

Original article

Distribution of *Bradypodion taeniabronchum* (Smith 1831) and other dwarf chameleons in the eastern Cape Floristic Region of South Africa

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Abstract.—The evolutionary relationships and taxonomic status of dwarf chameleons in the eastern Cape Floristic Region is not well understood. Through a combination of field observations and mitochondrial DNA sequencing (ND2 and 16S), it has become apparent that morphological and genetic evidence are often in conflict. In some instances, species that are morphologically quite different, show close genetic relationships, eg., *Bradypodion taeniabronchum* and *B. ventrale*. In other cases there are substantial genetic differences between lineages, with little morphological differentiation. For example, dwarf chameleons from the Kouga and the Baviaanskloof mountains were thought to represent a single undescribed species. However, chameleons from these two mountain ranges show large sequence divergence (6.8% for ND2) that is more than twice the value between *B. taeniabronchum* and *B. ventrale*. The evolutionary relationships within this group are reticulate in nature, making taxonomy difficult to resolve. Application of only mitochondrial data to resolve the taxonomy of this group is therefore insufficient, because mitochondrial DNA may only indicate historical isolation of lineages, and if used independently, may not be a good indicator of species definitions. Therefore our next step toward resolving the taxonomy of this group is the inclusion of nuclear DNA markers, in conjunction with a full morphological analysis.

Key words.—dwarf chameleons, *Bradypodion taeniabronchum*, distribution, mtDNA.

In the 150 years following its description in 1831, Smith's Dwarf Chameleon (*Bradypodion taeniabronchum*) was known from only three specimens. The type specimen was collected from an unidentified site, vaguely referred to as the "vicinity of Algoa Bay" of South Africa (Smith 1849; FitzSimons 1943). The other specimens were collected from "Schoenmakerskop" and "Vanstadens River" near Port Elizabeth (FitzSimons 1943), but more specific localities were not given. The discovery of a population at Lady's Slipper in the Vanstadensberg (Fig. 1) in 1980 was therefore of considerable herpetological interest and prompted further surveys of this locality and surrounding areas (see Branch 1988). Despite

this increased interest, by the time the 1988 version of the *South African Red Data Book - Reptiles and Amphibians* was published, the species was still thought to be limited to about 20 km² of the eastern summit and upper slopes of Vanstadensberg (3325CD). Due to the species' restricted distribution, and the potential threat of encroachment from adjacent pine plantations, it was listed as Endangered (Branch 1988). Currently it is listed as Critically Endangered on the IUCN Red List (following the listing criteria CR B1+2C; Hilton-Taylor 2000).

Since 1988, several additional records of dwarf chameleons from localities to the west of the

Vanstadensberg were also assigned to *B. taeniabronchum*. Burger & Smith (1992) recorded this species from the northern slopes of Tsitsikamma Mountains in the Formosa Conservation Area. This locality is adjacent to the farm Jagersbos, about 15 km west of Kareedouw (Fig. 1, Locality 8). The identification of specimens collected at this locality was primarily based on the presence of several dark gular grooves. This is a diagnostic character for *B. taeniabronchum* that is not shared with any other known *Bradypodion* species. Further references to this population were also made in other publications (Burger 1997; Burger *et al.* 1997).

In an atlas of South Africa's dwarf chameleons, Raw (1995) listed the following additional localities for *B. taeniabronchum*: Longmore Forest Reserve, Vanstadensberg (3325CC); Port Elizabeth, including Heatherbank and Lorraine (3325DC); Vanstadens River Heights

(3325CC); Vanstadensberg National Heritage Site (3325CC/CD); Vanstadens Wildflower Reserve (3325CC). Raw (1995) also lists Groot Winterhoek Wilderness Area (3219CC) as a locality, but this is presumably in error since this Wilderness Area is south of the Cederberg, approximately 600 km to the west of Vanstadensberg.

Later, in the *Field Guide to Snakes and Other Reptiles of Southern Africa* (Branch 1998), the distribution of *B. taeniabronchum* was given as: "Apparently isolated populations on Vanstadensberg near Port Elizabeth, around Churchill Dam and Kareedouw and on N. slopes of Tsitsikammaberg, near Misgund in Langkloof, E. Cape". The "Vanstadensberg" locality refers to the population at Lady's Slipper and surroundings, and the reference to "Kareedouw" is the same locality as that of Burger & Smith (1992), i.e., in the Formosa Conservation area near Jagersbos farm. The

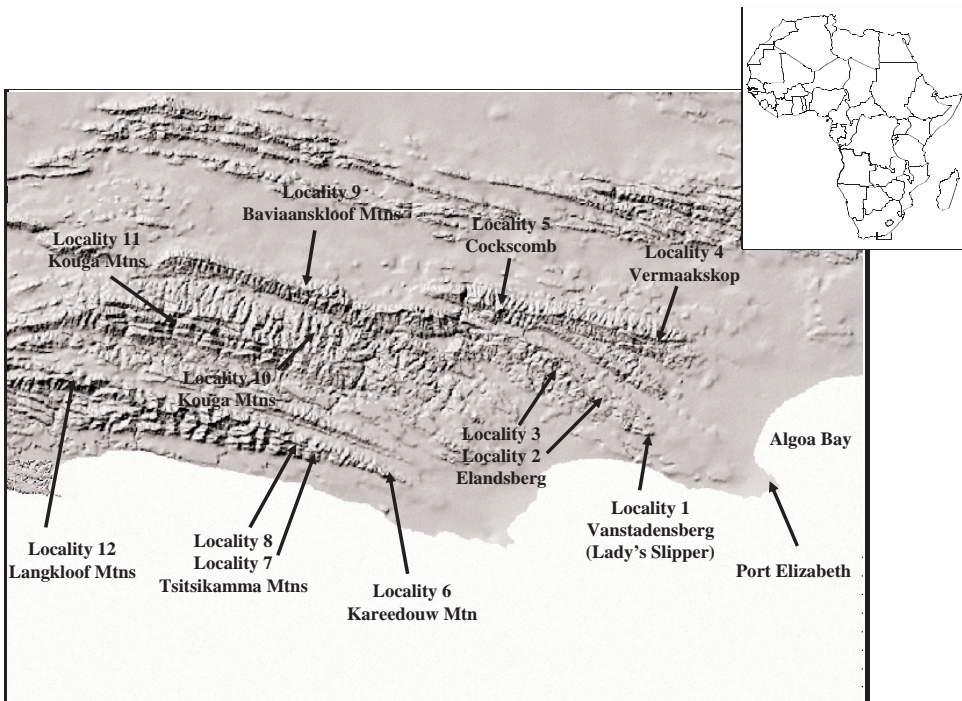


Figure 1. Sampling localities for *Bradypodion* from the eastern Cape Floristic Region, South Africa.

“Churchill Dam” record originates from specimens observed on Kareedouw Mountain north of the mountain pass Spoorbek se Nek (3424AB; approx. 34° 04' S 24° 27' E) and was presumed to be the same taxon as recorded from Jagersbos based on the presence of dark gular grooves (V. Egan and K. Kirkman pers. comm.). Most recently, *B. taeniabronchum* was also recorded from Cockscomb in the Groot Winterhoek Mountains (Cunningham *et al.* 2003). (Note that the Groot Winterhoek Mountains and the Groot Winterhoek Wilderness Area are not the same.)

A comprehensive phylogeographic analysis for *Bradypodion* from the Cape Floristic Region is currently in preparation (K.A. Tolley), but during the course of that investigation it became evident that special attention was required to clarify the distribution of Smith’s Dwarf Chameleon. Here we present a preliminary analysis specifically addressing this issue by making use of two mitochondrial DNA markers (ND2 peptide and 16S-rRNA genes). This analysis includes two previously published sequences from Lady’s Slipper (Tolley *et al.* 2004), plus additional DNA sequence data from several localities within the Groot Winterhoek, Elandsberg, Baviaanskloof, Kouga, Kareedouw, Tsitsikamma and Langkloof mountains (Appendix II, Fig. 1).

Chameleon tissue samples (1-2 mm tail clips) and/or voucher specimens were collected from twelve localities (Appendix II). Following laboratory and data analysis procedures for phylogenetic reconstruction outlined in Tolley *et al.* (2004), a 580 base pair portion of the mitochondrial ND2 gene and a 460 base pair portion of the 16S RNA gene were sequenced for each individual. A phylogenetic analysis using parsimony was run (Fig. 2). For full taxonomic representation, published sequences from multiple representatives of other members of the genus were also included (Appendix I). All sequences have been accessioned into

GenBank and voucher specimens have been deposited in the Port Elizabeth Museum (see Appendix I for accession numbers).

The 12 localities sampled, together with the respective clades of *Bradypodion* occurring at each, are presented below. Further details are given in Appendix II.

Locality 1.—Vanstadensberg. Two individuals were collected in the fynbos vegetation on the slopes of Lady’s Slipper, and were sequenced by Tolley *et al.* (2004). These can be regarded as *B. taeniabronchum* (Fig. 2, sub-clade F).

Locality 2-3.—Elandsberg Mountains 10-30 km NW of Lady’s Slipper. One chameleon was observed in the fynbos vegetation from the eastern part of the Elandsberg Mountains at Locality 2. Fourteen additional chameleons were discovered in fynbos vegetation in the central Elandsberg Mountains at Locality 3. These individuals were identified in the field as *B. taeniabronchum*. DNA samples were sequenced from nine individuals from these two localities. The identity of seven Elandsberg specimens was confirmed as *B. taeniabronchum* based on the phylogenetic tree, as these individuals formed a monophyletic group inclusive of the previously published sequences of *B. taeniabronchum*, with 100% bootstrap support (Fig. 2, sub-clade F). The two remaining Elandsberg specimens did not group with the other seven specimens, but instead were more closely related to seven other individuals collected from the Cockscomb and Vermaakskop, Groot Winterhoek Mountains (Localities 4 & 5, Fig. 2, J).

Locality 4.—Vermaakskop, Groot Winterhoek Mountains. All individuals lacked dark gular grooves. These individuals were related to those from the Cockscomb (Locality 5) and to two of the Elandsberg specimens (Locality 3; Fig. 2, J).

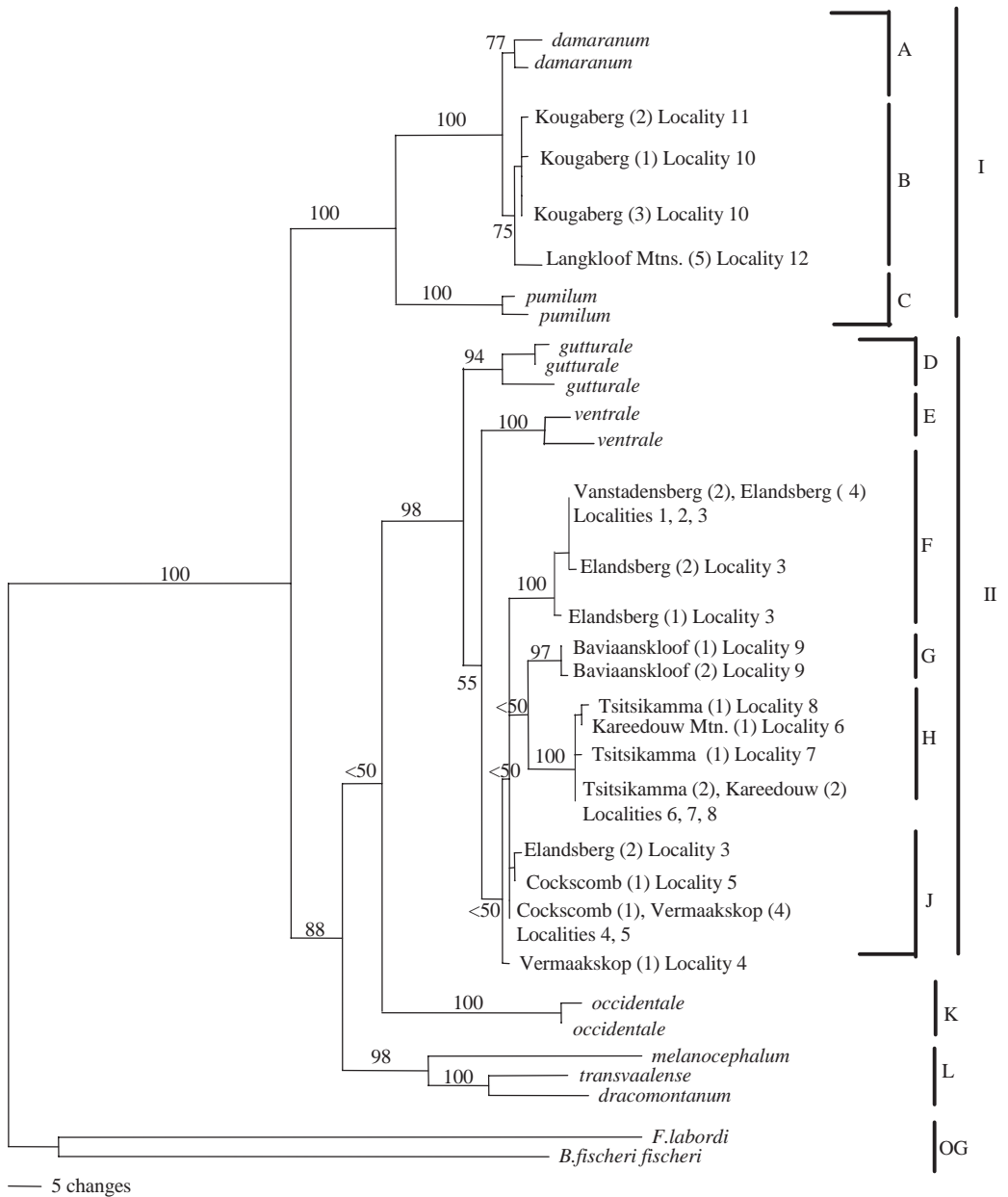


Figure 2. A parsimony phylogram (1000 bootstrap replicates with random addition of taxa) for *Bradypodion* from the eastern Cape Floristic Region, South Africa. Bootstraps are indicated at each node, and unsupported nodes are given as <50%. Included in the analysis were multiple representatives from additional *Bradypodion* species, plus two outgroup taxa (*B. fischeri* and *Furcifer labordi*). The number of identical sequences from a locality are indicated in parentheses after the locality name. Letters to the right (A-M, OG = outgroup) denote sub-clades referred to in the text and in Table 1. Two major clades of *Bradypodion* are also indicated (I & II).

Locality 5.—Cockscomb, Groot Winterhoek Mountains. Both individuals lacked dark gular grooves. These were related to the two individuals collected from the Elandsberg, and to the Vermaakskop specimens (Localities 3 & 4; Fig. 2, J).

Locality 6.—Kareedouw Mountain (northern slopes), south of Churchill Dam. Both individuals collected from this locality form a monophyletic group with individuals from Localities 7 & 8. Chameleons from this locality were previously identified as *B. taeniabronchum* (Branch 1998) as they possess the diagnostic character of dark gular grooves. However, the identification of these specimens does not conform well with our phylogenetic analysis (Fig. 2, J), as they do not form a monophyletic group with typical *B. taeniabronchum* (Fig. 2, sub-clade F).

Locality 7.—Northern slopes of the Tsitsikamma Mountains adjacent to the farm Hudsonvale, approximately 10 km west of Kareedouw. All individuals possessed the dark gular grooves characteristic of *B. taeniabronchum*. Three samples sequenced from this locality form a monophyletic group with Localities 6 & 8 (Fig. 2, sub-clade H), not with typical *B. taeniabronchum* (Fig. 2, sub-clade F).

Locality 8.—Northern slopes of the Tsitsikamma Mountains, adjacent to the farm Jagersbos approximately 15 km west of Kareedouw. Based on the presence of dark gular grooves, Burger & Smith (1992) identified chameleons from this locality as *B. taeniabronchum*. The phylogenetic analysis placed the two individuals from this locality in a sub-clade together with Localities 6 & 7 (Fig. 2, sub-clade H), but separate from typical *B. taeniabronchum* from Localities 1-3 (Fig. 2, sub-clade F).

Locality 9.—Baviaanskloof Mountains, along the Bosrug trail. All individuals lacked dark gular grooves. To date, three samples have been sequenced and the phylogenetic tree shows that the Bosrug individuals are in yet another separate monophyletic clade (Fig. 2, sub-clade G) that is related to *B. taeniabronchum*.

Locality 10.—Northern slopes of the Kouga Mountains, along the Geelhoutbos trail. Construction of the phylogenetic tree showed these sequences to be in a separate clade (Fig. 2, sub-clade B), closely related to *B. damaranum* (Fig. 2, sub-clade A), and only distantly related to the *taeniabronchum* group (Fig. 2, sub-clades D-J). These chameleons are morphologically very different from *B. damaranum*.

Locality 11.—Kouga Mountains on the slopes of Smitsberg. The phylogenetic tree shows that these chameleons are from the same clade (Fig. 2, sub-clade B) as those from Geelhoutbos in the Kouga Mountains (Locality 10) and Langkloof Mountains (Locality 12).

Locality 12.—Langkloof Mountains, south of Misgund. The phylogenetic tree shows that these chameleons are from the same clade (Fig. 2, sub-clade B) as the individuals from Geelhoutbos (Locality 10) and Smitsberg (Locality 11). Branch's (1998) reference to the locality "N. slopes of Tsitsikammaberg, near Misgund" as a locality for *B. taeniabronchum* is erroneous, and refers to this lineage of *Bradypodion*. They do not resemble *B. taeniabronchum* morphologically or genetically.

The taxonomy of dwarf chameleons has been notoriously difficult to assess because a good set of morphological characters for species identification is unavailable. Workers have long struggled to make sense of variation with respect to scalation, body proportions, coloration and patterning, especially since these

characters also vary amongst geographic regions, individuals, sexes and age groups (W.R. Branch, C.R. Tilbury & K.A. Tolley, unpublished data). When identifying dwarf chameleons using photos, field observations, or even vouchers, we have relied upon superficial and somewhat subjective characteristics to distinguish Recognisable Taxonomic Units (RTUs).

The phylogenetic analysis presented here shows that the evolutionary history of dwarf chameleons in the eastern Cape Floristic Region is more complex than previously believed. Chameleons from the eastern Cape Floristic Region fall into two different major clades (I & II; Fig. 2). Within the clade I, chameleons from the Kouga and Langkloof mountains (Localities 10-12) form a monophyletic group (sub-clade B) that is closely related to *B. damaranum* (sub-clade A). There is only 0.7% sequence divergence between them (Table 1), yet they are morphologically very distinct from each other. These chameleons are geographically adjacent, as clade B is known to occur along the northern slopes of the Tsitsikamma mountains (K.A. Tolley, unpub. data) while *B. damaranum* is found in the indigenous forest along the southern slopes of the Tsitsikamma and extending westward. Together with *B. pumilum* (sub-clade C) these chameleons form a strongly supported monophyletic group (Fig. 2). *Bradypodion pumilum* is allopatric with *B. damaranum*/sub-clade B, distributed in a region more than 300 km to the west. All other chameleons from the eastern Cape Floristic Region are members of a second major clade (clade II) that has several clades nested within (sub-clades D-J), and includes *B. taeniabronchum* (sub-clade F). These sub-clades are closely related, with approximately 1.3-3.0% sequence divergence between them (Table 1).

In several instances, these phylogenetic relationships do not conform with either the observed morphology, or taxonomic designations. Firstly, it has been known for a number of years that an undescribed species of dwarf chameleon occurs in the Baviaanskloof, Kouga and Tsitsikamma mountains (W.R. Branch, pers. comm.; M. Burger, pers. obs.). The phylogenetic analysis shows that chameleons from these localities actually fall into two different major clades. Chameleons from the Kouga (Localities 10-11) and Langkloof mountains (Locality 12) fall into sub-clade B (clade I), while those from the Baviaanskloof (Locality 9) fall into sub-clade G (clade II). These sub-clades differ from one another by 6.8% sequence divergence (Table 1). This is a particularly noteworthy example of a contradiction between morphological appearance and phylogenetic relationships. Here, chameleons are morphologically so similar that they were thought to be a single RTU, yet they show large sequence divergence and are in clades that are supported as highly divergent. Furthermore, sub-clade B is closely related to *B. damaranum* and *B. pumilum*, whereas sub-clade G clusters within the *B. taeniabronchum* group (sub-clade F). Given the large sequence divergence, the distant relationship of sub-clades B and G, and fact that these lineages are geographically adjacent, the possibility of these lineages being cryptic species seems likely.

In clade II, some of the individual sub-clades are distinguishable based on morphological differences. For example, *B. ventrale* falls exclusively into sub-clade E and on morphological grounds, is easily distinguishable from the others. Chameleons in group J are also distinguishable based on general appearance. Sub-clade G, while similar in appearance to sub-clade B (discussed above), does not resemble any of the other chameleons in clade II. In contrast, *B. taeniabronchum* falls into a monophyletic sub-clade (F) that is equally divergent (approximately 2.8%) from all other sub-clades

Table 1. Uncorrected p-distances (net sequence divergences) for ND2 among sub-clades of *Bradypodion* (see Fig. 2 for sub-clade designations).

	A	B	C	D	E	F	G	H	J	K	L	OG
A	0.008											
B	0.007	0.007										
C	0.044	0.044	0.005									
D	0.060	0.058	0.071	0.018								
E	0.081	0.075	0.088	0.027	0.000							
F	0.072	0.066	0.081	0.028	0.029	0.001						
G	0.073	0.068	0.080	0.025	0.029	0.030	0.001					
H	0.074	0.068	0.076	0.026	0.029	0.028	0.022	0.001				
J	0.067	0.062	0.077	0.016	0.017	0.013	0.017	0.017	0.003			
K	0.080	0.079	0.090	0.055	0.059	0.062	0.056	0.057	0.053	0.004		
L	0.055	0.055	0.063	0.043	0.052	0.052	0.049	0.051	0.043	0.054	0.055	
OG	0.074	0.075	0.086	0.067	0.085	0.084	0.070	0.077	0.073	0.085	0.069	0.195

in this group. However, chameleons from sub-clade H also have the dark gular grooves and the general appearance of *B. taeniabronchum*. In fact, they are so similar in appearance to *B. taeniabronchum*, that Burger & Smith (1992) assigned them to the same RTU. This raises the question as to whether sub-clade H simply represents a historically isolated and genetically divergent lineage of *B. taeniabronchum*, or whether it stands on its own as a taxonomic unit. From what is currently known of its geographic distribution, this clade is geographically isolated from *B. taeniabronchum*, separated by over 60 km of lowland grassy fynbos, valley bushveld and mesic thicket vegetation types. Up to this time, both lineages have been observed strictly in montane fynbos vegetation and although speculative, the habitat in the intervening 60 km of flats may be unsuitable, triggering and maintaining isolation of these two lineages.

A third instance where the phylogenetic patterns show unexpected results is for the two chameleons from Locality 3 (Elandsberg) that did not group with the other chameleons from the same locality. Instead, these two individuals fall into group J together with chameleons from Localities 4 & 5 (Fig. 2). Group J is not a monophyletic clade, but rather a group of individuals whose relationship to the other sub-

clades is unresolved. A haplotype network (not shown) indicates that group J chameleons have an intermediate position that is reticulate in nature between sub-clades E-H, a relationship that is not possible to recover in a bifurcating tree such as in Fig. 2 (Posada & Crandall 2001). Although they can be grouped together by a network, the phylogenetic analysis cannot recover their position with respect to the other lineages. Previously, the individuals from Locality 5 (Cockscomb) were identified as *B. taeniabronchum* (Cunningham *et al.* 2003). Superficially, these chameleons resembled *B. taeniabronchum*, but they lacked the dark gular grooves (note that this character was not confirmed for the two individuals from Locality 3). Our analysis shows that these Cockscomb individuals (group J) do not group with *B. taeniabronchum* (sub-clade F). Sequence divergence between group J and the other sub-clades in the eastern Cape Floristic Region is low (Table 1), ranging from 1.3% (with *B. taeniabronchum*) to 1.7% (with sub-clades G-H).

Given the results of this analysis, it appears that *B. taeniabronchum* (*sensu stricto*) occurs from the Vanstadensberg to the central Elandsberg. There are several sub-clades (D, E, G, H, J) that are closely related to *B. taeniabronchum* (sub-clade F). While a robust assessment regarding the status of these lineages cannot be made

without the inclusion of nuclear DNA markers and a full morphological analysis, several aspects of these patterns are noteworthy at this time. One viewpoint would be that these results suggest fewer species rather than more, and that sub-clades D-J could be simply historically divergent mtDNA lineages within a single morphologically variable species. While sequence divergence alone is not enough to describe any mitochondrial lineage as a "species" or not (Ferguson 2002; Cunningham & Cherry 2000), it is striking that sequence divergence among these lineages is rather low when compared to that among other *Bradypodion* species (Table 1). Nevertheless, the assumption of a single, morphologically variable species is unsatisfactory as it would mean that two morphologically distinct species, *B. ventrale* (Gray 1845) and *B. gutturale* (Smith 1849) would become synonymised with *B. taeniabronchum* (Smith 1831). The other lineages would simply be considered morphologically variable populations or geographically isolated lineages of *B. taeniabronchum* despite their own morphological distinctiveness.

Alternatively, these sub-clades (D-J) form a species complex of closely related, but separate species. The low divergence between all these sub-clades may indicate that they are recently diverged and the origins of these species are reticulate, resulting in low resolution among these sub-clades. Differential selection pressures on these lineages could have produced the varied morphological schemes that we have observed, regardless of the mitochondrial gene tree observed.

A third possibility, not mutually exclusive of reticulate evolution, is that some degree of introgression has occurred between lineages, slowing down the process of lineage sorting and genetic diversification. To address these alternative hypotheses, additional work is underway which will include a morphological

assessment, the use of nuclear markers, and an investigation into the chromosome structure of *Bradypodion*. This, together with additional geographic sampling, is necessary before we take a firm stand on which hypothesis best explains the observed patterns.

Finally, the IUCN conservation status of *B. taeniabronchum* as Critically Endangered should stand until a full conservation assessment can be conducted. The problem at hand is rather complex and any suggestions regarding the conservation status based on either the possibility that there is a single broadly distributed, morphology variable species, or that there is a complex of closely related species would be premature.

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LITERATURE CITED

- BRANCH, W.R. 1988. *Bradypodion taeniabronchum*: species account. pp. 43-45. In South Africa Red Data Book - Reptiles and Amphibians. W.R. Branch (Ed.), South African National Scientific Programmes Report 151. CSIR Pretoria.
- BRANCH, W.R. 1998. Field Guide to the Snakes and Other Reptiles of Southern Africa. Third Edition. 399 pp. Struik, Cape Town.
- BURGER, M. 1997. The current status of amphibians and reptiles in the Eastern Cape Province. The Naturalist 41: 31-34.
- BURGER, M., R.C. BOYCOTT & R.B.T. SMITH. 1997. The Herpetofauna of the Formosa Conservation Area. In: Proceedings of the Third H.A.A. Symposium, Van Wyk J.H. (Ed.): 135-139.
- BURGER, M. & R.B.T. SMITH. 1992. Geographical distribution: *Bradypodion taeniabronchum*. J. Herpetol. Assoc. Afr. 41:37.
- CUNNINGHAM, M. & M.I. CHERRY. 2000. Mitochondrial DNA divergence in southern African bufonids: are species equivalent entities? Afr. J. Herpetol. 49: 9-22.
- CUNNINGHAM, M., C.L. HENDERSON & K.A. TOLLEY. 2003. Herpetological surveys: Cockscomb (T'Numqa) mountain. Afr. Herp News 36: 22-25.
- FITZSIMONS, V.F.M. 1943. The Lizards of South Africa. Transv. Mus. Mem. 1: 1-528.
- FERGUSON, J.W.H. 2002. On the use of genetic divergences for identifying species. Biol. J. Linn. Soc. 75: 509-516.
- HILTON-TAYLOR, C. (compiler) 2000. 2000 IUCN Red List of Threatened Species. IUCN, Gland, Switzerland and Cambridge, UK.
- POSADA, D. & K.A. CRANDALL. 2001. Intraspecific gene genealogies: trees grafting into networks. TREE 16: 37-45.
- RAW, L.R.G. 1995. Biodiversity, biogeography and conservation of South Africa's endemic dwarf chameleons. INR Investigational Report No. 117, University of Natal.
- SMITH, A. 1849. Illustrations of the Zoology of South Africa. Smith, Elder and Co., London.
- TOLLEY, K.A., C.R. TILBURY, W.R. BRANCH & C.A. MATTHEE. 2004. Phylogenetics of the southern African dwarf chameleons, *Bradypodion*. Mol. Phylogenet. Evol. 30: 354-365.
- TOWNSEND, T., & A. LARSON. 2002. Molecular phylogenetics and mitochondrial genomic evolution in the Chamaeleonidae (Reptilia, Squamata). Mol. Phylogenet. Evol. 23: 22-26.
- VENCES, M. 2000. Phylogenetic studies of ranoid frogs (Amphibia:Anura), with a discussion of the origin and evolution of the vertebrate clades of Madagascar. Ph.D. Thesis. Universitaet Bonn, Germany.

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APPENDIX I

Sampling localities, clades and GenBank accession numbers (ND2 & 16S) for *Bradypodion* used in this study. Voucher numbers for specimens deposited in the Port Elizabeth Museum (PEM R) are given.

Field #	Locality/species	Clade	ND2	16S	PEM R
CT76	Locality 1	F	AY289883 ^a	AY289827 ^a	5697
CT77	Locality 1	F	AY289884 ^a	AY289828 ^a	5698
KT33	Locality 2	F	AY309228	AY555197	no specimen
KT34	Locality 3	J	AY309229	AY555198	no specimen
KT35	Locality 3	F	AY309230	AY555199	no specimen
KT36	Locality 3	F	AY309231	none	no specimen
KT37	Locality 3	J	AY309232	AY555200	no specimen
KT38	Locality 3	F	AY309233	AY555201	no specimen
KT39	Locality 3	F	AY309234	AY555202	no specimen
KT40	Locality 3	F	AY309235	AY555203	no specimen
KT41	Locality 3	F	AY309236	AY555204	no specimen
KTH140	Locality 4	J	AY615413	AY615400	5858
KTH161	Locality 4	J	AY615414	AY615401	5859
KTH165	Locality 4	J	AY615415	AY615402	5860
KTH169	Locality 4	J	AY615416	AY615403	no specimen
KTH182	Locality 4	J	AY615417	AY605404	no specimen
KT43	Locality 5	J	AY555221	AY555205	no specimen
KT44	Locality 5	J	AY555222	AY555206	no specimen
KTH180	Locality 6	H	AY615418	AY615405	5861
KTH187	Locality 6	H	AY615419	AY615406	no specimen
KTH150	Locality 7	H	AY615420	AY615407	no specimen
KTH164	Locality 7	H	AY615421	AY615408	5862
KTH168	Locality 7	H	AY615422	AY615409	no specimen
KTH094	Locality 8	H	AY555225	AY555208	5864
KTH108	Locality 8	H	AY555232	AY555215	5863
KT29	Locality 9	G	AY615424	AY615411	no specimen
KT30	Locality 9	G	AY615425	AY615412	no specimen
KTH099	Locality 9	G	AY615423	AY615410	5865
KTH088	Locality 10	B	AY555223	AY570759	5866
KTH095	Locality 10	B	AY555226	AY555209	no specimen
KTH100	Locality 10	B	AY555229	AY555212	no specimen
KTH103	Locality 10	B	AY555230	AY555213	no specimen
PEM R134	Locality 11	B	AY289904 ^a	AY289846 ^a	134
PEM R124	Locality 11	B	AY289905 ^a	AY289847 ^a	124
KTH092	Locality 12	B	AY555224	AY555207	no specimen
KTH096	Locality 12	B	AY555227	AY555210	no specimen
KTH098	Locality 12	B	AY555228	AY555211	5868
KTH105	Locality 12	B	AY555231	AY555214	5867
KTH111	Locality 12	B	AY555233	AY555216	no specimen
BS1	<i>B. damaranum</i>	A	AY555220	AY555196	no specimen
CT06	<i>B. damaranum</i>	A	AY289805 ^a	AY289861 ^a	5725
CT99	<i>B. pumilum</i>	C	AY289891 ^a	AY289835 ^a	5730
E2	<i>B. pumilum</i>	C	AY289903 ^a	AY289845 ^a	no specimen
CT14	<i>B. occidentale</i>	K	AY289868 ^a	AY289812 ^a	5713
CT94	<i>B. occidentale</i>	K	AY289888 ^a	AY289832 ^a	5716
CT73	<i>B. ventrale</i>	E	AY289882 ^a	AY289826 ^a	5704
CT11	<i>B. ventrale</i>	E	AY289866 ^a	AY289810 ^a	5703
CT91	<i>B. gutturale</i>	D	AY289887 ^a	AY289831 ^a	5727
CT07	<i>B. gutturale</i>	D	AY289862 ^a	AY289806 ^a	5685
KT14	<i>B. gutturale</i>	D	AY555235	AY555218	no specimen
CT17	<i>B. melanocephalum</i>	L	AY289870 ^a	AY289814 ^a	5694
CT26	<i>B. transvaalense</i>	L	AY289873 ^a	AY289817 ^a	5719
CPBD01	<i>B. dracomontanum</i>	L	AY555234	AY555217	no specimen
CT104	<i>B. fischeri fischeri</i>	OG	AY555236	AY555219	N/A
	<i>F. labordi</i>	OG	AF448767 ^b	AF215264 ^c	N/A

^aTolley *et al.* 2004, ^bTownsend & Larson 2002, ^cVences 2000

APPENDIX II

Details of sampling localities for *Bradypodion* from the eastern Cape Floristic Region, South Africa. QDGC = Quarter-degree Grid Cell. Number of vouchers from each locality, and the number of DNA samples that were sequenced are also given. MB - M. Branch; MIB - M. Burger; DC - D. Clark; MC - M. Cunningham; CLH - C.L. Henderson; AP - A. Page; CRT - C.R. Tilbury; KAT - K.A. Tolley; KAW - K.A. Whitaker.

Locality	QDGC	Lat/Long	Alt. (m)	Date	Collectors	Vouchers	DNA samples
1-Vanstadensberg, on Lady's Slipper	3325CD	33°53'14"S; 25°15'51"E	572	13/1/1999	CRT	2	2
2-Eastern part of Elandsberg, N of Longmore plantation (MTO Forestry)	3325CC	33°47'52"S; 25°09'53"E	480	2/12/2002	MC, CLH, KAT	0	1
3-Central part of Elandsberg, NW of Longmore, adjacent to border of Otterford State Forest	3324DB, 3325CA	33°42'15"S - 33°42'56"S 24°59'48"E - 25°00'24"E	800	3/12/2002	MC, CLH, KAT	0	8
4-Groot Winterhoek Mts, in Groendal Wilderness Area, on Vermaakskop	3325CB	33°37'47"S; 25°16'44"E	890	17/4/2004	MC, KAT, KAW	4	5
5-Groot Winterhoek Mts, on Cockscomb	3324DB	33°34'15"S; 24°47'41"E	1241	7/12/2002	MC, CLH, AP, KAT	0	2
6-Kareedouw Mt, S of Churchill Dam	3424AB	34°00'33"S; 24° 29'01"E	175	13/4/2004	MC, CLH, KAT, KAW	1	2
7-Tsitsikamma Mts, in Formosa Conservation Area, adjacent to farm Hudsonvale, c. 10 km W of Kareedouw	3324CC	33°56'57"S; 24°11'22"E	415	12/4/2004	MC, CL, KAT, KAW	2	3
8-Tsitsikamma Mts, in Formosa Conservation area, adjacent to farm Jagersbos, c. 15 km W of Kareedouw	3324CC	33°55'42"S; 24°08'13"E	413	28/11/2003	MIB, KAT	2	2
9-Baviaanskloof Mts, in Baviaanskloof Wilderness Area, along Bosrug trail	3324CA	33°32'03"S; 24°05'01"E 33°31'50"S; 24°05'13"E	1060	30/11/2002 1/12/2003	MIB, DC, MC, CLH, KAT	4	3
10-Kouga Mts, in Baviaanskloof Wilderness Area, Geelhoutbos trail	3324CA	33°40'07"S; 24°12'37"E 33°41'14"S; 24°12'07"E	960-980	2/12/2003	MIB, KAT	1	4
11-Kouga Mts, in Baviaanskloof Wilderness Area, on Smitsberg	3323DB	33°37'34"S; 23°45'08"E		2/1998	MB, DC	3	2
12-Langkloof Mts, Formosa Conservation area, S of Misgund	3323CD	33°47'12"S - 33°47'11"S 23°27'03"E - 23°25'00"E	1170-1285	28/11/2003	MIB, KAT	2	5