




Multilocus phylogenetics in a widespread African anuran lineage (Brevicipitidae: *Breviceps*) reveals patterns of diversity reflecting geoclimatic change

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Abstract

Aim: To investigate models assessing the influence of geomorphology and climatic shifts on species diversification in sub-Saharan Africa by reconstructing the pattern and timing of phylogenetic relationships of rain frogs (Brevicipitidae: *Breviceps*).

Location: Sub-Saharan Africa, south of the Congo Basin.

Methods: Multilocus sequence data were generated for near complete species-level sampling of the genus *Breviceps*. Phylogenetic relationships were inferred via Bayesian inference and maximum likelihood analyses on both concatenated and single-gene datasets. Network analyses identified locus-specific reticulate relationships among taxa. Bayesian methods were used to infer dates of divergence among *Breviceps* lineages, and niche modelling was used to identify possible adaptive divergence.

Results: *Breviceps* is monophyletic and comprised of two major, largely allopatric subclades. Diversity within each subclade is concentrated in two areas with contrasting geologic and climatic histories: the arid/semiarid winter rainfall zone in the south-western (SW) Cape, and the semitropical East Coast that receives predominantly summer rainfall. Recognized species diversity in the SW Cape based on phenotypic variation is consistent with observed genetic patterns whereas the East Coast is shown to harbour unexpectedly high genetic diversity and up to seven putative, cryptic species. Niche models show significant overlap between closely related species.

Main conclusions: Dating analyses indicate that diversification of *Breviceps* occurred rapidly within the Miocene, with only a moderate decline over the Plio-Pleistocene, suggesting that this process might be slowed but ongoing. Our findings suggest that a combination of two models, a landscape barrier model and climate fluctuation model, can explain patterns of diversification in *Breviceps*. This demonstrates that Miocene epeirogenic events and climatic shifts may have had a considerable influence on contemporary patterns of biodiversity. Topographic complexity and relative geoclimatic stability in the East have promoted cryptic diversification in allopatry, and this area clearly harbours numerous undescribed taxa and is in need of detailed biotic investigation.

KEYWORDS

climate fluctuation model, cryptic species, distribution modelling, Great Escarpment, landscape barrier model, Miocene, rain frogs

1 | INTRODUCTION

To comprehend the biogeographical patterns we observe today, one must be cognizant of the evolutionary history of species and the palaeoenvironmental conditions under which they evolved, especially as these may differ from the present (Tolley, Chase, & Forest, 2008). The generation and persistence of biogeographical patterns is often region-specific. For example, the Southern Hemisphere remained largely unglaciated during the late Cenozoic, preserving biogeographically informative genetic signal that would otherwise have been erased by recurrent episodes of Plio-Pleistocene glaciation (Hewitt, 2000). Whereas temperatures gradually decreased in the Northern Hemisphere, southern Africa became more warm and arid (van Zinderen Bakker & Mercer, 1986), relegating widespread, mesic floral and faunal lineages to allopatric, climatically stable refugia, promoting diversification and adaptation to xeric environments (Barlow et al., 2013; Bauer, 1999; Heinicke, Jackman, & Bauer, 2017; ; Lorenzen, Heller, & Siegmund, 2012; Matthee & Flemming, 2002; Verboom et al., 2009). Reconstructing the evolutionary history of Southern Hemisphere organisms, therefore, presents a potentially informative system for understanding how contemporary communities were influenced by pre-Quaternary environmental change.

Since the breakup of southern Gondwana in the late Jurassic and early Cretaceous, southern Africa has experienced many epeirogenic events that have contributed to contemporary topographical relief. Uplifts at the Oligocene–Miocene and Pliocene–Pleistocene boundaries formed most modern mountain ranges and the Kalahari Depression (Birkenhauer, 1991; Clark, Barker, & Mucina, 2011; Dingle, Siesser, & Newton, 1983). These uplifts also created the Great Escarpment, a feature that influences climate as well as the distribution and diversification of organisms (Clark et al., 2011). Along the east coast of southern Africa, the warm Agulhas current combined with the Great Escarpment (specifically the Drakensberg Mountains) to create a rain shadow maintaining a subtropical climate along the Escarpment's eastern slopes (Neumann & Bamford, 2015). Consequently, patches of forest habitat have persisted here since the Miocene while the rest of the subcontinent underwent aridification (Sepulchre et al., 2006). This aridification was driven by a Miocene shift in circumpolar air circulation in the Southern Ocean combined with the nascent Benguela upwelling system off the south-western African coast (Figure 1; Siesser, 1980; McCarthy & Rubidge, 2005; Neumann & Bamford, 2015). Wind patterns then brought winter moisture to the south-western Cape, creating discrete rainfall zones with contrasting seasonality (Chase & Meadows, 2007). Additional post-Miocene epeirogeny (including significant crustal flexuring) increased topo-edaphic heterogeneity.

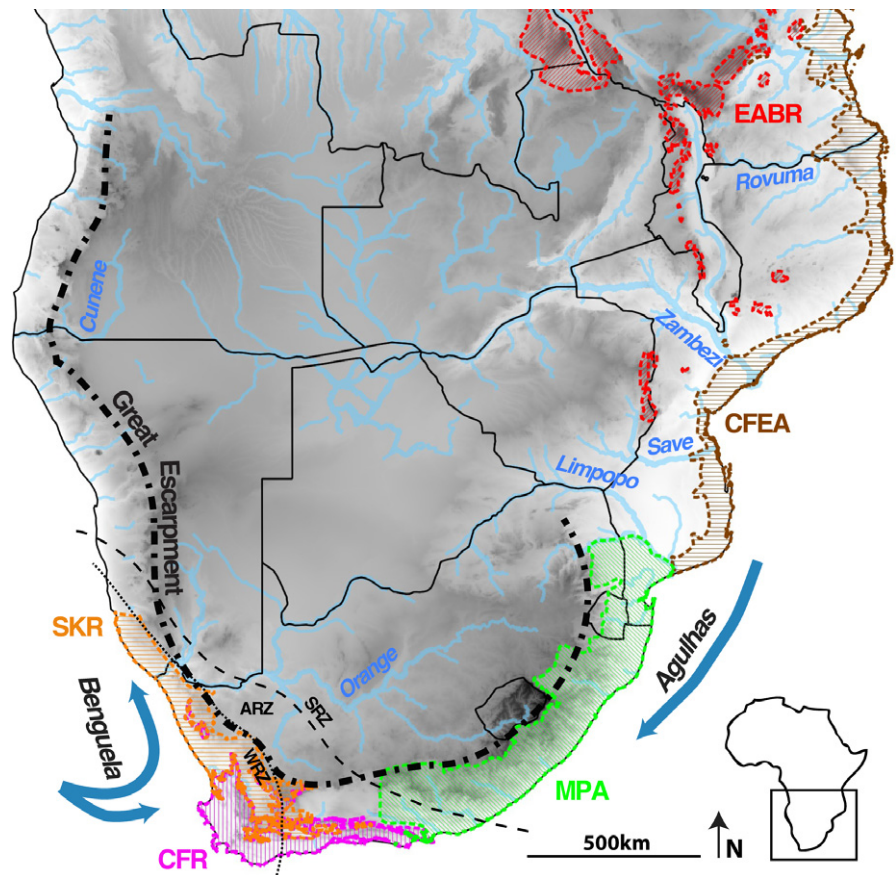
The continued evolution of landscape features and climate over time, combined with landscape and climate heterogeneity across space, has likely stimulated floral and faunal diversification in southern Africa (Cowling, Procheş, & Partridge, 2009; Moore, 1999). The region's long history of aridification has also generated a largely arid-adapted and endemic biota (Bauer, 1999; Brain, 1985). Indeed, a commonly observed biogeographical pattern in the southern African fauna is recent (late Miocene to Pleistocene) diversification driven by climate fluctuation, promoting allopatric speciation (Barlow et al., 2013; Daniels, Mouton, & Du Toit, 2004; Diedericks & Daniels, 2014; Furman et al., 2015; Lorenzen et al., 2012; Swart, Tolley, & Matthee, 2009; Tolley et al., 2008). However, Africa is an old continent and modern lineages vary in age. Thus, many older lineages have persisted alongside recently diversifying ones, and the historical biogeographical factors that promoted diversification in these older lineages may have differed.

The genus *Breviceps* (rain frogs) represents one such older lineage. *Breviceps* is an ancient African genus, having diverged from their sister clade (the East African brevicipitids) in the early Palaeogene (Loader et al., 2014). Species accumulation within the East African radiation of Brevicpitids occurred predominantly in the Miocene and has been attributed to the long-term persistence of forests across the Eastern Afrotropical Biodiversity Region (EABR; Loader et al., 2014) (Figure 1). Diversification of *Breviceps* in southern Africa has thus far remained unstudied. However, they are an ideal exemplar group for studying the historical biogeography of more ancient lineages in southern Africa, for a number of reasons. First, they are widely distributed across eastern and southern Africa. *Breviceps* can be found from Angola eastward through southern Democratic Republic of the Congo and Tanzania, and southward to South Africa (Minter, 2004), only absent from the most arid regions of the Namib/Pro-Namib (Namibia) and Nama-Karoo (South Africa) and areas that experience winter frost and low annual average rainfall (Karoo/Highveld Grassland of South Africa). Second, their ecology, life history and morphology likely limit gene flow (i.e. they are largely nocturnal, fossorial, small-bodied insectivores with poor dispersal ability and reproduce via direct-development [no free-swimming tadpole stage]), potentially fostering allopatric divergence. Third, southern African *Breviceps* are varied in habitat choice, occurring in afro-montane and coastal forest, as well as Fynbos (Mediterranean-like shrub or heathland), vegetated dune, savanna and grassland habitats, where they prefer sandy/loamy, well-drained soils (Channing, 2001; du Preez & Carruthers, 2009; Minter, 2004). Thus, niche divergence could have played a role in diversification.

Here, we incorporate multilocus molecular data and broad taxonomic sampling to reconstruct the pattern and timing of diversification of *Breviceps*. These data are combined with niche models to



FIGURE 1 Map of sub-Saharan Africa south of the Congo basin, highlighting relevant biodiversity hotspots: Cape Floristic Region (CFR, magenta); Succulent Karoo Region (SKR, orange); Maputa-Pondoland-Albany (MPA, green); Coastal Forests of East Africa (CFEA, brown); and Eastern Afromontane Biodiversity Region (EABR, red). Biogeographically relevant features are indicated, including rivers, rainfall zones (winter [WRZ], summer [SRZ], and aseasonal [ARZ]), and ocean currents (see main text for details) [Color figure can be viewed at wileyonlinelibrary.com]



deduce what has shaped contemporary patterns of diversity, distribution and biogeography of *Breviceps* in Africa south of the Congo Basin. We use these data to evaluate three alternative models of diversification caused by allopatric divergence. In the climate fluctuation model, climate change isolates populations in patches of suitable habitat, allowing allopatric divergence to occur. This is the same model suggested for many faunal groups in southern Africa, as described above. Under this model, we expect most divergences among *Breviceps* taxa to be relatively recent and for there to be little niche separation among closely related species, as niche conservatism would be the primary factor isolating populations. Species distribution models would imply large areas of overlapping suitable habitat for close relatives. In a landscape barrier model, divergences are caused by barriers formed by geological processes (e.g. deposition of the Kalahari sands, uplift associated with formation of the Great Escarpment, river capture and formation of resulting gorges, etc.). Under this model, we expect divergences to be older, and coincident in time with periods of geological change. Closely related species would vary in their degree of niche overlap, as ecological differentiation would not affect population divergence. The third model is an adaptive change model. In this model, we expect divergences among species to vary in age, but closely related species will have significant divergence in their ecological niche, with nonoverlapping species distribution models as divergence would be driven by adaptation to different ecological conditions.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling and laboratory protocols

We obtained tissue from 77 individual frogs representing 16 of the 18 recognized species and downloaded partial sequence data from GenBank for the two remaining species (see Table S1 in Appendix S1). For two species, where voucher identification is difficult and/or ambiguous (*B. sopranus* and *B. bagginsi*), we include a *cf.* designation to represent identification uncertainty. Furthermore, topotypic material was not available for all species. Although the phylogenetic position of *Breviceps* is well established within the greater Afrobatrachian radiation (Pyron & Wiens, 2011), there have been no studies exploring the monophyly of *Breviceps* sensu stricto. Thus, we include complete sampling of brevicipitid genera to test the monophyly of this genus. Outgroups include species from other members of Brevicipitidae (e.g. *Callulina*, *Balebreviceps*, *Probreviceps* and *Spaeleophryne*), as well as more distantly related Afrobatrachian and microhylid taxa (see Table S1).

We isolated genomic DNA from ethanol preserved (95%) liver, skin and/or muscle tissue samples via salt extraction (Aljanabi & Martinez, 1997). We performed polymerase chain reaction (PCR) to amplify partial sequences of two mitochondrial (12S and 16S ribosomal rRNA genes) and three nuclear loci (recombination activating protein 1, RAG1; brain-derived neurotrophic factor, BDNF; and solute

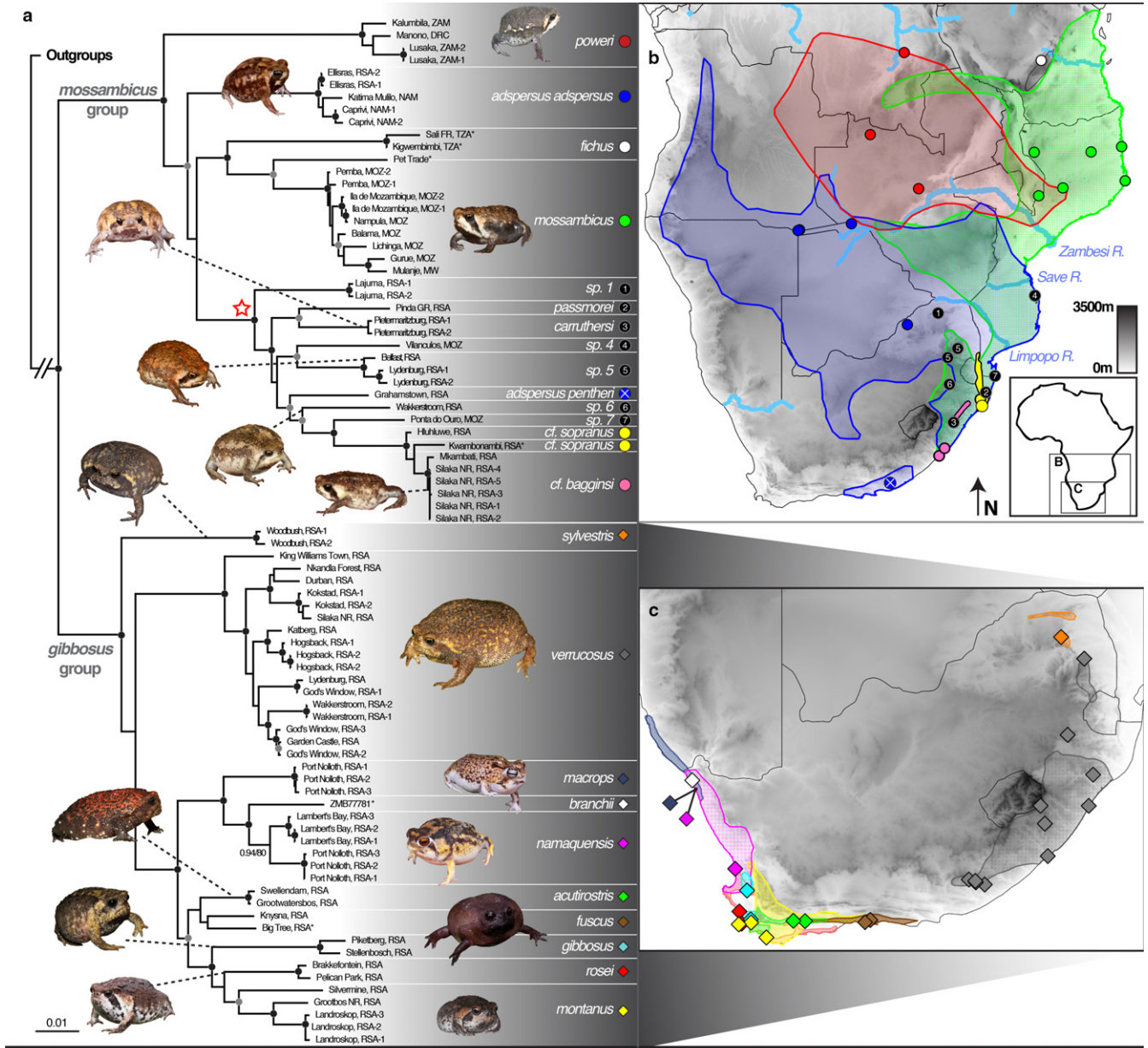




FIGURE 2 (a) Phylogenetic relationships of *Breviceps* species-level relationships with representative photographs of each species in life (not size standardized), as well as maps of the geographical distributions of the two major groups: (b) the *mossambicus*-group; (c) the *gibbosus*-group). Coloured polygons on the maps coordinate with the small coloured circles and diamonds following the taxon names on the tree. The latter relate to approximate geographic sampling localities indicated on the associated maps. Black circles with numbers represent the seven putatively novel lineages within the *mossambicus*-group (including two recently described taxa). Within the tree, a solid, black circle indicates nodal support values $\geq 70\%$ for Maximum Likelihood bootstraps (BS) and ≥ 0.95 for Bayesian inference posterior probabilities (PP), whereas a grey circle represents PP ≥ 0.95 , but BS $< 70\%$. (D) A gene network of phased *RAG1* haplotype sequences. (E) Time-calibrated ultrametric tree of *Breviceps* lineages based on concatenated, partitioned nuclear data, with support and 95% confidence intervals (blue bars) indicated at each node. Asterisks indicate high (PP ≥ 0.95) support. The lineage-through time plot (red line) was generated using the LTT function in the R package 'ape' [Color figure can be viewed at wileyonlinelibrary.com]

carrier family 8 member 3, *SLC8A3*). PCR primers are reported in Appendix S1 (Table S2). We viewed and purified PCR products via 1.5% agarose gel electrophoresis and ExoSAP-IT[®] (Affymetrix, Santa Clara, CA, USA), respectively. Sequencing reactions used the BigDye TERMINATOR v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), which were then sent to the DNASU Sequencing Core (Arizona State University) for purification and sequencing using an Applied Biosystems 3730XL automated sequencer.

2.2 | Sequence alignment, model selection and phylogenetic reconstruction

The forward and reverse raw sequence reads were edited, assembled and then aligned using GENEIOUS v.6 (Biomatters <http://www.geneious.com>). We also checked the amino-acid translation of protein-coding loci to verify open reading frames. Sequences were submitted to GenBank and their accession numbers can be found in Table S1. Sequence data from GenBank were used for the two missing species mentioned above, as well as representatives from outgroup taxa for rooting purposes. Uncorrected mean *p* sequence divergence values were calculated for both 12S and 16S (Table S3) using MEGA v.6.0 (Tamura, Stecher, Peterson, Filipiński, & Kumar, 2013).

Datasets (concatenated, mitochondrial [mtDNA]-only and nuclear [nuDNA]-only) of all samples were analysed using Bayesian Markov chain Monte Carlo (MRBAYES v.3.2; Ronquist & Huelsenbeck, 2003) and maximum likelihood methods (RAxML v.8.1.11; Stamatakis, 2006) using the CIPRES Science Gateway 3.1 for online phylogenetic analysis (<http://www.phylo.org/index.php/portal/>). An appropriate partitioning strategy and molecular models specifically for Bayesian analyses were chosen using PARTITIONFINDER v.1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012), which assessed all possible candidate positions (e.g. each codon in the nuDNA) using the Bayesian information criterion (Lanfear et al., 2012). The resulting partitioning scheme is listed in Table S4. Final Bayesian analyses ran for 50 million generations with four independent chains, and sampled every 50,000 generations. We checked for stationarity using TRACER v.1.6 (Rambaut, Drummond, & Suchard, 2007), after which a 25% burn-in was removed, leaving 750 trees for posterior analysis. Maximum likelihood analyses were performed using the default settings for RAxML using the GTRGAMMA model of sequence evolution (Stamatakis, 2006) and ceasing bootstrapping when extended majority rule bootstrapping criteria had been reached.

For comparison with tree-based methods and in order to view gene tree (haplotype) relationships among the ingroup, networks for

each nuDNA locus and combined mtDNA were constructed using SPLITSTREE v.4.12.3 (Huson & Bryant, 2006) with the Neighbour-net algorithm. We used an algorithmic approach to phase nuDNA alleles using PHASE v2.1.1 (Scheet & Stephens, 2006; Stephens, Smith, & Donnelly, 2001) prior to building splitstrees.

2.3 | Estimating divergence times

Using a lognormal relaxed molecular clock, the age of the *Breviceps* radiation and its constituent lineages was estimated in BEAST v.1.8 (Drummond, Suchard, Xie, & Rambaut, 2012). Analyses were performed using a concatenated alignment split into two partitions (nuclear vs. mitochondrial DNA), and limited to a single individual (or chimera of individuals) per species representing a complete sampling of loci. Missing data could yield potentially problematic results (Blanners, Townsend, Pepe, Reeder, & Wiens, 2013), thus we excluded taxa with high proportion of missing data, particularly nuclear loci (i.e. *B. fichus* and *B. branchi*). Secondary calibrations employed in these analyses followed Loader et al. (2014) and were based on the fossil calibrated study of Roelants et al. (2007) exploring relationships among the Amphibia. Specifically, we used the following as normally distributed constraints of node ages: MRCA of the crown group including *Arthroleptis*, 92.8 (84.5–111.8) million years ago [Ma]; *Hemiusus* + *Brevicipitidae*, 65.9 (54.1–84.9) Ma; *Breviceps*, 45.4 (32.9–63.4) Ma; and *Callulina*, 29.6 (19.5–44.5) Ma (see Loader et al., 2014 for more detail). Direct fossil calibration was not possible due to the lack of pre-Quaternary fossil material attributable to *Brevicipitidae* (Matthews, van Dijk, Roberts, & Smith, 2015). The BEAST analysis ran for 100 million generations, sampling every 10,000th gen., with a 20% burn-in, and using a Yule prior. Stationarity was always reached well before the end of the burn-in. TRACER confirmed that all runs had converged on similar model parameters (estimated sample size > 200 for all parameters). To view the relative timing of diversification with respect to the accumulation of lineages, a lineage-through-time (LTT) plot was generated using the 'LTT' function in the R package 'ape' (Paradis, Claude, & Strimmer, 2004).

2.4 | Species distribution modelling

We estimated species distribution models (SDMs) using the program MAXENT v. 3.3.2 (Phillips, Anderson, & Schapire, 2006) to provide a measure of climatic niche divergence among species. Estimates used the default settings as implemented in the 'dismo' and 'SDMTools' R

packages. Models were trained using collection localities attached to the samples used for genetic analysis, as well as supplementary data points obtained from published sources (e.g. Channing & Wahlberg, 2011) or the Virtual Museum (Animal Demography Unit, University of Cape Town; <http://vmus.adu.org.za/>). The museum data required some manual filtering and we omitted any unverified data points (e.g. those represented by unrecorded nuptial calls only). The assignment of museum samples to widespread species was, at times, untenable (due to widespread paraphyly within *B. adspersus* and *B. mossambicus*; see below). Therefore, our distribution modelling included only samples for which museum locality data were relatively certain (i.e. the *gibbosus*-group exclusive of *B. branchi* [a recently described species known only from a single sample]) and for which our results did not suggest the presence of cryptic species (Table S5).

Our sample size for *B. sylvestris* fell to four points postfiltering. Modelling with so few points is not ideal. However, when we added three additional points for which GPS data exist (based on unrecorded calls) and reran the SDM the results were comparable (results not shown), suggesting a reasonably reliable model. A recent theoretical study (van Proosdij, Sosef, Wieringa, & Raes, 2016) also suggested that as few as three presence samples from a narrow-range taxon will provide accurate SDMs.

SDMs were generated based on the 19 bioclimatic data layers available from the Worldclim database (www.worldclim.org) that were resampled to the WGS1984 Transverse Mercator projection and cropped to a geographically relevant window (xlim = 10 to 36; ylim = -35 to -22) using the 'mapproj' R package. Following Elith et al. (2011), we retained all bioclimatic variables, even if correlated, and allowed the MAXENT algorithm to determine the required predictors for each model. MAXENT performed 100 bootstrapped replicates, using 75% of the locality data to train and 25% to test the models, with a regularization multiplier of 1. 'Area under the curve' (AUC) scores of the receiver-operating characteristic were calculated to assess the accuracy of the models. Jack-knife tests were also used to determine individual variable contributions for the final SDMs. To assess the degree of overlap in predicted SDMs among the taxa, we estimated Schoener's *D* (a measure of overlap) using ENMtools (Warren, Glor, & Turelli, 2010). We were keen to assess whether closely related taxa differed significantly with respect to niche (as defined by the 19 bioclimatic variables), so we performed pairwise identity and similarity tests in ENMtools to generate values of Schoener's *D*. We generated a distribution of pseudoreplicates ($n = 100$) to ascertain whether the observed value of *D* is significantly different from random.

3 | RESULTS

3.1 | Phylogenetic relationships

The concatenated, aligned ingroup dataset totalled 3,434 characters (containing 550 parsimony-informative characters out of 660

variable sites). The optimized Maximum Likelihood (ML) tree had a log-likelihood score of $-23,500.1$ and Bayesian Inference (BI) produced an optimal tree with a mean likelihood score of $-21,730.8$. Both reconstructions based on the concatenated dataset produced nearly identical consensus tree topologies (Appendix S2, Fig. S1A-B), and any discordance exhibited poor support. Many of the nodes receiving low support are associated with distal nodes and do not affect our biogeographical conclusions. Mitochondrial and individual nuclear gene trees produced largely congruent patterns of relationship (not illustrated) and any disagreements occurred were associated with low statistical support.

We confirm the monophyly of *Breviceps*, as all material ascribed to this genus (rooted with the non-Afrobatrachian *Kaloula pulchra*) fell within this well-supported clade (Figure 2a; posterior probability [pp] 1.0, bootstrap support [bs] 100%). All phylogenetic analyses recovered two well-supported subclades: (i) the *mossambicus*-group, composed of more northerly/easterly distributed species that occur both above and below the Great Escarpment (GE), and are largely restricted to savanna (*B. mossambicus*, *B. adspersus*, *B. poweri*, *B. cf. sopranus* and *B. cf. bagginsi*) or montane grassland (*B. fichus*) (Figure 2b); and (ii) the *gibbosus*-group composed of species distributed exclusively in the Cape, on or below the GE and either restricted to the Cape Floristic Region (CFR) and Succulent Karoo Region (SKR) hotspots in the southwest Cape (*B. macrops*, *B. branchi*, *B. namaquensis*, *B. acutirostris*, *B. fuscus*, *B. gibbosus*, *B. rosei* and *B. montanus*), the Maputo-Pondoland-Albany (MPA) hotspot (*B. verrucosus*), or in isolated patches of afro-montane forest in northeast South Africa (*B. sylvestris*) (Figure 2c). Monophyly of these two subclades is well supported by both BI and ML (pp 1.0, bs >90%) and average uncorrected mtDNA p-distances ranged from 10% to 12% (see Appendices 1 and 2). Furthermore, there are group-specific amino-acid differences in both RAG-1 and SLC8A3. Inter-relationships within these groups are largely topologically congruent between the two optimality criteria, although statistical support varies and is often highly supported by BI alone.

We recovered substantial structure within the two major subclades. Within the *mossambicus*-group, for example, we recovered a clade of up to 11 genetically distinct lineages (Figure 2a, indicated with a red star; based on the operational criteria of the general lineage concept of species (De Queiroz, 1998)), which includes four of the five most recently described *Breviceps* species, including *B. caruthersi* and *B. passmorei* described just last year (Minter, Netherlands, & Du Preez, 2017), while the present paper was in review (comparative data not shown). This subgroup (hereafter the *pentheri*-complex) was previously ascribed to a subspecies of *B. adspersus* (i.e. *B. a. pentheri*), or field identified as *B. adspersus* or *B. mossambicus*. These lineages are deeply divergent (interlineage uncorrected 12S p-distance 2%-9% [mean = 6%]) and are broadly distributed in south-eastern southern Africa, including the MPA. This level of divergence is comparable to interspecific distances in the southwest Cape species of the *gibbosus*-group (interlineage uncorrected 12S p-distance 4%-11% [mean = 6.6%]). Geographical substructure was recovered

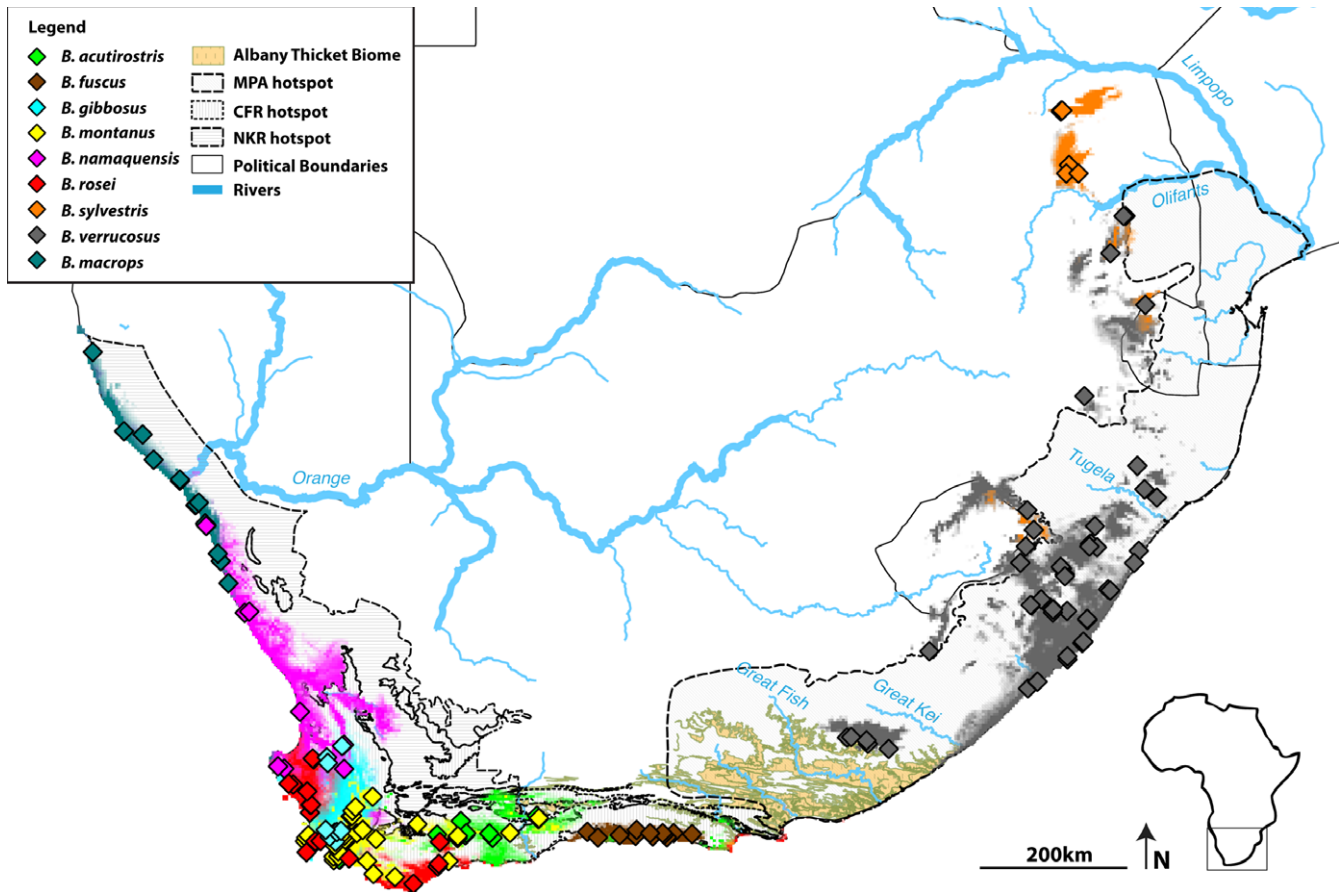


FIGURE 3 Composite map of the species distribution models (SDMs) for each species of the *gibbosus*-group across the Cape of southern Africa, overlaid with pertinent biogeographical features mentioned in the main text. Habitat suitability was calculated using MAXENT from 100 bootstrap replicates and only the top 20% of maximum habitat suitability is displayed for each taxon. Diamonds indicate museum and/or collection localities used to generate each model [Colour figure can be viewed at wileyonlinelibrary.com]

in widespread species (e.g. *B. verrucosus*, *B. namaquensis*), as well as in species represented by limited samples collected from relatively proximate localities (e.g. *B. montanus*, *B. fuscus*).

3.2 | Network structure

Network analyses based on single nuclear loci (Figures 2d, S2A-B) resemble those recovered using mtDNA (Figure S2C), and are largely congruent with results of phylogenetic analyses based on concatenated data (Figure 2a), suggesting that there is little to no locus-specific discordance.

3.3 | Divergence times

We date the split separating the two major *Breviceps* groups to the mid-Oligocene (27.5 Ma; 95% confidence interval [CI] = 21–34 Ma), and contemporary lineage accumulation occurred throughout the Miocene (Figure 2e). The most recent divergence event in our tree (1.3 Ma) was between the geographically proximate *B. cf. sopranus* and *B. cf. bagginsi*. The slope of the line representing lineage accumulation through time illustrates a dramatic increase in species-level diversity during the Miocene.

3.4 | Distribution modelling

The SDMs based on contemporary climatic conditions and the recorded localities of nine members of the *gibbosus*-group are shown in Figures 3 and S3. Certain bioclimatic variables had disproportional impact on the models (Table S5), suggesting species/species-groups and biogeographical regions are being influenced by shared stimuli. For example, SDMs of the species predominantly distributed in the winter rain dominated CFR (*B. acutirostris*, *B. gibbosus*, *B. montanus*, *B. rosei*) are disproportionately influenced by winter precipitation. However, the most important variables affecting the SDMs for species found in the hot, arid SKR were more variable, such as annual temperature variation and summer precipitation (*B. macrops*) or altitude (*B. namaquensis*). For *B. sylvestris* and *B. verrucosus*, distributed across the subtropical, mesic MPA/east, SDMs were largely influenced by precipitation (summer & annual precipitation, respectively). The most influential variable for *B. fuscus* was precipitation during the driest time of the year (the Knysna-Amatole ecoregion receives rainfall year-round, with spring and fall being the most predictable; Figures 3, S3; Table 1). SDM-specific AUC values were all ≥ 0.99 .

Niche overlap tests rejected the null hypotheses of random differences in Schoener's *D* values for most, but not all, pairwise

TABLE 1 Pairwise Schoener's *D* values (below the diagonal; and their associated *p*-values above) from Niche Identity Tests performed in MAXENT using the 'phyloclim' package in R. Cells with warmer colours indicate the highest values, and with asterisks indicate when the measured overlap falls within the distribution of pseudoreplicates

	<i>B. sylvestris</i>	<i>B. verrucosus</i>	<i>B. namaquensis</i>	<i>B. macrops</i>	<i>B. acutirostris</i>	<i>B. fuscus</i>	<i>B. gibbosus</i>	<i>B. rosei</i>	<i>B. montanus</i>	<i>P</i> -val
<i>B. sylvestris</i>		0.371	0.012	<0.01	<0.001	0.015	<0.001	<0.001	<0.001	
<i>B. verrucosus</i>	0.420*		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
<i>B. namaquensis</i>	0.042	0.000		<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	
<i>B. macrops</i>	0.004	0.065	0.395*		<0.001	<0.001	<0.001	<0.001	<0.001	
<i>B. acutirostris</i>	0.010	0.015	0.022	0.021		<0.001	<0.001	<0.001	<0.01	
<i>B. fuscus</i>	0.022	0.029	0.001	0.002	0.139		<0.001	<0.001	<0.001	
<i>B. gibbosus</i>	0.030	0.004	0.317	0.079	0.222	0.007		<0.01	<0.001	
<i>B. rosei</i>	0.046	0.031	0.312	0.087	0.251	0.065	0.546		<0.01	
<i>B. montanus</i>	0.022	0.021	0.086	0.017	0.648*	0.137	0.437	0.568*		
Schoener's <i>D</i>										

comparisons (Table 1 [nonbold values]). The SDMs for the allopatric *B. sylvestris* and *B. verrucosus* seem to reflect similar niche space (as seen in Figure 3), a pattern confirmed by the high, though nonsignificant ($p = 0.371$), niche overlap (Schoener's $D = 0.420$). We recovered similar results when comparing *B. macrops* with *B. namaquensis*, and *B. montanus* with *B. acutirostris* and *B. rosei*, although these comparisons included partially sympatric species distributions. Other comparisons, however, with particularly high Schoener's D values are both significant and can be explained by overlapping distribution. We consistently recovered low measures of overlap between species living in the MPA/east (*B. sylvestris* + *B. verrucosus*) or the south Cape (*B. fuscus*), confirming that these species occupy climatically distinct niche space.

4 | DISCUSSION

Our results suggest that no single model of allopatric divergence can fully account for patterns of diversification in *Breviceps*. Instead, the data are most consistent with a combination of the climate fluctuation and landscape barrier models. The earliest divergence occurred between two largely allopatric subclades near the Oligocene-Miocene boundary (Figure 2; Loader et al., 2014), followed by a surge of lineage accumulation in each of the daughter clades during the Miocene. These two subclades (hereafter the *mossambicus*- and *gibbosus*-groups) are largely allopatric (Figure 2b,c), with the *gibbosus*-group occupying most of South Africa and the *mossambicus*-group occurring to the north. The initial divergence in the genus, along with divergences in the *mossambicus*-group, best fits predictions of the landscape barrier model. In contrast, the climate fluctuation model is a better fit for the *gibbosus*-group.

The landscape barrier model predicted that divergences would be relatively deep, ecological differentiation would be unimportant in separating species, and the formation of landscape barriers could explain cladogenic events. The major split producing the *gibbosus*- and *mossambicus*-groups occurred between the late Eocene to early Miocene (21–34 Ma 95% CI), coincident with renewed epeirogenic

uplift of the Great Escarpment (i.e. early Miocene) (King, 1978; Moore, Blenkinsop, & Cotterill, 2009; Partridge & Maud, 1987). Even if this cladogenic event occurred earlier and under different stimuli, the dramatic uplift (up to 1,000 m) almost certainly reinforced geographical separation. Furthermore, global cooling trends led to the fragmentation of pan-African forest and promoted the emergence of savanna and grassland (Couvreur, Chatrou, Sosef, & Richardson, 2008; Zachos, Pagani, Sloan, Thomas, & Billups, 2001), which in turn has sculpted modern species distributions (Medina et al., 2016). Fossil and pollen records suggest widespread expansion of these habitats (coinciding with the shift from C3 to C4 grasses), rather than contraction, beginning in the Miocene (Jacobs, 2004; Sepulchre et al., 2006). Members of the *mossambicus*-group are found in savanna habitats, suggesting that climate-caused fragmentation of habitat does not explain their diversification. Likewise, although we were unable to perform distribution modelling in this clade due to uncertainty in species assignment, they lack obvious ecological or morphological differentiation expected in a clade undergoing adaptive divergence.

Landscape barriers are the best-supported model for diversification in the *mossambicus*-group. The phylogenetic data show that divergent taxa are concentrated in the south-eastern portion of the group's range. Within this region, the Great Escarpment meets a series of major rivers flowing from the interior plateau to the Indian Ocean, including the Olifants, Limpopo, Save, Zambezi, and Shire rivers. These rivers form substantial gorges as they escape the Escarpment, at least some of which clearly delimit the ranges of *Breviceps* species. For example, the deeply incised canyon of the Olifants River in Limpopo Province, South Africa demarcates the distributions of two species from the *gibbosus*-group, *B. verrucosus* and *B. sylvestris* (Lawes, Eeley, Findlay, & Forbes, 2007; Minter, 1998) (Figure 3). The SDMs for both species reciprocally predict suitable habitat across either side of this putative barrier (Table 1). We expect the same would be the case if we had enough data points to develop SDMs for *mossambicus*-group species. The Olifants River has received little attention as a biogeographical barrier in the literature (e.g. Jacobsen, Kuhn, Jackman, & Bauer, 2014; Maswanganye, Cunningham,



Bennett, Chimimba, & Bloomer, 2017; Stanley & Bates, 2014), but both the steep gorge and river itself are likely insurmountable obstacles for *Breviceps* species, which are poorly suited for either climbing or swimming. For the major eastward-flowing rivers as a whole, periods of uplift from the Oligocene to Pleistocene spurred large-scale reorganization of river systems that involved capture of the Upper Zambezi system by the Lower Zambezi (away from the Limpopo system), erosion of major river gorges, and periodic formation of large internal basins (Moore & Larkin, 2001). The timing of these events is consistent with divergences in the *mossambicus*-group.

The data suggest a different history for most species in the *gibbosus*-group, in which evidence for divergence caused by climatic fluctuation is strong. The *gibbosus*-group inhabits recognized floral biodiversity hotspots (Mittermeier et al., 2004), although the delimitation of such does not necessarily reflect vertebrate diversity (see Perera, Ratnayake-Perera, & Procheş, 2011). Eastern South Africa (i.e. the MPA hotspot) is a mosaic of vegetation types, slope, soils, and levels of precipitation, particularly as one moves inland from the coast towards the escarpment (Perera et al., 2011), and substantial species-level diversification has been noted in other regional vertebrate taxa thought to have limited dispersal ability (da Silva & Tolley, 2017). In the south-western Cape, a period of uplift in the early Miocene, gradual climatic deterioration (Zachos et al., 2001), and the onset of the Benguela upwelling system caused a switch to winter-dominant rainfall (Roberts & Brink, 2002; Siesser, 1980), and produced significant changes in the resident flora and fauna. The unique biotic composition of the CFR effectively delimits the area affected by these climatic and epeirogenic changes (Daniels, Gouws, & Crandall, 2006; Daniels, Picker, Cowlin, & Hamer, 2009; Matthee & Flemming, 2002; Tolley, Burger, Turner, & Matthee, 2006; Tolley, de Villiers, Cherry, & Measey, 2010).

The times of divergence we estimate for species in the *gibbosus*-group are consistent with isolation of populations being caused by this climatic deterioration. SDMs also support the climate fluctuation model. An adaptive divergence model would predict that closely related species would have significantly different niches (Schroeder's D values near zero), and the landscape barrier model would predict geographical barriers between close relatives, rather than the range overlap seen between close relatives in the *gibbosus*-group (Figure 3). Instead, closely related species in the *gibbosus*-group display substantial overlap in niche, especially the most closely related species from the SW Cape (Figures 3, S3; Table 1), and it is less closely related species that show significant niche divergence. This would be expected if *gibbosus*-group populations became fragmented in situ as climate changed and eventually adapted as local climates continued to diverge. For example, the split of *B. namaquensis*, *B. macrops* and *B. branchi* from the rest of the SW Cape species (e.g. *B. gibbosus*, *B. montanus*, etc.) occurred in the mid- to late Miocene, which broadly corresponds to the regional climate shift in the SW Cape that promoted advanced aridity and generated the winter rainfall zone. This shift restricted the previously widespread subtropical forest to disjunct, relictual patches along the southern slopes of the Cape Fold mountains (e.g. Knysna-Amatole ecoregion, habitat of

B. fuscus, Figure 3) and replaced it with the dominant, contemporary Fynbos and Succulent Karoo Biomes (Cowling et al., 2009; Neumann & Bamford, 2015; although see Matthews, Measey, & Roberts, 2016). The SKR, or more specifically Namaqualand, receives limited rainfall but benefits from inland-penetrating, coastal fog (Olivier, 2002). The continued input of moisture has likely been crucial in maintaining populations of *Breviceps* in South Africa and allowing them to diversify rather than go extinct as a result of climate deterioration.

While ecological preferences and geological barriers shape diversification, the SDMs suggest that they also impact contemporary distributions of *Breviceps*. The Albany Thicket biome (Figure 3) is a distinct intersection between dramatically different climatic and vegetative zones between the MPA and CFR hotspots and represents an example of an ecological barrier. It has been shown to be a natural break in the distributions of numerous faunal taxa (e.g. velvet worms, Daniels et al., 2009; and chameleons, Tolley et al., 2006). This gap marks the south-western limit of the broadly distributed *B. verrucosus* and the eastern distribution of the Cape taxa (represented by the easternmost species, *B. fuscus*), reflected in the lack of reciprocal overprediction between species specific SDMs (Table 1; Figure 3). Moreover, the habitat of *B. fuscus* falls within (presumably) relictual, temperate, coastal afro-montane forest of the Aseasonal Rainfall Zone (Figure 1; although it uniquely receives rainfall year round, with spring and fall rainfall being the most consistent). This area is home to other range-restricted frogs (e.g. *Africalus knysnae*, *Heleophryne hewitti*) and lizards (e.g. *Bradypodion damaranum*, *Ninurta coeruleopunctatus*), suggesting that this is a unique area for herpetofauna (i.e. the Cape Temperate Fauna sensu Poynton & Broadley, 1978). The Orange River is an example of a potential geological barrier (Matthee & Flemming, 2002; but see Portik & Bauer, 2012) and could play a role in the distribution of *B. namaquensis*. The SDM for *B. namaquensis* overpredicts suitable habitat north of this river, but the species is only known from the south.

5 | CONCLUSIONS

As shown in this study, a single model of divergence cannot fully explain diversification in *Breviceps*. Instead, a combination of isolation resulting from geological processes and climatic deterioration best explains the current diversity in the group. We found no evidence for diversification driven by adaptive divergence. This pattern is broadly consistent with patterns seen in more recently diverging amphibian and reptile lineages of southern Africa, as described in the Introduction. Thus, we can conclude that the processes promoting diversification in the region have remained consistent throughout the Cenozoic; the main change over time is that specific events causing divergences may differ. In the case of *Breviceps*, these events were mostly Miocene in age and included geological evolution of the Great Escarpment, major changes to river systems, and climate change that included both aridification and establishment of the winter rain system in the SW Cape.

Two future extensions to this study may allow the primary conclusions to be refined. First, broader sampling in the *mossambicus*-group would allow range limits of species in this group to be better demarcated and allow development of SDMs to better assess niche overlap. Second, additional variables could be coded into niche models as data become available. For example, although we recovered high *D* values between three similarly distributed SW Cape taxa that all utilize (to some degree) the Fynbos biome (Table 1), *B. montanus* generally prefer mountain tops (Minter, 2004), as opposed to mountain slopes (*B. gibbosus*) or the surrounding Cape flats (*B. rosei*). Likewise, to the northwest, *B. macrops* is found exclusively in white sands close to the coast, and *B. namaquensis* is generally found in red sands further inland (Channing & Wahlberg, 2011). Substrate specificity has been suggested as a significant driver of divergence in other southern African taxa (e.g. Bauer, 1999; Heinicke, Turk, & Bauer, 2017) and combining such ecological data with climate data could provide new insight into the degree of niche overlap in *Breviceps*.

This study also has implications beyond identifying factors promoting diversification of the African biota. Over ninety years have passed since the last major systematic revision of *Breviceps* (Power, 1926). Our phylogenetic results identified numerous cryptic lineages, two of which were described while this manuscript was in review (Figure 2; Minter et al., 2017). Such findings were previously predicted (Channing, 2001), and in some cases supported by allozyme and acoustic data (Engelbrecht & Mulder, 2000; Minter, 1998), but so far no modern, comprehensive taxonomic revision of this group has been attempted (although work is underway to rectify that deficiency).

This study mirrors previous findings suggesting that our knowledge of African amphibian diversity is still a work in progress (Bittencourt-Silva et al., 2016; Channing, Schmitz, Burger, & Kielgast, 2013; Loader et al., 2014; Tolley et al., 2010; Turner & Channing, 2008). The “cryptic” lineages we have identified are concentrated in an area that is renowned for high herpetofaunal diversity (Channing et al., 2013; Jacobsen et al., 2014; Stanley & Bates, 2014; Travers, Jackman, & Bauer, 2014), yet also as a region that will experience rapid amphibian decline due to habitat loss (Stuart et al., 2004). Immediate conservation action is needed to preserve this distinct evolutionary radiation whose diversification has been shaped by the interwoven changes of geology and climate in southern Africa.

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BIOSKETCH

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SUPPORTING INFORMATION

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