

**No Ecological Opportunity Signal on a Continental Scale?
Diversification and Life-History Evolution of African True
Toads (Anura: Bufonidae)**

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Keywords:	Amphibia, evolutionary rate dynamics, disparity, BMM, bGMYC, molecular phylogeny

1 **No Ecological Opportunity Signal on a Continental Scale? Diversification and Life-History**
2 **Evolution of African True Toads (Anura: Bufonidae)**

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28 phylogeny

29

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30 Abstract

31 The niche-filling process predicted by the ‘ecological opportunity’ (EO) model is an often-invoked
32 mechanism for generating exceptional diversity in island colonizers. Whether the same process
33 governs the lineage accumulation and trait disparity during continental colonization events is less
34 clear. Here we test this prediction by investigating the rate dynamics and trait evolution of one of
35 Africa’s most widespread amphibian colonizers, the true toads (Bufonidae). By reconstructing the
36 most complete molecular phylogeny of African Bufonidae to date, we find that the diversification of
37 lineages in Africa has been constant throughout time and across subclades, with little support for
38 early-burst diversification. Evolutionary rates of life history traits have similarly been constant over
39 time. However, an analysis of generalists and specialists showed a shift towards higher speciation
40 rates associated with habitat specialization. The overall lack of EO signal can be interpreted in a
41 number of ways and we propose several not mutually exclusive explanations. Firstly, methodological
42 issues might preclude the detection of EO, secondly colonizers might not experience true EO
43 conditions and due to the size, ecological heterogeneity and age of landmasses, the diversification
44 processes might be more complex, thirdly lower speciation rates of habitat generalists may have
45 affected overall proliferation of lineages.

46

47 Introduction

48 How species and species assemblages respond to a release from ecological competition is a key
49 question in evolutionary biology (Simpson 1953; Schluter 2000; Losos 2010; Yoder et al. 2010). The
50 colonization of islands (Robichaux et al. 1990; Grant 1999; Whittaker and Fernandez-Palacios 2007),
51 mass extinction events (Sepkoski 1998), the availability of new resources (McKenna et al. 2009) or
52 the evolution of key innovations (Hunter and Jernvall 1995; Jönsson et al. 2012) are classic examples
53 of where a sudden intrinsic or extrinsic change has presented organisms with an ‘ecological
54 opportunity’ (EO; Simpson 1953). In response to this opportunity, lineages are predicted to rapidly

55 diversify, unimpeded by competition until an ecological saturation point is reached inducing a
56 slowdown in diversification (Nee et al. 1992; Rabosky 2009a). Phylogenies are a powerful tool for
57 the inference of macroevolutionary processes (Mooers and Heard 1997) and the detection of diversity
58 dependent lineage accumulation patterns in response to competitive release has been interpreted as a
59 signal for EO, especially in relation to adaptive radiations (Losos and Mahler 2010). Diversifying
60 into new niche space when presented with EO should also be reflected in the diversification and
61 disparity of phenotypes (Simpson 1953; Schluter 2000; Harmon et al. 2003; Slater et al. 2010;
62 Jönsson et al. 2012), especially in traits relevant to adaptation (Steelman and Danley 2003).
63 Reconstructing the evolutionary history of phenotypes can therefore strongly complement our
64 understanding of diversification from studying phylogenies (Mahler et al. 2010; Harmon et al. 2010;
65 Slater et al. 2010).

66 Ecological opportunity is often cited as an important precondition for generating exceptional
67 levels of biodiversity (Schluter 2000; Losos 2010), but most empirical studies on EO are focused on
68 insular (Grant 1999; Harmon et al. 2008a; Jönsson et al. 2012), or localized mainland systems
69 (Hughes and Eastwood 2006; Kozak and Wiens 2006; Pinto et al. 2008; Rabosky and Lovette 2008a;
70 Slingsby et al. 2014; Price et al. 2014b). Yet, continental systems are often more diverse than their
71 island counterparts (Whittaker and Fernandez-Palacios 2007; Pinto et al. 2008) and whether the same
72 processes can generate bursts in biodiversity on a continent-wide scale has only recently begun to
73 receive attention. Some of these studies have yielded support for EO as a key mechanism for
74 producing exceptional biodiversity (Burbrink and Pyron 2009; Barker et al. 2013; Schenk et al. 2013,
75 although not always; Price et al. 2014a), even showing multiple EO events nested across subclades
76 (Drummond et al. 2012b; McGuire et al. 2014), while others have not detected EO signals
77 (Derryberry et al. 2011; Claramunt et al. 2012b; Day et al. 2013; Schweizer et al. 2014; Alhajeri et al.
78 2015), and a general consensus on the role of ecological limits for diversification is lacking (Harmon
79 and Harrison 2015; Rabosky and Hurlbert 2015).

80 In continent-wide studies of EO, detections of both rapid and early lineage and trait
81 diversification has been attributed to the biogeographic transition to new, underutilized areas as a
82 result of a colonization event (Burbrink and Pyron 2009; Barker et al. 2013; Schenk et al. 2013; Price
83 et al. 2014a). Conversely, a lack of signal has been attributed to the geographic and ecological
84 complexity of continents, with ecological saturation unlikely to occur on such a scale (Derryberry et
85 al. 2011; Day et al. 2013; Schweizer et al. 2014). Furthermore, the dispersal ability of ancestors that
86 may have led to the continent-wide colonization of new habitats may in itself inhibit rapid speciation
87 by preventing ecological isolation (Claramunt et al. 2012b). EO as a result of expansion into new
88 geographic or ecological space may therefore be as much a driver for generating biodiversity in
89 continent-wide clades as is the case for island or localized mainland radiations, but high dispersal
90 ability or the magnitude of the ecological carrying capacity of continents could equally mean that the
91 EO model is less applicable to such geographically and ecologically more complex systems.

92 Alternatively, if indeed EO played a role in shaping diversification, these systems may be too old for
93 early burst signals to be detectable if changes in rate over time were not drastic or if too much time
94 has passed since rate equilibrium has been reached (Liow et al. 2010; Rabosky and Hurlbert 2015).

95 With 586 currently recognized species worldwide, Bufonidae is the third most species-rich
96 family of amphibians (Frost 2016). Unlike most other amphibians, bufonids were able to colonize
97 most parts of the world (Duellman 1999) and this species-rich and world-wide diversification across
98 entire continents offers an excellent system for investigating how biodiversity accumulates on
99 continents and whether early bursts in both lineage accumulation and trait disparity has occurred as a
100 response to EO. African bufonids in particular are suitable for addressing EO, firstly, due to extreme
101 trait disparity observed across species. Variable phenotypic traits include body size (19-163 mm;
102 Liedtke et al. 2014), which is correlated with ecological factors and under strong selection in many
103 systems (Davis 1938; Peters 1986). Furthermore, diverse modes of life history strategies (biphasic
104 aquatic breeding to viviparity) are evident in African bufonids (Liedtke 2014; Liedtke et al. 2014)

105 and components of these, such as fecundity (clutch size) and parental investment per offspring (egg
106 size), are good indicators for adaptation to extrinsic factors (Dobzhansky 1950; Duellman and Trueb
107 1994; Roff 2002; Räsänen et al. 2008). A second qualification is the biogeographic transition African
108 bufonids underwent. Both fossil and molecular evidence point to a Neotropical origin of Bufonidae
109 (Tihen 1962; Blair 1972; Pramuk et al. 2008) at around 80-60 Ma (Pramuk et al. 2008; Van Bocxlaer
110 et al. 2010) followed by a rapid global diversification earliest in the Late Eocene (40-30 Ma; Pramuk
111 et al. 2008; Van Bocxlaer et al. 2010; Portik and Papenfuss 2015). By the Oligocene (~30 Ma)
112 bufonids were established on all continents except Australia and Antarctica (Van Bocxlaer et al.
113 2010), neither of which host endemic bufonid lineages at present. Van Bocxlaer et al. (2010)
114 proposed that the evolution of an ‘optimal range-expansion phenotype’ (robust, explosive breeders
115 with high dispersal abilities) was crucial for their success, a phenotype they estimated as
116 characteristic of the first lineage to colonize Africa as well. Their broad ecological tolerance may
117 have been advantageous for allowing this group to disperse widely on the continent, but such habitat
118 generalism may ultimately result in lower overall lineage proliferation (Claramunt et al. 2012a).
119 Nonetheless, African bufonids display a rich array of phenotypes, reproductive behaviour and habitat
120 preferences (Tandy and Keith 1972; Clarke 2001; Liedtke et al. 2014), which raises the question
121 whether this diversity was spurred by EO.

122 By assembling the largest molecular and trait dataset for African bufonids to date, including
123 numerous candidate taxa so far not formally described, we test whether the colonization of Africa by
124 toads has left signals characteristic of the EO model. Under this model we expect to find an early
125 burst of lineage accumulation and life history trait disparity with a subsequent slowdown in rates. We
126 also investigate whether habitat generalists have experienced different speciation rates compared to
127 habitat specialists.

128

129 **Methods**

130 An extended version of the methods employed can be found as Supporting Information S1 and is
131 summarized in brief here.

132

133 *Taxon Sampling and DNA Sequencing*

134 The number of currently recognized species of African Bufonidae (101, see extended methods S1) is
135 unlikely to be the true number of species due to the questionable taxonomic validity of some (Tandy
136 and Keith 1972; Poynton 1997; Rödel 2000; Rödel and Branch 2003) and the large number of
137 candidate species awaiting formal taxonomic treatment (Tandy and Keith 1972; Poynton and
138 Broadley 1988; Tolley et al. 2010). Taxon sampling has therefore been extensive to include at least
139 one representative of every African genus and as many geographic localities as possible per species.
140 In total, 1676 sequences from 432 individuals were generated *de novo* for this study, and in
141 combination with sequence data from GenBank, the complete dataset includes 591 individuals of at
142 least 112 species including non-African outgroups. This covers almost 70% of all described African
143 species (69 out of 101), 14 out of 18 Eurasian genera and a selection of New World bufonids to
144 provide wider phylogenetic context and to allow for the inclusion of more fossil calibration points.

145 DNA was extracted from preserved tissue, using a Qiagen DNeasy Blood and Tissue Kit
146 (Qiagen Inc., CA, USA) and the default protocol. A total of ~3439 base pairs comprising five
147 markers including partial sequences of two ribosomal RNA genes; 12S and 16S rRNA (~380 and
148 ~575 bp), and three coding regions: cytochrome-oxidase subunit 1 (COI; mitochondrial, ~840 bp), C-
149 X-C chemokine receptor type 4 (CXCR4; nuclear, 711 bp), and recombination activating gene-1
150 (RAG1; nuclear, ~933 bp) were amplified via Polymerase Chain Reaction (PCR) using Illustra
151 puReTaq Ready-To-Go PCR beads (GE healthcare, Buckinghamshire, UK). PCR products were
152 sequenced by Microsynth AG (Balgris, CH), complementary strands were sequenced for
153 proofreading and all sequences were deposited in GenBank (Supporting Information S2).

154

155 *Phylogenetic Inferences*

156 Sequences were processed using the Codoncode Aligner v4.4.1 (Codoncode Cooperation, MA, USA)
157 and Geneious Pro v5.6.7 (www.geneious.com; Kearse et al. 2012). Each gene region was aligned
158 separately with MAFFT v7.017 (Kato and Standley 2013), and GBlocks (Castresana 2000) was
159 used to remove poorly aligned, ambiguous nucleotide and gap positions in the 12S and 16S
160 alignments. The coding genes were realigned and translated using TranslatorX (Abascal et al. 2010),
161 and an optimal partitioning scheme and nucleotide substitution models for a concatenated alignment
162 were determined using PartitionFinder v1.1.1 (Lanfear et al. 2012).

163 Previous molecular phylogenetic inferences have not recovered African bufonid species as
164 monophyletic (Graybeal 1997; Frost et al. 2006; Pramuk et al. 2008; Van Bocxlaer et al. 2010; Pylon
165 and Wiens 2011; Portik and Papenfuss 2015). To gain clarity on the phylogenetic relationship of
166 African species and to allow for geological time calibration, a ‘global tree’ inference was conducted
167 first. Along with African species, representatives of Eurasian and New World genera were included
168 in this inference, but only samples for which sequence data of all five gene regions was available
169 (with the exception of *Incilius* spp. and *Bufo* spp., included for calibration purposes despite
170 missing COI sequences). Although only 60 of the 101 currently recognized species are covered, all
171 African genera are represented in this tree, with the exception of *Laurentophryne*, a monotypic genus
172 from eastern Democratic Republic of the Congo that has not been sighted since its original collection
173 and description (Laurent 1950), despite recent efforts (Greenbaum and Kusamba 2012; IUCN SSC
174 Amphibian Specialist Group 2013). For the purpose of getting a more complete understanding of the
175 diversity of African lineages, a second alignment and phylogenetic reconstruction, restricted to
176 include only African species, was conducted, using sequence data for as many individuals as
177 possible, even if not all five genes were available. Due to the paraphyletic nature of African bufonids
178 (see results), this reconstruction excluded genera that were not part of the first African radiation
179 (FAR; applies to Central African genera: *Nectophryne*, *Werneria* and *Wolterstorffina*), because their

180 inclusion would a) violate a number of assumptions related to monophyly and complete taxon
181 sampling for downstream analyses and b) an EO signal in diversification is not expected for
182 subsequent colonization events where the assumption of vacant niches no longer holds true (Schenk
183 et al. 2013). The resulting nucleotide matrix for this second inference favours taxon sampling
184 (covering 60 of the 89 currently recognized species of the FAR clade), but at the cost of missing
185 sequence data, fossil calibration points and species not belonging to the FAR clade.

186 Joint posterior distributions of all model parameters for both trees were estimated using
187 Bayesian Markov Chain Monte Carlo (MCMC) searches in BEAST v1.7.5 (Drummond et al. 2012a).
188 Molecular clock models were estimated for a linked set of the mitochondrial markers and for CXCR4
189 and RAG1 separately using uncorrelated lognormal relaxed clock (ucl) priors (Drummond et al.
190 2006) and birth-death tree prior (Gernhard 2008). The global tree was calibrated to geological units
191 of time by including four fossil node constraints: the estimated origin of the *Rhinella marina* species-
192 group (11.8 Ma ; Estes and Wassersug 1963), the most recent common ancestor of *Anaxyrus* and
193 *Incilius* (20 Ma; Tihen 1951), the oldest unambiguously identified member of the *Bufo bufo* group
194 (9.6 Ma; Rage and Roček 2003) and the estimated age of the *Bufo viridis* lineage (18 Ma; Martín
195 et al. 2012). As these fossils are not contained within the FAR clade, the crown age of the FAR tree
196 ingroup was calibrated using the crown age distribution of the FAR clade in the global tree. A total of
197 three MCMC searches with 100 million generations and three with 50 million generations, sampling
198 every 2000th iteration, were conducted. For chain and parameter diagnostics, an additional MCMC
199 search on priors only was conducted, convergence and effective sample sizes (ESS) of parameters in
200 the log files as well as prior distributions were visually inspected using Tracer, and Are We There
201 Yet (AWTY; Wilgenbusch et al. 2004) was used to assess convergence of tree topologies. Posterior
202 trees were resampled and combined using LogCombiner v1.7.5 (Rambaut and Drummond 2012a)
203 and summarized as a maximum clade credibility (MCC) tree using TreeAnnotator v1.7.5 (Rambaut
204 and Drummond 2012b).

205

206 *Species Discovery*

207 Two pruning methods were employed for deriving a tree with single representative tips per species.
208 First, the FAR phylogeny was pruned to include only a single representative per currently recognized
209 species (CRS; based on Frost 2016). However, extensive field and lab work by the authors and
210 collaborators has revealed a large number of undescribed species of African bufonids. Investigating
211 diversification rates using only currently recognized species is therefore not a true representation of
212 their phylogenetic diversity and to objectively obtain a tree that includes undescribed, but distinct,
213 taxa, the Bayesian implementation of the General Mixed Yule-Coalescent model (bGMYC; Pons et
214 al. 2006; Reid and Carstens 2012) was used to identify suitable delimitation points on the
215 chronogram to generate a second tree. Using the bGMYC package v1.0.2 (Reid 2014) in R (R core
216 team 2013), the algorithm was run for 1 million MCMC iterations, sampling every 10000th iteration
217 after an initial 10000 repetition burn-in. Point estimates for species limits were derived using a 0.01
218 posterior probability cutoff threshold and the FAR MCC tree as well as a random subset of 1000
219 posterior trees were pruned to include only a single representative terminal per delimited element.
220 This pruning collapsed all divergences younger than 1.508 Ma resulting in an artificial flat-lining of
221 diversification. As this may not be biologically meaningful, all analyses were repeated on the
222 bGMYC tree with terminal branches truncated by 1.508 Myr (Supporting Information, Fig. S1.1e and
223 S7). The results did not differ substantially to those when using the bGMYC tree and are thus not
224 discussed further.

225

226 *Lineage Diversification*

227 Temporal and topological lineage diversification rate dynamics in the FAR clade (using both the
228 bGMYC and the CRS tree) were investigated in order to detect an early burst followed by a
229 slowdown in rate over time. The γ statistic (Pybus and Harvey 2000) was calculated to test whether

230 the net diversification of a given phylogeny departs from an exponential, pure-birth-like
231 accumulation of lineages using the ape package v3.2 (Paradis et al. 2004). To account for missing
232 taxa in the CRS tree, we employed a Monte Carlo Constant Rate (MCCR) test using the laser
233 package v 2.4-1 (Rabosky 2006).

234 Using a likelihood approach, we then compared two constant rate models (a pure-birth [PB]
235 and birth-death [BD] model with constant rates), to four variable rate models (PB with an exponential
236 speciation rate [PB λ exp], BD with a constant speciation rate and exponential extinction rates
237 [BD λ cst- μ exp], BD with an exponential speciation rate and constant extinction rate [BD λ exp- μ cst]
238 and BD with both exponential speciation and extinction rates [BD λ exp- μ exp]), using the fit_bd
239 function of the RPANDA package v1.1 (Morlon et al. 2011, 2015). Model-fit was compared using
240 Akaike Information Criterion adjusted for small sample sizes (AICc) and Akaike weights (Aw).

241 Bayesian Analysis of Macroevolutionary Mixtures software (BAMM; Rabosky 2014) in
242 combination with the R package BAMMtools v.2.0 (Rabosky et al. 2014) was used to test whether
243 subclades diversify under distinct rate regimes. BAMM was allowed to sample every 1000th
244 generation of 5 million MCMC iterations, priors were configured based on the setBAMMprior
245 function in BAMMtools. The analysis using the bGMYC tree assumed complete sampling (see
246 extended methods; S1), whereas the analysis using the CRS tree was supplied with sampling
247 proportion information for each genus. For each analysis, four independent runs were executed to
248 check for convergence of the posterior probability densities, and Bayes factors were calculated to
249 compare the relative support of one rate regime model over another.

250

251 *Life History Diversification*

252 To explore how life history characters diversified over time, the rates of evolution and disparity of
253 body, clutch, and egg size were investigated. Mature female body size (snout-vent length in mm),
254 clutch size (number of eggs per clutch), and egg size (diameter of eggs in mm) were log₁₀

255 transformed to better conform to normality and size-free residuals were subsequently calculated for
256 clutch and egg size data by regressing traits on body size using phylogenetic generalized least squares
257 (pGLS) regressions. Measurements were taken from Liedtke et al. (2014) and references therein, with
258 new measurements for *Churamiti maridadi*. Traits were mapped on the CRS tree and species for
259 which traits were unknown were pruned from the tree, resulting in a dataset of 60, 46 and 42 species
260 for body, clutch, and egg size respectively.

261 A likelihood approach was used to compare the fit of a series of six evolutionary models to the
262 continuous trait data. Three constant rate models (Brownian motion [BM; Felsenstein 1973],
263 Ornstein-Uhlenbeck [OU; Butler and King 2004], and Pagel's lambda [Pagel 1999]) were compared
264 to three variable rate models (early-burst [EB; exponential rate change through time; Harmon et al.
265 2010], linear [LIN; linear rate change through time], and Pagel's time-dependent [Pagel 1999]
266 model). Models were fitted using the fitContinuous function of the geiger package v2.0 (Harmon et
267 al. 2008b) and comparisons were based on AIC and A_w . We further investigated the temporal rate
268 dynamics and rate heterogeneity of trait evolution using BAMM. For each trait, the rate shift
269 configuration with the highest posterior probability was determined and the rate profile through time
270 of the phenotypic rate parameter β was plotted. In addition, the disparity of traits within and between
271 sub clades were investigated using the dtt function in the geiger package, and by calculating the
272 morphological disparity index (MDI; Harmon et al. 2003) to test for deviation from a Brownian
273 motion model.

274

275 *Speciation Rates of Habitat Generalists versus Specialists*

276 Species were scored as being either habitat generalists or habitat specialists based on the habitat
277 description provided by the IUCN red list database and the authors' first-hand experience
278 (www.iucnredlist.org; Table S1.3; Fig. 7). Our evaluation of habitat comprised of a two-step process:
279 (1) Constructing Table S1.3 based on IUCN data and checking for anomalies and/or mistakes. All

280 authors participated in this process and have considerable experience working in the field
281 observing/collecting African amphibians, and (2) Filtering data according to evaluation from experts
282 and grouping according to being generalist (more than two distinct habitat types) or specialist (two or
283 less habitat types). It should be noted that certain habitat types were grouped giving the ambiguity in
284 the habitats being really distinct from one another. The Binary State Speciation and Extinction
285 (BiSSE) model implemented in the diversitree v.0.9-6 package (FitzJohn 2012) was used to examine
286 whether shifts in habitat specialization are associated with shifts in speciation rate using a maximum
287 likelihood (ML) and Bayesian approach (10000 iterations with 1000 iterations discarded as burn-in).
288 The analysis run with the CRS tree included sampling fraction information to correct for biased
289 undersampling (sampling 0.72 of habitat generalists and 0.66 of habitat specialist) and the bGMYC
290 tree, coding undescribed species based on sampling locality and habitat preferences of their closest
291 relative. This method is known to be problematic when the number of terminals are low or when
292 character ratios are biased (Davis et al. 2013; Rabosky and Goldberg 2015), and so simulation tests
293 were performed (following Onstein et al. 2015) to ensure that there was sufficient power in the data
294 to avoid type I and type II errors (S1).

295

296 **Results**

297 *Phylogenetic Inferences*

298 The global tree (Fig. 1; Supporting Information S3) supports previous claims that African bufonids
299 are paraphyletic (Pramuk et al. 2008; Van Bocxlear et al. 2010; Portik et al. 2015), here recovering
300 two independent colonization events into Africa. Most relationships of Eurasian groups are poorly
301 resolved, but for both African radiations, internal nodes are generally well supported (posterior
302 probabilities >0.9). The global tree reconstruction dates the origin of the Old World radiation at 30.4
303 Ma (95% Highest Posterior Density interval; HPD=23.2-38.5), with the two colonization events into
304 Africa occurring shortly after, at 29.4 Ma (95% HPD=22.8-37.5) and 21.7 Ma (95% HPD=15.8-29.4)

305 respectively. An unexpectedly high degree of genetic divergences, especially within *Nectophryne*,
306 *Wolterstorffina*, *Nectophrynoides*, *Mertensophryne* and in the *Sclerophrys gracilipes-kisoloensis-*
307 *villiersi* complex were recovered, highlighting the need for taxonomic revisions of these groups. All
308 major relationships were congruent in the global tree and the FAR tree (Supporting Information S3
309 and S4). When pruning the FAR tree to only include a single representative of each currently
310 recognized species (CRS tree; Supporting Information S5), 60 out of the 89 known species are
311 represented with the missing 29 belonging to the following genera: *Sclerophrys*–15 (out of 38),
312 *Mertensophryne*–6 (out of 11), *Nectophrynoides*–2 (out of 15) and *Poyntonophrynus*–6 (out of 10).

313

314 *Species Discovery*

315 The bGMYC species discovery with a posterior probability threshold of 0.01 recovered 102
316 delimited entities (Supporting Information S4 and S6). Almost all currently recognized species of this
317 clade were delimited consistently, with the exception of three species pairs: *Mertensophryne howelli*
318 and *M. usambarae*, *Poyntonophrynus damaranus* and *P. dombensis* and *Sclerophrys pardalis* and *S.*
319 *pantherinus*, which were not identified as distinct entities. This echoes previous difficulties in
320 discerning the species status of at least the latter species pair (Poynton and Lambiris 1998; Measey
321 and Tolley 2009). In addition, units phylogenetically distinct from currently recognized species were
322 recovered in the following genera: *Schismaderma*–2, *Nectophrynoides*–13, *Capensibufo*–4,
323 *Mertensophryne*–8 and *Sclerophrys*–18. Cryptic diversity has previously been recognized (Poynton
324 and Broadley 1988; Tolley et al. 2010), and qualitative and quantitative assessments (acoustic calls,
325 distribution, genetics and morphology) of the entities recovered suggest that overall, delimited
326 elements are likely to represent valid species. We investigated the degree to which potential over-
327 splitting would affect our results by tracing the erosion of Pagel's γ as delimited units are sequentially
328 dropped from the bGMYC tree to approach the CRS tree (supporting information S8). From this, we
329 can conclude that only if ~58% or more of the delimited units are not true species, does Pagel's γ

330 deviate significantly from the below reported results. It is therefore unlikely that our estimation of
331 cryptic species is impacting the diversification results.

332

333 *Lineage Diversification*

334 Lineage through time plots for the bGMYC tree (102 terminals), the CRS tree (60 terminals) were
335 compared to a simulated set of 1000 pure-birth trees based on the total number of currently
336 recognized species (89 terminals; Fig. 2). For the bGMYC tree, a negative γ was recovered, but the
337 relative distribution of splitting events was not significantly different from the null hypothesis of
338 constant rates through time ($\gamma_{\text{MCC}}=-1.061$, $p_{\text{MCC}}=0.144$; $\text{mean}\pm\text{SD } \gamma_{\text{posterior}}=-0.951\pm 0.537$, $p=0.171$).
339 Similarly, the observed γ recovered for the CRS tree was negative ($\gamma_{\text{MCC}}=-1.834$; $\text{mean}\pm\text{SD } \gamma_{\text{posterior}}=-$
340 1.826 ± 0.447), but again was not significantly different from the null distribution (MCCR test 5%
341 critical value=-2.449; $p_{\text{MCC}}=0.158$; $p_{\text{posterior}}=0.153$).

342 For both trees (bGMYC and CRS tree), the best fitting models to describe lineage
343 diversification were constant rate, pure-birth models (Table 1). Fit over variable rate models was not
344 always substantial however. For the bGMYC tree, this model was a considerably better fit than any
345 variable rate model ($A_w=0.501$; $\Delta\text{AICc}>2.069$; Table 1), but for the CRS tree, support for constant
346 diversification over variable rate models was less substantial ($A_w=0.452$; $\Delta\text{AICc}>1.460$; Table 1).

347 For both the bGMYC and CRS tree, BAMM found strong support for rate homogeneity. A
348 model with a single evolutionary rate regime had the highest posterior probability (PP=0.650 and
349 0.630 for the two trees respectively; Fig. 3a) with posterior odds ratios of 2.390 (bGMYC) and 2.234
350 (CRS) and Bayes Factor scores of 1.624 (bGMYC) and 1.519 (CRS) over the next best models,
351 which in both cases were two-rate regime models (i.e. one rate shift). Support diminished with
352 complexity of the models (Fig. 3a). BAMM estimated extinction rates to be more or less constant
353 (and low) over time for both the bGMYC and CRS tree, with a slight increase in extinction rates in
354 recent history in the CRS tree (Fig. 3b). The CRS tree showed a marginal decline in speciation rate

355 over time, whereas the bGMYP tree showed an initial increase followed by a flattening out of the
356 curve over time (Fig. 3b).

357

358 *Life-history Diversification*

359 Likelihood model fitting for rates of body, clutch, and egg size evolution, consistently recovered
360 constant rate models outperforming variable rate models (Table 2). For body and egg size, Pagel's λ
361 model performed best, but only for body size was this a noticeably better fit than the next best model
362 (body size $A_{W\lambda}=0.950$ over $A_{W_{OU}}=0.019$; egg size $A_{W\lambda}=0.280$ over $A_{W_{OU}}=0.226$; Table 2). For
363 clutch size, a Brownian motion model was the best fit ($A_{W_{BM}}=0.328$ over the next best $A_{W\lambda}=0.171$).
364 Despite marginal differences in top model performances, the early burst model was consistently
365 ranked lowest for all three traits (body size $A_{W_{EB}}=0.003$; clutch size $A_{W_{EB}}=0.121$; egg size
366 $A_{W_{EB}}=0.042$). BAMM also recovered single-rate regimes as the best shift configurations for body,
367 clutch, and egg size although differences from more complex regime models were marginal
368 (posterior probabilities: 0.38; 0.53; 0.50, Bayes factors: 1.29; 0.84; 0.84 respectively). In line with the
369 likelihood model fitting, all three characters show relatively constant rates over time, with the
370 arguable exception of an initial increase in clutch size (Fig. 4).

371 The trait disparity analysis corroborates homogeneity in trait evolution. The average subclade
372 disparities of all three investigated life-history traits did not significantly deviate from expectation
373 under Brownian motion (MDI scores of -0.022, -0.095 and 0.077 for body, clutch, and egg size
374 respectively), but clutch size, and to some degree body size, show a more defined drop in subclade
375 disparity early on in the history of bufonids than expected (Fig. 5). The disparity plots for body size
376 and egg size indicate peaks during the last 5 million years, where disparity is greater than expected
377 under a BM model, which may be an artefact of under-sampling recent nodes (Harmon et al. 2003)
378 and unlikely to be a biological signal (see similar pattern in: Burbrink and Pyron 2009; Slater et al.
379 2010; Derryberry et al. 2011; Rowe et al. 2011).

380

381 *Speciation Rates of Habitat Generalists versus Specialists*

382 Based on our coding, habitat specialists are more common than habitat generalists (bGMYC tree:
383 73%, CRS tree: 83%), but habitat generalists are present in all major subclades except in
384 *Nectophrynoidea*. Both the ML and Bayesian approaches in BiSSE suggested a shift towards higher
385 speciation rates associated with shifts from habitat generalists to habitat specialists (Fig. 6). The ML
386 approach recovers the shift in speciation rates as significant when using the CRS tree (and
387 incorporating sampling fraction information), but not when using the bGMYC tree (CRS tree:
388 $\chi^2=4.779$, $p=0.029$; bGMYC tree: $\chi^2=0.508$, $p=0.576$). Transitions from generalist to specialists (q_{01})
389 were higher than vice versa (q_{10}) for both the bGMYC (post burn-in MCMC medians: $q_{01}=0.060$;
390 $q_{10}=0.019$) and the CRS tree (medians: $q_{01}=0.113$; $q_{10}=0.074$).

391

392 **Discussion**

393 According to the EO model, expansion into new geographic areas should lead to a rapid
394 diversification both in lineages and in phenotypic traits. Once niches become saturated in the newly
395 colonized areas, rates should decrease in a diversity-dependent manner. Studies testing the EO model
396 have predominantly focused on young lineages restricted to small, isolated areas such as islands.
397 Whether the same niche filling principles can lead to bursts in biodiversity in continent-wide systems,
398 or if such burst can even be detected is less clear. By investigating the diversification history of one
399 of Africa's most species-rich amphibian colonizers, we tested whether signals characteristic of the
400 EO model can be recovered for this geographically and ecologically more complex, continental
401 system.

402

403 *Lineage and Trait Diversification of African Bufonids*

404 Key for accurately estimating diversification rates is the thorough sampling of species (Cusimano and
405 Renner 2010; Brock et al. 2011), which is difficult when dealing with a geographically expansive
406 radiation. Our extensive sampling and analyses of bufonids have revealed a sizable number of
407 undescribed species, up to 45 phylogenetically delimited units. These undescribed, mostly cryptic
408 lineages represent recent, species-level divergences and their exclusion from diversification analyses
409 creates an erroneous overestimation of early divergences relative to recent ones (Figs. 2, 3b;
410 Cusimano and Renner 2010). Critically, the inclusion of this cryptic diversity shows more apparently
411 that the lineage accumulation curve of African toads does not significantly differ from a simulated
412 constant rate curve (Pagels's γ close to zero; Fig. 2) and that the diversification rate is best described
413 by a constant, pure-birth process (slightly outperforming a variable pure-birth process with a
414 marginally exponentially decreasing speciation rate over time; Table 1). Furthermore, there are no
415 significant rate regime shifts between subclades and thus, our data suggests that the first wave of
416 African bufonids as a whole, or any subclades therein, have not experienced a period of rapid lineage
417 expansion followed by a subsequent slowdown as expected under an EO mode (Fig. 3).

418 An early burst in diversification under the EO scenario would indicate the rapid filling of
419 available niches through adaptation and speciation. Analyses of trait evolution should reflect this in
420 the form of early partitioning of traits (Simpson 1953; Mahler et al. 2010), which has even been
421 suggested to be a more reliable signal for EO than lineage diversification (e.g. Slater et al. 2010;
422 Schweizer et al. 2014; but see Harmon et al. 2010). In African bufonids we find little deviation from
423 constant rates and subclade disparity through time in the evolution of life history traits (Table 2; Figs.
424 4, 5). At most, clutch size is partitioned more rapidly than expected, indicating that a division
425 between explosive and low-fecundity breeders likely occurred early on in the history of African
426 toads. However it should be noted that here too that deviation in trait evolution from expectations
427 under Brownian motion is not substantial. This is mirrored in the rate estimates over time that show a
428 steeper increase in rates closer to the root of the tree, but the overall rate change is minimal. Despite

429 Simpson's predictions (Simpson 1953; shown also in more recent comparative studies e.g. Rabosky
430 et al. 2013), trait and lineage diversification need not always be coupled (Ruta et al. 2013) and
431 whether the constant lineage and trait diversification rates are correlated in bufonids requires further
432 testing. Nonetheless, the combined molecular and trait evidence from both analyses provides stronger
433 support for gradual evolutionary patterns on the continent of Africa – not an intuitively clear outcome
434 from previous studies and predictions (e.g. Van Bocxlaer et al. 2010).

435 High dispersal ability can facilitate geographic expansions while maintaining gene flow among
436 populations and thereby inhibit speciation (Claramunt et al. 2012a). Given the high-dispersal nature
437 of many bufonids (Van Bocxlaer et al. 2010), we predicted that the colonization of Africa by toads
438 need not result in the proliferation of ecological specialists, but instead can result in a lower number
439 of generalists. Based on our coding, the majority of species (at least 73%) show narrow habitat
440 preferences and such specialists have experienced (albeit marginally) higher speciation rates than
441 their generalist counterparts (Fig. 6), a pattern frequently observed in other groups too (Hernández et
442 al. 2005 and references therein), and these specialists are most frequently derived from generalist
443 ancestors. We note such analyses are strongly subjected to the coding of traits, and determining
444 whether species are indeed habitat specialists is not trivial and our analysis provides only the first
445 assessment of this. Nonetheless, the BAMM analysis places the highest probability for rate shifts to
446 have occurred along the basal branches of *Nectophrynoidea* (data not shown), a highly specialized
447 genus restricted to montane environments (Müller and Liedtke et al. 2013) and the largest subclade
448 with no habitat generalists. Because generalist lineages have persisted in most other subclades of
449 African bufonids they may have contributed to a buffering of the explosive early niche-filling that is
450 central to the EO model. However, we caution against an over interpretation of this finding, as
451 differences in speciation rates between generalists and specialists were marginal, and the overall
452 number of generalist species was comparatively low. Nonetheless, a similar scenario has been

453 proposed for furnariid birds of South America (Claramunt et al. 2012a,b) and the exceptional ability
454 of some organisms to colonize large areas may ultimately inhibit their lineage proliferation.

455
456 *Is the EO Model a Good Framework for explaining Diversification in Colonizers of Continental*
457 *Systems?*

458 A growing number of studies on a diverse range of vertebrates have failed to find evidence for
459 diversity-dependent rate curves (Neotropical birds: Derryberry et al. 2011; Claramunt et al. 2012b;
460 Schweizer et al. 2014; African catfish: Day et al. 2013; murid rodents: Schenk et al. 2013). A trend is
461 seemingly emerging that EO, frequently considered a precursor for generating diversity in insular
462 systems (Arbogast et al. 2006; Hughes and Eastwood 2006; Harmon et al. 2008a; Jönsson et al. 2012;
463 but see Esselstyn et al. 2009; Losos and Mahler 2010) may not always be an appropriate model for
464 explaining diversification patterns in continent-wide radiations. Here, we discuss, using our data on
465 toads, possible reasons why constant diversification rates may indeed be more common for
466 continental systems and why some studies continue to find EO signals. Reasons why EO might
467 simply not be detectable are discussed in the ensuing sections.

468 *Tabula Rasa?*—Most African amphibian families are endemic and relatively old (Andreone et
469 al. 2008), with molecular (Cannatella and de Sá 1993; Duellman 1993; Vences et al. 2003; Van
470 Bocxlaer et al. 2006; Roelants et al. 2007; Barej et al. 2014) and fossil (Duellman 1999; Blackburn et
471 al. 2015; Gardner and Rage 2016) data suggesting that there is a long history of amphibian
472 assemblages on the continent. Bufonids were late arrivers on the continent of Africa and may not
473 have been presented with the opportunity of ‘vacant’ niches. Furthermore, competition for resources
474 need not be restricted to interactions only with other amphibians; macroinvertebrates for example,
475 directly compete for resources with tadpoles (Morin et al. 1988). Schenk et al. (2013), comparing
476 successive colonization events in rodents, have shown that indeed, secondary lineages, on average,
477 diversify to a lesser extent regardless of whether or not primary colonizers exhibit early burst

478 diversification patterns. In our system too, the second African toad radiation (although not treated in
479 detail here) appears to be much less diverse than the first, despite comparable clade ages (12, relative
480 to 89 recognized species resulting in 0.553 compared to 3.027 species per million years respectively,
481 when dividing number of extant species by clade age). The concept of ‘vacant’ niches may therefore
482 be unrealistic for continental systems that tend to have much older histories of supporting life than
483 unbalanced assemblages in insular systems. Only in cases where a specific ‘ecomorph’ is
484 underrepresented may colonizers continue to be truly presented with EO on continental systems (e.g.
485 Burbrink and Pyron 2009).

486 *Scale and complexity.*—The ecological limits for diversity may not easily be reached if an area
487 is large (Kisel et al. 2011) and dispersal ability of organisms is high (Fritz et al. 2011). Continents
488 tend to be larger than insular systems, and high dispersal ability would be important for successful,
489 continent-wide colonisation. With an area of approximately 30 million km², the potential carrying
490 capacity of Africa dictated by the species-area relationship alone (MacArthur and Wilson 1967;
491 Lomolino 2000) is exceedingly high and it seems plausible that a saturation point of resources,
492 causing a diversity dependent decline in diversification, may never be reached by toads, or at least
493 has not been reached yet, as has been speculated for African catfish (Day et al. 2013).

494 The dynamic geographic changes that impact the history of life are likely to produce highly
495 complex conditions for diversification of lineages under the assumptions of an EO model. For
496 example, the successive rising of new islands in Southeast Asia is thought to have produced repeated
497 EO for shrews to diversify, contra to expectations sustaining a constant diversification rate, instead of
498 reaching the expected diversity-dependent slowdown (Esselstyn et al. 2009). Since the Oligocene,
499 Africa has experienced a fluctuating climate (Richards 1973; Flenley 1979; Livingstone 1993;
500 Parmentier et al. 2007) and one can imagine that the resulting expansion and contraction of habitats
501 and species ranges (Nakazawa and Peterson 2015) could equally have resulted in a pattern of
502 repeated regional opportunity, concealing any singular continent-wide EO signal. Geographic range

503 expansions into the Andes for example has been attributed to promoting repeated bursts of
504 diversification within continent-wide radiations of legumes (Drummond et al. 2012b) and
505 hummingbirds (McGuire et al. 2014). In line with the notion of continuous opportunity, the slow
506 decline of competing lineages (as seen in Quental and Marshall 2013) may have similarly presented
507 bufonids with gradual niche-filling opportunities over its entire history, not just immediately after its
508 colonization of Africa.

509 *Broad habitat tolerance limits EO diversification.*— Habitat generalists are likely to be less
510 hindered by ecological barriers to dispersal, which is an important trait for successful long distance
511 dispersal and colonization (Baur and Bengtsson 1987; Van Bocxlaer et al. 2010; Dennis et al. 2012).
512 This dispersal ability of habitat generalists would however, potentially limit genetic isolation caused
513 by geographic fragmentation through fluctuating ecological conditions or geographic barriers,
514 resulting in lower diversification than in specialists (Price and Wagner 2004; Phillimore et al. 2006;
515 Von Rintelen et al. 2010; Claramunt et al. 2012a; Salisbury et al. 2012; but see Moyle et al. 2009).
516 Therefore, an alternative to an explosive, niche-filling diversification history resulting in large
517 numbers of habitat specialists as predicted by the EO model may be a diversification history
518 dominated by less explosive, habitat generalist lineages.

519

520 *Caveats*

521 Based on rates of evolution in traits and lineages our data suggest bufonids did not experience an
522 early burst followed by a diversity dependent slowdown. Simpson (1953) pointed out that
523 opportunity alone may not be sufficient to promote invasion of new ecological space. As Simpson
524 outlined, if an evolutionary lineage is constrained or unable to ‘take advantage’ of evolutionary
525 opportunities (Simpson 1953; Schluter 2000) some radiations may simply fail to be explosive if the
526 necessary traits do not exist/evolve (Steelman and Danley 2003; Losos 2010; Yoder et al. 2010).
527 Studies on the selective pressures acting on toads and competition with other species are required to

528 better evaluate potential constraints on EO, and whether such factors form the basis for explaining
529 non-EO evolutionary patterns.

530 Alternatively, EO may indeed have been presented to bufonids upon colonizing Africa, but we
531 have failed to detect it. Diversification rate estimates require a number of assumptions concerning
532 estimates of speciation and extinction, which question the veracity of interpreting diversification
533 patterns outlined in this study. For example, high rates of extinction can erode signals of early bursts
534 in phylogenies (Rabosky and Lovette 2008b). Although we included models that fit varying
535 extinction rates through time, estimating this parameter from molecular phylogenies is problematic
536 (Rabosky 2009b) and both γ and the MCCR test are known to be conservative with respect to
537 extinction, and produce high type II errors (Pybus and Harvey 2000; Brock et al. 2011). If extinction
538 rates were indeed low (as estimated by our analyses), but sufficient time has elapsed since the
539 equilibrium diversity has been reached, traces of initial diversity-dependent lineage accumulation
540 may again be lost (Liow et al. 2010; Rabosky and Hurlbert 2015). With a limited fossil record for
541 African bufonids, direct evidence for estimating extinction rates is lacking, but Raven and Axelrod
542 (1974) suggested that angiosperms in Africa have experienced high extinction rates during the
543 Tertiary and Quaternary, a history that if shaped by climate, might have been similar for amphibians.
544

545 **Conclusion**

546 Bufonids are renowned as one of the few amphibian radiations that have achieved a near global
547 distribution, with peaks in diversification rates during dispersal periods to new continents facilitated
548 by the evolution of the ‘toad-like’ phenotype. Yet despite impressive present-day diversity, upon
549 arriving in Africa, lineage and trait diversification rates appear to have been constant over time,
550 showing no early burst signal as might be expected under an EO model. Based on the findings
551 presented here and recent studies in other groups, we conclude that constant-rate and trait
552 diversification might be the more pervasive model for continent-scale radiations in general. The

553 constant overall rate might be due to more complex geographic and climatic histories of continents
554 coupled with lineage specific traits, such as those promoting habitat generalism, which might buffer
555 against rapid EO-driven diversification. Limitations of current methods to detect early burst signals
556 for old radiations and a depauperate fossil record makes an adequate evaluation of these factors
557 difficult at this point, but our initial investigations into the role habitat generalism may have in
558 buffering speciation is encouraging for further investigations into understanding how habitat
559 tolerance affects large scale colonization success and diversification rates.

560

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582

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939 **Figure legends**

940 Figure 1: MCC tree for Bufonidae recovered from time-calibrated Bayesian MCMC tree searches
941 using BEAST under a birth-death uncorrelated lognormal relaxed clock model. Node support below
942 posterior probabilities of 0.9 are indicated by grey squares and node bars show the 95% highest
943 posterior density of divergence times for key nodes; the origin of the two African clades (red) and the
944 fossil calibration points (green), A: the origin of the *Rhinella marina* clade, B: the most recent
945 common ancestor for *Anaxyrus* and *Incilius* C: the origin of the *Bufo bufo* group and D: the origin of
946 the *Bufo viridis* group. The first African radiation (FAR) is colour-coded blue and the second
947 African radiation (SAR) is colour-coded green, with the insert depicting the geographic distribution
948 of these two clades and a representative per genus (sizes approximately to relative scale).

949
950 Figure 2: Lineage through time plots for the bGMYC tree (green) the CRS tree (yellow/orange) and
951 the median of 1000 Yule simulations for a tree with 89 taxa (grey/black). Shading marks the 95%
952 quantiles of posterior and simulated trees.

953
954 Figure 3: Diversification dynamics for the bGMYC (green) and the CRS tree (yellow) using the
955 BAMM software package. a) Posterior distribution of regimes with different numbers of rate
956 processes (including the root process). b) Speciation and extinction rates through time, where shaded
957 areas denote the 95% quantiles on the posterior distribution of the rates.

958
959 Figure 4: Rate dynamics (beta) through time for body size (full line) and size-free clutch (long
960 dashes) and egg (short dashes) size. Shaded areas denote the 95% quantiles on the posterior
961 distribution of rates.

962

963 Figure 5: Disparity through time (DTT) plots for a) body size and b) size-corrected clutch size and c)
964 size-corrected egg size. Solid lines represent the observed DTT using the MCC tree and grey lines are
965 the observed values for a subsample of 1000 post-burnin posterior trees. Dashed lines represent the
966 median DTT under a Brownian motion model simulation with 95% confidence intervals as the light
967 grey translucent polygon.

968
969 Figure 6: Probability density plots of posterior distribution of speciation rates associated with shifts
970 from habitat generalists (dark) to specialists (light), estimated using MCMC-BiSSE using the a)
971 bGMYC tree and b) CRS tree. Dashed lines are maximum likelihood rate estimates and inserted
972 phylogenies depict the coding of tip states.

973

974 **Supporting information**

975 Supporting Information S1: Extended version of Methods section

976

977 Supporting Information S2: GenBank numbers and voucher information for individuals included in
978 the phylogenetic reconstructions.

979

980 Supporting Information S3: MCC tree for Bufonidae (Global Tree) recovered from time-calibrated
981 Bayesian MCMC tree searches using BEAST under a birth-death uncorrelated lognormal relaxed
982 clock model. Nodes are annotated with posterior probabilities.

983

984 Supporting Information S4: MCC tree for the first African radiation (FAR tree) of bufonids,
985 recovered from time-calibrated Bayesian MCMC tree searches using BEAST under a birth-death
986 uncorrelated lognormal relaxed clock model. Nodes are annotated with posterior probabilities and
987 clades in green are delimited units based on a bGMYC species discovery analysis at a 0.01 threshold.

988

989 Supporting Information S5: Phylogenetic tree recovered from pruning the FAR tree to include only a
990 single representative of each currently recognized species (CRS tree).

991

992 Supporting Information S6. Tree recovered from pruning the FAR tree to include only a single
993 representative of each bGMYC delimited element (bGMYC tree).

994

995 Supporting Information S7. Repeat of rate dynamics analyses (BAMM and BiSSE) using truncated
996 bGMYC tree.

997

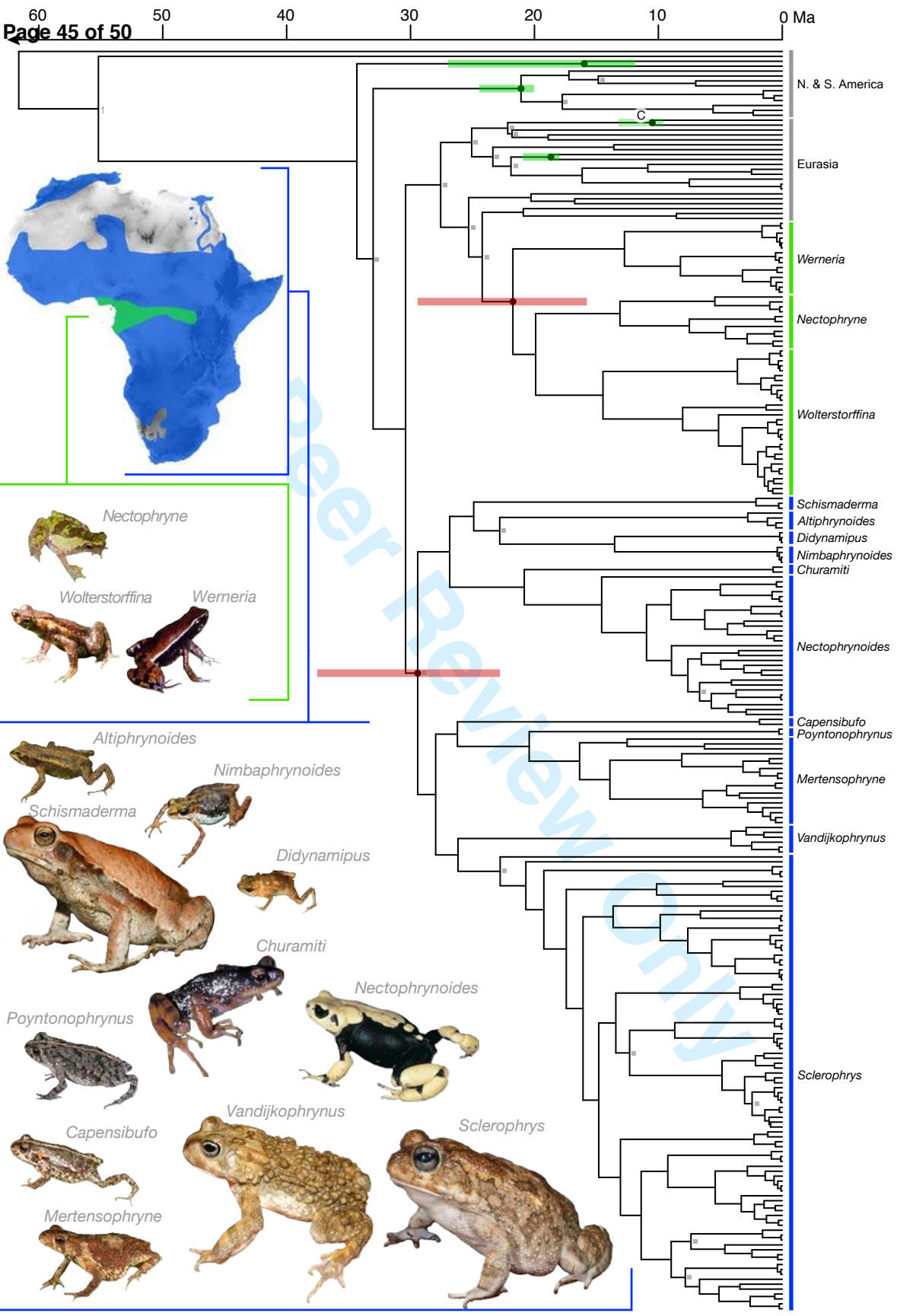
998 Supporting Information S8. Investigating the effects of bGMYC oversplitting.

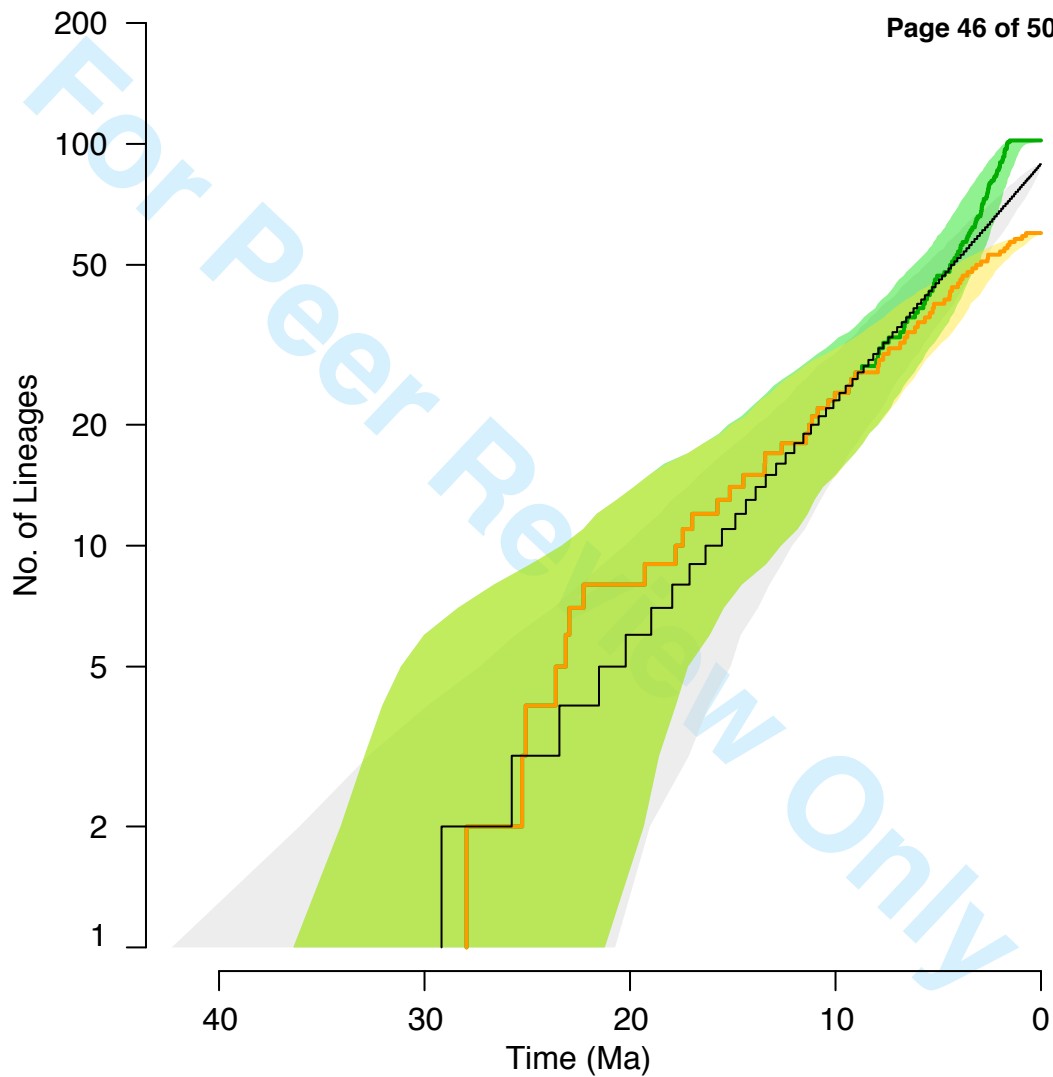
1 Table 1. Summary statistic of diversification models fitted to the branching times of the a) species
 2 delimited bGMYC tree and b) CRS tree. The models tested are pure-birth (PB) and birth-death (BD)
 3 with constant rates, PB with an exponential speciation rate (PB λ_{exp}), BD with a constant speciation
 4 rate and exponential extinction rates (BD λ_{cst} - μ_{exp}), BD with an exponential speciation rate and
 5 constant extinction rate (BD λ_{exp} - μ_{cst}) and BD with both exponential speciation and extinction rates
 6 (BD λ_{exp} - μ_{exp}). Parameters refer to the estimated rates at the tips and the corresponding time-
 7 variation parameter.

Model	λ Parameters	μ Parameters	LH	AICc	Δ AICc	Aw
A) bGMYC tree						
<i>Constant Rate Models</i>						
PB	0.137		-298.691	599.421	-	0.501
BD	0.137	<0.001	-298.691	601.503	2.081	0.177
<i>Variable Rate Models</i>						
PB λ_{exp}	0.136; 0.002		-298.685	601.491	2.069	0.178
BD λ_{cst} - μ_{exp}	0.137	<0.001; -0.218	-298.691	603.626	4.205	0.061
BD λ_{exp} - μ_{cst}	0.136; 0.002	<0.001	-298.685	603.614	4.193	0.062
BD λ_{exp} - μ_{exp}	0.135; 0.002	<0.001; -0.056	-298.685	605.782	6.360	0.021
B) CRS tree						
<i>Constant Rate Models</i>						
PB	0.121		-191.348	384.765	-	0.452
BD	0.121	<0.001	-191.348	386.907	2.142	0.155
<i>Variable Rate Models</i>						
PB λ_{exp}	0.106; 0.015		-191.008	386.226	1.460	0.218
BD λ_{cst} - μ_{exp}	0.146	378.188; -2225.174	-190.891	388.210	3.445	0.081
BD λ_{exp} - μ_{cst}	0.106; 0.015	<0.001	-191.008	388.444	3.679	0.072
BD λ_{exp} - μ_{exp}	0.106; 0.015	<0.001; 0.005	-191.008	390.743	5.977	0.023

Table 2. Model fit comparison for evolutionary dynamics of life history traits a) body size, b) clutch size and c) egg size. The models tested are Brownian motion (BM), Ornstein-Uhlenbeck (OU), Pagel's lambda (λ), early burst (EB), linear variable rate (LIN) and Pagel's delta (δ). Parameters refer to trait evolution rate estimates at the root (z_0), attraction strength of the OU model (α), Pagel's branch length transformation (λ), rate change parameter for the EB model (a), slope parameter for the LIN model (b) and Pagel's delta (δ).

Model	Rate	Parameters	lnL	AIC	Δ AIC	AW
<i>a) Body size</i>						
BM	constant	$z_0=1.692$	13.392	-22.785	9.666	0.008
OU	constant	$z_0=1.697; \alpha=0.034$	15.339	-24.677	7.774	0.019
λ	constant	$z_0=1.689; \lambda=0.808$	19.226	-32.451	-	0.950
EB	variable	$z_0=1.692; a=-1e-6$	13.392	-20.785	11.666	0.003
LIN	variable	$z_0=1.698; b=0.217$	14.745	-23.490	8.961	0.011
δ	variable	$z_0=1.702; \delta=1.849$	14.635	-23.271	9.180	0.010
<i>b) Clutch size</i>						
BM	constant	$z_0=5.160e-11$	-18.984	41.969	-	0.328
OU	constant	$z_0=0.005; \alpha=0.007$	-18.908	43.817	1.848	0.130
λ	constant	$z_0=-0.001; \lambda=0.965$	-18.635	43.270	1.301	0.171
EB	variable	$z_0=-3.169e-7; a=-1e-6$	-18.984	43.969	2.000	0.121
LIN	variable	$z_0=0.004; b=0.015$	-18.92	43.840	1.871	0.129
δ	variable	$z_0=0.003; \delta=1.079$	-18.97	43.940	1.971	0.122
<i>c) Egg size</i>						
BM	constant	$z_0=-1.707e-11$	37.909	-71.818	1.785	0.115
OU	constant	$z_0=-0.013; \alpha=0.041$	39.588	-73.175	0.428	0.226
λ	constant	$z_0=-0.001; \lambda=0.8$	39.801	-73.603	-	0.280
EB	variable	$z_0=-1.180e-7; a=-1e-6$	37.909	-69.818	3.785	0.042
LIN	variable	$z_0=-0.013; b=98.913$	39.271	-72.542	1.061	0.165
δ	variable	$z_0=-0.016; \delta=2.254$	39.31	-72.620	0.983	0.171





Posterior probability

0.8

Posterior probability

0.0

1

No. of rate regimes

No. of rate regimes

2

3

4

5

1

2

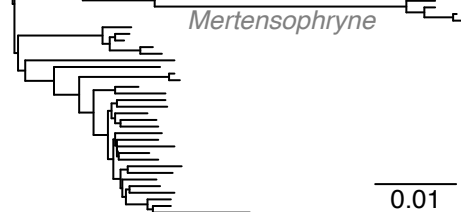
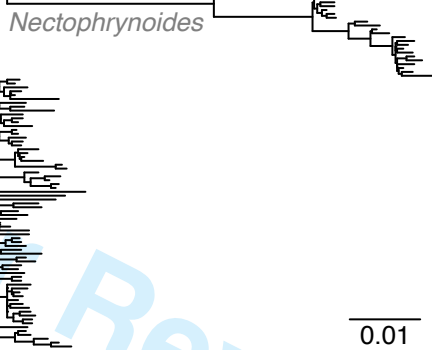
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6

b)



c)

Speciation Rate (λ)

0.6

Extinction Rate (μ)

0.00

30

Time (Ma)

25

20

15

10

5

0

0.0

0.1

0.2

0.3

0.4

0.5

0.6

0.00

0.05

0.10

0.15

0.20

0.25

0.30

0.35

0.40

0.45

0.50

0.55

0.60

Time (Ma)

