

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29

DR. STUART V NIELSEN (Orcid ID : 0000-0002-3114-1469)

DR. SAVEL R DANIELS (Orcid ID : 0000-0003-2956-3256)

Article type : Research Paper

Original Article (Revised; for consideration in the Journal of Biogeography)

Title: Multi-locus phylogenetics in a widespread African anuran lineage (*Brevicipitidae*:
Breviceps) reveals patterns of diversity reflecting geo-climatic change

S.V. Nielsen^{1*}, S.R. Daniels², W. Conradie^{3,4}, M.P. Heinicke⁵, B.P. Noonan⁶

¹*Department of Biological Sciences, Marquette University, Wehr Life Sciences 109,
Milwaukee, WI 53233 USA*

²*Department of Zoology and Botany, University of Stellenbosch, P/Bag XI, Matieland
7602, South Africa*

³*Port Elizabeth Museum/Bayworld, P.O. Box 13147, Humewood, 6013, South Africa*

⁴*Research Associate: School of Natural Resource Management, George Campus, Nelson
Mandela University, George, South Africa*

⁵*Department of Natural Sciences, University of Michigan-Dearborn, 4901 Evergreen
Road, Dearborn, MI 48128 USA*

⁶*Department of Biology, University of Mississippi, 214 Shoemaker Hall, University, MS
38677 USA*

*Correspondence: Stuart V. Nielsen, Dept. of Biological Sciences, P.O. Box 1881,
Milwaukee, WI 53201 USA. E-mail: stuart.nielsen@marquette.edu

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/jbi.13394](https://doi.org/10.1111/jbi.13394)

This article is protected by copyright. All rights reserved

30 Running head: Pre-Quaternary diversification in rain frogs.

31 Word count: 5,225 (excluding abstract/acknowledgements)

32 **ABSTRACT**

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

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

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

43 **Results** *Breviceps* is monophyletic and comprised of two major, largely allopatric sub-
44 clades. Diversity within each sub-clade is concentrated in two areas with contrasting
45 geologic and climatic histories: the arid/semi-arid winter rainfall zone in the southwestern
46 (SW) Cape, and the semi-tropical East Coast that receives predominantly summer rainfall.
47 Recognized species diversity in the SW Cape based on phenotypic variation is consistent
48 with observed genetic patterns whereas the East Coast is shown to harbor unexpectedly
49 high genetic diversity and up to seven putative, cryptic species. Niche models show
50 significant overlap between closely related species.

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

60

61

62 **Keywords**

63 Great Escarpment, rain frogs, Miocene, distribution modeling, cryptic species, landscape
64 barrier model, climate fluctuation model

65

66

67 **Main Text:**

68 **INTRODUCTION**

69 To comprehend the biogeographical patterns we observe today, one must be
70 cognizant of the evolutionary history of species and the paleo-environmental conditions
71 under which they evolved, especially as these may differ from the present (Tolley et al.,
72 2008). The generation and persistence of biogeographic patterns is often region specific.
73 For example, the Southern Hemisphere remained largely un-glaciated during the late
74 Cenozoic, preserving biogeographically informative genetic signal that would otherwise
75 have been erased by recurrent episodes of Plio-Pleistocene glaciation (Hewitt, 2000).

76 Whereas temperatures gradually decreased in the Northern Hemisphere, southern Africa
77 became more warm and arid (van Zinderen Bakker & Mercer, 1986), relegating
78 widespread, mesic floral and faunal lineages to allopatric, climatically-stable refugia,
79 promoting diversification and adaptation to xeric environments (Bauer, 1999; Matthee &
80 Flemming, 2002; Verboom et al., 2009; Lorenzen et al., 2012; Barlow et al., 2013;
81 Heinicke et al., 2017a). Reconstructing the evolutionary history of Southern Hemisphere
82 organisms, therefore, presents a potentially informative system for understanding how
83 contemporary communities were influenced by pre-Quaternary environmental change.

84 Since the breakup of southern Gondwana in the late Jurassic and early Cretaceous,
85 southern Africa has experienced many epeirogenic events that have contributed to
86 contemporary topographical relief. Uplifts at the Oligocene–Miocene and Pliocene–
87 Pleistocene boundaries formed most modern mountain ranges and the Kalahari
88 Depression (Dingle et al., 1983; Birkenhauer, 1991; Clark et al., 2011). These uplifts also
89 created the Great Escarpment, a feature that influences climate as well as the distribution
90 and diversification of organisms (Clark et al., 2011). Along the east coast of southern
91 Africa, the warm Agulhas current combined with the Great Escarpment (specifically the

92 Drakensberg Mountains) to create a rain shadow maintaining a subtropical climate along
93 the Escarpment's eastern slopes (Neumann & Bamford, 2015). Consequently, patches of
94 forest habitat have persisted here since the Miocene while the rest of the sub-continent
95 underwent aridification (Sepulchre et al., 2006). This aridification was driven by a
96 Miocene shift in circumpolar air circulation in the Southern Ocean combined with the
97 nascent Benguela upwelling system off the south-western African coast (Fig. 1; Siesser,
98 1980; McCarthy & Rubidge, 2005; Neumann & Bamford, 2015). Wind patterns then
99 brought winter moisture to the south-western Cape, creating discrete rainfall zones with
100 contrasting seasonality (Chase & Meadows, 2007). Additional post-Miocene epeirogeny
101 (including significant crustal flexuring) increased topo-edaphic heterogeneity.

102 The continued evolution of landscape features and climate over time, combined
103 with landscape and climate heterogeneity across space, has likely stimulated floral and
104 faunal diversification in southern Africa (Moore, 1999; Cowling et al., 2009). The
105 region's long history of aridification has also generated a largely arid-adapted and
106 endemic biota (Brain, 1985; Bauer, 1999). Indeed, a commonly observed biogeographic
107 pattern in the southern African fauna is recent (late Miocene to Pleistocene)
108 diversification driven by climate fluctuation, promoting allopatric speciation (Daniels et
109 al., 2004; Tolley et al., 2008; Swart et al., 2009; Lorenzen et al., 2012; Barlow et al.,
110 2013; Diedericks & Daniels, 2014; Furman et al., 2015). However, Africa is an old
111 continent and modern lineages vary in age. Thus, many older lineages have persisted
112 alongside recently diversifying ones, and the historical biogeographic factors that
113 promoted diversification in these older lineages may have differed.

114 The genus *Breviceps* (rain frogs) represents one such older lineage. *Breviceps* is
115 an ancient African genus, having diverged from their sister clade (the East African
116 brevicipitids) in the early Paleogene (Loader et al., 2014). Species accumulation within
117 the East African radiation of Brevicipitids occurred predominantly in the Miocene and
118 has been attributed to the long-term persistence of forests across the Eastern Afromontane
119 Biodiversity Region (EABR; Loader et al. 2014) (Fig. 1). Diversification of *Breviceps* in
120 southern Africa has thus far remained unstudied. However, they are an ideal exemplar
121 group for studying the historical biogeography of more ancient lineages in southern
122 Africa, for a number of reasons. First, they are widely distributed across eastern and

123 southern Africa. *Breviceps* can be found from Angola eastward through southern
124 Democratic Republic of the Congo and Tanzania, and southward to South Africa (Minter,
125 2004), only absent from the most arid regions of the Namib/Pro-Namib (Namibia) and
126 Nama-Karoo (South Africa) and areas that experience winter frost and low annual
127 average rainfall (Karoo/Highveld Grassland of South Africa). Second, their ecology, life
128 history and morphology likely limit gene flow (i.e. they are largely nocturnal, fossorial,
129 small-bodied insectivores with poor dispersal ability and reproduce via direct-
130 development [no free-swimming tadpole stage]), potentially fostering allopatric
131 divergence. Third, southern African *Breviceps* are varied in habitat choice, occurring in
132 afro-montane and coastal forest, as well as Fynbos (Mediterranean-like shrub or
133 heathland), vegetated dune, savannah, and grassland habitats, where they prefer
134 sandy/loamy, well-drained soils (Channing, 2001; Minter, 2004; du Preez & Carruthers,
135 2009). Thus, niche divergence could have played a role in diversification.

136 Here we incorporate multi-locus molecular data and broad taxonomic sampling to
137 reconstruct the pattern and timing of diversification of *Breviceps*. These data are
138 combined with niche models to deduce what has shaped contemporary patterns of
139 diversity, distribution, and biogeography of *Breviceps* in Africa south of the Congo Basin.
140 We use these data to evaluate three alternative models of diversification caused by
141 allopatric divergence. In the climate fluctuation model, climate change isolates
142 populations in patches of suitable habitat, allowing allopatric divergence to occur. This is
143 the same model suggested for many faunal groups in southern Africa, as described above.
144 Under this model, we expect most divergences among *Breviceps* taxa to be relatively
145 recent and for there to be little niche separation among closely related species, since
146 niche conservatism would be the primary factor isolating populations. Species
147 distribution models would imply large areas of overlapping suitable habitat for close
148 relatives. In a landscape barrier model, divergences are caused by barriers formed by
149 geological processes (e.g., deposition of the Kalahari sands, uplift associated with
150 formation of the Great Escarpment, river capture and formation of resulting gorges, etc.).
151 Under this model, we expect divergences to be older, and coincident in time with periods
152 of geological change. Closely related species would vary in their degree of niche overlap,
153 since ecological differentiation would not affect population divergence. The third model

154 is an adaptive change model. In this model, we expect divergences among species to vary
155 in age, but closely related species will have significant divergence in their ecological
156 niche, with non-overlapping species distribution models as divergence would be driven
157 by adaptation to different ecological conditions.

158

159 **MATERIALS AND METHODS**

160 **Taxon sampling and laboratory protocols**

161 We obtained tissue from 77 individual frogs representing 16 of the 18 recognized
162 species and downloaded partial sequence data from GenBank for the two remaining
163 species (see Table S1 in Appendix 1). For two species, where voucher identification is
164 difficult and/or ambiguous (*B. sopranus* and *B. bagginsi*), we include a *cf.* designation to
165 represent identification uncertainty. Furthermore, topotypic material was not available for
166 all species. Although the phylogenetic position of *Breviceps* is well-established within the
167 greater Afrobatrachian radiation (Pyron & Wiens, 2011), there have been no studies
168 exploring the monophyly of *Breviceps sensu stricto*. Thus, we include complete sampling
169 of brevicipitid genera to test the monophyly of this genus. Outgroups include species
170 from other members of Brevicipitidae (e.g. *Callulina*, *Balebreviceps*, *Probreviceps*, and
171 *Spaeleophryne*), as well as more distantly related Afrobatrachian and microhylid taxa
172 (see Table S1).

173 We isolated genomic DNA from ethanol preserved (95%) liver, skin and/or
174 muscle tissue samples via salt extraction (Aljanabi & Martinez, 1997). We performed
175 polymerase chain reaction (PCR) to amplify partial sequences of two mitochondrial (*12S*
176 and *16S* ribosomal rRNA genes) and three nuclear loci (recombination activating protein
177 1, *RAG1*; brain derived neurotrophic factor, *BDNF*; and solute carrier family 8 member 3,
178 *SLC8A3*). PCR primers are reported in Appendix 1 (Table S2). We viewed and purified
179 PCR products via 1.5% agarose gel electrophoresis and ExoSAP-IT[®] (Affymetrix, Santa
180 Clara, CA, USA), respectively. Sequencing reactions used the BigDye TERMINATOR V.3.1
181 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), which were then
182 sent to the DNASU Sequencing Core (Arizona State University) for purification and
183 sequencing using an Applied Biosystems 3730XL automated sequencer.

184

185 **Sequence alignment, model selection, and phylogenetic reconstruction**

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

194 Datasets (concatenated, mitochondrial [mtDNA]-only and nuclear [nuDNA]-only)
195 of all samples were analyzed using Bayesian Markov chain Monte Carlo (MRBAYES v.3.2;
196 Ronquist & Huelsenbeck, 2003) and maximum likelihood methods (RAXML v.8.1.11;
197 Stamatakis, 2006) using the CIPRES Science Gateway 3.1 for online phylogenetic
198 analysis (<http://www.phylo.org/index.php/portal/>). An appropriate partitioning strategy
199 and molecular models specifically for Bayesian analyses were chosen using
200 PARTITIONFINDER v.1.1.1 (Lanfear et al., 2012), which assessed all possible candidate
201 positions (e.g. each codon in the nuDNA) using the Bayesian information criterion
202 (Lanfear et al., 2012). The resulting partitioning scheme is listed in Table S4. Final
203 Bayesian analyses ran for 50 million generations with four independent chains, and
204 sampled every 50,000 generations. We checked for stationarity using TRACER v.1.6
205 (Rambaut et al., 2014), after which a 25% burn-in was removed, leaving 750 trees for
206 posterior analysis. Maximum likelihood analyses were performed using the default
207 settings for RAXML using the GTRGAMMA model of sequence evolution (Stamatakis,
208 2006) and ceasing bootstrapping when extended majority rule bootstrapping criteria had
209 been reached.

210 For comparison with tree-based methods and in order to view gene tree
211 (haplotype) relationships among the ingroup, networks for each nuDNA locus and
212 combined mtDNA were constructed using SPLITSTREE v.4.12.3 (Huson & Bryant, 2006)
213 with the Neighbor-net algorithm. We used an algorithmic approach to phase nuDNA
214 alleles using PHASE v2.1.1 (Stephens et al., 2001; Scheet & Stephens, 2006) prior to
215 building splittrees.

216

217 **Estimating divergence times**

218 Using a lognormal relaxed molecular clock, the age of the *Breviceps* radiation and
219 its constituent lineages was estimated in BEAST v.1.8 (Drummond et al., 2012). Analyses
220 were performed using a concatenated alignment split into two partitions (nuclear vs.
221 mitochondrial DNA), and limited to a single individual (or chimera of individuals) per
222 species representing a complete sampling of loci. Missing data could yield potentially
223 problematic results (Blankers et al., 2013), thus we excluded taxa with high proportion of
224 missing data, particularly nuclear loci (i.e. *B. fichus* and *B. branchi*). Secondary
225 calibrations employed in these analyses followed Loader et al. (2014) and were based on
226 the fossil calibrated study of Roelants et al. (2007) exploring relationships among the
227 Amphibia. Specifically, we used the following as normally-distributed constraints of
228 node ages: MRCA of the crown group including *Arthroleptis*, 92.8 (84.5–111.8) million
229 years ago [Ma]; *Hemisus* + Brevicipitidae, 65.9 (54.1–84.9) Ma; *Breviceps*, 45.4 (32.9–
230 63.4) Ma; and *Callulina*, 29.6 (19.5–44.5) Ma (see Loader et al. 2014 for more detail).
231 Direct fossil calibration was not possible due to the lack of pre-Quaternary fossil material
232 attributable to Brevicipitidae (Matthews et al., 2015). The BEAST analysis ran for 100
233 million generations, sampling every 10000th gen., with a 20% burn-in, and using a Yule
234 prior. Stationarity was always reached well before the end of the burn-in. TRACER
235 confirmed that all runs had converged on similar model parameters (estimated sample
236 size >200 for all parameters). To view the relative timing of diversification with respect
237 to the accumulation of lineages, a lineage-through-time (LTT) plot was generated using
238 the ‘LTT’ function in the R package ‘ape’ (Paradis et al., 2004).

239

240 **Species distribution modeling**

241 We estimated species distribution models (SDMs) using the program MAXENT v.
242 3.3.2 (Phillips et al., 2006) to provide a measure of climatic niche divergence among
243 species. Estimates used the default settings as implemented in the ‘dismo’ and
244 ‘SDMTools’ R packages. Models were trained using collection localities attached to the
245 samples used for genetic analysis, as well as supplementary data points obtained from
246 published sources (e.g. Channing & Wahlberg, 2011) or the Virtual Museum (Animal

247 Demography Unit, University of Cape Town; <http://vmus.adu.org.za/>). The museum data
248 required some manual filtering and we omitted any unverified data points (e.g. those
249 represented by unrecorded nuptial calls only). The assignment of museum samples to
250 widespread species was, at times, untenable (due to widespread paraphyly within *B.*
251 *adpersus* and *B. mossambicus*; see below). Therefore our distribution modeling included
252 only samples for which museum locality data was relatively certain (i.e. the *gibbosus*-
253 group exclusive of *B. branchi* [a recently described species known only from a single
254 sample]) and for which our results did not suggest the presence of cryptic species (Table
255 S5).

256 Our sample size for *B. sylvestris* fell to four points post-filtering. Modeling with
257 so few points is not ideal. However, when we added three additional points for which
258 GPS data exist (based on unrecorded calls) and re-ran the SDM the results were
259 comparable (results not shown), suggesting a reasonably reliable model. A recent
260 theoretical study (van Proosdij et al., 2016) also suggested that as few as three presence
261 samples from a narrow-range taxon will provide accurate SDMs.

262 SDMs were generated based on the 19 bioclimatic data layers available from the
263 Worldclim database (www.worldclim.org) that were re-sampled to the WGS1984
264 Transverse Mercator projection and cropped to a geographically relevant window
265 ($xlim=10$ to 36 ; $ylim=-35$ to -22) using the ‘maptools’ R package. Following Elith et al.
266 (2011), we retained all bioclimatic variables, even if correlated, and allowed the MAXENT
267 algorithm to determine the required predictors for each model. MAXENT performed 100
268 bootstrapped replicates, using 75% of the locality data to train and 25% to test the models,
269 with a regularization multiplier of 1. ‘Area under the curve’ (AUC) scores of the
270 receiver-operating characteristic were calculated to assess the accuracy of the models.
271 Jackknife tests were also used to determine individual variable contributions for the final
272 SDMs. To assess the degree of overlap in predicted SDMs among the taxa, we estimated
273 Schoener’s *D* (a measure of overlap) using ENMtools (Warren et al., 2010). We were
274 keen to assess whether closely related taxa differed significantly with respect to niche (as
275 defined by the 19 bioclimatic variables), so we performed pairwise identity and similarity
276 tests in ENMtools to generate values of Schoener’s *D*. We generated a distribution of

277 pseudoreplicates (n=100) to ascertain whether the observed value of *D* is significantly
278 different from random.

279

280 RESULTS

281 Phylogenetic relationships

282 The concatenated, aligned in-group dataset totaled 3434 characters (containing
283 550 parsimony-informative characters out of 660 variable sites). The optimized
284 Maximum Likelihood (ML) tree had a log-likelihood score of -23500.1 and Bayesian
285 Inference (BI) produced an optimal tree with a mean likelihood score of -21730.8. Both
286 reconstructions based on the concatenated dataset produced nearly identical consensus
287 tree topologies (Appendix 2, Fig. S1A-B), and any discordance exhibited poor support.
288 Many of the nodes receiving low support are associated with distal nodes and do not
289 affect our biogeographical conclusions. Mitochondrial and individual nuclear gene trees
290 produced largely congruent patterns of relationship (not illustrated) and any
291 disagreements occurred were associated with low statistical support.

292 We confirm the monophyly of *Breviceps*, as all material ascribed to this genus
293 (rooted with the non-Afrobatrachian *Kaloula pulchra*) fell within this well-supported
294 clade (Fig. 2A; posterior probability [pp] 1.0, bootstrap support [bs] 100%). All
295 phylogenetic analyses recovered two well-supported sub-clades: i) the *mossambicus*-
296 group, composed of more northerly-/easterly-distributed species that occur both above
297 and below the Great Escarpment (GE), and are largely restricted to savannah (*B.*
298 *mossambicus*, *B. adpersus*, *B. poweri*, *B. cf. sopranus*, and *B. cf. bagginsi*) or montane
299 grassland (*B. fichus*) (Fig. 2B); and ii) the *gibbosus*-group composed of species
300 distributed exclusively in the Cape, on or below the GE and either restricted to the Cape
301 Floristic Region (CFR) and Succulent Karoo Region (SKR) hotspots in the southwest
302 Cape (*B. macrops*, *B. branchi*, *B. namaquensis*, *B. acutirostris*, *B. fuscus*, *B. gibbosus*, *B.*
303 *rosei*, and *B. montanus*), the Maputo-Pondoland-Albany (MPA) hotspot (*B. verrucosus*),
304 or in isolated patches of afro-montane forest in northeast South Africa (*B. sylvestris*) (Fig.
305 2C). Monophyly of these two sub-clades is well supported by both BI and ML (pp 1.0, bs
306 > 90%) and average uncorrected mtDNA p-distances ranged from 10-12% (see
307 Appendices 1 and 2). Furthermore, there are group-specific amino acid differences in

308 both *RAG-1* and *SLC8A3*. Inter-relationships within these groups are largely
309 topologically congruent between the two optimality criteria, although statistical support
310 varies and is often highly supported by BI alone.

311 We recovered substantial structure within the two major sub-clades. Within the
312 *mossambicus*-group, for example, we recovered a clade of up to 11 genetically distinct
313 lineages (Fig. 2A, indicated with a red star; based on the operational criteria of the
314 general lineage concept of species (De Queiroz, 1998)), which includes four of the five
315 most recently described *Breviceps* species, including *B. carruthersi* and *B. passmorei*
316 described just last year (Minter et al., 2017), while the present paper was in review
317 (comparative data not shown). This sub-group (hereafter the *pentheri*-complex) was
318 previously ascribed to a subspecies of *B. adspersus* (i.e. *B. a. pentheri*), or field identified
319 as *B. adspersus* or *B. mossambicus*. These lineages are deeply divergent (inter-lineage
320 uncorrected 12S p-distance 2-9% [mean = 6%]) and are broadly distributed in
321 southeastern southern Africa, including the MPA. This level of divergence is comparable
322 to interspecific distances in the southwest Cape species of the *gibbosus*-group (inter-
323 lineage uncorrected 12S p-distance 4-11% [mean = 6.6%]). Geographic substructure was
324 recovered in widespread species (e.g. *B. verrucosus*, *B. namaquensis*), as well as in
325 species represented by limited samples collected from relatively proximate localities (e.g.
326 *B. montanus*, *B. fuscus*).

327

328 **Network structure**

329 Network analyses based on single nuclear loci (Figs. 2D, S2A-B) resemble those
330 recovered using mtDNA (Fig. S2C), and are largely congruent with results of
331 phylogenetic analyses based on concatenated data (Fig. 2A), suggesting that there is little
332 to no locus-specific discordance.

333

334 **Divergence times**

335 We date the split separating the two major *Breviceps* groups to the mid-Oligocene
336 (27.5 Ma; 95% confidence interval [CI] = 21-34 Ma), and contemporary lineage
337 accumulation occurred throughout the Miocene (Fig. 2E). The most recent divergence
338 event in our tree (1.3 Ma) was between the geographically proximate *B. cf. sopranus* and

339 *B. cf. bagginsi*. The slope of the line representing lineage accumulation through time
340 illustrates a dramatic increase in species-level diversity during the Miocene.

341

342 **Distribution modeling**

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

358 Niche overlap tests rejected the null hypotheses of random differences in
359 Schoener's *D* values for most, but not all, pairwise comparisons (Table 1 [non-bold
360 values]). The SDMs for the allopatric *B. sylvestris* and *B. verrucosus* seem to reflect
361 similar niche space (as seen in Fig. 3), a pattern confirmed by the high, though non-
362 significant ($p=0.371$), niche overlap (Schoener's $D=0.420$). We recovered similar results
363 when comparing *B. macrops* with *B. namaquensis*, and *B. montanus* with *B. acutirostris*
364 and *B. rosei*, although these comparisons included partially sympatric species
365 distributions. Other comparisons, however, with particularly high Schoener's *D* values
366 are both significant and can be explained by overlapping distribution. We consistently
367 recovered low measures of overlap between species living in the MPA/east (*B. sylvestris*
368 + *B. verrucosus*) or the south Cape (*B. fuscus*), confirming that these species occupy
369 climatically distinct niche space.

370

371 DISCUSSION

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

383 The landscape barrier model predicted that divergences would be relatively deep,
384 ecological differentiation would be unimportant in separating species, and the formation
385 of landscape barriers could explain cladogenic events. The major split producing the
386 *gibbosus*- and *mossambicus*-groups occurred between the late Eocene to early Miocene
387 (21–34 Mya 95% CI), coincident with renewed epeirogenic uplift of the Great
388 Escarpment (i.e. early Miocene) (King, 1978; Partridge & Maud, 1987; Moore et al.,
389 2009). Even if this cladogenic event occurred earlier and under different stimuli, the
390 dramatic uplift (up to 1000m) almost certainly reinforced geographic separation.
391 Furthermore, global cooling trends led to the fragmentation of pan-African forest and
392 promoted the emergence of savannah and grassland (Zachos et al., 2001; Couvreur et al.,
393 2008), which in turn has sculpted modern species distributions (Medina et al., 2016).
394 Fossil and pollen records suggest widespread expansion of these habitats (coinciding
395 with the shift from C3 to C4 grasses), rather than contraction, beginning in the Miocene
396 (Jacobs, 2004; Sepulchre et al., 2006). Members of the *mossambicus*-group are found in
397 savannah habitats, suggesting that climate-caused fragmentation of habitat does not
398 explain their diversification. Likewise, although we were unable to perform distribution
399 modeling in this clade due to uncertainty in species assignment, they lack obvious

400 ecological or morphological differentiation expected in a clade undergoing adaptive
401 divergence.

402 Landscape barriers are the best-supported model for diversification in the
403 *mossambicus*-group. The phylogenetic data show that divergent taxa are concentrated in
404 the southeastern portion of the group's range. Within this region the Great Escarpment
405 meets a series of major rivers flowing from the interior plateau to the Indian Ocean,
406 including the Olifants, Limpopo, Save, Zambezi, and Shire rivers. These rivers form
407 substantial gorges as they escape the Escarpment, at least some of which clearly delimit
408 the ranges of *Breviceps* species. For example, the deeply incised canyon of the Olifants
409 River in Limpopo Province, South Africa demarcates the distributions of two species
410 from the *gibbosus*-group, *B. verrucosus* and *B. sylvestris* (Minter, 1998; Lawes et al.,
411 2007) (Fig. 3). The SDMs for both species reciprocally predict suitable habitat across
412 either side of this putative barrier (Table 1). We expect the same would be the case if we
413 had enough data points to develop SDMs for *mossambicus*-group species. The Olifants
414 River has received little attention as a biogeographic barrier in the literature (e.g.
415 Jacobsen et al., 2014; Stanley & Bates, 2014; Maswanganye et al., 2017), but both the
416 steep gorge and river itself are likely insurmountable obstacles for *Breviceps* species,
417 which are poorly suited for either climbing or swimming. For the major eastward-flowing
418 rivers as a whole, periods of uplift from the Oligocene to Pleistocene spurred large scale
419 reorganization of river systems that involved capture of the Upper Zambezi system by the
420 Lower Zambezi (away from the Limpopo system), erosion of major river gorges, and
421 periodic formation of large internal basins (Moore & Larkin, 2001). The timing of these
422 events is consistent with divergences in the *mossambicus*-group.

423 The data suggest a different history for most species in the *gibbosus*-group, in
424 which evidence for divergence caused by climatic fluctuation is strong. The *gibbosus*-
425 group inhabits recognized floral biodiversity hotspots (Mittermeier et al., 2004), although
426 the delimitation of such does not necessarily reflect vertebrate diversity (see Perera et al.,
427 2011). Eastern South Africa (i.e. the MPA hotspot) is a mosaic of vegetation types, slope,
428 soils, and levels of precipitation, particularly as one moves inland from the coast towards
429 the escarpment (Perera et al., 2011), and substantial species-level diversification has been
430 noted in other regional vertebrate taxa thought to have limited dispersal ability (da Silva

431 & Tolley, 2017). In the southwestern Cape, a period of uplift in the early Miocene,
432 gradual climatic deterioration (Zachos et al., 2001), and the onset of the Benguela
433 upwelling system caused a switch to winter-dominant rainfall (Siesser, 1980; Roberts &
434 Brink, 2002), and produced significant changes in the resident flora and fauna. The
435 unique biotic composition of the CFR effectively delimits the area affected by these
436 climatic and epeirogenic changes (Matthee & Flemming, 2002; Daniels et al., 2006, 2009,
437 Tolley et al., 2006, 2010).

438 The times of divergence we estimate for species in the *gibbosus*-group are
439 consistent with isolation of populations being caused by this climatic deterioration. SDMs
440 also support the climate fluctuation model. An adaptive divergence model would predict
441 that closely related species would have significantly different niches (Schroeder's D
442 values near zero), and the landscape barrier model would predict geographic barriers
443 between close relatives, rather than the range overlap seen between close relatives in the
444 *gibbosus*-group (Fig. 3). Instead, closely related species in the *gibbosus*-group display
445 substantial overlap in niche, especially the most closely related species from the SW
446 Cape (Figs. 3, S3; Table 1), and it is less closely related species that show significant
447 niche divergence. This would be expected if *gibbosus*-group populations became
448 fragmented *in situ* as climate changed and eventually adapted as local climates continued
449 to diverge. For example, the split of *B. namaquensis*, *B. macrops* and *B. branchi* from the
450 rest of the SW Cape species (e.g. *B. gibbosus*, *B. montanus*, etc.) occurred in the mid- to
451 late-Miocene, which broadly corresponds to the regional climate shift in the SW Cape
452 that promoted advanced aridity and generated the winter rainfall zone. This shift
453 restricted the previously widespread subtropical forest to disjunct, relictual patches along
454 the southern slopes of the Cape Fold mountains (e.g. Knysna-Amatole ecoregion, habitat
455 of *B. fuscus*, Fig. 3) and replaced it with the dominant, contemporary Fynbos and
456 Succulent Karoo Biomes (Cowling et al., 2009; Neumann & Bamford, 2015; although
457 see Matthews et al., 2016). The SKR, or more specifically Namaqualand, receives limited
458 rainfall but benefits from inland-penetrating, coastal fog (Olivier, 2002). The continued
459 input of moisture has likely been crucial in maintaining populations of *Breviceps* in South
460 Africa and allowing them to diversify rather than go extinct as a result of climate
461 deterioration.

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

482

483 **Conclusions**

484 As shown in this study, a single model of divergence cannot fully explain
485 diversification in *Breviceps*. Instead, a combination of isolation resulting from geological
486 processes and climatic deterioration best explains the current diversity in the group. We
487 found no evidence for diversification driven by adaptive divergence. This pattern is
488 broadly consistent with patterns seen in more recently diverging amphibian and reptile
489 lineages of southern Africa, as described in the Introduction. Thus, we can conclude that
490 the processes promoting diversification in the region have remained consistent
491 throughout the Cenozoic; the main change over time is that specific events causing
492 divergences may differ. In the case of *Breviceps*, these events were mostly Miocene in

493 age and included geological evolution of the Great Escarpment, major changes to river
494 systems, and climate change that included both aridification and establishment of the
495 winter rain system in the SW Cape.

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

510 This study also has implications beyond identifying factors promoting
511 diversification of the African biota. Over ninety years have passed since the last major
512 systematic revision of *Breviceps* (Power, 1926). Our phylogenetic results identified
513 numerous cryptic lineages, two of which were described while this manuscript was in
514 review (Fig. 2; Minter et al., 2017). Such findings were previously predicted (Channing,
515 2001), and in some cases supported by allozyme and acoustic data (Minter, 1998;
516 Engelbrecht & Mulder, 2000), but so far no modern, comprehensive taxonomic revision
517 of this group has been attempted (although work is underway to rectify that deficiency).

518 This result mirrors previous findings suggesting that our knowledge of African
519 amphibian diversity is still a work in progress (Turner & Channing, 2008; Tolley et al.,
520 2010; Channing et al., 2013; Loader et al., 2014; Bittencourt-Silva et al., 2016). These
521 'cryptic' lineages are concentrated in an area that is renowned for high herpetofaunal
522 diversity (Channing et al., 2013; Jacobsen et al., 2014; Stanley & Bates, 2014; Travers et
523 al., 2014), yet also identified as a region that will experience rapid amphibian decline due

524 to habitat loss (Stuart et al., 2004), thus immediate conservation action is needed to
525 preserve this distinct evolutionary radiation whose diversification has been shaped by the
526 interwoven changes of geology and climate in southern Africa.

527

528 **ACKNOWLEDGEMENTS**

529 We particularly wish to thank Roger Bills (South African Institute of Aquatic
530 Biodiversity), James Harvey (Harvey Ecological), Dan Portik, Jim McGuire and Carol
531 Spencer (Museum of Vertebrate Zoology), William R. Branch (Port Elizabeth Museum at
532 Bayworld), David Kizirian and David Dickey (American Museum of Natural History),
533 Jose Rosado (Museum of Comparative Zoology), Aaron Bauer (Villanova University),
534 Johan Marais (African Snakebite Institute), and Eli Greenbaum (UTEP Biodiversity
535 Collections) for loans of tissues in their collections, which greatly improved the scope of
536 this study. Louis du Preez kindly provided the pre-print sequence data for *B. carruthersi*
537 and *B. passmorei*. WC wishes to thank Jan Venter (Eastern Cape Parks and Tourism
538 Agency) and the Eastern Cape Department of Economic Development, Environmental
539 Affairs and Tourism (permit nos. CRO 84/11CR and CRO 85/11CR) for issuing
540 collecting permits. We are indebted to Trevor Hardaker, Cliff Dorse, and Vincenzo
541 Mercurio for allowing us the use of their excellent photographs. We thank Scott Bingham
542 and the staff of the DNASU sequencing facility (Arizona State University, Tempe, AZ)
543 for assistance troubleshooting sequencing issues. SVN thanks Paul Oliver, Tim J. Colston,
544 R. Drew Hataway, Ed Stanley, Megan Smith, Aaron Bauer, and the members of the
545 Noonan and Gamble labs for encouragement, discussion and suggestions that improved
546 earlier versions of this manuscript. We also appreciate the comments of Simon Loader
547 and two anonymous reviewers, as well as the editorial advice of Krystal Tolley. Funding
548 for this project was provided by the US National Science Foundation (Grants DEB
549 0841963 & DEB 1407294 to BPN and DEB 1556585 to MPH) and the US Department of
550 State Fulbright Student Fellowship Program to SVN.

551

552 **REFERENCES**

553

554 Aljanabi S.M. & Martinez I. (1997) Universal and rapid salt-extraction of high quality

555 genomic DNA for PCR-based techniques. *Nucleic Acids Research*, **25**, 4692–4693.

556 Barlow A., Baker K., Hendry C.R., Peppin L., Phelps T., Tolley K.A., Wüster C.E., &
557 Wüster W. (2013) Phylogeography of the widespread African puff adder (*Bitis*
558 *arietans*) reveals multiple Pleistocene refugia in southern Africa. *Molecular Ecology*,
559 **22**, 1134–57.

560 Bauer A.M. (1999) Evolutionary scenarios in the *Pachydactylus* Group geckos of
561 southern Africa: new hypotheses. *African Journal of Herpetology*, **48**, 53–62.

562 Birkenhauer J. (1991) *The great escarpment of Southern Africa and its coastal forelands.*
563 *A re-appraisal.* Institut für Geographie der Universität, München.

564 Bittencourt-Silva G.B., Conradie W., Siu-Ting K., Tolley K.A., Channing A.,
565 Cunningham M., Farooq H.M., Menegon M., & Loader S.P. (2016) The
566 phylogenetic position and diversity of the enigmatic mongrel frog *Nothophryne*
567 Poynton, 1963 (Amphibia, Anura). *Molecular Phylogenetics and Evolution*, **99**, 89–
568 102.

569 Blankers T., Townsend T.M., Pepe K., Reeder T.W., & Wiens J.J. (2013) Contrasting
570 global-scale evolutionary radiations: Phylogeny, diversification, and morphological
571 evolution in the major clades of iguanian lizards. *Biological Journal of the Linnean*
572 *Society*, **108**, 127–143.

573 Brain C.K. (1985) Temperature-induced environmental changes as biological isolating
574 mechanisms in southern Africa. *Species and Speciation* (ed. by E.S. Vrba), pp. 45–
575 52. Transvaal Museum, Pretoria.

576 Channing A. (2001) *Amphibians of central and southern Africa.* Comstock Publishing
577 Associates, London.

578 Channing A., Schmitz A., Burger M., & Kielgast J. (2013) A molecular phylogeny of
579 African Dainty Frogs, with the description of four new species (Anura:
580 Pyxicephalidae: *Cacosternum*). *Zootaxa*, **3701**, 518–550.

581 Channing A. & Wahlberg K. (2011) Distribution and conservation status of the desert
582 rain frog *Breviceps macrops*. *African Journal of Herpetology*, **60**, 101–112.

583 Chase B.M. & Meadows M.E. (2007) Late Quaternary dynamics of southern Africa's
584 winter rainfall zone. *Earth-Science Reviews*, **84**, 103–138.

585 Clark V.R., Barker N.P., & Mucina L. (2011) The Great Escarpment of southern Africa:

- 586 a new frontier for biodiversity exploration. *Biodiversity and Conservation*, **20**,
587 2543–2561.
- 588 Couvreur T.L.P., Chatrou L.W., Sosef M.S.M., & Richardson J.E. (2008) Molecular
589 phylogenetics reveal multiple tertiary vicariance origins of the African rain forest
590 trees. *BMC Biology*, **6**, 54.
- 591 Cowling R.M., Procheş Ş., & Partridge T.C. (2009) Explaining the uniqueness of the
592 Cape flora: Incorporating geomorphic evolution as a factor for explaining its
593 diversification. *Molecular Phylogenetics and Evolution*, **51**, 64–74.
- 594 Daniels S.R., Gouws G., & Crandall K.A. (2006) Phylogeographic patterning in a
595 freshwater crab species (Decapoda: Potamonautidae: *Potamonautes*) reveals the
596 signature of historical climatic oscillations. *Journal of Biogeography*, **33**, 1538–
597 1549.
- 598 Daniels S.R., Picker M.D., Cowlin R.M., & Hamer M.L. (2009) Unravelling evolutionary
599 lineages among South African velvet worms (Onychophora: *Peripatopsis*) provides
600 evidence for widespread cryptic speciation. *Biological Journal of the Linnean
601 Society*, **97**, 200–216.
- 602 Dingle R. V., Siesser W.G., & Newton A.R. (1983) *Mesozoic and Tertiary geology of
603 southern Africa*. A.A. Balkema, Rotterdam.
- 604 Drummond A.J., Suchard M.A., Xie D., & Rambaut A. (2012) Bayesian phylogenetics
605 with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–73.
- 606 Elith J., Phillips S.J., Hastie T., Dudík M., Chee Y.E., & Yates C.J. (2011) A statistical
607 explanation of MaxEnt for ecologists. *Diversity and Distributions*, **17**, 43–57.
- 608 Engelbrecht G.D. & Mulder P.F.S. (2000) A biochemical genetic comparison of four
609 populations of *Breviceps adspersus* (Anura: Microhylidae). *African Zoology*, **35**,
610 217–221.
- 611 Furman B.L.S., Bewick A.J., Harrison T.L., Greenbaum E., Gvozdik V., Kusamba C., &
612 Evans B.J. (2015) Pan-African phylogeography of a model organism, the African
613 clawed frog “*Xenopus laevis*.” *Molecular Ecology*, **24**, 909–925.
- 614 Heinicke M.P., Jackman T.R., & Bauer A.M. (2017) The measure of success: geographic
615 isolation promotes diversification in *Pachydactylus* geckos. *BMC Evolutionary
616 Biology*, **17**, 9.

- 617 Hewitt G. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.
- 618 Huson D.H. & Bryant D. (2006) Application of phylogenetic networks in evolutionary
619 studies. *Molecular Biology and Evolution*, **23**, 254–267.
- 620 Jacobs B.F. (2004) Palaeobotanical studies from tropical Africa: relevance to the
621 evolution of forest, woodland and savannah biomes. *Philosophical Transactions of
622 the Royal Society B: Biological Sciences*, **359**, 1573–1583.
- 623 Jacobsen N.H.G., Kuhn A.L., Jackman T.R., & Bauer A.M. (2014) A phylogenetic
624 analysis of the southern African gecko genus *Afroedura* Loveridge (Squamata:
625 Gekkonidae), with the description of nine new species from Limpopo and
626 Mpumalanga provinces of South Africa. *Zootaxa*, **3846**, 451–501.
- 627 King L.C. (1978) The geomorphology of central and southern Africa. *Biogeography and
628 Ecology of Southern Africa*. (ed. by M.J.A. Werger), pp. 3–17. W. Junk, The Hague,
629 Netherlands.
- 630 Lanfear R., Calcott B., Ho S.Y.W., & Guindon S. (2012) PartitionFinder: Combined
631 selection of partitioning schemes and substitution models for phylogenetic analyses.
632 *Molecular Biology and Evolution*, **29**, 1695–1701.
- 633 Lawes M.J., Eeley H.A.C., Findlay N.J., & Forbes D. (2007) Resilient forest faunal
634 communities in South Africa: A legacy of palaeoclimatic change and extinction
635 filtering? *Journal of Biogeography*, **34**, 1246–1264.
- 636 Loader S.P., Sara Ceccarelli F., Menegon M., Howell K.M., Kassahun R., Mengistu A. a.,
637 Saber S. a., Gebresenbet F., de Sá R., Davenport T.R.B., Larson J.G., Müller H.,
638 Wilkinson M., & Gower D.J. (2014) Persistence and stability of Eastern
639 Afromontane forests: Evidence from brevicipitid frogs. *Journal of Biogeography*, **41**,
640 1781–1792.
- 641 Lorenzen E.D., Heller R., & Siegismund H.R. (2012) Comparative phylogeography of
642 African savannah ungulates. *Molecular Ecology*, **21**, 3656–3670.
- 643 Maswanganye K.A., Cunningham M.J., Bennett N.C., Chimimba C.T., & Bloomer P.
644 (2017) Life on the rocks: Multilocus phylogeography of rock hyrax (*Procapra
645 capensis*) from southern Africa. *Molecular Phylogenetics and Evolution*, **114**, 49–62.
- 646 Matthee C.A. & Flemming A.F. (2002) Population fragmentation in the southern rock
647 agama, *Agama atra*: More evidence for vicariance in Southern Africa. *Molecular*

648 *Ecology*, **11**, 465–471.

649 Matthews T., van Dijk E., Roberts D.L., & Smith R.M.H. (2015) An early Pliocene (5.1
650 Ma) fossil frog community from Langebaanweg, south-western Cape, South Africa.
651 *African Journal of Herpetology*, **64**, 39–53.

652 Matthews T., Measey G.J., & Roberts D.L. (2016) Implications of summer breeding
653 frogs from Langebaanweg, South Africa: Regional climate evolution at 5.1 mya.
654 *South African Journal of Science*, **112**, 1–7.

655 McCarthy T. & Rubidge B. (2005) *The Story Of Earth & Life: A Southern African*
656 *Perspective on a 4.6-Billion-Year Journey*. Struik Publishers, Cape Town.

657 Medina M.F., Bauer A.M., Branch W.R., Schmitz A., Conradie W., Nagy Z.T., Hibbitts
658 T.J., Ernst R., Portik D.M., Nielsen S. V., Colston T.J., Kusamba C., Behangana M.,
659 Rödel M.O., & Greenbaum E. (2016) Molecular phylogeny of [i]Panaspis[/i] and
660 *Afroablepharus* skinks (Squamata: Scincidae) in the savannas of sub-Saharan Africa.
661 *Molecular Phylogenetics and Evolution*, **100**, 409–423.

662 Minter L.R. (1998) *Aspects of the reproductive biology of Breviceps*. University of
663 Witwatersrand,

664 Minter L.R. (2004) Genus *Breviceps* Merrem, 1820 (Family Microhylidae). (eds.).
665 SI/MAB Series 9. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho*
666 *and Swaziland*. (ed. by L. Minter, M. Burger, J. Harrison, H. Braack, P. Bishop, and
667 D. Kloepfer), pp. 168–170.

668 Minter L.R., Netherlands E.C., & Du Preez L.H. (2017) Uncovering a hidden diversity:
669 two new species of *Breviceps* (Anura: Brevicipitidae) from northern KwaZulu-Natal,
670 South Africa. *Zootaxa*, **4300**, 195–216.

671 Mittermeier R.A., Robles-Gil P., Hoffmann M., Pilgrim J.D., Brooks T.B., Mittermeier
672 C.G., Lamoreux J.L., & Fonseca G.A.B. (2004) *Hotspots Revisited: Earth's*
673 *Biologically Richest and Most Endangered Ecoregions*. CEMEX, Mexico City,
674 Mexico.

675 Moore A., Blenkinsop T., & Cotterill F. (2009) Southern African topography and erosion
676 history: Plumes or plate tectonics? *Terra Nova*, **21**, 310–315.

677 Moore A.E. (1999) A reappraisal of epeirogenic flexure axes in southern Africa. *South*
678 *African Journal of Geology*, **102**, 363–376.

- 679 Neumann F.H. & Bamford M.K. (2015) Shaping of modern southern African biomes:
680 Neogene vegetation and climate changes. *Transactions of the Royal Society of South*
681 *Africa*, 1–18.
- 682 Olivier J. (2002) Fog-water harvesting along the West Coast of South Africa : A
683 feasibility study. *Water South Africa*, **28**, 349–360.
- 684 Paradis E., Claude J., & Strimmer K. (2004) APE: Analyses of phylogenetics and
685 evolution in R language. *Bioinformatics*, **20**, 289–290.
- 686 Partridge T.C. & Maud R.R. (1987) Geomorphic evolution of southern Africa since the
687 Mesozoic. *South African Journal of Geology*, **90**, 179–208.
- 688 Perera S.J., Ratnayake-Perera D., & Procheş Ş. (2011) Vertebrate distributions indicate a
689 greater Maputaland-Pondoland-Albany region of endemism. *South African Journal*
690 *of Science*, **107**, 1–15.
- 691 Phillips S., Anderson R., & Schapire R. (2006) Maximum entropy modeling of species
692 geographic distributions. *Ecological Modelling*, **190**, 231–259.
- 693 Portik D. & Bauer A. (2012) Untangling the complex: molecular patterns in *Trachylepis*
694 *variegata* and *T. punctulata* (Reptilia: Scincidae). *African Journal of Herpetology*,
695 **4574**, 37–41.
- 696 Power J.H. (1926) A monographic revision of the genus *Breviceps*, with distribution
697 records and descriptions of new species. *Annals of the South African Museums*, **20**,
698 451–471.
- 699 Poynton J.C. & Broadley D.G. (1978) The herpetofauna. *Biogeography and ecology of*
700 *southern Africa* (ed. by M.J.A. Werger), pp. 925–948. Springer, Netherlands.
- 701 du Preez L. & Carruthers V. (2009) *A complete guide to the frogs of southern Africa*.
702 Struik Nature, Cape Town.
- 703 van Proosdij A.S.J., Sosef M.S.M., Wieringa J.J., & Raes N. (2016) Minimum required
704 number of specimen records to develop accurate species distribution models.
705 *Ecography*, **39**, 542–552.
- 706 Pyron R.A. & Wiens J.J. (2011) A large-scale phylogeny of Amphibia including over
707 2800 species, and a revised classification of extant frogs, salamanders, and
708 caecilians. *Molecular Phylogenetics and Evolution*, **61**, 543–583.
- 709 De Queiroz K. (1998) The general lineage concept of species, species criteria, and the

710 process of speciation: A conceptual unification and terminological recommendations.
711 *Endless Forms: Species and Speciation* (ed. by D.J. Howard and S.H. Berlocher), pp.
712 57–75. Oxford University Press, New York, NY.

713 Rambaut A., Suchard M.A., Xie D., & Drummond A.J. (2014) Tracer v1.6. .
714 Roberts D.L. & Brink J.S. (2002) Dating and correlation of neogenecoastal deposits in
715 the Western Cape (South Africa): implications for neotectonism. *South African*
716 *Journal of Geology*, **105**, 337–352.

717 Roelants K., Gower D.J., Wilkinson M., Loader S.P., Biju S.D., Guillaume K., Moriau L.,
718 & Bossuyt F. (2007) Global patterns of diversification in the history of modern
719 amphibians. *Proceedings of the National Academy of Sciences*, **104**, 887–892.

720 Ronquist F. & Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference
721 under mixed models. *Bioinformatics*, **19**, 1572–1574.

722 Scheet P. & Stephens M. (2006) A fast and flexible statistical model for large-scale
723 population genotype data: applications to inferring missing genotypes and
724 haplotypic phase. *American Journal of Human Genetics*, **78**, 629–644.

725 Sepulchre P., Ramstein G., Fluteau F., Schuster M., Tiercelin J.-J., & Brunet M. (2006)
726 Tectonic Uplift and Eastern Africa Aridification. *Science*, **313**, 1419–1423.

727 Siesser W.G. (1980) Late Miocene origin of the Benguela upswelling system off northern
728 Namibia. *Science*, **208**, 283–285.

729 da Silva J.M. & Tolley K.A. (2017) Diversification through ecological opportunity in
730 dwarf chameleons. *Journal of Biogeography*, **44**, 834–847.

731 Stamatakis A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic
732 analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

733 Stanley E.L. & Bates M.F. (2014) Here be dragons: a phylogenetic and biogeographical
734 study of the *Smaug warreni* species complex (Squamata: Cordylidae) in southern
735 Africa. *Zoological Journal of the Linnean Society*, **172**, 892–909.

736 Stephens M., Smith N.J., & Donnelly P. (2001) A new statistical method for haplotype
737 reconstruction from population data. *American Journal of Human Genetics*, **68**,
738 978–989.

739 Stuart S.N., Chanson J.S., Cox N.A., Young B.E., Rodrigues A.S.L., Fischman D.L., &
740 Waller R.W. (2004) Status and Trends of Amphibian Declines and Extinctions

741 Worldwide. *Science*, **306**, 1783–1786.

742 Tamura K., Stecher G., Peterson D., Filipinski A., & Kumar S. (2013) MEGA6: Molecular
743 Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, **30**,
744 2725–2729.

745 Tolley K.A., Burger M., Turner A.A., & Matthee C.A. (2006) Biogeographic patterns and
746 phylogeography of dwarf chameleons (Bradypodion) in an African biodiversity
747 hotspot. *Molecular Ecology*, **15**, 781–793.

748 Tolley K.A., Chase B.M., & Forest F. (2008) Speciation and radiations track climate
749 transitions since the Miocene Climatic Optimum: A case study of southern African
750 chameleons. *Journal of Biogeography*, **35**, 1402–1414.

751 Tolley K.A., de Villiers A.L., Cherry M.I., & Measey G.J. (2010) Isolation and high
752 genetic diversity in dwarf mountain toads (*Capensibufo*) from South Africa.
753 *Biological Journal of the Linnean Society*, **100**, 822–834.

754 Travers S.L., Jackman T.R., & Bauer A.M. (2014) A molecular phylogeny of
755 Afromontane dwarf geckos (*Lygodactylus*) reveals a single radiation and increased
756 species diversity in a South African montane center of endemism. *Molecular*
757 *Phylogenetics and Evolution*, **80**, 31–42.

758 Verboom G.A., Archibald J.K., Bakker F.T., Bellstedt D.U., Conrad F., Dreyer L.L.,
759 Forest F., Galley C., Goldblatt P., Henning J.F., Mummenhoff K., Linder H.P.,
760 Muasya A.M., Oberlander K.C., Savolainen V., Snijman D.A., Niet T. van der, &
761 Nowell T.L. (2009) Origin and diversification of the Greater Cape flora: Ancient
762 species repository, hot-bed of recent radiation, or both? *Molecular Phylogenetics*
763 *and Evolution*, **51**, 44–53.

764 Warren D.L., Glor R.E., & Turelli M. (2010) ENMTools: A toolbox for comparative
765 studies of environmental niche models. *Ecography*, **33**, 607–611.

766 Zachos J., Pagani M., Sloan L., Thomas E., & Billups K. (2001) Trends, rhythms, and
767 aberrations in global climate 65 Ma to present. *Science*, **292**, 686–693.

768 van Zinderen Bakker E.M. & Mercer J.H. (1986) Major late Cainozoic climatic events
769 and palaeoenvironmental changes in Africa viewed in a world wide context.
770 *Palaeogeography, Palaeoclimatology, Palaeoecology*, **56**, 217–235.

771

772 **BIOSKETCH**

773 Stuart V. Nielsen is an avid gastro-gnome, nomad, and evolutionary biologist
 774 interested in the systematics, diversification, and biogeography of Gondwanan fauna,
 775 particularly reptiles and amphibians, that dreams of one day living in a hobbit-hole.

776
 777 Author contributions: S.R.D. and S.V.N. conceived the idea; S.R.D. and W.C.
 778 performed fieldwork; S.V.N. produced the sequence data and conducted the analyses,
 779 S.V.N. led the writing although all authors contributed to and approved the final version
 780 of this manuscript. We declare no conflicts of interest and this work is ours alone.

781

782 **TABLES**

783 **Table 1.** Pairwise Schoener’s *D* values (below the diagonal; and their associated *p*-values
 784 above) from Niche Identity Tests performed in MAXENT using the ‘phyloclim’ package in
 785 R. Cells with warmer colors indicate the highest values, and values are in bold when the
 786 measured overlap falls within the distribution of pseudoreplicates.

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

787

788

789 **FIGURES**

790 **Figure 1.** Map of sub-Saharan Africa south of the Congo basin, highlighting relevant
 791 biodiversity hotspots: Cape Floristic Region (CFR, magenta); Succulent Karoo Region
 792 (SKR, orange); Maputa-Pondoland-Albany (MPA, green); Coastal Forests of East Africa
 793 (CFEA, brown); and Eastern Afromontane Biodiversity Region (EABR, red).

794 Biogeographically relevant features are indicated, including rivers, rainfall zones (winter
 795 [WRZ], summer [SRZ], and aseasonal [ARZ]), and ocean currents (see main text for
 796 details).

797

798 **Figure 2.** (A) Phylogenetic relationships of *Breviceps* species-level relationships with
799 representative photographs of each species in life (not size standardized), as well as maps
800 of the geographic distributions of the two major groups: B) the *mossambicus*-group; C)
801 the *gibbosus*-group). Colored polygons on the maps coordinate with the small colored
802 circles and diamonds following the taxon names on the tree. The latter relate to
803 approximate geographic sampling localities indicated on the associated maps. Black
804 circles with numbers represent the eight putatively novel lineages within the
805 *mossambicus*-group (including two recently described taxa). Within the tree, a solid,
806 black circle indicates nodal support values $\geq 70\%$ for Maximum Likelihood bootstraps
807 (BS) and ≥ 0.95 for Bayesian inference posterior probabilities (PP), whereas a grey circle
808 represents PP ≥ 0.95 , but BS $< 70\%$. (D) A gene network of phased *RAG1* haplotype
809 sequences. (E) Time-calibrated ultrametric tree of *Breviceps* lineages based on
810 concatenated, partitioned nuclear data, with support and 95% confidence intervals (blue
811 bars) indicated at each node. Asterisks indicate high PP support. The lineage-through
812 time plot (red line) was generated using the LTT function in the R package ‘ape’.

813

814 **Figure 3.** Composite map of the species distribution models (SDMs) for each species of
815 the *gibbosus*-group across the Cape of southern Africa, overlaid with pertinent
816 biogeographic features mentioned in the main text. Habitat suitability was calculated
817 using MAXENT from 100 bootstrap replicates and only the top 20% of maximum habitat
818 suitability is displayed for each taxon. Diamonds indicate museum and/or collection
819 localities used to generate each model.

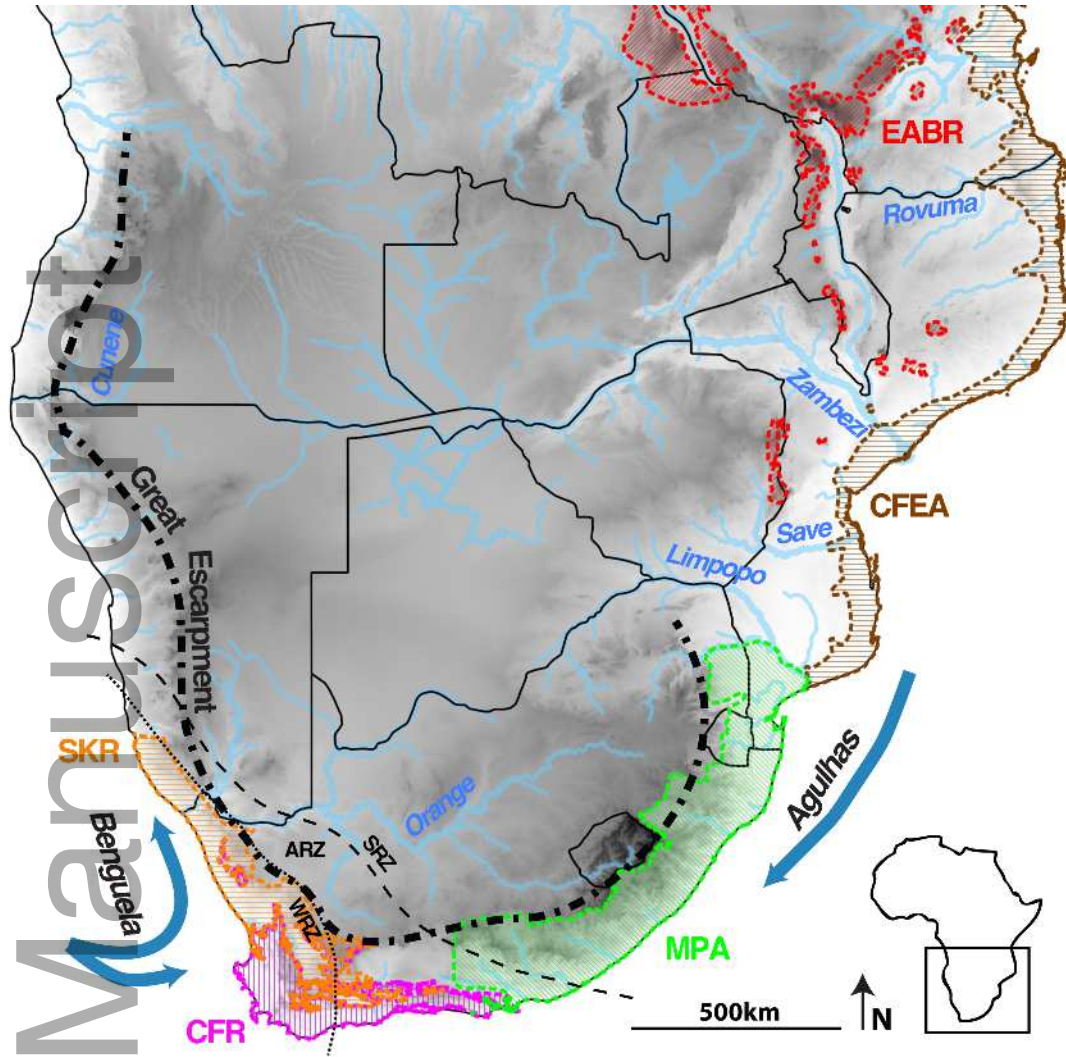
820

821 SUPPORTING INFORMATION

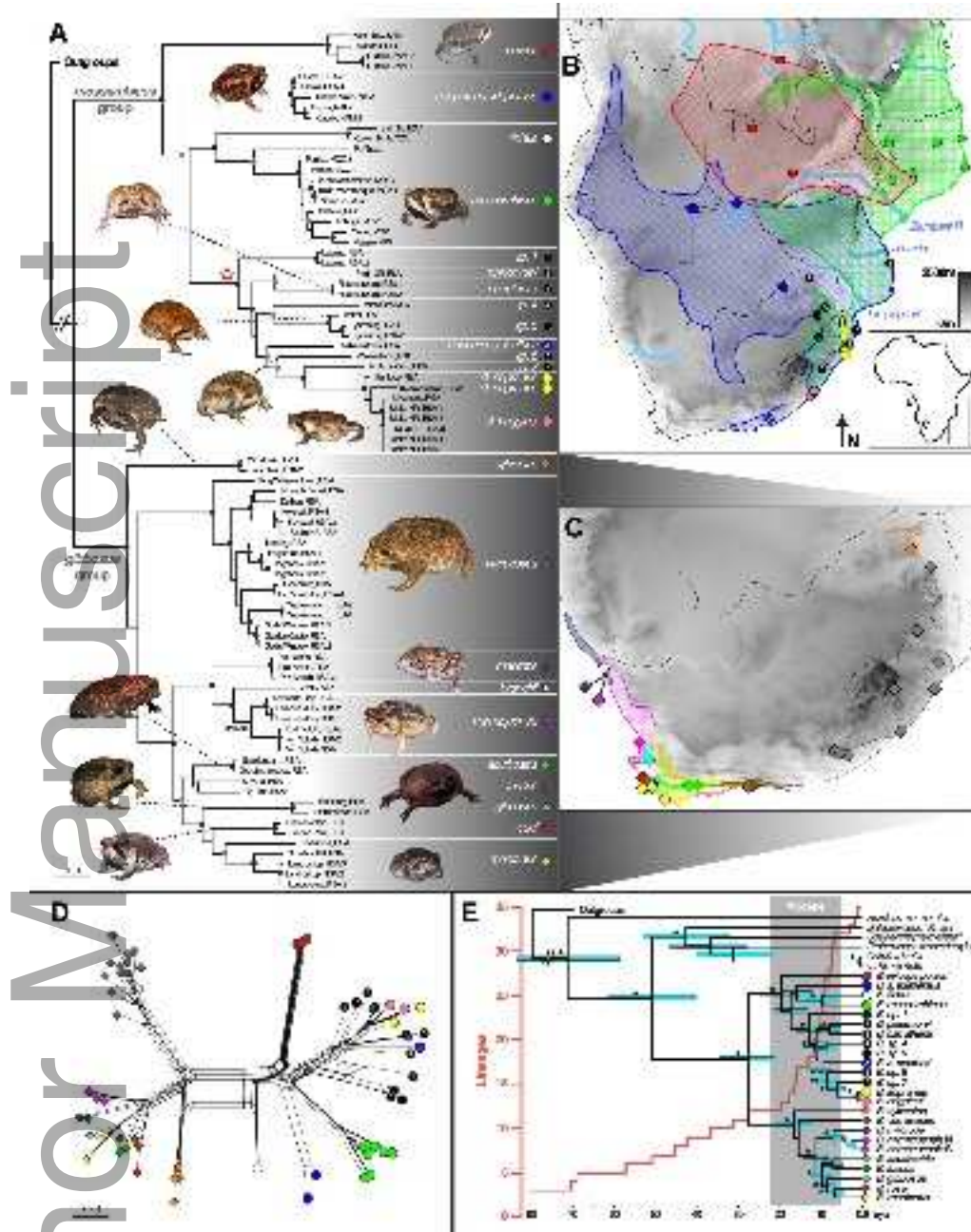
822 **Appendix 1** Supplementary tables including taxon sampling, primer information, genetic
823 distance, substitution models, bioclimatic variable contribution, and pseudoreplicate data
824 for each niche identity test.

825 **Appendix 2** Supplementary figures including additional phylogenetic trees, gene
826 networks, and niche model output.

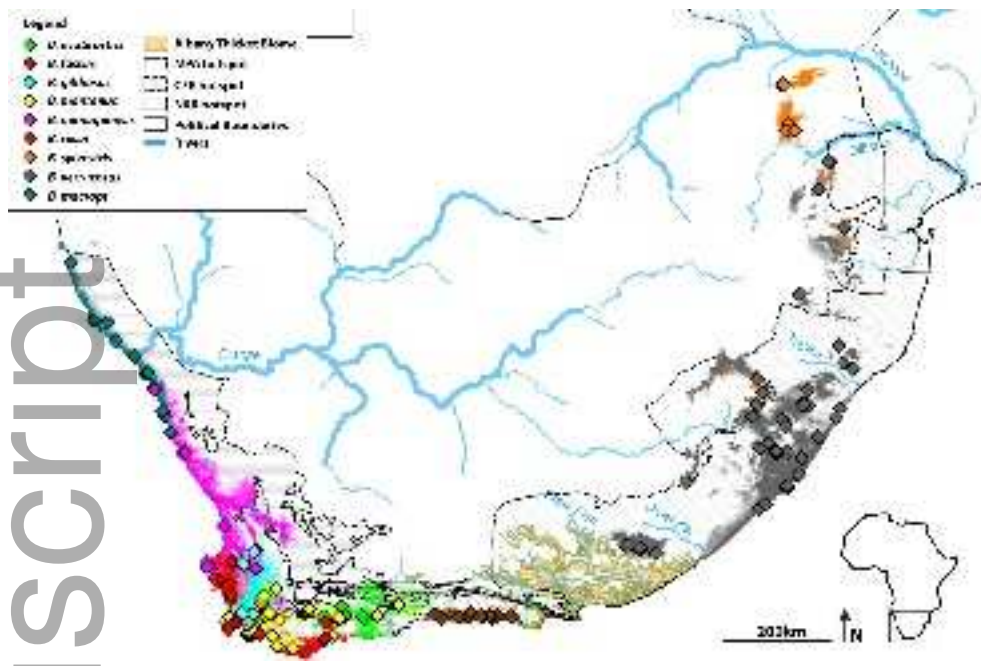
	<i>sylvestris</i>	<i>verrucosus</i>	<i>namaquensis</i>	<i>macrops</i>	<i>acutirostris</i>	<i>fuscus</i>	<i>gibbosus</i>	<i>rosei</i>	<i>montanus</i>	<i>p-val</i>
<i>sylvestris</i>		0.371	0.012	<0.01	<0.001	0.015	<0.001	<0.001	<0.001	
<i>verrucosus</i>	0.420		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001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



jbi_13394_f1a.tif



jbi_13394_f2.tif



jbi_13394_f3.tif