994). For example in the present study, *P. burchelli* is restricted to predominantly rocky montane habitats and, due to limited dispersal, might show patterns of genetic structure between different mountain ranges. Although there is potential for phylogeographic structure in this species, no morphologically distinct populations have yet been identified.

Phylogeography provides insights into understanding the evolutionary processes, history and origins of populations and species (Avise, 1994, 1998, 2000). Evolutionary processes such as range expansion, gene flow, or long distance migration leave their imprints in the distribution of genetic variation within and among populations (Crandall & Templeton,

1993). Several studies on southern African reptiles have demonstrated phylogeographic structure within a species or closely related species. For example, in *Pachydactylus namaquensis,* three separately isolated lineages were identified in southern African region (Branch *et al.,* 1996), while Branch *et al.* (1995) found five well-defined lineages in *Phyllodactylus lineatus*. More recently Matthee and Flemming (2002) defined three distinct geographic assemblages within *Agama atra* in the region and subsequent fine scale analyses distinguished at least another four separate assemblages (Swart, 2006). Clear geographic partitioning of lineages has also been detected in cordylid lizards (*Cordylus*; Daniels *et al.,* 2004), dwarf chameleons (*Bradypodion;* Tolley *et al.,* 2004, 2006) and geckos (*Pachydactylus*; Lamb and Bauer, 2000). These patterns are not only restricted to South African reptiles but extend to mammals (Prinsloo & Robinson, 1992; Matthee & Robinson, 1996, 1999; Rambau *et al.,* 2003), birds (Bowie *et al.,* 2003; Bowie *et al.,* 2005) and invertebrates (Daniels *et al.,* 2001; Gouws & Daniels, 2003). Plants also show fragmented distribution patterns in some instances (Hughes *et al.,* 2004). Congruent phylogeographic patterns among multiple unrelated taxa can provide strong evidence for vicariance, which in turn is critical to help explain patterns of regional diversity.

Mitochondrial DNA (mtDNA) has long been used as the marker of choice in phylogeographic studies (Riddle, 1996). Data from mtDNA allow the estimation of only one specific component of the pedigree, namely the maternal phylogeny. A maternally inherited genome is valuable for phylogeographic studies because of the relatively quick rate of fixation due to the uniparental mode of inheritance. (Avise *et al.,* 1987; Avise, 1994).

3. 2. Aims

In this study mitochondrial DNA (ND2) is used to determine the:

(i) phylogeographic structure in *P. burchelli* in South Africa

(ii) historical factors influencing genetic structure in *P. burchelli*.

3. 3. Materials and Methods

3. 3. 1. Sampling

In total, 96 samples were collected across the distribution range of the species (Fig. 5; Table 2). Tissues (tail clipping or liver, in the case of voucher specimens) were preserved in 95% ethanol. Individuals of *P. laticeps* and *P. lineoocellata* were included as outgroup taxa (see chapter 2). Specimesn were identified by the respective collectors. When there was potential doubt, specimen identitity was re-confirmed by Bill Branch or LeFras Mouton.

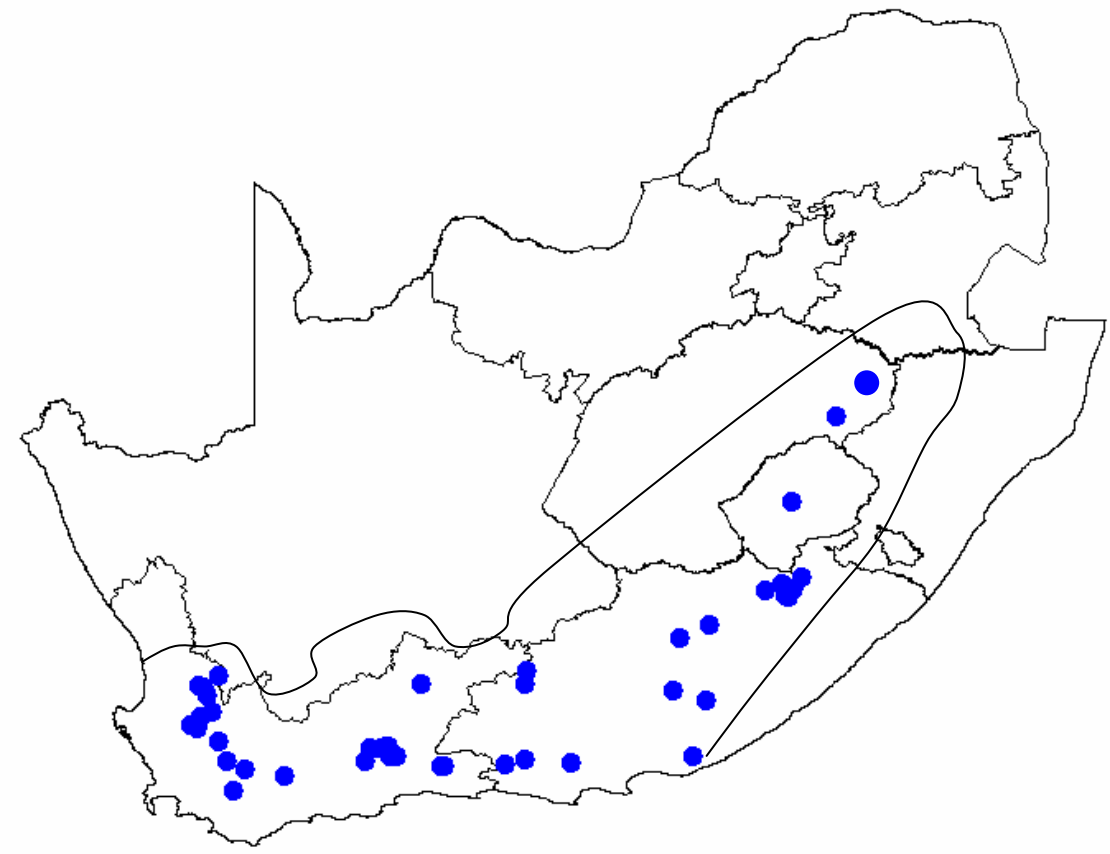


Fig. 5. The distribution of *P. burchelli* indicated by black line (Branch, 1998) and the sampling localities from the present study indicated by blue dots.

Table 2. Specimens used in this study with their locality information. Also geven are geographical co-ordinates in decimal degrees, altitutude in meters (if available) and GeneBank accession numbers for each haplotype. Collectors code: KTH & KAT = Krystal Tolley (tissue accessioned at the South African National Biodiversity Institute), CF, LF, MH, V, VC, EL & ELN = Cape Fold Herp project (tissue only, no voucher specimens); JSM = Jane S. Makokha (voucher specimens pending accession in Port Elizabeth Museum, South Africa). The selected outgroups are *P. laticeps* (first six) and *P. lineoocellata* (next two) to *P. burchelli* (ingroup).

Collectors Code	**Locality**				**GenBank Accession**
		Dlong	Dlat	Alt	
	Outgroup				
KTH222	Tankwa Karoo, Western Cape Province	-32.4906	19.2656	389	**DQ871069**
JSM015	Tankwa Karoo, Western Cape Province	-32.1184	19.4286	362	**DQ871066**
JSM016	Tankwa Karoo, Western Cape Province	-32.1184	19.4286	362	-
JSM029	Touwsfontein Anysberg Nature Reserve, Western Cape Province	-33.3234	20.2979	627	-
JSM032	Touwsfontein Anysberg Nature Reserve, Western Cape Province	-33.3234	20.2979	627	-
JSM039	Allermogan siding, Anysberg Nature Reserve, Western Cape Province	-33.3099	20.2517	616	-
JSM019	Ceres Karoo, Bezantsgat Farm, Western Cape Province	-32.4955	19.5927	616	-
JSM095	Quaggas drift farm, Murraysburg, Western Cape Province	-32.17611	24.1228	1633	-
	Ingroup				
KAT11	Kammanassie Mountains, Western Cape Province	-33.61917	22.85722	1500	**DQ925390**
PBCC1	Cockscomb, Groot Winterhoek Mountains, Eastern Cape Province	-33.57222	24.80639	1155	**DQ925381**
KTH015	Groot Winterhoek Wilderness area, Western Cape Province	-32.99556	19.10083	1049	**DQ925408**
KTH031	Groot Winterhoek Wilderness area, Western Cape Province	-33.04917	19.15111	1329	**DQ925405**
KTH032	Groot Winterhoek Wilderness area, Western Cape Province	-33.04917	19.15111	1329	**DQ925407**
KTH038	Groot Winterhoek Wilderness area, Western Cape Province	-33.03306	19.14083	1144	-
KTH137	Waboomberg near Ceres, Western Cape Province	-33.2519	19.47920	1429	**DQ871064**
LF046	Semonkong, Central Lesotho	-29.7944	28.0378	2270	**DQ925379**
LF002	Semonkong, Central Lesotho	-29.8575	28.0442	2227	**DQ925378**
PBBK1	Smutsberg, Kouga Mountains, Eastern Cape Province	-33.60694	23.80694	1145	**DQ925388**
PBBK2	Smutsberg, Kouga Mountains, Eastern Cape Province	-33.525	24.08944	1262	**DQ925389**

V324	Wyeneck plateau, Groot Swartberg Mountains, Western Cape Province	-33.36833	21.76194	1535	-
VC013	Wyeneck plateau, Groot Swartberg Mountains, Western Cape Province	-33.38306	21.76583	1410	-
VC184	Upperdiepkloof, Kammanassie Mountains,	-33.56080	21.70250	1230	**DQ925415**
MH0706	Bushman's Kloof, Die Galg Langeberg Mountains Western Cape Province	-34.0122	19.71139	720	**DQ925409**
MH0758	Turret Peak, Kouebokkeveld Mountains, Western Cape Province	-32.8725	19.19139	1562	-
MH0771	Die Trap, Cederberg Mountains, Western Cape Province	-32.4422	19.25056	1543	-
MH0334	Tafelberg, Cederberg Mountains, Western Cape Province	-32.3983	19.16333	1476	**DQ871062**
MH0870	Keeromsberg, Hex River Mountains, Western Cape Province	-33.56111	19.60417	2030	**DQ925392**
ENL04	Outeniqua Mountains, Western Cape Province	-33.3292	22.0342	1479	**DQ925382**
EL026	Swartberg, Western Cape Province	-33.34278	22.02389	-	**DQ925387**
EL037	Swartberg, Western Cape Province	-33.33500	22.03806	-	-
KTH344	Qwaqwa, Free State Province	-	-	-	**DQ925377**
KTH345	Qwaqwa, Free State Province	-	-	-	-
KTH343	Qwaqwa, Free State Province	-	-	-	**DQ925376**
KTH346	Qwaqwa, Free State Province	-	-	-	**DQ871065**
JSM01	Groot Winterhoek Mountains, Western Cape Province	-33.01530	19.05000	955	-
JSM04	Prince Albert side, Swartberg, Western Cape Province	-33.46970	22.18139	1380	**DQ925383**
JSM05	Prince Albert side Swartberg Western Cape Province	-33.46970	22.18139	1380	**DQ925384**
JSM07	Prince Albert side Swartberg Western Cape Province	-33.46970	22.18139	1380	**DQ925385**
JSM014	Swartberg, Western Cape Province	-33.46970	22.18139	1388	-
KTH526	Naudesberg, Langeberg Mountains, near Montagu, Western Cape Province	-33.69190	19.87750	1386	-
JSM044	Grahamstown, Howiesonspoort, Glenthorpe Farm Eastern Cape Province	-33.48611	26.65694	622	**DQ925375**
JSM045-54	Katberg Pass, junction to Adelaide & Post Retief, Didima range, Eastern Cape Province	-32.62917	26.85167	1611	**DQ925373**
JSM052	“	“	“	“	**DQ925374**

JSM056	Rd to Farm Ravinia from Molteno, Bamborsberg, Eastern Cape Province	-31.67167	26.43944	1641	**DQ925401**
JSM057-62	Rd to Smutspass, Stormsberg, Eastern Cape Province	-31.48861	26.89611	1870	**DQ925398**
JSM058	"	"	"	"	**DQ925399**
JSM060	"	"	"	"	**DQ925400**
JSM063-65	Barkly East Rd, 15 km before Rhodes, Eastern Cape Province	-31.02750	28.04722	1952	**DQ925397**
JSM066-69	Naudesnek, Barkly East, Eastern Cape Province	-30.76667	28.28778	2556	**DQ925395**
JSM070-76	Barlky East Rd, 10 km before Rhodes, Eastern Cape Province	-31.05111	28.10917	1943	-
JSM077-78	Glenfollan towards Lundean's Nek, Barkly East, Eastern Cape Province	-30.84500	27.98778	1895	-
JSM079-82	Farm Cloverley, Barkly East, Eastern Cape Province	-30.94889	27.74556	1783	**DQ925396**
JSM083-92	Debeers Pass, Winterberg, Eastern Cape Province	-32.48722	26.35083	1528	**DQ925402**
JSM088	"	"	"	"	**DQ925403**
JSM089	"	"	"	"	**DQ925404**
JSM093-94	Quaggas drift farm, Murraysburg, Western Cape Province	-32.17611	24.12278	1633	**DQ925393**
JSM096	Btn Farm Koueveld and Onderhougte, Murraysburg Western Cape Province	-32.37778	24.10028	1667	**DQ925394**
JSM097-98	Puttersvlei, Beaufort West, Western Cape Province	-32.37222	22.53833	1600	**DQ925391**
JSM099	Top of Anysberg Nature Reserve, Western Cape Province	-33.3169	20.3356	1457	**DQ925414**
MH0514	Matroosberg, Smitt Rd, Western Cape Province	-33.3748	19.6717	2059	**DQ925413**
MH0512	Matroosberg, Smitt Rd, Western Cape Province	-33.3748	19.6717	2059	**DQ925412**
MH0553	Matroosberg, Sonklip sinkel, Western Cape Province	-33.3627	19.6717	1952	**DQ925411**
MH0420	Swartberg, Western Cape Province	-	-	-	**DQ925386**
MH1114	Witkope, Upper sand stone slope, Free State Province	-27.7794	29.3531	2240	**DQ925380**

3. 3. 2. DNA amplification, sequencing and phylogenetic analysis

For the phylogeography study the mitochondrial ND2 gene was sequenced following the procedures for amplification, sequencing and alignment as outlined in chapter 2 of this thesis. To obtain a crude estimation of the evolutionary associations among *P. burchelli* samples, parsimony and Bayesian analyses were employed following the same methodology as specified in chapter two of this document.

3. 3. 3. Population level analysis

A median-joining network of haplotypes was constructed using Network v. 4.1 (Bandelt *et al.,* 1999) to investigate population processes and to make predictions about the ancestor-descendent relationships among haplotypes (Hudson, 1990). Haplotype and nucleotide diversity were estimated in the program ARLEQUIN v. 3.01 (Excoffier *et al.,* 2005). The amount of differentiation among and within populations was estimated by making use of an analysis of molecular variance (AMOVA) (Excoffier *et al.,* 1992). The overall AMOVA and corresponding pairwise comparisons were run using geographic groupings identified *a priori* by the median joining network. Both F_{ST} and Φ_{ST} values were estimated and their significance values were estimated using 1000 permutations. To examine potential group structure among sampling areas, spatial analysis of molecular variance (SAMOVA) was performed using SAMOVA 1.0 (Dupanloup *et al.,* 2002). The method examines and defines partitions of genetic diversity among the different geographic areas maximally and in the process determines whether there are any historically isolated lineages among geographic sampling areas.

The corrected genetic distances between groups and associated standard errors were computed in MEGA v. 3.1 (Kumar *et al.,* 2004) using the Kimura 2-parameter model with gamma distribution of the substitution rates and the shape parameter obtained in PAUP* 4.0b10 (Swofford, 2002). To determine whether genetic distance is a function of geographic distance, a Mantel test (Mantel, 1967) was used to investigate isolation by distance among clades. The test was implemented in the program Mantel (http://life.bio.sunysb.edu/morph/) using 10000 permutations. The corrected mean genetic distance and straight-line geographic distances (Km) were estimated in MEGA v. 3.1 (Kumar *et al.,* 2004) and ArcView GIS 3.2 respectively. To determine whether there were any recent demographic changes, a mismatch distribution analysis (Roger & Harpending, 1992) was done in Arlequin (Excoffier *et al.,* 2005). Fu's F_S (Fu, 1997) and Tajima's D (Tajima, 1989) tests of selective neutrality were performed in Arlequin to determine potential departure from neutrality, which could be interpreted as demographic changes in population numbers. Only clades with ten or more samples were considered for the mismatch distribution and selective neutrality tests.

To obtain further insights into the processes underlying the identified phylogeographic structure, a coalescent based program, MDIV (Nielson & Wakeley, 2001; Nielson, 2002) was used to simultaneously estimate parameters Theta ($\theta = 2N_{ef}\mu$), migration rate ($M = 2N_{ef}M$), time of population divergence ($T = t/N_{ef}$) and time to the most recent common ancestor (TMRCA = $t\mu$). Unlike the conventional F_{ST} statistics, this method allows for the assessment of the role of migration and isolation as the probable cause of structure between two given populations. Only *P. burchelli* clades with sufficient sampling (≥ 10

individuals) were considered for this analysis. Pairwise parameters were estimated using a MCMC analysis where each pairwise comparison was run twice for 5 million generations and a burn-in of 10% using the infinite sites model (Nielson & Wakeley, 2001). A mutation rate of 0.65% per million years for this gene region within the agamid lizards is available (Macey *et al.* 1998). The generation time of 2.09 years within lacertid lizards (Pedro, 1999) and the latter mutation rate were used to calculate parameters T and TMCA. The output values of θ, M, and T were plotted in Excel and the mode of their posterior distribution was selected as the most likely estimate. The 95% credibility intervals were estimated (for only θ and M values). To estimate T and TMRCA, each estimate was multiplied by θ for the particular pairwise comparison.

3. 4. Results

3. 4. 1. Phylogenetic relationships

The ND2 matrix consisted of 600 characters (305 constant, 47 variable but uninformative and 248 parsimony informative). Parsimony analysis retrieved 631 equally parsimonious trees (TL = 642; CI = 0.5935; HI = 0.4409; RI = 0.9358). In the Bayesian inference analysis the average split frequencies after the five million generation run was 0.005596. Bayesian results were largely congruent with parsimony analysis and rooted using *P. lineoocellata*. At least 50% of the nodes on the tree are supported by both parsimony bootstrap support (BS) (≥ 75%) as well as Bayesian posterior probability (PP) (≥ 0.95) (Fig. 6a).

The phylogenetic tree identifies six clades along the Cape Fold Mountains (CFM) and the Great Escarpment (GE) (Figs. 6a and 6b). The first group (eastern GE / Clade I) is monophyletic (100% BS; 1.0 PP) and consists of individuals from Grahamstown, Katberg, Witkope-Drakensberg, Lesotho-Drakensberg, and Qwaqwa-Drakensberg. This clade is basal to all other samples of *P. burchelli* (100% BS; 1.0 PP). The second well supported (100% BS; 1.0 PP) clade is from north-west CFM / Clade II (Cederberg, Groot Winterhoek Wilderness area, Turret Peak in the Kouebokkeveld). The third, central CFM / Clade III (Anysberg, Bushmanskloof-Langaberg, on Sneeukop in Koubokkeveld, Groot Swartberg in Wyneck Plateau, Waboomberg, Matroosberg, Upperdiepkloof-Kammanasie) forms the third essemblage (100% BS; 1.0 PP), while the fourth is located in the eastern CFM / Clade IV (85% BS; 1.0 PP) (Klein Swartberg, Kammanasie

mountains, Smutsberg-Kouga mountains, Outeniqua mountains, Cockscomb -Groot Winterhoek mountains). The fifth clade consists of individuals from central CFM (Langeberg and Keeromsberg) as well as the Great Escarpment (GE) (Beaufort West-Nuweveldberg). This clade is not supported by either the parsimony or Bayesian inference analyses. The last clade is from eastern central GE / Clade VI (Murraysburg, Stormsberg, Winterberg, Bamboorsberg, Barkely East- Drakensberg) is well supported in Baysian analysis (0.98 PP) but not parsimony (63% BS). The sister relationship between clade II and third clade III from CFM is supported in the Bayesian analysis (0.95 PP). However, the relationship among the other clades is not supported in the two phylogenetic analyses used in this study.

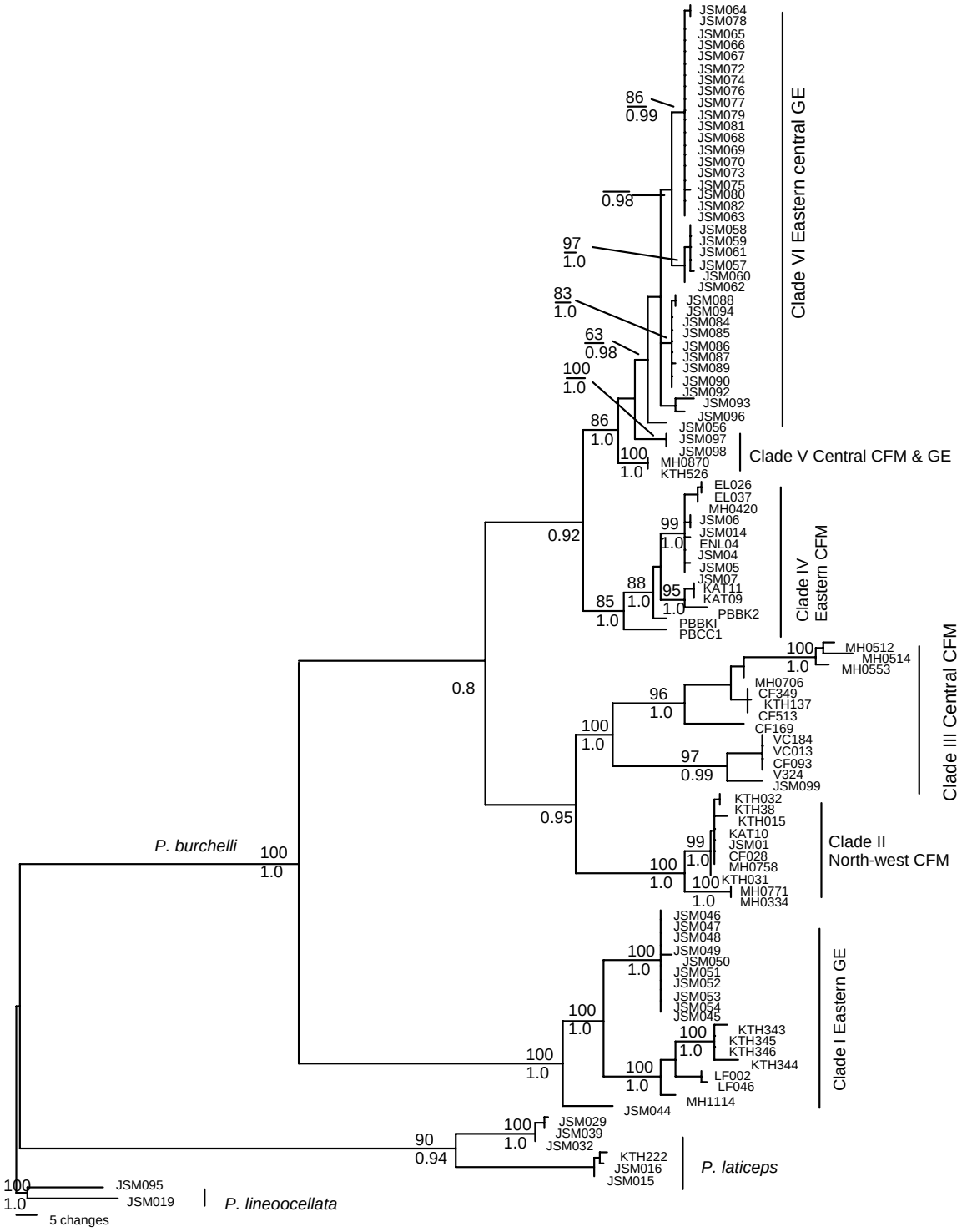


Fig. 6a. A phylogram from parsimony analysis showing the phylogenetic relationships among individuals of *P.burchelli* sampled in South Africa. Parsimony bootstrap support values are indicated above and Bayesian posterior probability below the nodes.

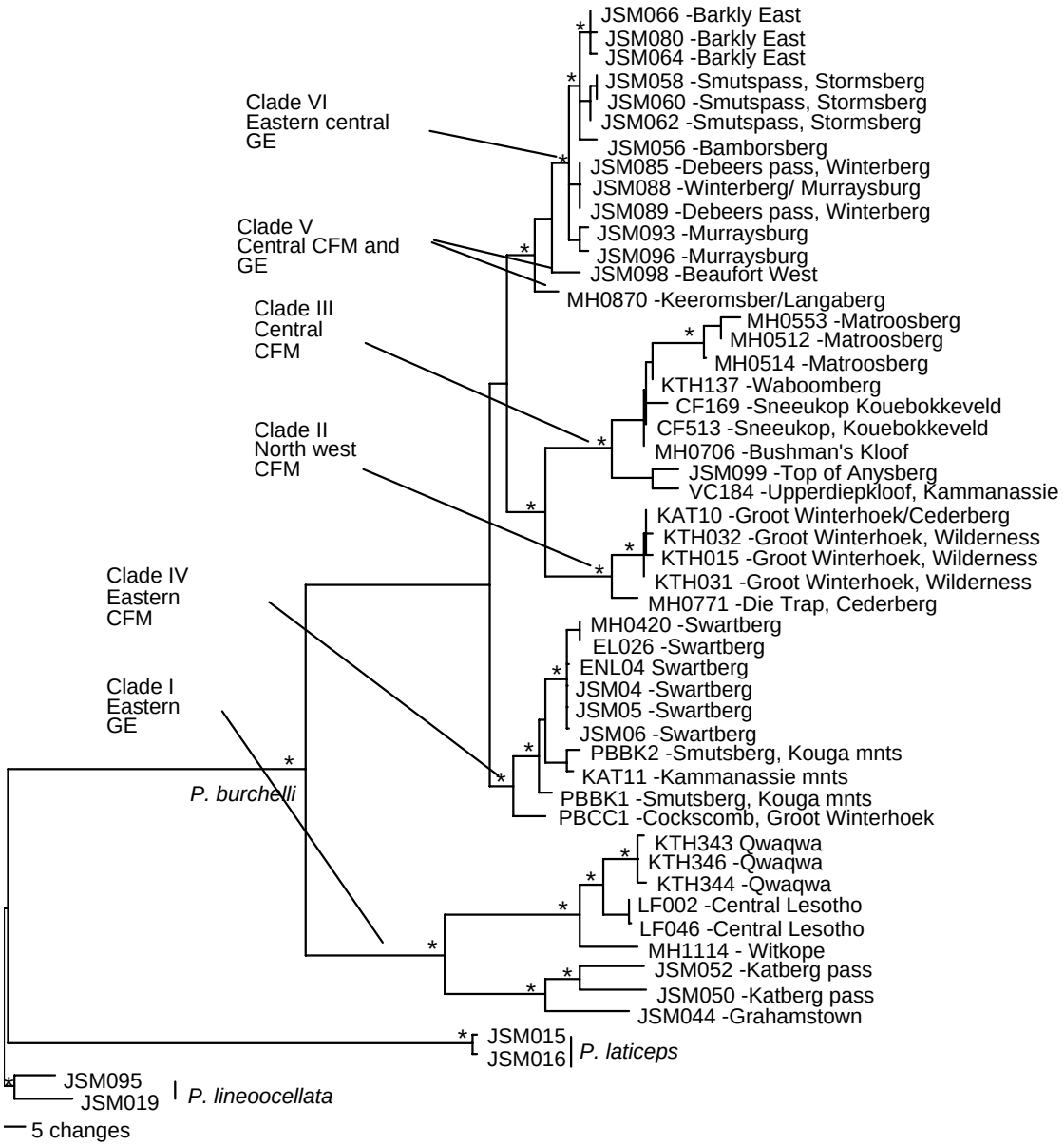


Fig. 6b. One of the most parsimonious trees phylogram based on 47 unique haplotypes with their localities obtained from all individuals of *P. burchelli* sampled. Asterisk indicates nodes that are significantly (≥ 75% BS, ≥ 0.95 PP) supported in either or both parsimony and Bayesian analyses as indicated in Fig. 6a.

3. 4. 2. Median-joining network

Overal, 47 unique haplotypes were identified from the 96 individual sequences. The haplotype network (Fig. 7a) shows the same six groups (clades) revealed by the phylogentic analyses (Figs. 6a and 6b), with a minimum of 15-20 mutational steps between any two clades. This means that these six clades could not be connected parsimoniously with the 95% confidence level, making the branching pattern subjective. However, the clades correspond with geography with limited overlap in spatial distribution (Fig. 7b). On a finer geographic scale, haplotypes were shared between sampling localities within clades: for example one haplotype was shared between the Cederberg and Groot Winterhoek sampling sites in clade II, while another haplotype was shared between Murraysburg and Winterberg in clade VI. There is also a large amount of intraclade variation, probably reflecting the retention of ancient polymorphisms. For example, within clade I, the Grahamstown sample is 28 steps away from the closest haplotype in the group.

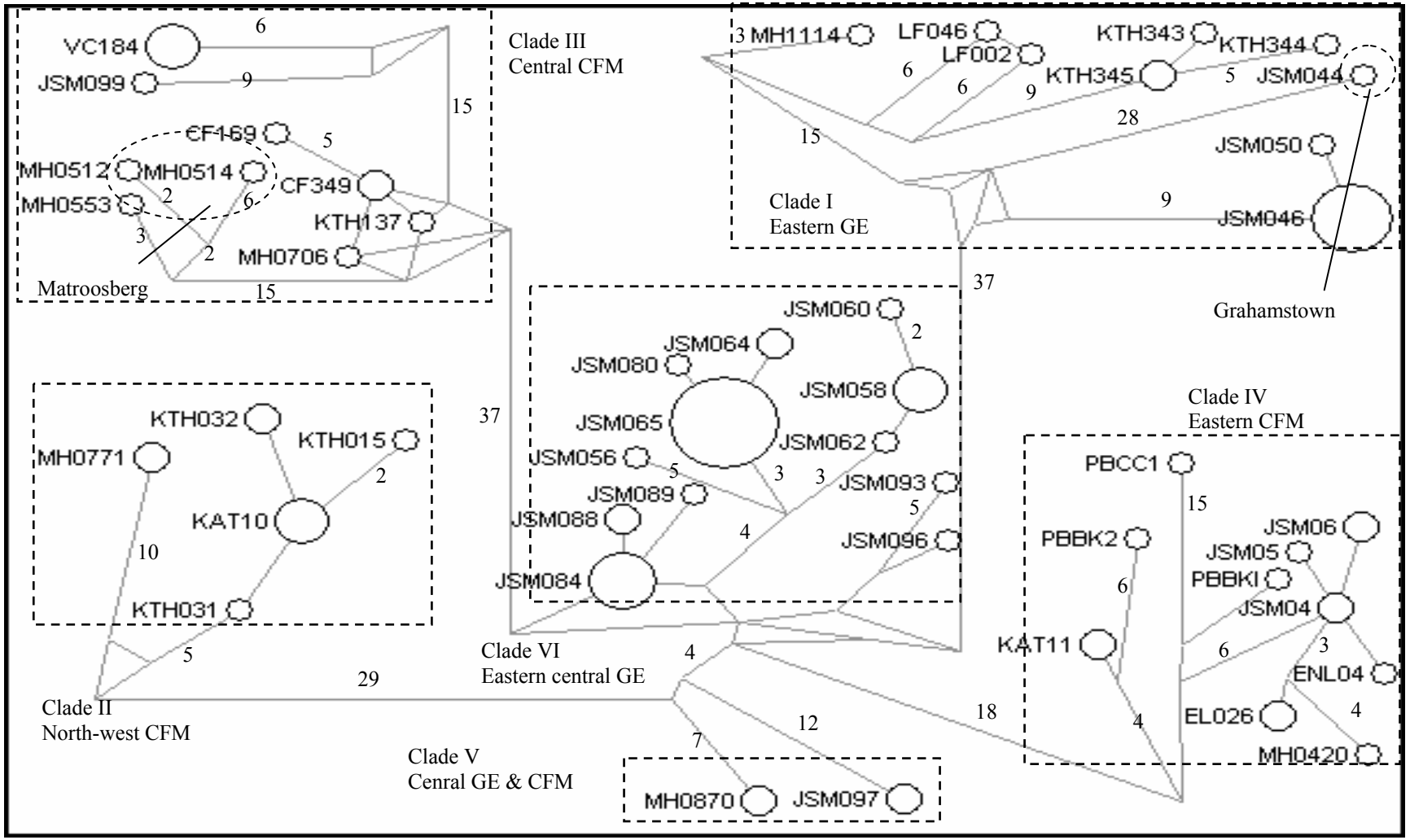


Fig. 7a. A median joining network of all the sequences analysed in this study with the circle size approximately proportional to the haplotype frequency. Connecting lines are proportional to single site changes unless otherwise indicated along the branches. The network shows six geographic assemblages (clades I-VI) indicated by the number of site changes alond the branches by dashed rectangles. Clade names correspond with Fig. 6a and 6b.

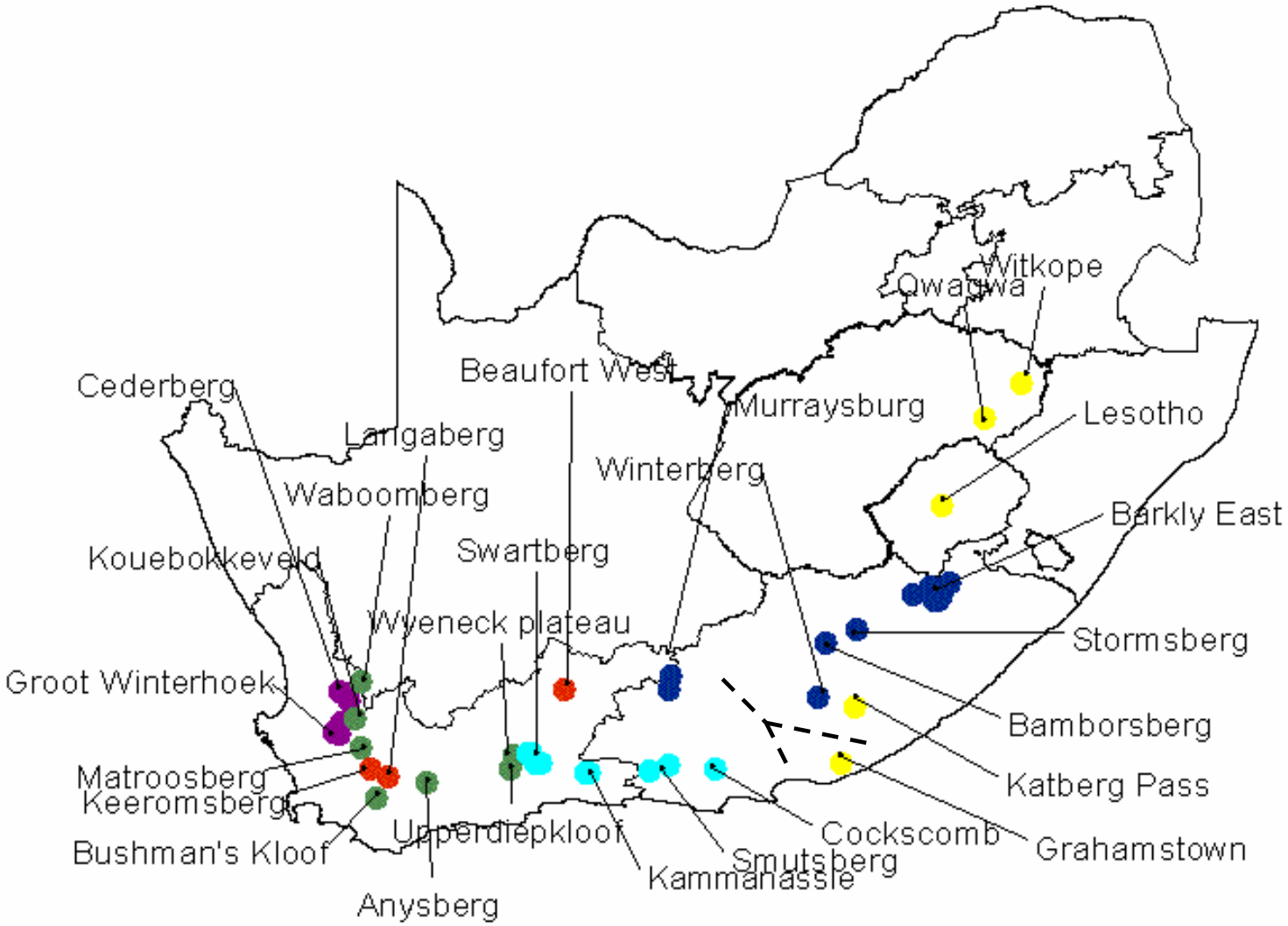


Fig. 7b. The spatial distribution of the six identified clades of *P. burchelli*; yellow is clade I, purple clade II, green clade III, light blue clade IV, red clade V and navy blue is clade VI. The black dotted lines indicate the isolation of the Grahamstown locality by Karoo vegetation along the "Bedford Gap".

3. 4. 3. Genetic structure and diversity

Haplotype as well as nucleotide diversity were rather high and ranged between 0.82 - 0.96 and 0.01 - 0.03 respectively (Table 3); this is comparable to other studies of lizards from this region, for instance in *Agama atra* (Swart, 2006). This suggests old stable clades with a lot of divergent haplotypes and is probably indicative of the absence of any recent bottlenecks. The range of the corrected genetic distance between clades was high (3% - 12.2%) (Table 4). The overall AMOVA of the six clades shows significant geographic structure for both F_{ST} and Φ_{ST} ($F_{ST} = 0.76$, $p < 0.01$; $\Phi_{ST} = 0.78$, $p < 0.01$). Pairwise comparisons were high between clades (Table 5) with the highest between clades II and VI ($F_{ST} = 0.88$, $\Phi_{ST} = 0.89$). All the pairwise comparisons were significant.

The SAMOVA indicated that variance among geographic regions is greatest when the samples are partitioned into eight groups (Fig. 8 and Table 6), whereas the phylogenetic analysis and network revealed only six clades. This could highlight the sensitivity of SAMOVA in identifying more geographic groupings relative to Network and phylogenetic methods. Within the CFM geographic region, SAMOVA separates Langaberg/Keeromsberg and Wyneck Plateau/Anysberg as two additional groups. In the GE, clade 1 is divided further into two separate groups: Katberg/Grahamstown and Qwaqwa/Lesotho/Witkope. The highest increase in F_{CT} values occured when eight groups were specified (bold Fig. 8), and although it increases further when more groups are specified, this is not substantially. This could be due to the high genetic variance within and between populations sampled in this study.

Table 3. The number of individuals, haplotypes, haplotype diversity and nucleotide diversity with their corresponding confidence intervals in each of the six clades of *P. burchelli*.

Clade	No. of individuals	No. of haplotypes	Haplotype diversity	Nucleotide diversity
I	18	9	0.863 +/- 0.080	0.039 +/- 0.020
II	10	5	0.822 +/- 0.097	0.011 +/- 0.006
III	13	9	0.923 +/- 0.069	0.032 +/- 0.018
IV	14	10	0.956 +/- 0.038	0.017 +/- 0.009
V	4	2	0.833 +/- 0.222	0.020 +/- 0.014
VI	37	12	0.875 +/- 0.042	0.009 +/- 0.005

Table 4. The corrected genetic distance (diagonal below) and the standard error (diagonally above) among the six *P. burchelli* clades estimated using Kimura 2-parameter model ($\alpha = 1.78$) in the program MEGA.

Clade	I	II	III	IV	V	VI
I		0.015	0.013	0.014	0.013	0.013
II	0.121		0.013	0.012	0.012	0.012
III	0.122	0.101		0.011	0.012	0.012
IV	0.121	0.081	0.092		0.009	0.009
V	0.109	0.085	0.090	0.058		0.006
VI	0.109	0.090	0.090	0.056	0.03	

Table 5. Pairwise AMOVA of the six clades of *P. burchelli* identified by the network with F_{ST} (below diagonal) values and Φ_{ST} (above diagonal) values estimated using Tamura & Nei distances ($\alpha = 1.78$) in Arlequin. All the values were significant ($p < 0.001$).

Clade	I	II	III	IV	V	VI
I		0.748	0.693	0.750	0.658	0.809
II	0.729		0.762	0.820	0.830	0.889
III	0.663	0.740		0.737	0.662	0.818
IV	0.726	0.806	0.713		0.693	0.796
V	0.636	0.817	0.636	0.677		0.608
VI	0.792	0.878	0.800	0.784	0.601	

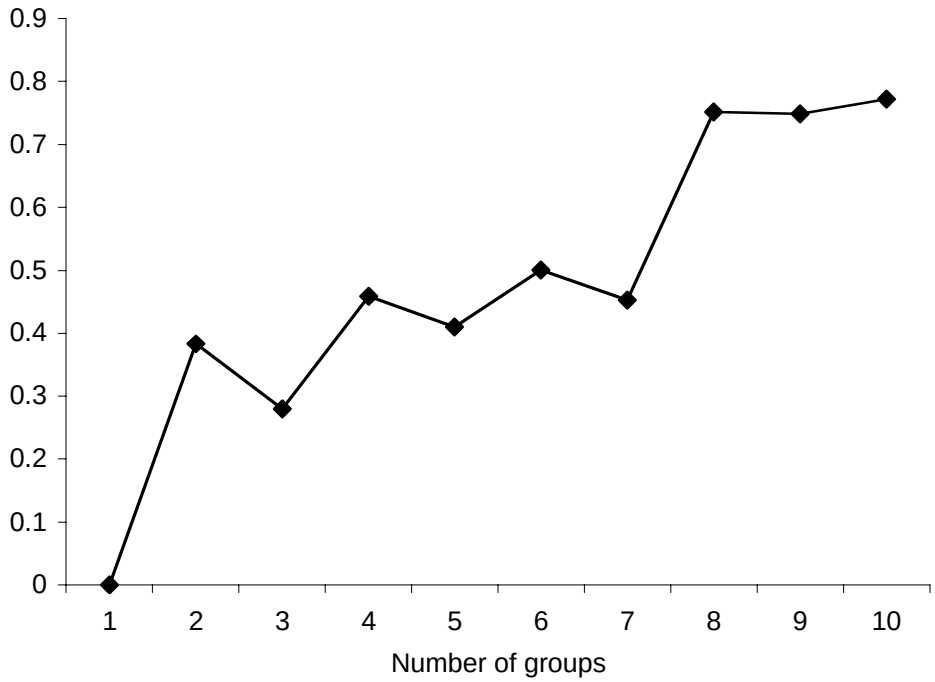


Fig. 8. Line graph showing the F_{CT} values plotted against the number of groups. The highest increase in F_{CT} value was when eight groups were specified. Also see Table 6 below.

Table 6. Statistics generated from SAMOVA based on 100 simulations where $P < 0.05$. The highest increase in the F_{CT} value was at eight groups (bold).

Number and name Populations in each group	**Significance test Values**	**% Source of variation**		
		Va	Vb	Vc
1. Katberg, Grahamstown, Cederberg, Groot Winterhoek, Wyeneck Plateau, Anysberg, Sneeukop, Waboomsberg, Bushmanskloof, Matroosberg, Cockscomb, Smutsberg, Kammanasie, Swartberg, Outeniqua Mnts, Beaufort West, Langerberg, Keeromsberg, Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg. 2. Qwaqwa, Lesotho, Witkope	$F_{SC} = 0.95796$ $F_{ST} = 0.97408$ $F_{CT} = 0.38343$	38.34	59.06	2.59
1. Qwaqwa, Lesotho, Witkope, Cederberg, Groot Winterhoek, Wyeneck Plateau, Anysberg, Sneeukop, Waboomsberg, Bushmanskloof, Matroosberg, Beaufort West, Langerberg, Keeromsberg, Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg 2. Cockscomb, Smutsberg, Kammanasie, Swartberg, Outeniqua Mnts 3. Katberg, Grahamstown	$F_{SC} = 0.95467$ $F_{ST} = 0.96733$ $F_{CT} = 0.27921$	27.92	68.81	3.27
1. Katberg, Grahamstown, Cockscomb, Smutsberg, Kammanasie Mnts, Swartberg, Outeniqua Mnts, Beaufort West, Langerberg, Keeromsberg, Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg 2. Qwaqwa, Lesotho, Witkope 3. Wyeneck Plateau, Anysberg, Sneeukop, Waboomsberg, Bushmanskloof, Matroosberg 4. Cederberg, Groot Winterhoek	$F_{SC} = 0.94387$ $F_{ST} = 0.96965$ $F_{CT} = 0.45930$	45.93	51.03	3.04
1. Katberg, Qwaqwa, Lesotho, Witkope, Grahamstown, Beaufort West, Langerberg, Keeromsberg, Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg 2. Cockscomb, Smutsberg, Kammanasie Mnts, Swartberg, Outeniqua Mnts 3. Wyeneck Plateau, Anysberg, Sneeukop, Waboomsberg, Bushmanskloof 4. Cederberg, Groot Winterhoek 5. Matroosberg	$F_{SC} = 0.94451$ $F_{ST} = 0.96731$ $F_{CT} = 0.41080$	41.68	55.65	3.27
1. Katberg, Grahamstown, Beaufort West, Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg 2. Cockscomb, Smutsberg, Kammanasie Mnts, Swartberg, Outeniqua Mnts 3. Wyeneck Plateau, Anysberg, Sneeukop, Waboomsberg, Bushmanskloof, Matroosberg 4. Qwaqwa, Lesotho, Witkope 5. Cederberg, Groot Winterhoek 6. Langerberg, Keeromsberg	$F_{SC} = 0.93304$ $F_{ST} = 0.96660$ $F_{CT} = 0.50118$	50.12	46.54	3.34

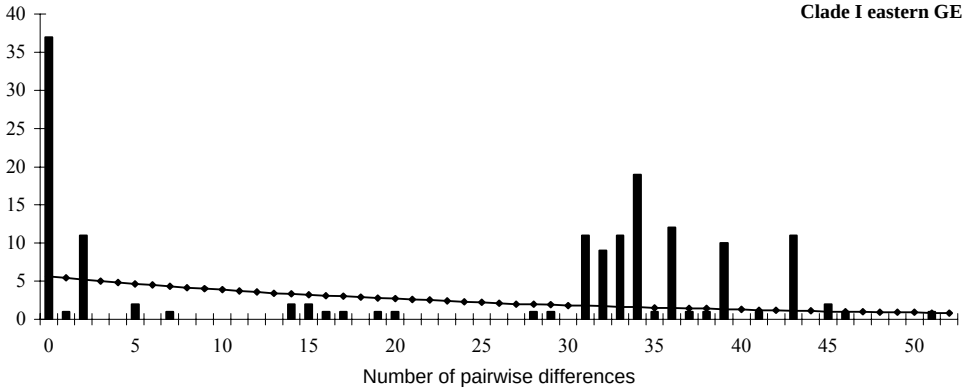
1. Katberg, Grahamstown, Cockscomb, Smutsberg, Kammanasie Mnts, Swartberg, Outeniqua Mnts, Beaufort West, Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg 2. Sneeukop, Matroosberg 3. Wyeneck Plateau, Anysberg 4. Cederberg, Groot Winterhoek 5. Qwaqwa, Lesotho, Witkope 6. Langerberg, Keeromsberg 7. Waboomsberg, Bushmanskloof	F_{SC} = 0.94294 F_{ST} = 0.96879 F_{CT} = 0.45303	45.30	51.58	3.12
1. Beaufort West, Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg 2. Qwaqwa, Lesotho, Witkope 3. Cockscomb, Smutsberg, Kammanasie Mnts, Swartberg, Outeniqua Mnts 4. Katberg, Grahamstown 5. Wyeneck Plateau, Anysberg 6. Sneeukop, Waboomsberg, Bushmanskloof, Matroosberg 7. Langerberg, Keeromsberg 8. Cederberg, Groot Winterhoek	F_{SC} = 0.86278 F_{ST} = 0.96592 **F_{CT} = 0.75165**	75.16	21.43	3.41
1. Beaufort West, Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg 2. Cederberg, Groot Winterhoek 3. Qwaqwa, Lesotho, Witkope 4. Wyeneck Plateau, Anysberg 5. Katberg, Grahamstown 6. Waboomsberg, Bushmanskloof 7. Langerberg, Keeromsberg 8. Cockscomb, Smutsberg, Kammanasie Mnts, Swartberg, Outeniqua Mnts 9. Sneeukop, Matroosberg	F_{SC} = 0.86402 F_{ST} = 0.96582 F_{CT} = 0.74864	74.86	21.72	3.42
1. Murraysburg, Winterberg, Stormsberg, Barklyeast, Bamborsberg 2. Sneeukop, Waboomsberg, Bushmanskloof 3. Matroosberg 4. Cockscomb, Smutsberg, Kammanasie Mnts, Swartberg, Outeniqua Mnts 5. Qwaqwa, Lesotho, Witkope 6. Katberg, Grahamstown 7. Cederberg, Groot Winterhoek 8. Wyeneck Plateau, Anysberg 9. Beaufort West 10. Langerberg, Keeromsberg	F_{SC} = 0.84825 F_{ST} = 0.96541 F_{CT} = 0.77207	77.21	19.33	3.46

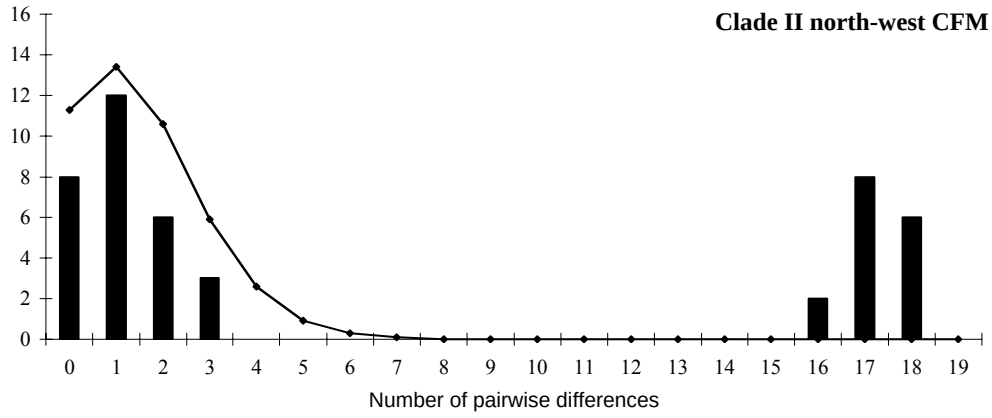
3. 4. 4. Demographic history, selective neutrality and isolations by distance

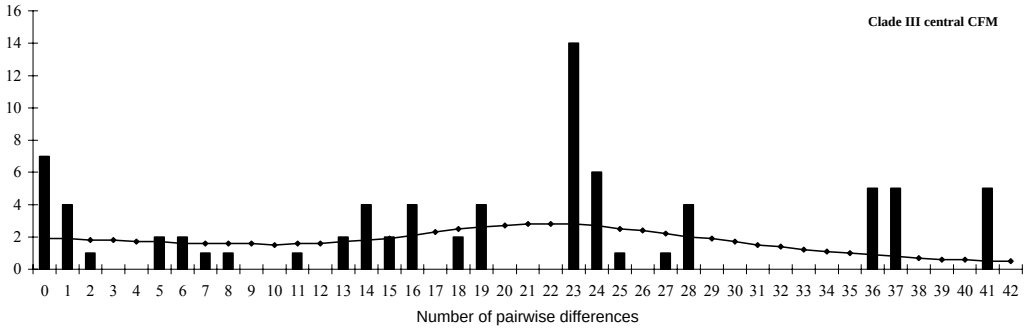
Mismatch distribution results of five clades separately demonstrate multimodal distributions (Fig. 9; Table 7). There was no significant difference detected between the observed distribution and that expected under a model of demographic change. The lack of significance does not necessarily suggest there has been a demographic change but that the null hypothesis of observed equals expected cannot be rejected. The more powerful tests of selective neutrality, Fu's and Tajima's tests of selective neutrality, were not significant for any of the five clades (Table 7). This could imply that either the populations have not undergone recent demographic changes (such as population expansion) and therefore the mitochondrial gene investigated has evolved in neutral fashion, or that selective swaps are not severely affecting the genetic signature. Ramos-Onsins and Rozas (2002) comparison of a number of statistical tests for detecting population growth found Fu's F_S to be one of the most powerful test for demographic changes in population numbers while the SSD (sum of square differences) test associated with the mismatch distribution to be the least powerful and conservative in a variety of cases. This could explain the contrasting results found between these two types of tests, and given the high level of mtDNA variation within clades, it is likely that there is no good evidence for any population expansion in the recent past.

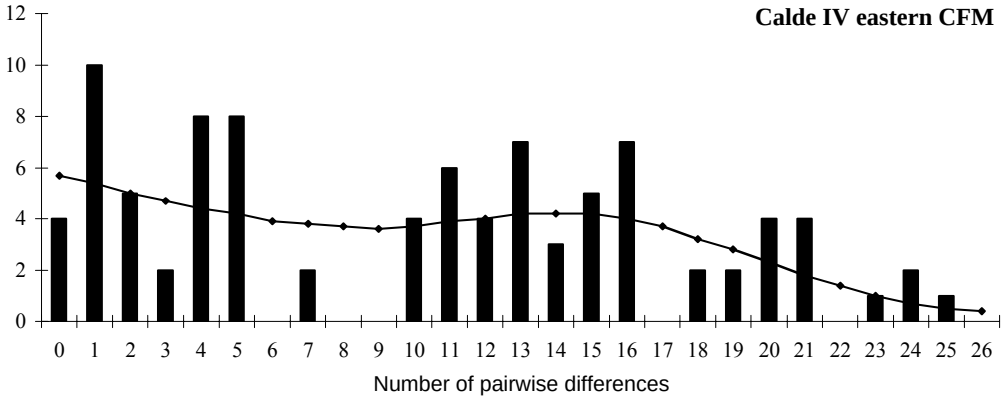
The Mantel test suggests that isolation by distance is not present (Fig. 10), as there was no correlation between genetic and geographic distance ($r = 0.31$, $z = 1143.1$ and $p = 0.89$). Clades I and VI were examined separately, because they have a wider geographic

coverage in comparison to other clades in this study, but also do not show isolation by distance (Clade I: r = 0.68, z = 294.2 p = 0.25; Clade VI: r = 0.42, z = 77.3, p = 0.85) (Fig. 10). This means that geographic distance did not play a significant role in the genetic structure among and within these clades.









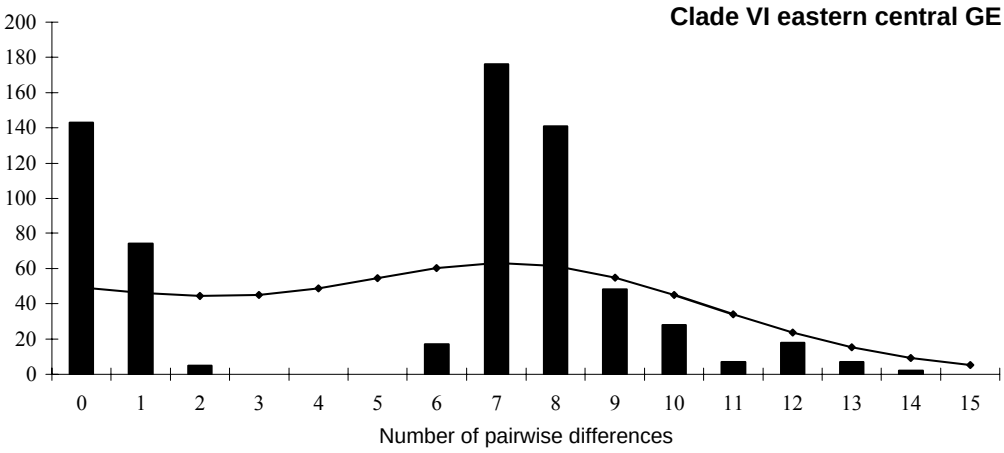
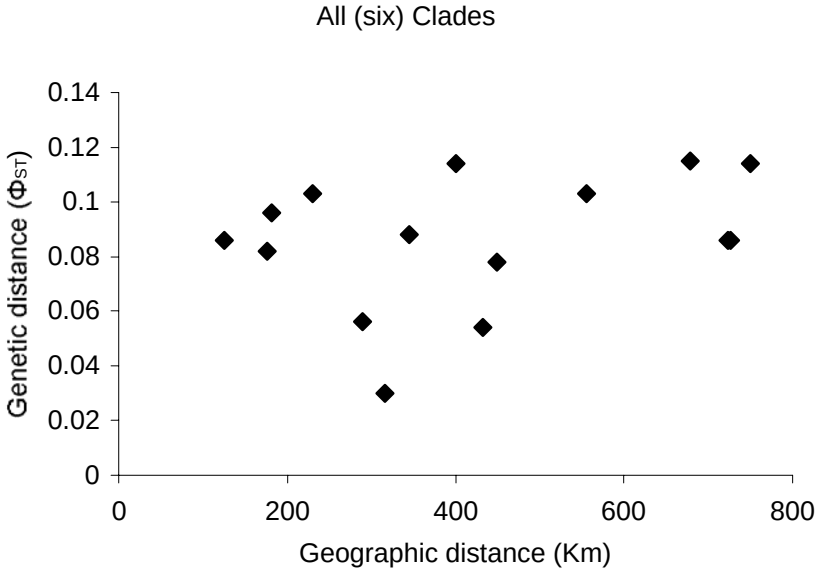


Fig. 9. Mismatch distributions of the five identified (n ≥ 10 individuals) *P. burchelli* clades (indicated in the right upper corner). The bars represent the observed and the line the expected differences.

Table 7. The SSD values from mismatch distribution and selective neutrality test using Tajima's D, Fu's F_S tests for five *P. burchelli* clades that had sufficient sample size (n ≥ 10 individuals) with their corresponding *p* value in parentheses.

Clade	SDD	D	F_S
I	0.027 (0.750)	-0.328 (0.412)	5.859 (0.985)
II	0.055 (0.89)	0.105 (0.576)	2.449 (0.875)
III	0.054 (0.17)	0.551 (0.756)	2.018 (0.819)
IV	0.019 (0.78)	-0.853 (0.206)	-0.369 (0.416)
VI	0.059 (0.20)	-0.618 (0.300)	0.094 (0.554)



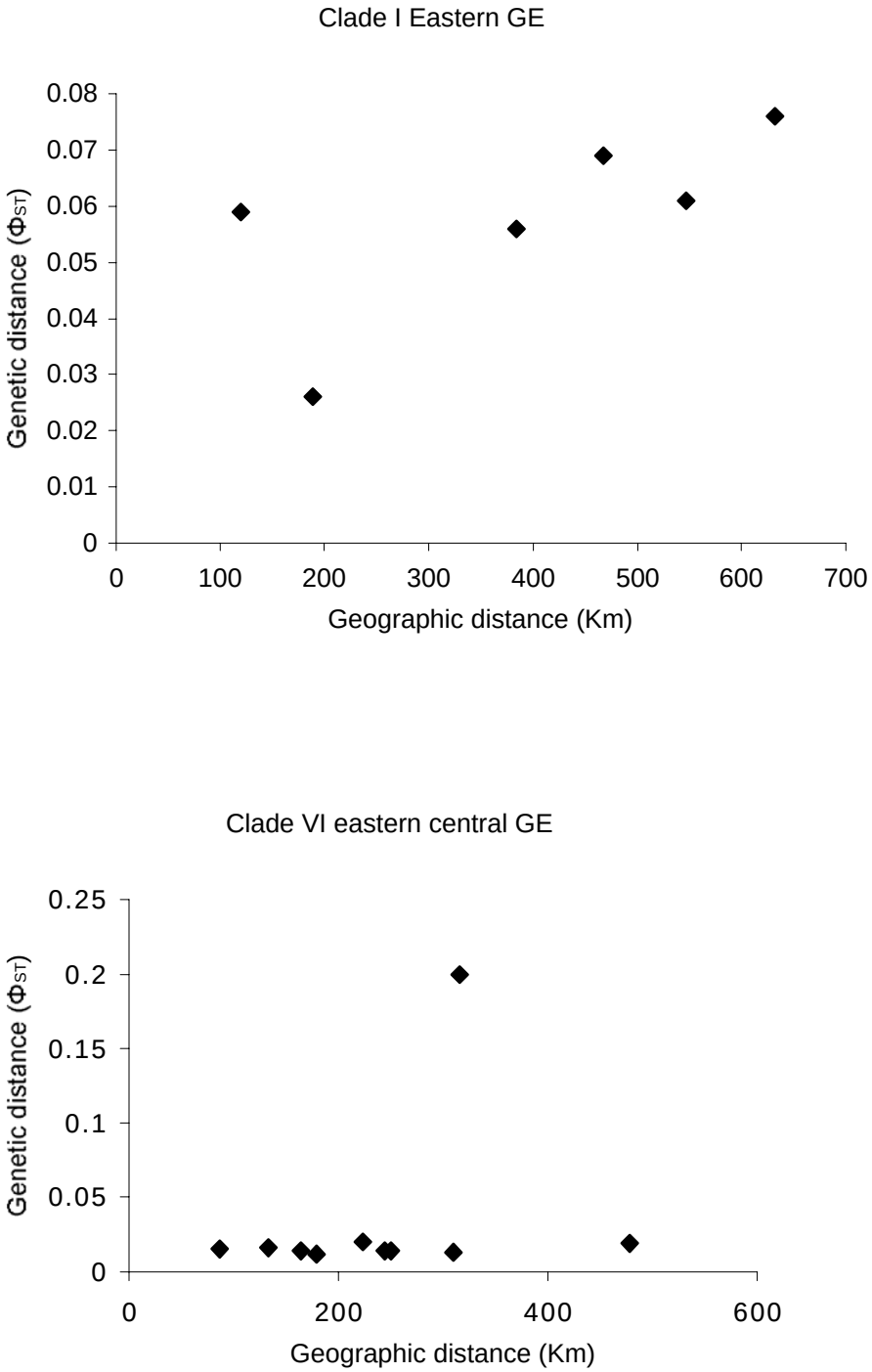


Fig. 10. The relationship between geographic and genetic distances between: all clades (I-VI), among sampling sites within clade I eastern GE (Katberg, Grahamstown, Qwaqwa and Lesotho) and among sampling sites within clade VI eastern central (Stormsberg, Bambosberg, Barkly East, Winterberg and Murraysburg).

3. 4. 5. Estimated time of divergence between *P. burchelli* clades

In the coalescent analysis, there is generally a huge disparity between the values of T and TMRCA for each of the comparisons, which could be an indication that although the respective populations began to diverge much earlier (Pliocene), there has been gene flow between them until the time the respective populations actually diverged in the Pleistocene (Table 8). The Clade I, (eastern GE) seems to have diverged first from the remaining CFM populations (4.2 - 4.7 MYA BP) and then the eastern-central, clade VI diverged around 3.9 MYA BP. The Cape Fold mountains clades diverge more or less at the same time between 3.4 - 2.7 MYA BP. The rate of migration among the five clades was very low, almost zero (Table 8).

Table 8. Pairwise estimates of female effective population size (θ) and migration rate (M) with the 95% credibility intervals in parantheses. The time of population divergence (T) and time to the most recent common ancestor (TMRCA) in millions of years ago before present (MYA BP) calculated using a mutation rate of 0.65% per million years and generation time of 2.09 years.

Clade	I	II	III	IV
II	θ =15.25 (10.32-26.41) M = 0.02 (0.0-1.12) T = 1.1 MYA BP TMRCA = 4.2 MYA BP			
III	θ = 24.89 (15.89-38.38) M = 0.02 (0.0-0.50) T = 0.49 MYA BP TMRCA = 4.7 MYA BP	θ = 15.53 (10.78-20.50) M = 0.02 (0.00-1.28) T = 0.55 MYA BP TMRCA = 3.2 MYA BP		
IV	θ = 19.71 (13.14-32.12) M = 0.02 (0.0-0.62) T = 0.72 MYA BP TMRCA = 4.2 MYA BP	θ = 10.72 (6.73-19.59) M = 0.02 (0.00-0.84) T = 0.91 MYA BP TMRCA = 2.6 MYA BP	θ = 20.41 (13.05-34.50) M = 0.02 (0.00-0.52) T = 0.42 MYA BP TMRCA = 3.4 MYA BP	
VI	θ = 13.93 (9.79-20.09) M = 0.02 (0.0-0.46) T = 1.2 MYA BP TMRCA = 3.9 MYA BP	θ = 7.29 (4.96-12.13) M = 0.02 (0.00-0.38) T = 2.1 MYA BP TMRCA = 2.7 MYA BP	θ = 13.00 (9.39-20.53) M = 0.02 (0.00-0.46) T = 0.84 MYA BP TMRCA = 3.1 MYA BP	θ = 10.67 (7.21-16.11) M = 0.02 (0.00-0.44) T = 0.49 MYA BP TMRCA = 1.9 MYA BP

3. 5. Discussion

3. 5. 1. Geographic patterns and genetic structure

Pedioplanis burchelli forms a monophyletic group that is well supported by both phylogenetic (chapter 2) and phylogeographic analyses. Within the species, at least six genetic clades were detected and these correspond to spatial distribution. The clades are well supported and separated by a large number of mutational steps. The distinct genetic structure is not entirely surprising given the habitat preference of the species, i.e. high altitude mountainous areas. Although FitzSimons (1943) suggests that this species also occur at low altitudes in the Cape Agulhas area, this has not been confirmed with any records in recent years. A similar disjunct pattern was obtained in other lacertid lizards occupying similar habitats, for instant Iberian rock lizard, *Iberolacerta* (Crochet *et al.*, 2004). The estimated rate of migration among female individuals of the respective clades was found to be virtually absent. Although very little is known about the dispersal capabilities of *P. burchelli*, the high level of divergence between these clades suggests fairly persistent barriers to gene flow among the clades. The genetic divergence between identified clades is estimated to have taken place during the Pliocene-Pleistocene period.

3. 5. 1. 1. Great Escarpment (GE)

Clade I is vastly divergent from the other clades, with corrected genetic distances of 10.9% – 12.2%. Genetic distance values among haplotypes within this clade were relatively high (3.9%), and this can be interpreted as either having been isolated from each other for a long time or due to sparse missing intermediate haplotypes. Clade I is widely distributed in the eastern mountains of the Great Escarpement in South Africa and

Lesotho. It is sister to all the other clades in the phylogenetic analysis. There is clear structure within this clade with populations from Grahamstown, Katberg, Qwaqwa, Witkope and Lesotho being separated from each other by several mutational steps (Fig. 6a). All these populations occupy grassland and summer rainfall regions. The Grahamstown sample is highly divergent from the other populations in the clade (with 28 steps), suggesting that individuals in this region are also geographically isolated from the other localities in this clade. The isolation by distance analysis among populations in this clade (Fig. 10) did not show a correlation between geographic distance and genetic distance. Therefore, the high genetic diversity between the Grahamstown and other populations in the clade could be attributed to isolation over time and not geographic distance. The exact reason for the isolation is uncertain but it is likely that the unsuitable habitat (Karoo vegetation) that extends from the interior along the 'Bedford Gap' into a fork (Lawes, 1990), can act as isolating barrier to the Grahmstown population, distinguishing them from the CFM in the west and grassland biome in the north (Fig. 7b).

The other GE clade (VI) is found more in the eastern-central part of the Great, Escarpment with populations from the Drakensberg Mountains in Barkely East, Stormsberg, Bamboorsberg, Winterberg and Murraysburg. Except for the Barkly East population, the rest are found on the mountain-tops in montane grasslands isolated by Karoo and Thicket vegetation in the surrounding areas not preferred by this species. One shared haplotype was found between Murraysburg and Winterberg. A separate analysis of isolation by distance performed for clade VI indicated there was no relationship between geographic and genetic distance. This could point to past fragmentation as the

most probable explanation for the observed divergence levels among sampling localities within this clade. In apparent constrast the other samples, localities from the Drakensberg mountain range (Lesotho and Qwaqwa) clustered in Clade I were highly divergent from the ones from the same mountain range in clade VI (corrected genetic distance of 10.9%). This might indicate no or restricted gene flow between the Drakensberg montain range localities or could be an artefact of inadequate sampling. The two clades (I and VI) from the GE only diverged from each other in the late pliocene (0.49 MYA BP).

3. 5. 1. 2. Cape Fold Mountains (CFM)

Clades II, III and IV are all from the CFM, although they do not form a monophyletic clade in the phylogenetic analysis. However, they are embedded between the two clades from the GE, meaning that some of the clades within the CFM *e.g.,* clade IV are more closely related to that from the GE than to those from the CFM. The sister relationship between clade II and III is well supported in Bayesian analysis (0.95 PP). Geographically, clades II (North-west CFM) and III (Central CFM) are also nearer to each other and these two are sister to clade IV (Eastern CFM). The reciprocal monophyly of each of these three clades could indicate long standing isolation. The main habitat in the CFM clades is fynbos. Clade II consists of populations from the Cederberg, Groot Winterhoek and Turret Peak in the Kouebokkeveld mountains, which are more to the North-west part of the CFM associated with winter rainfall caused by westerly winds from the Atlantic Ocean. Clade III consists of populations from the south-central CFM including Matroosberg, which can be classified in both winter and all year rainfall. Although Matroosberg samples were assigned subspecific status by Hewitt (1926), they were not

found to be distinct in relation to all other *P. burchelli* samples, which is in agreement with FitzSimons (1943). However, within clade III the Matroosberg population is 15 mutation steps away from the other populations in this clade. Clade IV is more to the east of CFM and receives precipitation all year. In general there was high genetic distances between these three CFM clades (8.1% – 10.1%) despite their geographic proximity. This could indicate that the structure observed in the CFM is a result of repeated episodes of colonisation, extinction and expansion from several refugial populations. However, Fu's F_S and Tajima's D tests suggests these populations have not undergone any recent demographic changes, such as population expansion. All these clades diverged from each other roughly the same time during the Pliocene. Other phylogeographic studies of reptiles in the CFM have found similar genetic patterns, for instance, a northern, central and eastern CFM clades were also identified in *Agama atra* (Swart, 2006). Species in the chameleon genus *Bradypodiom* also showed a strong geographic structure in the region, mainly due to habitat changes resulting from climatic shifts in the past (Tolley *et al.*, 2004, 2006).

Clade V, with only three sampling localities (Langeberg, Keeromsberg, and Beaufort West), shows a very interesting pattern. The first two localities are part of the CFM, but they group closely together to the Beaufort West (Nuweveldberg), which is part of the Great Escarpment. Although not well supported in the phylogenetic analysis, the network indicates that the Langeberg/Keeromsberg population is more closely related to the Beaufort West population in clade V than to any other CFM population (although they are separated from each other by 20 mutational steps). This could also indicate that there

has been gene flow in the past and or ancestral polymorphism, although no shared haplotype was found between the CFM and GE localities sampled. These two populations could form the link between Clade VI and CFM clades or broadly between the CFM and the Great Escarpment populations. Interestingly however, other studies in South Africa have shown that there is an east-west isolation *i.e.,* in leopards (Martins, N per. Comm.), shrews (Willows-Munro, 2006) and chameleons (Tolley *et al.,* 2004, 2006).

The distinct phylogeographic structure in this species might be attributed to several factors. The genetic divergence among clades is relatively old (Pliocene), although the MDIV analyses suggest that there seems to have been gene flow until the time of population divergence in the late Pleistocene. The fact that the species is restricted mainly to montane habitats might have played an important role in the divergence of populations. This might have been accelerated by lack of suitable habitats in between such that there is no gene flow between populations in neighbouring mountains. According to Avise (1994), a strong evolutionary clustering of haplotypes in a geographic region and haplotype clusters separated by long branch lengths in phylogentic trees with missing and or extinct intermediates in populations network is often interpreted as evidence of past fragmentation events. A number of studies in sourthern Africa have shown that habitat fragmentation has had an influence on the deep divergence of reptile species and/or populations in the region (Matthee & Flemming, 2002, Swart, 2006; Tolley *et al.,* 2004, 2006). Climate changes, especially during the Pliocene-Pliostocene period, might also have had an effect on the structure observed dispersal and gene flow between populations during the cooler periods and isolation during the warmer periods. Major habitat changes

have been ascribed to climate change during the Quaternary (Vrba, 1995; Partridge *et al.*, 1995; Van Zinderen Bakker & Mercer, 1986). These events lead to the isolation and fragmentation of the habitats, shifting populations continuously as a consequence (Axelrod & Raven, 1978).

3. 5. 2. Conservation implications

The sequence divergence between the clades in *P. burchelli* is high and is comparable to ND2 divergence between the species in the genus *Pedioplanis* (chapter 2), other lacertids (Crochet *et al.,* 2004) and other lizard taxa (Macey *et al.,* 1998; Gifford *et al.,* 2004; Matthee & Flemming, 2002). The question is whether these clades represent extremely high genetic polymorphism or separate evolving lineages within a species? The level of divergence (Pliocene – Pliestocene) indicates a very old species that is in the process of incipient speciation. Sequence divergence by itself is not a good measure of separately evolving lineages especially in view of the fact that no obvious morphological differentiation has been previously found among these clades. The argument for several species within this one taxon can be clarified with more extensive morphological investigations and the inclusion of nuclear DNA markers, for instance microsatelites. Other studies on lacertid lizards (*Lacerta schreiberi*) that have tried to contrast the patterns observed for mtDNA nuclear have shown substantial admixture with nuclear markers (Godinhno *et al.,* 2006).

3. 6. Conclusions

Although *P. burchelli* is widely distributed in South Africa in montane and other habitats, it exhibits a deep phylogeographic structuring, with at least six distinct groups along the Great Escarpment and Cape Fold Mountains in South Africa, including Lesotho. There is limited geographic overlap among these clades, which represents very old asssembleges that may have started to differentiate during the Pliocene. Reasons for the existence of these distinct clades are not yet clear but are most probably attributable to the habitat preference of the species and regional habitat fragmentation. The distinct genetic structuring in *P. burchelli* has also been demonstrated for other reptiles and other groups in the region. However, for a comprehensive picture of the species evolutionary history in the region, a more fine scale study of the mtDNA pattern using nuclear as well as morphology is desirable.

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