Phylogenetics of the southern African dwarf chameleons, *Bradypodion* (Squamata: Chamaeleonidae)

Krystal A. Tolley, a,* Colin R. Tilbury, b William R. Branch, c and Conrad A. Matthee a

a Molecular Zoology Laboratory, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa
b 112A 3rd Avenue, Fairland, Johannesburg 2935, South Africa
c Port Elizabeth Museum, Port Elizabeth, South Africa

Received 9 October 2002; revised 12 May 2003

Abstract

The taxonomic relationships within the dwarf chameleons (*Bradypodion*) of southern Africa have long been controversial. Although informal phenotypic groups have been suggested, the evolutionary relationships among the 15 recognised species in southern Africa have not been previously investigated. To investigate the relationships among species within this genus, fragments of two mitochondrial genes (16S ribosomal RNA and ND2) were sequenced and analysed using maximum parsimony, maximum likelihood and Bayesian inference. All analyses showed congruent topologies, revealing at least 5 well-supported clades distributed across distinct geographic regions. The mtDNA gene tree indicated that in many instances, geographic location has played a role in shaping the evolution of this group, and that the previously suggested phenotypic groupings do not adequately reflect evolutionary relationships. Furthermore, it appears that some of the currently recognised species (described on morphology) are polyphyletic for mitochondrial sequences, most notably those occurring in the isolated forest patches of north-eastern South Africa, near the Drakensberg Escarpment.

© 2003 Elsevier Inc. All rights reserved.

Keywords: *Bradypodion*; Chamaeleonidae; mtDNA; Phylogeny; South Africa

1. Introduction

Chameleons are endemic to Madagascar, Africa, and some associated Indian Ocean islands, with restricted distributions in Saudi Arabia, India, Sri Lanka, and parts of coastal Mediterranean Europe. Based on morphological characteristics, there are six genera (*Bradypodion*, *Chamaeleo*, *Furcifer*, *Calumma*, *Rhampholeon*, and *Brookesia*) and approximately 140 species (Branch, 1998; Klaver and Böhme, 1986). The taxonomic composition, and thus evolutionary affinities, of the dwarf chameleons (genus *Bradypodion*) has been contentious. Raw (1976, 2001) has reviewed the nomenclatural history and diverging views on the recognition of the genus. Whilst describing four new taxa from southern Africa, he treated all taxa previously described from the subcontinent as separate species (Raw, 1976, 1978).

Conversely, some authors (e.g., Hillenius, 1959; Mertens, 1966) have conservatively recognised only a single species (*Chamaeleo pumilus*), with numerous subspecies. Despite recent recognition of the validity of the genus *Bradypodion* (e.g., Branch, 1998; Jacobsen, 1990; Riepapel, 1997), there remains considerable disagreement on the evolutionary relationships within the genus.

Presently, the taxonomy of the group is based largely on morphological characteristics, yet the distinctiveness of many *Bradypodion* species has been difficult to confirm due to a lack of a comprehensive assessment of sexual ontogenetic and/or population variation for these characters. Because most of the characters currently used to define *Bradypodion* species (casque development, scale shape and size in crests, lateral tubercles, etc.) may be subject to these factors, it is possible that some species have been inadequately defined.

Perhaps the most contentious recent action was the transfer of nine east and central African species from *Chamaeleo* to *Bradypodion* (Klaver and Böhme, 1986).
This action was not based on any synapomorphies between these nine species and the South African *Bradypodion*, but rather the lack of synapomorphies linking them to *Chamaeleo* or other tropical genera. In effect, *Bradypodion* became a catch-all for species unallocated elsewhere, and Branch (1998) specifically rejected the transfer. Townsend and Larson (2002) found strong molecular support for the monophyly of the southern African species, and like Hofman et al. (1991) found no support for the inclusion of the east and central African taxa within *Bradypodion*. Later, Klaver and Böhme (1997) recognised only five southern African species within *Bradypodion*, i.e., Raw’s (1976, 1978) four new species (*Bradypodion dracomontanum*, *Bradypodion nemorale*, *Bradypodion setaroi*, and *Bradypodion thamnobates*) and an expanded *Bradypodion pumilum* containing all other taxa as subspecies. This action contradicted the views of South African herpetologists familiar with the genus (e.g., Branch, 1988; Jacobsen, 1990; Raw, 1976, 1978), who recognised at least 15 endemic species with limited and mostly allopatric distributions (Branch, 1998; Fig. 1, inset). It should be noted that Raw (2001) considers many more species worthy of recognition, based on morphological characters.

Branch (1998) has suggested that two ‘general’ phenotypic groups can be recognised. The first comprises larger-bodied species associated with montane forests plus other habitats in the Western Cape of South Africa. Within the larger-bodied forms, short-tailed, cryptic taxa tend to occur in the semi-arid regions (*Bradypodion ventrale*, *Bradypodion karrooicum*, *Bradypodion gutturale*, and *Bradypodion occidentale*), whereas the large bodied, long tailed, brilliantly coloured taxa occur in a variety of habitats, including forest, grassland, bush, and “fynbos” (fine-leaved, evergreen shrub-land) (*B. pumilum*, *Bradypodion damaranum*, *B. thamnobates*, *B. dracomontanum*, and *B. transvaalense*). The second phenotypic group appears to be restricted to escarpment forests, coastal thickets, or montane fynbos vegetation and consists of smaller-bodied species (*Bradypodion taeniabronchum*, *B. setaroi*, *Bradypodion melanocephalum*, *B. nemorale*, *Bradypodion kentanicum*, and *Bradypodion caffrum*). Although Raw (2001) has described phenotypic relationships within southern African...
Bradypodion, it has been noted that the evolutionary relationships of these phenotypic groups are unknown (Branch, 1998).

The objective of this study was to investigate the evolutionary relationships among the southern African dwarf chameleons using two mitochondrial DNA genes. We hypothesised that their evolutionary relationships would correspond to either geographical proximity, or alternatively, to the general phenotypic groupings. From a broader perspective, a robust molecular phylogeny, coupled with more clearly defined species boundaries for the taxa, can be of significant value for conservation planning in the region. All South African species are endemic (Branch, 1998); one is listed as Critically Endangered (B. taeniabronchum), one as Endangered (B. setaroi), and two as Near Threatened (B. thamnobates and B. nemorale) on the International IUCN Red List (Hilton-Taylor, 2000).

2. Materials and methods

2.1. Taxon sampling and laboratory methods

Muscle or blood samples were obtained from 55 dwarf chameleon specimens (Appendix A). With the exception of B. nemorale and B. kentanicum, topotypic voucher specimens were included in the study (B. kentanicum was not collected). Individuals were identified by their morphological characteristics according to Branch (1998) and where possible multiple representatives of each species were included (Appendix A: Fig. 1). In addition, a number of specimens that could not easily be taxonomically assigned but whose geographic origin was known, were included in the genetic analysis.

Samples were preserved in 70% ethanol or 20% DMSO saturated with NaCl. Total genomic DNA was extracted according to standard procedures with a proteinase K digestion followed by a phenol/chloroform procedure (Sambrook et al., 1989). Portions of two genes in the mitochondrial DNA were amplified; ND2 using primers L4437b (Macey et al., 1997a) and H5934 (Macey et al., 1997b), and 16S using primers L2510 and H3080 (Palumbi, 1996). For amplification, 50 ng genomic DNA were added to a reaction containing a thermophilic buffer (50 mM KCl, 10 mM Tris–HCl, pH 9.0), 1.5 mM MgCl2, 0.2 μM of each primer, 0.2 mM dNTPs, and 0.025 U/μl Taq polymerase. Cycling profile included an initial denaturing step at 95°C for 1 min, followed by 35 cycles of 95°C for 1 min, 55°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 5 min.

Cycle sequencing was carried out for ND2 using two forward primers L4437b and L4882 (Macey et al., 2000) to obtain a 987 bp consensus segment. For 16S, the forward primer was used to obtain a 452 bp segment. Cycle sequencing products were purified with sephadex spin columns, and analysed on an ABI 3100 automatic sequencer. Sequences were edited with Sequence Navigator v1.01 (Perkin–Elmer) and aligned using Clustal X (Thompson et al., 1997) with the default alignment parameters. All alignments were checked manually for misalignments. A 21 bp fragment of the 16S gene could not be aligned unambiguously, so this segment was omitted from the analyses. ND2 sequences were translated to protein codons, and examined for the presence of stop codons. All sequences have been deposited in GenBank (Accession Nos. AY289805–AY289918).

2.2. Phylogenetic analyses

Sequence divergences corrected for within group variation (ΔA; Nei, 1987) were estimated in MEGA Version 2.1 (Kumar et al., 2001) using the Tamura-Nei model of evolution to adjust for differences in nucleotide frequencies, substitution rate heterogeneity, plus the proportion of AG and TC transitions (Nei and Kumar, 2000). Maximum likelihood estimates for the gamma distribution were estimated in PAUP*4.0b10 (Swofford, 2002) for 16S and ND2 separately (respectively, $\alpha = 0.45$ and $\alpha = 0.83$).

Three additional taxa were included in the outgroup for this study. Bradypodion mlanjense (a central African species) and Chamaeleo dilepis (the flap-neck chameleon) were sequenced as part of this study, and included together with Rhampoleon breviceudatus (GenBank Accession Nos. AF448771 and AF121961). Furthermore, because the outgroup taxa were rather distantly related to the ingroup, midpoint rooting was also examined to test for topological congruence.

Analyses for phylogenetic inference were conducted using three methods: maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). To examine whether the sequences from the two genes could be combined in a single analysis, a partition homogeneity test was run in PAUP*4.0b10 (Swofford, 2002), and significance was estimated by 100 repartitions. This test indicated no conflicting phylogenetic signals between the datasets ($p = 0.68$) and given that the mtDNA genes are linked, the entire dataset was analysed together.

2.3. Maximum parsimony

Saturation plots were investigated for both genes at all sites, and for transitions and transversions separately. In addition, each codon position was investigated for ND2. The transition/transversion ratio estimated by ML was low for ND2 (3.3), and saturation among ingroup taxa was not evident for either transitions, or transversions, nor when all sites were considered for either gene (data not shown). In contrast, due to high divergence values between the ingroup and outgroup, saturation
was clearly evident when considering the outgroup taxa. Because saturation was not observed within the ingroup, and weighting schemes may decrease phylogenetic resolution (Kluge, 1997; Sennblad and Bremer, 2000; Wenzel and Siddall, 1999), an unweighted maximum parsimony analysis using an heuristic search was performed on the combined DNA sequence dataset. Characters were unordered, and TBR branch swapping was in effect, with a random addition of taxa (1000 replicates). All alignment gaps in the 16S rRNA gene were treated as missing characters. Confidence in the nodes was assessed by 1000 bootstrap replicates (Felsenstein, 1985) with random addition of taxa. Competing phylogenetic hypotheses were tested using the Shimodaira–Hasegawa test (S-H test) for maximum likelihood (1000 replicates) in PAUP*4.0b10 (Swofford, 2002), and these alternative topologies were constructed using MacClade 4.0 (Maddison and Maddison, 2000).

2.4. Maximum likelihood

Due to computational constraints, a sub-set of 31 taxa was used for the ML analysis. This included one individual from each species, all unassigned taxa, and one outgroup taxon (R. brevicaudatus). Modeltest Ver. 3.06b (Posada and Crandall, 1998) was used to estimate the evolutionary model which best described the data. The general time reversible model + I + G had the lowest likelihood score (−lnL = 8910.84) and showed a significantly better fit than the other less complicated models (model parameters: GTR + I + G, I = 0.28, G = 0.57; base frequencies A = 0.392, C = 0.274, G = 0.100, T = 0.234; rate matrix A−C = 1.08, A−G = 8.16, A−T = 1.18, C−G = 0.38, C−T = 8.16, G−T = 1.00). A heuristic search was used as described for MP (except 1000 replicates of random addition of taxa were not performed), and support for the nodes was assessed by 100 bootstrap replicates.

2.5. Bayesian inference

Bayesian inference was used to investigate optimal tree space using the program MrBayes 3.0 (Huelsenbeck and Ronquist, 2001) on the same sub-set of the taxa used in ML, and on the full dataset. The GTR model of evolution was used for this analysis. For both the full and the reduced datasets, the search was repeated four separate times (4 runs of 1 million generations). For each search, four simultaneous chains were run (one cold, three heated) starting with a random tree, and trees were sampled every 50 generations. The initial ‘burn-in’ was estimated by determining the point at which the likelihood scores of the trees sampled did not continue to decrease (‘stationarity’). This occurred between 10,000 and 15,000 generations for the reduced dataset, and between 15,000 and 20,000 generations for the full dataset (data not shown). To ensure that all the burn-in trees were removed, the first 400 trees (20,000 generations) were removed from all runs. A 50% majority rule consensus tree was generated from the trees retained from each run, with posterior probabilities for each node estimated by the percentage of times the node was recovered.

3. Results

3.1. Sequence variation

Of the 1418 sites examined, there were 520 variable ND2 sites, 379 of which were parsimony informative (38%), and 121 variable 16S rRNA sites, 53 of which were parsimony informative (12%). When the protein coding ND2 gene was partitioned into codon positions, the third position showed the most parsimony informative sites (first position, 10%; second position, 5%; and third position, 23%). For 16S rRNA, inter-clade sequence divergences ranged from 0.5 to 4.0%, with B. setaroi (single representative indicated by H, Fig. 2) showing the highest divergences (Table 1). ND2 sequence divergences ranged between ~2 and 16.5%, with the largest sequence distance between B. pumilum (clade B) and B. setaroi (lineage H). The three outgroup taxa (B. mlanjense, C. dilepis, and R. brevicaudatus) were particularly divergent from the South African Bradypodion (Table 1). Most notably, the estimates of sequence divergence between the central African species of Bradypodion (B. mlanjense) and the southern African Bradypodion were as high as the divergences between the other outgroup taxa and the southern African Bradypodion.

3.2. Phylogenetic analyses

Maximum parsimony (MP) analysis recovered 3886 equally parsimonious trees with a length of 1397 steps (Fig. 2). The large number of equally parsimonious solutions was largely due to terminal branch swapping, particularly among taxa belonging to the same species. Numerous nodes had bootstrap support greater than or equal to 78%. Given the large number of taxa and characters used in this study, there was a relatively low amount of homoplasy in the dataset (CI = 0.617, RI = 0.841, rescaled CI = 0.519). Regardless of the rooting method (outgroup rooted or mid-point rooting) none of the supported nodes in the topology were affected.

For the Bayesian inference method (BI), identical topologies were recovered for each of the 4 runs with the full dataset, although posterior probabilities for some of the nodes differed slightly between each of the Bayesian runs (Fig. 2). There was congruence between the BI and
the MP analyses, as they showed the same basic topology and well-supported nodes (≥78% bootstrap, ≥95% posterior probabilities).

The maximum likelihood (ML) analysis of the sub-set of data recovered an identical tree topology (not shown) to the BI analysis on the sub-set of data (−ln L = 6454.9). Nodes recovered for the sub-set of data showed similar support to the full dataset for both MP and BI.

In all, there were five well-supported clades (A, B, C, E, and F; Fig. 2), corresponding to separate geographic regions throughout southern Africa (Fig. 1). Within 2 of these clades (C and F), several sub-clades were found with fairly high statistical support. These latter sub-clades either represent a distinct geographic origin for taxa (cf. Figs. 1 and 2) or are congruent with previous taxonomic designations based on morphology (e.g.,
Table 1
Net sequence divergences within and among the main mtDNA clades/lineages (A–I) of dwarf chameleons, for (a) 16S and (b) ND2

(a) A B C1 C2 C3 C4 C5 D E F1 F2 F3 G H I OUT1 OUT2 OUT3
A 0.001
B 0.018 0.004
C1 0.025 0.027 0.003
C2 0.031 0.031 0.008 0.005
C3 0.032 0.033 0.011 0.011 n/c
C4 0.033 0.033 0.011 0.013 n/c
C5 0.032 0.031 0.026 0.029 0.028 0.026 n/c
D 0.027 0.025 0.026 0.029 0.028 0.025 0.031 n/c
E 0.032 0.027 0.027 0.033 0.029 0.033 0.031 0.004
F1 0.021 0.018 0.016 0.019 0.019 0.016 0.016 0.015 0.014 0.005
F2 0.032 0.036 0.031 0.034 0.033 0.025 0.026 0.033 0.032 0.014 n/c
F3 0.019 0.026 0.021 0.024 0.023 0.021 0.026 0.022 0.020 0.007 n/c
G 0.024 0.025 0.023 0.026 0.025 0.023 0.023 0.023 0.019 0.005 0.022 0.007 n/c
H 0.027 0.028 0.034 0.039 0.040 0.037 0.025 0.037 0.031 0.018 0.030 0.018 0.020 n/c
I 0.022 0.025 0.017 0.024 0.025 0.026 0.026 0.026 0.023 0.011 0.022 0.018 0.017 0.026 n/c
OUT1 0.126 0.131 0.135 0.118 0.133 0.135 0.135 0.139 0.133 0.116 0.118 0.120 0.130 0.126 0.133 n/c
OUT2 0.272 0.297 0.261 0.296 0.291 0.297 0.277 0.278 0.272 0.279 0.289 0.284 0.284 0.284 0.277 n/c
OUT3 0.126 0.131 0.128 0.120 0.134 0.126 0.128 0.136 0.138 0.118 0.129 0.122 0.129 0.122 0.183 0.297 n/c

(b) A B C1 C2 C3 C4 C5 D E F1 F2 F3 G H I OUT1 OUT2 OUT3
A 0.011
B 0.073 0.005
C1 0.089 0.120 0.016
C2 0.101 0.130 0.025 0.007
C3 0.106 0.129 0.034 0.023 0.002
C4 0.093 0.108 0.026 0.041 0.048 n/c
C5 0.101 0.128 0.063 0.076 0.088 0.073 0.011
D 0.124 0.137 0.111 0.118 0.131 0.107 0.107 n/c
E 0.122 0.143 0.108 0.109 0.129 0.092 0.105 0.128 0.010
F1 0.103 0.125 0.090 0.093 0.098 0.088 0.097 0.106 0.093 0.018
F2 0.112 0.125 0.090 0.095 0.102 0.090 0.098 0.115 0.090 0.047 n/c
F3 0.099 0.107 0.081 0.089 0.100 0.089 0.090 0.091 0.065 0.038 0.041 0.025
G 0.110 0.123 0.106 0.113 0.125 0.104 0.102 0.105 0.095 0.056 0.064 0.053 0.002
H 0.140 0.165 0.104 0.113 0.132 0.124 0.110 0.151 0.122 0.114 0.120 0.095 0.091 n/c
I 0.112 0.132 0.111 0.115 0.130 0.117 0.109 0.135 0.101 0.103 0.103 0.087 0.107 0.120 n/c
OUT1 0.503 0.488 0.515 0.493 0.496 0.406 0.551 0.533 0.524 0.519 0.510 0.450 0.522 0.514 0.517 n/c
OUT2 0.326 0.325 0.370 0.335 0.354 0.297 0.362 0.383 0.369 0.372 0.358 0.335 0.371 0.370 0.344 0.515 n/c
OUT3 0.320 0.326 0.380 0.354 0.378 0.327 0.359 0.374 0.402 0.401 0.377 0.338 0.377 0.354 0.368 0.507 0.507 0.001

The outgroup taxa are listed separately (OUT1, R. brevicaudatus; OUT2, C. dilepis; and OUT3, B. mlanjense). Bolded values on diagonal are within clade sequence divergences. No values were calculated (n/c) where only one individual was sampled.
clade C5 represents the monophyletic B. occidentale. Several evolutionary lineages represented by a single individual could not be placed with certainty (these include lineage D represented by B. caffrum, lineage G represented by individuals from the Nkandla forest, lineage H represented by B. setaroi, and lineage I represented by a single specimen sampled in the Dlinza forest). Our limited sample size for these latter individuals precludes any definitive statements on their phylogenetic affinities.

Clade A was comprised of B. damaranum, plus several unassigned individuals from the southeastern montane forests of the Tsitsikamma and the adjacent Kouga Mountains. The second clade (B) formed a monophyletic assemblage comprised of B. pumilum from the south-western coast of South Africa (Fig. 1). Because bootstrap support was low for the monophyly of clades A and B, support for this node was also assessed by the S–H test. Although these two clades are united by 11 synapomorphies, this test did not significantly support their monophyly at the 0.05 confidence level (p = 0.08). Subsequently, we also investigated the possibility that either clade A or B were more closely related to clades C-I, but in both instances the presented topology (Fig. 2) showed a significantly better fit than the alternative hypotheses (S–H test; clade A with C-I, p < 0.000; clade B with C-I, p < 0.000).

The monophyly of clades/lineages C-I was supported by high bootstrap (94%), but only moderate posterior probabilities (90–97%). Twenty-eight synapomorphies unite these taxa. Within this group, clade C was comprised of dwarf chameleons from the southern and western regions of South Africa (Fig. 1). Clade C could be further sub-divided into well supported monophyletic groups (sub-clades) of dwarf chameleons from the central Cape Fold Mountains (C1), the Karoo biome (C2), the south coast (C3), the Swartberg Mountains (C4), and the west coast (C5). These five sub-clades were all well supported with bootstrap values between 78 and 100% (MP) and posterior probabilities greater than 90% (BI). The single individual collected in the Swartberg Mountains (C4) was previously identified as a probable new taxon (Plate 1, Branch, 1998). In our study this taxon shows a strong phylogenetic affinity to sub-clades C1–C3 (represented by B. gutturale, B. ventrale, B. karrooicum, and B. taeniabronchum). B. occidentale, which is widely distributed along the west coast of South Africa, forms a monophyletic group (C5) basal to all other representatives of clade C.

Groups D-I consisted of dwarf chameleons found in the scattered montane forests and adjacent grasslands of the north-eastern region of South Africa. In general, clade E consisted of individuals collected east of the Drakensberg escarpment. Clade F was well supported (84% bootstrap, 99–100% posterior probabilities), and could be sub-divided into three evolutionary lineages (F1–F3) that correspond with geographic location. All individuals from clade F1 were collected in the northern part of the Drakensberg escarpment, while both individuals in clade F3 were collected in the southern part of the Drakensberg escarpment. The status of the single individual from the Ngome forest was unresolved (F2). Two individuals from the Nkandla forest (clade G) which lies south of the Ngome forest were basal to clade F, although the relationship was not consistently supported (65% bootstrap, 100% posterior probability).

To test competing hypotheses of phylogenetic relationships, the two phenotypic groupings (large body and small body) were enforced to be monophyletic. A mtDNA gene tree reflecting these associations would require an additional 239 steps and showed a significantly worse fit than the topology presented (p < 0.000, − ln L difference between trees = 582.8). As an alternative, the enforced monophyly of large body forest/fynbos taxa, large body arid taxa, and small body taxa was tested, but was also unsupported (p < 0.000, − ln L difference = 618.7) and the topology reflecting these associations would require 244 synapomorphic changes.

4. Discussion

Phylogenetic analyses suggest the presence of at least five clades of southern African Bradypodion, generally supported by high bootstraps and posterior probabilities. All the methods used (ML, MP, and BI) produced trees with the same internal branching pattern. Sequence divergences among the clades were higher than within the clades (Table 1), suggesting diverse evolutionary histories within members of this genus. The overall results showed a strong correspondence between clade and geographic location (Fig. 1), while the phenotypic groupings (large-bodied arid, large-bodied forest/fynbos, and small-bodied forms) were not supported by the present phylogenetic analysis.

4.1. Taxonomic considerations

Bradypodion pumilum, B. transvaalense, B. gutturale, and B. ventrale were each found to be monophyletic OTUs, supporting the taxonomic status of these species. It appears that this analysis also supports the status of B. damaranum, the Knysna dwarf chameleon (see clade A). Although there was only one sample available for this species, that individual was quite divergent from the other clades. In addition, clade A contained three previously unassigned individuals from nearby mountain ranges (Kouga and Tsitsikamma) that clustered together in a well-resolved monophyletic group within clade A. This could suggest that this monophyletic group represents a separate, previously undescribed sister species to B. damaranum. However, comprehensive sampling and
assessment of morphological characters must be carried out to confirm this hypothesis.

The status of B. occidentale as a separate species has been uncertain (originally described as a subspecies of B. ventrale), but this group was also found to be a monophyletic OTU (clade C5). This suggests that it is distinct from B. ventrale (clade C2), and its’ recognition as a separate species (see Branch, 1998) may be warranted. The status of the Karoo dwarf chameleon (B. karrooicum) and the Robertson’s dwarf chameleon (B. gutturale) as separate species also have been problematic in the past. Branch (1998) suggested that these two taxa may simply be subspecies or races of the southern dwarf chameleon (B. ventrale). All three taxa clustered together in clade C, but it appears the situation is more complex than previously thought. Individuals sampled from within the range of B. ventrale and B. karrooicum all clustered in clade C2 forming an unresolved polytomy, suggesting that B. karrooicum indeed may be only a local race of B. ventrale. Conversely, all specimens identified as B. gutturale formed a well-supported monophyletic group (clade C1). This included several unassigned individuals from outside the recognised range of B. gutturale. Hence, B. gutturale appears to be more distinct than simply a local race of B. ventrale and is separated from the ventrale clade (C2) by approximately 2.5% sequence divergence for ND2, a value that falls within the range of other recognised Bradypodion species (Table 1). Given that these specimens are representative of B. gutturale, their inclusion in the clade substantially increases the range of this species (from near Porterville to the Montagu Pass).

Smith’s dwarf chameleon (B. taeniabronchum) has been thought to occur in two isolated patches, one in the Van Stadensberg Mountains near Port Elizabeth, and the other on the northern slopes of the Tsitsikamma Mountains (Branch, 1998). However, specimens from these two localities were placed in two different clades (A and C3) and showed high sequence divergences (>10% for ND2). The specimen collected in Tsitsikamma clusters with B. damaranum from the nearby Knysna region (clade A). As B. taeniabronchum is listed as Critically Endangered on the IUCN Red List (Hilton-Taylor, 2000; CR – B1 + 2c), its’ unique status is of conservation importance. We argue that should B. taeniabronchum exist in only one location near Port Elizabeth (the type locality), then its’ genetic distinctiveness renders this taxon important for the long term conservation of regional biodiversity.

Basal to the B. gutturale, B. ventrale, and B. taeniabronchum clades is a single undescribed individual (lineage C4) collected in the Swartberg Mountains bordering the Karoo biome. Although showing some morphological similarities to B. karrooicum, it was substantially smaller than the usual B. karrooicum and it has been suggested that this is a new species (Branch, 1998; plate 1). Sequence divergence estimates suggest that it may be closely related to B. gutturale (Table 1). Although unconfirmed at this point, it is possible that this region does contain a new species that is morphologically convergent with B. ventrale (B. karrooicum form), although genetically more similar to B. gutturale.

Clade E presents an interesting problem for the taxonomy of dwarf chameleons. The monophyly of this clade was well supported (100% bootstrap, 100% posterior probabilities) but the genetic variation within the clade was low as demonstrated by the short branch lengths (Fig. 2) and low sequence divergences within the clade (Table 1). The individuals forming the clade were all collected from a contiguous geographic region, but were presumed to be several different species (Fig. 1). Chiefly, B. thamnobates and B. melanocephalum were classified as different species based on morphological variation, but were clearly polyphyletic for the mtDNA genes used in this study. Furthermore, there were several individuals that could not be pre-assigned to any taxonomic group, but were collected from within the presumed geographic range of B. dracomontanum (specimen CT71) and B. melanocephalum (specimens CT16, CT17, and CT69; compare Fig. 1 to inset). Despite the reported phenotypic diversity in this region corresponding to described species (Raw, 1995, 2001), there were a number of polytomies in the phylogenetic tree. Because none of these species form a monophyletic assemblage, the status of these taxa as separate species should be re-examined, inclusive with more comprehensive geographic sampling. Information on distribution, habitat preferences, morphology, and an assessment of both nuclear and mtDNA markers on a fine scale would assist in establishing the validity of these taxa at the specific level.

Clade F formed a well-supported group of taxa from the Drakensberg Mountains. The clade could be subdivided into three evolutionary lineages, two of which are consistent with described species (B. transvaalense and B. dracomontanum). Additionally, an unassigned individual from the Ngome forest clusters within this clade but the sister taxon relationship of this individual is unresolved.

Bradypodion nemorale (Zululand dwarf chameleon) poses an interesting challenge from a taxonomic standpoint. This species is thought to occur in a limited region in the northern KwaZulu-Natal Province (Fig. 1, inset). However, individuals collected in this region formed separate OTUs (clade G, Nkandla forest; lineage I, Dlinza forest), that were highly divergent from all other clades/lineages (Table 1). These results support Raw’s (2001) finding that these forests contain discrete groups based on phenotypic distinctiveness. Hence, it appears that although these specimens were collected from within the recognised range of B. nemorale, mtDNA divergences suggest that they do not share a recent
common ancestry with this species. It should be noted however, that the type locality of *B. nemorale* is the Quedeni forest (not sampled) and it is possible that the Nkandla and Dlinza forms are not representative of this taxon.

Setaro’s dwarf (*B. setaroi*) and the Transkei dwarf chameleon (*B. caffrum*) each formed separate evolutionary lineages (D and H) that were unresolved and separated by high sequence divergences from the other clades (Table 1). Even with a lack of duplicate samples for these taxa, the results suggest that both these species have highly distinct evolutionary histories. Both have restricted distributions in forested areas on the east coast, and have probably been historically isolated from other dwarf chameleons.

The mitochondrial phylogeny supports the taxonomic status of most *Bradypodion* species, but does call into question the status of some taxa. The analysis does not support the phenotypic groupings (large body, small body) informally suggested by Branch (1998) and it appears that the mtDNA phylogeny more closely reflects geographic distribution, rather than the phenotypic groupings. Comparing Fig. 1 to the inset, it appears that some species boundaries may be ill-defined at present. These discrepancies probably contribute to the taxonomic uncertainties in this genus, not to mention the conflict between the current taxonomy and the mtDNA data. Furthermore, these results lend additional support to Townsend and Larson’s (2002) speculation that the east and southern African *Bradypodion* are not monophyletic. Specifically, the east African *Bradypodion* used as an outgroup taxon in the present study showed very high sequence divergences from the southern African *Bradypodion* (Table 1). Although a full evaluation of the central African taxa will be necessary to determine the taxonomic status of that group, it is possible that the transfer of *B. mlanjense* from *Chamaeleo* to *Braypodion* by Klaver and Böhme (1986) was premature.

4.2. Phylogeography

Generally, the clades and lineages corresponded well with geographic location, and in some cases this correspondence could be extended to differences in habitats. For example, the Knysna dwarf chameleon, *B. damarum* (clade A), is found in the wet montane forests along the seaward-facing slopes of the south coast. Other members of clade A (undescribed individuals) that formed a well resolved, monophyletic group within clade A were found in the montane fynbos of the nearby Kouga and Tsitsikamma Mountains. Although these regions are geographically close, there is a clear habitat difference between the regions which seems to correspond with the monophyly of the undescribed individuals.

The Cape dwarf chameleon, *B. pumilum* (clade B), is usually found in the thickets, reed beds, and fynbos vegetation of the extreme south-western Cape (Fig. 1). All individuals formed a single monophyletic assemblage that showed no within-clade geographic pattern, and low sequence divergence among individuals (range from 0 to 0.01). Although both *B. pumilum* and *B. damarum* are considered to be large bodied and brilliantly coloured, there is a difference in habitat preference for these two species and it is possible that long term isolation may have played a role in shaping the genetic divergence between these clades. Paleoclimatic data suggest this entire region has had long-term climatic stability, remaining cool temperate fynbos, and montane forest, although the extent of these habitats has shifted in response to changes temperatures and moisture (Kingdon, 1990; Scott et al., 1997).

Most taxa in clade C are distributed across a broad zone from the west coast, eastwards to Port Elizabeth (Fig. 1). This area generally consists of a mixture of thicket, succulent and semi-arid Karoo vegetation, and fynbos (Cowling et al., 1997). Clade C lacks substantial phenotypic diversity, as most taxa are large bodied and cryptic. The exception to this is *B. taeoniabronchum* which is morphologically divergent from the other taxa, and occurs in montane fynbos of the eastern Cape Fold Mountains. The general lack of morphological variation could suggest that divergent selection is not especially strong in this clade, possibly because most members are distributed across semi-arid biomes, despite some variation in vegetation type across the region. Paleoclimatic data suggests that, despite some fluctuations, the vast majority of this region has remained semi-arid for an extended period of time (Kingdon, 1990; Scott et al., 1997). Depending on long-term rainfall patterns, the size of the arid region appears to expand and contract, but does not become fragmented. Instead, encroachment/retreat of woodlands, savannah, and grasslands from the north, west, and south appear to constrict/expand the arid region (Kingdon, 1990; Scott et al., 1997), possibly causing the geographic range of these semi-arid adapted taxa to shift in response. In general, chameleons show a high level of dependency on adequate moisture for survival, while species inhabiting semi-arid biomes show appropriate adaptations to climatic demands. This could suggest that there is a need for phenotypic rigidity in these semi-arid regions, but that long-term geographic isolation within the semi-arid biomes (e.g., succulent Karoo, fynbos) and at different elevations (e.g., parts of the Cape Fold Mountains) has promoted genetic divergence of this clade.

The taxa in clade E were all obtained from isolated montane forest patches in the region east of the central Drakensberg escarpment. Overall, the region is particularly heterogeneous with respect to altitude and major biomes, forming a mosaic of mixed lowland forest,
montane forest, savannah, and grasslands (Acocks, 1953). Thus, the taxa in clade E are distributed in isolated patches that are essentially 'islands' in an otherwise diverse habitat. Paleoclimatic data shows the region to have experienced bouts of both warmer/wetter periods, and cooler/drier periods over at least the last ca. 100,000 years (Lindesay, 1998), with corresponding responses in the size of forested areas (Deacon and Lancaster, 1988). Although speculative without a comprehensive genetic and morphological analysis, these habitat shifts could have resulted in bouts of isolation, promoting the observed diversity in local phenotypic adaptations. During climatic periods that favoured repeated formation of contact zones, episodes of increased gene flow would have been possible, shaping the observed phylogenetic pattern.

As a whole, the examination of mtDNA lineages in the southern African dwarf chameleons will contribute substantially to refining the taxonomic status for several species. Phylogenetic information can now be added to what is known regarding their morphology and distribution to produce a more accurate taxonomic framework for this group. On the basis of the evidence inferred from the present study, it appears that there are now sufficient grounds to support a diverse phylogeny of species, in contradiction to the views of Hillenius (1959) and Klaver and Böhme (1997) of a single species complex (B. pumilum group). What remains is an examination on a fine geographic scale along the clade/species boundaries for both the southern and central/east African Bradypodion using mtDNA and nuclear markers. Coupled with more detailed information on distribution, plus comparative phylogeography of co-distributed species, it may be possible to produce a comprehensive evaluation of the regions central to the maintenance of biodiversity in southern Africa.

**Acknowledgments**

We would like to thank T. Robinson for financial and logistical support, and R. Bowie, T. Townsend, and the members of the Laboratory for Molecular Zoology (University of Stellenbosch) for assistance with this project. Thanks also go to M. Cunningham, S. Daniels, G. Gouws, C. Henderson, and B. Jansen van Vuuren for many useful discussions and comments. M. Burger, G. Haagner, and E. Thomas assisted in collecting tissues. Funding was provided by the South African National Research Foundation, and the Laboratory for Molecular Zoology (University of Stellenbosch). We are also grateful for the GIS map data provided by the Western Cape Nature Conservation, and Les Powrie at the National Botanical Institute in Cape Town.

**Appendix A. Bradypodion specimens used in the present study**

Laboratory ID numbers are given, species name (unless unassigned), collecting location, and reference number for the Port Elizabeth Museum (PEM-R). Topotypic specimens are indicated with a (T) after the species name.

<table>
<thead>
<tr>
<th>Lab ID</th>
<th>Species</th>
<th>Location</th>
<th>PEM-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT6</td>
<td>B. damaranum (T)</td>
<td>Knysna</td>
<td>5725</td>
</tr>
<tr>
<td>CT7</td>
<td>B. sp.</td>
<td>Montagu Pass</td>
<td>5685</td>
</tr>
<tr>
<td>CT8</td>
<td>B. sp.</td>
<td>Tsitsikamma</td>
<td>5686</td>
</tr>
<tr>
<td>CT9</td>
<td>B. caffrum (T)</td>
<td>Port St. Johns</td>
<td>5692</td>
</tr>
<tr>
<td>CT10</td>
<td>B. ventrale</td>
<td>Aliwal North</td>
<td>5709</td>
</tr>
<tr>
<td>CT11</td>
<td>B. ventrale</td>
<td>Zastron</td>
<td>5703</td>
</tr>
<tr>
<td>CT13</td>
<td>B. gutturale</td>
<td>De Wet</td>
<td>5718</td>
</tr>
<tr>
<td>CT14</td>
<td>B. occidentale</td>
<td>Rocher Pan</td>
<td>5713</td>
</tr>
<tr>
<td>CT16</td>
<td>B. sp.</td>
<td>Hilton</td>
<td>5693</td>
</tr>
<tr>
<td>CT17</td>
<td>B. sp.</td>
<td>Hilton</td>
<td>5694</td>
</tr>
<tr>
<td>CT18</td>
<td>B. thamnobates (T)</td>
<td>Nottingham Road</td>
<td>5721</td>
</tr>
<tr>
<td>CT19</td>
<td>B. thamnobates (T)</td>
<td>Nottingham Road</td>
<td>5722</td>
</tr>
<tr>
<td>CT26</td>
<td>B. transvaalense (T)</td>
<td>Haenertsburg</td>
<td>5719</td>
</tr>
<tr>
<td>CT62</td>
<td>B. sp.</td>
<td>Hendriksdal</td>
<td>5687</td>
</tr>
<tr>
<td>CT63</td>
<td>B. sp.</td>
<td>Hendriksdal</td>
<td>5688</td>
</tr>
<tr>
<td>CT65</td>
<td>B. sp.</td>
<td>Ngome Forest</td>
<td>5690</td>
</tr>
<tr>
<td>CT66</td>
<td>B. setaroi (T)</td>
<td>Dukuduku Forest</td>
<td>5702</td>
</tr>
<tr>
<td>CT68</td>
<td>B. nemorale</td>
<td>Dlinza Forest</td>
<td>5701</td>
</tr>
<tr>
<td>CT69</td>
<td>B. sp.</td>
<td>Greytown</td>
<td>5695</td>
</tr>
<tr>
<td>CT71</td>
<td>B. sp.</td>
<td>Giants Castle</td>
<td>5691</td>
</tr>
</tbody>
</table>
### Appendix A (continued)

<table>
<thead>
<tr>
<th>Lab ID</th>
<th>Species</th>
<th>Location</th>
<th>PEM-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT72</td>
<td>B. melanocephalum (T)</td>
<td>Durban</td>
<td>5696</td>
</tr>
<tr>
<td>CT73</td>
<td>B. ventrale (T)</td>
<td>Port Elizabeth</td>
<td>5704</td>
</tr>
<tr>
<td>CT75</td>
<td>B. ventrale (T)</td>
<td>Port Elizabeth</td>
<td>5706</td>
</tr>
<tr>
<td>CT76</td>
<td>B. taeniabronchium (T)</td>
<td>Van Stadensberg</td>
<td>5697</td>
</tr>
<tr>
<td>CT77</td>
<td>B. taeniabronchium (T)</td>
<td>Van Stadensberg</td>
<td>5698</td>
</tr>
<tr>
<td>CT83</td>
<td>B. dracomontanum</td>
<td>Tendeni</td>
<td>5726</td>
</tr>
<tr>
<td>CT91</td>
<td>B. gutturalé</td>
<td>Skurweberge, Porterville</td>
<td>5727</td>
</tr>
<tr>
<td>CT94</td>
<td>B. occidentale (T)</td>
<td>Namaqualand</td>
<td>5716</td>
</tr>
<tr>
<td>CT97</td>
<td>B. pumilum</td>
<td>Franschhoek</td>
<td>5728</td>
</tr>
<tr>
<td>CT98</td>
<td>B. pumilum</td>
<td>Franschhoek</td>
<td>5729</td>
</tr>
<tr>
<td>CT99</td>
<td>B. pumilum (T)</td>
<td>Cape Town</td>
<td>5730</td>
</tr>
<tr>
<td>CT130</td>
<td>B. dracomontanum (T)</td>
<td>Cathedral Peak</td>
<td>5739</td>
</tr>
<tr>
<td>CT132</td>
<td>B. nemorale</td>
<td>Nkandla Forest</td>
<td>5740</td>
</tr>
<tr>
<td>CT133</td>
<td>B. nemorale</td>
<td>Nkandla Forest</td>
<td>5741</td>
</tr>
<tr>
<td>CT136</td>
<td>B. karrooicum (T)</td>
<td>Beaufort West</td>
<td>5742</td>
</tr>
<tr>
<td>CT137</td>
<td>B. karrooicum (T)</td>
<td>Beaufort West</td>
<td>5743</td>
</tr>
<tr>
<td>CT138</td>
<td>B. sp.</td>
<td>Swartberg Pass</td>
<td>5744</td>
</tr>
<tr>
<td>B304</td>
<td>B. sp.</td>
<td>Gilboa Forest</td>
<td>5416</td>
</tr>
<tr>
<td>B305</td>
<td>B. sp.</td>
<td>Gilboa Forest</td>
<td>5415</td>
</tr>
<tr>
<td>KT1</td>
<td>B. pumilum</td>
<td>Stellenbosch</td>
<td>No specimen</td>
</tr>
<tr>
<td>KT2</td>
<td>B. pumilum</td>
<td>Stellenbosch</td>
<td>No specimen</td>
</tr>
<tr>
<td>KT3</td>
<td>B. pumilum</td>
<td>Stellenbosch</td>
<td>No specimen</td>
</tr>
<tr>
<td>E2</td>
<td>B. pumilum</td>
<td>Stellenbosch</td>
<td>No specimen</td>
</tr>
<tr>
<td>E6</td>
<td>B. sp.</td>
<td>Kougaberg</td>
<td>134</td>
</tr>
<tr>
<td>E7</td>
<td>B. sp.</td>
<td>Kougaberg</td>
<td>124</td>
</tr>
<tr>
<td>E18</td>
<td>B. pumilum</td>
<td>Franschhoek</td>
<td>190</td>
</tr>
<tr>
<td>E27</td>
<td>B. occidentale</td>
<td>Saldanha</td>
<td>No specimen</td>
</tr>
<tr>
<td>E31</td>
<td>B. occidentale</td>
<td>Saldanha</td>
<td>No specimen</td>
</tr>
<tr>
<td>E32</td>
<td>B. occidentale</td>
<td>Saldanha</td>
<td>No specimen</td>
</tr>
<tr>
<td>E97</td>
<td>B. occidentale</td>
<td>Rocher Pan</td>
<td>18000</td>
</tr>
<tr>
<td>E98</td>
<td>B. occidentale</td>
<td>Rocher Pan</td>
<td>18001</td>
</tr>
<tr>
<td>E99</td>
<td>B. gutturalé</td>
<td>Worcester</td>
<td>18003</td>
</tr>
<tr>
<td>E100</td>
<td>B. gutturalé</td>
<td>De Wet</td>
<td>18004</td>
</tr>
<tr>
<td>E101</td>
<td>B. pumilum (T)</td>
<td>Cape Town</td>
<td>18002</td>
</tr>
<tr>
<td>E106</td>
<td>B. gutturalé</td>
<td>Porterville</td>
<td>No specimen</td>
</tr>
<tr>
<td>E4</td>
<td>C. dilepis</td>
<td>N. Mozambique</td>
<td>105</td>
</tr>
<tr>
<td>CT54</td>
<td>B. mlanjense</td>
<td>Malawi</td>
<td>5745</td>
</tr>
<tr>
<td>CT55</td>
<td>B. mlanjense</td>
<td>Malawi</td>
<td>5746</td>
</tr>
</tbody>
</table>

### References


