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

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Complete List of Authors:	Liedtke, Christoph; Doñana Biological Station EBD-CSIC, Department of Wetland Ecology; University of Basel, Institute of Biogeography Müller, Hendrik; Friedrich-Schiller-Universität, Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum Rodel, Mark-Oliver; Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science Menegon, Michele; MUSE- Museo delle Scienze, Tropical Biodiversity Section Gonwouo, LeGrand; Cameroon Herpetology-Conservation Biology Foundation Barej, Michael; Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science Gvoždík, Václav; Institute of Vertebrate Biolog, Czech Academy of Sciences Schmitz, Andreas; Muséum d'Histoire Naturelle, Department of Herpetology and Ichthyology Channing, Alan; University of the Western Cape, Biodiversity and Conservation Biology Department Nagel, Peter; University of Basel, Department of Environmental Science (Biogeography) Loader, Simon; University of Basel, Institute of Biogeography; University of Roehampton, Department of Life Sciences
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No Ecological Opportunity Signal on a Continental Scale? Diversification and Life-History Evolution of African True Toads (Anura: Bufonidae)

H. Christoph Liedtke[1,2], Hendrik Müller[3], Mark-Oliver Rödel[4], Michele Menegon[5], LeGrand Nono Gonwouo[6], Michael F. Barej[4], Václav Gvoždík[7], Andreas Schmitz[8], Alan Channing[9], Peter Nagel[1], Simon P. Loader[1,10]

[1]*Department of Environmental Science (Biogeography), University of Basel, Klingelbergstrasse 27, 4056 Basel, Switzerland*

[2]*Ecology, Evolution and Developmental Group, Department of Wetland Ecology, Estación Biológica de Doñana (CSIC), 41092 Sevilla, Spain*

[3]*Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum, Friedrich-Schiller-Universität Jena, Erbertstraße 1, 07743 Jena, Germany*

[4]*Museum für Naturkunde Berlin, Leibniz Institute for Evolution and Biodiversity Science, Invalidenstraße 43, 10115 Berlin, Germany*

[5]*Tropical Biodiversity Section, MUSE- Museo delle Scienze, Corso del Lavoro e della Scienza 3, Trento 38123, Italy*

[6]*Cameroon Herpetology-Conservation Biology Foundation, P.O. Box 8218, Yaoundé, Cameroon*

[7]*Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, 603 65 Brno, Czech Republic*

[8]*Natural History Museum of Geneva, Department of Herpetology and Ichthyology, C.P. 6434, 1211 Geneva 6, Switzerland*

[9]*Biodiversity and Conservation Biology Department, University of the Western Cape, Private Bag X17, Bellville, 7535, South Africa*

[10]*Department of Life Sciences, University of Roehampton, London SW15 4JD, United Kingdom*

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Abstract

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

Introduction

How species and species assemblages respond to a release from ecological competition is a key question in evolutionary biology (Simpson 1953; Schluter 2000; Losos 2010; Yoder et al. 2010). The colonization of islands (Robichaux et al. 1990; Grant 1999; Whittaker and Fernandez-Palacios 2007), mass extinction events (Sepkoski 1998), the availability of new resources (McKenna et al. 2009) or the evolution of key innovations (Hunter and Jernvall 1995; Jønsson et al. 2012) are classic examples of where a sudden intrinsic or extrinsic change has presented organisms with an ‘ecological opportunity’ (EO; Simpson 1953). In response to this opportunity, lineages are predicted to rapidly diversify, unimpeded by competition until an ecological saturation point is reached inducing a slowdown in diversification (Nee et al. 1992; Rabosky 2009a). Phylogenies are a powerful tool for the inference of macroevolutionary processes (Mooers and Heard 1997) and the detection of diversity dependent lineage accumulation patterns in response to competitive release has been interpreted as a signal for EO, especially in relation to adaptive radiations (Losos and Mahler 2010). Diversifying into new niche space when presented with EO should also be reflected in the diversification and disparity of phenotypes (Simpson 1953; Schluter 2000; Harmon et al. 2003; Slater et al. 2010; Jønsson et al. 2012), especially in traits relevant to adaptation (Steelman and Danley 2003). Reconstructing the evolutionary history of phenotypes can therefore strongly complement our understanding of diversification from studying phylogenies (Mahler et al. 2010; Harmon et al. 2010; Slater et al. 2010).

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

In continent-wide studies of EO, detections of both rapid and early lineage and trait diversification has been attributed to the biogeographic transition to new, underutilized areas as a result of a colonization event (Burbrink and Pyron 2009; Barker et al. 2013; Schenk et al. 2013; Price et al. 2014a). Conversely, a lack of signal has been attributed to the geographic and ecological complexity of continents, with ecological saturation unlikely to occur on such a scale (Derryberry et al. 2011; Day et al. 2013; Schweizer et al. 2014). Furthermore, the dispersal ability of ancestors that may have led to the continent-wide colonization of new habitats may in itself inhibit rapid speciation by preventing ecological isolation (Claramunt et al. 2012b). EO as a result of expansion into new geographic or ecological space may therefore be as much a driver for generating biodiversity in continent-wide clades as is the case for island or localized mainland radiations, but high dispersal ability or the magnitude of the ecological carrying capacity of continents could equally mean that the EO model is less applicable to such geographically and ecologically more complex systems. Alternatively, if indeed EO played a role in shaping diversification, these systems may be too old for early burst signals to be detectable if changes in rate over time were not drastic or if too much time has passed since rate equilibrium has been reached (Liow et al. 2010; Rabosky and Hurlbert 2015).

With 586 currently recognized species worldwide, Bufonidae is the third most species-rich family of amphibians (Frost 2016). Unlike most other amphibians, bufonids were able to colonize most parts of the world (Duellman 1999) and this species-rich and world-wide diversification across entire continents offers an excellent system for investigating how biodiversity accumulates on continents and whether early bursts in both lineage accumulation and trait disparity has occurred as a response to EO. African bufonids in particular are suitable for addressing EO, firstly, due to extreme trait disparity observed across species. Variable phenotypic traits include body size (19-163 mm; Liedtke et al. 2014), which is correlated with ecological factors and under strong selection in many systems (Davis 1938; Peters 1986). Furthermore, diverse modes of life history strategies (biphasic aquatic breeding to viviparity) are evident in African bufonids (Liedtke 2014; Liedtke et al. 2014) and components of these, such as fecundity (clutch size) and parental investment per offspring (egg size), are good indicators for adaptation to extrinsic factors (Dobzhansky 1950; Duellman and Trueb 1994; Roff 2002; Räsänen et al. 2008). A second qualification is the biogeographic transition African bufonids underwent. Both fossil and molecular evidence point to a Neotropical origin of Bufonidae (Tihen 1962; Blair 1972; Pramuk et al. 2008) at around 80-60 Ma (Pramuk et al. 2008; Van Bocxlaer et al. 2010) followed by a rapid global diversification earliest in the Late Eocene (40-30 Ma; Pramuk et al. 2008; Van Bocxlaer et al. 2010; Portik and Papenfuss 2015). By the Oligocene (~30 Ma) bufonids were established on all continents except Australia and Antarctica (Van Bocxlaer et al. 2010), neither of which host endemic bufonid lineages at present. Van Bocxlaer et al. (2010) proposed that the evolution of an 'optimal range-expansion phenotype' (robust, explosive breeders with high dispersal abilities) was crucial for their success, a phenotype they estimated as characteristic of the first lineage to colonize Africa as well. Their broad ecological tolerance may have been advantageous for allowing this group to disperse widely on the continent, but such habitat generalism may ultimately result in lower overall lineage proliferation (Claramunt et al. 2012a). Nonetheless, African bufonids display a rich array of phenotypes, reproductive behaviour and habitat preferences (Tandy and Keith 1972; Clarke 2001; Liedtke et al. 2014), which raises the question whether this diversity was spurred by EO.

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

Methods

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

Taxon Sampling and DNA Sequencing

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

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

Phylogenetic Inferences

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

Previous molecular phylogenetic inferences have not recovered African bufonid species as monophyletic (Graybeal 1997; Frost et al. 2006; Pramuk et al. 2008; Van Bocxlaer et al. 2010; Pyron and Wiens 2011; Portik and Papenfuss 2015). To gain clarity on the phylogenetic relationship of African species and to allow for geological time calibration, a 'global tree' inference was conducted first. Along with African species, representatives of Eurasian and New World genera were included in this inference, but only samples for which sequence data of all five gene regions was available (with the exception of *Incilius* spp. and *Bufotes surdus*, included for calibration purposes despite missing COI sequences). Although only 60 of the 101 currently recognized species are covered, all African genera are represented in this tree, with the exception of *Laurentophryne*, a monotypic genus from eastern Democratic Republic of the Congo that has not been sighted since its original collection and description (Laurent 1950), despite recent efforts (Greenbaum and Kusamba 2012; IUCN SSC Amphibian Specialist Group 2013). For the purpose of getting a more complete understanding of the diversity of African lineages, a second alignment and phylogenetic reconstruction, restricted to include only African species, was conducted, using sequence data for as many individuals as possible, even if not all five genes were available. Due to the paraphyletic nature of African bufonids (see results), this reconstruction excluded genera that were not part of the first African radiation (FAR; applies to Central African genera: *Nectophryne*, *Werneria* and *Wolterstorffina*), because their inclusion would a) violate a number of assumptions related to monophyly and complete taxon sampling for downstream analyses and b) an EO signal in diversification is not expected for subsequent colonization events where the assumption of vacant niches no longer holds true (Schenk et al. 2013). The resulting nucleotide matrix for this second inference favours taxon sampling (covering 60 of the 89 currently recognized species of the FAR clade), but at the cost of missing sequence data, fossil calibration points and species not belonging to the FAR clade.

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

Species Discovery

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

Lineage Diversification

Temporal and topological lineage diversification rate dynamics in the FAR clade (using both the bGMYC and the CRS tree) were investigated in order to detect an early burst followed by a slowdown in rate over time. The γ statistic (Pybus and Harvey 2000) was calculated to test whether the net diversification of a given phylogeny departs from an exponential, pure-birth-like accumulation of lineages using the ape package v3.2 (Paradis et al. 2004). To account for missing taxa in the CRS tree, we employed a Monte Carlo Constant Rate (MCCR) test using the laser package v 2.4-1 (Rabosky 2006).

Using a likelihood approach, we then compared two constant rate models (a pure-birth [PB] and birth-death [BD] model with constant rates), to four variable rate models (PB with an exponential speciation rate [PBλexp], BD with a constant speciation rate and exponential extinction rates [BDλcst-μexp], BD with an exponential speciation rate and constant extinction rate [BDλexp-μcst] and BD with both exponential speciation and extinction rates [BDλexp-μexp]), using the fit_bd function of the RPANDA package v1.1 (Morlon et al. 2011, 2015). Model-fit was compared using Akaike Information Criterion adjusted for small sample sizes (AICc) and Akaike weights (Aw).

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

Life History Diversification

To explore how life history characters diversified over time, the rates of evolution and disparity of body, clutch, and egg size were investigated. Mature female body size (snout-vent length in mm), clutch size (number of eggs per clutch), and egg size (diameter of eggs in mm) were log10 transformed to better conform to normality and size-free residuals were subsequently calculated for clutch and egg size data by regressing traits on body size using phylogenetic generalized least squares (pGLS) regressions. Measurements were taken from Liedtke et al. (2014) and references therein, with new measurements for *Churamiti maridadi*. Traits were mapped on the CRS tree and species for which traits were unknown were pruned from the tree, resulting in a dataset of 60, 46 and 42 species for body, clutch, and egg size respectively.

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

Speciation Rates of Habitat Generalists versus *Specialists*

Species were scored as being either habitat generalists or habitat specialists based on the habitat description provided by the IUCN red list database and the authors' first-hand experience (www.iucnredlist.org; Table S1.3; Fig. 7). Our evaluation of habitat comprised of a two-step process: (1) Constructing Table S1.3 based on IUCN data and checking for anomalies and/or mistakes. All authors participated in this process and have considerable experience working in the field observing/collecting African amphibians, and (2) Filtering data according to evaluation from experts and grouping according to being generalist (more than two distinct habitat types) or specialist (two or less habitat types). It should be noted that certain habitat types were grouped giving the ambiguity in the habitats being really distinct from one another. The Binary State Speciation and Extinction (BiSSE) model implemented in the diversitree v.0.9-6 package (FitzJohn 2012) was used to examine whether shifts in habitat specialization are associated with shifts in speciation rate using a maximum likelihood (ML) and Bayesian approach (10000 iterations with 1000 iterations discarded as burn-in). The analysis run with the CRS tree included sampling faction information to correct for biased undersampling (sampling 0.72 of habitat generalists and 0.66 of habitat specialist) and the bGMYC tree, coding undescribed species based on sampling locality and habitat preferences of their closest relative. This method is known to be problematic when the number of terminals are low or when character ratios are biased (Davis et al. 2013; Rabosky and Goldberg 2015), and so simulation tests were performed (following Onstein et al. 2015) to ensure that there was sufficient power in the data to avoid type I and type II errors (S1).

Results

Phylogenetic Inferences

The global tree (Fig. 1; Supporting Information S3) supports previous claims that African bufonids are paraphyletic (Pramuk et al. 2008; Van Bocxlear et al. 2010; Portik et al. 2015), here recovering two independent colonization events into Africa. Most relationships of Eurasian groups are poorly resolved, but for both African radiations, internal nodes are generally well supported (posterior probabilities >0.9). The global tree reconstruction dates the origin of the Old World radiation at 30.4 Ma (95% Highest Posterior Density interval; HPD=23.2-38.5), with the two colonization events into Africa occurring shortly after, at 29.4 Ma (95% HPD=22.8-37.5) and 21.7 Ma (95% HPD=15.8-29.4) respectively. An unexpectedly high degree of genetic divergences, especially within *Nectophryne*, *Wolterstorffina*, *Nectophrynoides*, *Mertensophryne* and in the *Sclerophrys gracilipes-kisoloensis-villiersi* complex were recovered, highlighting the need for taxonomic revisions of these groups. All major relationships were congruent in the global tree and the FAR tree (Supporting Information S3 and S4). When pruning the FAR tree to only include a single representative of each currently recognized species (CRS tree; Supporting Information S5), 60 out of the 89 known species are represented with the missing 29 belonging to the following genera: *Sclerophrys*–15 (out of 38), *Mertensophryne*–6 (out of 11), *Nectophrynoides*–2 (out of 15) and *Poyntonophrynus*–6 (out of 10).

Species Discovery

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

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

Lineage Diversification

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

For both trees (bGMYC and CRS tree), the best fitting models to describe lineage diversification were constant rate, pure-birth models (Table 1). Fit over variable rate models was not always substantial however. For the bGMYC tree, this model was a considerably better fit than any variable rate model (Aw=0.501; ΔAICc>2.069; Table 1), but for the CRS tree, support for constant diversification over variable rate models was less substantial (Aw=0.452; ΔAICc>1.460; Table 1).

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

Life-history Diversification

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

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

Speciation Rates of Habitat Generalists versus *Specialists*

Based on our coding, habitat specialists are more common than habitat generalists (bGMYC tree: 73%, CRS tree: 83%), but habitat generalists are present in all major subclades except in *Nectophrynoides*. Both the ML and Bayesian approaches in BiSSE suggested a shift towards higher speciation rates associated with shifts from habitat generalists to habitat specialists (Fig. 6). The ML approach recovers the shift in speciation rates as significant when using the CRS tree (and incorporating sampling fraction information), but not when using the bGMYC tree (CRS tree: $\chi^2=4.779$, p=0.029; bGMYC tree: $\chi^2=0.508$, p=0.576). Transitions from generalist to specialists (q01) were higher than vice versa (q10) for both the bGMYC (post burn-in MCMC medians: q01=0.060; q10=0.019) and the CRS tree (medians: q01=0.113; q10=0.074).

Discussion

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

Lineage and Trait Diversification of African Bufonids

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

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

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

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

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

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

Tabula Rasa?—Most African amphibian families are endemic and relatively old (Andreone et al. 2008), with molecular (Cannatella and de Sá 1993; Duellman 1993; Vences et al. 2003; Van Bocxlaer et al. 2006; Roelants et al. 2007; Barej et al. 2014) and fossil (Duellman 1999; Blackburn et al. 2015; Gardner and Rage 2016) data suggesting that there is a long history of amphibian assemblages on the continent. Bufonids were late arrivers on the continent of Africa and may not have been presented with the opportunity of 'vacant' niches. Furthermore, competition for resources need not be restricted to interactions only with other amphibians; macroinvertebrates for example, directly compete for resources with tadpoles (Morin et al. 1988). Schenk et al. (2013), comparing successive colonization events in rodents, have shown that indeed, secondary lineages, on average, diversify to a lesser extent regardless of whether or not primary colonizers exhibit early burst diversification patterns. In our system too, the second African toad radiation (although not treated in detail here) appears to be much less diverse than the first, despite comparable clade ages (12, relative to 89 recognized species resulting in 0.553 compared to 3.027 species per million years respectively, when dividing number of extant species by clade age). The concept of ‘vacant’ niches may therefore be unrealistic for continental systems that tend to have much older histories of supporting life than unbalanced assemblages in insular systems. Only in cases where a specific ‘ecomorph’ is underrepresented may colonizers continue to be truly presented with EO on continental systems (e.g. Burbrink and Pyron 2009).

Scale and complexity.—The ecological limits for diversity may not easily be reached if an area is large (Kisel et al. 2011) and dispersal ability of organisms is high (Fritz et al. 2011). Continents tend to be larger than insular systems, and high dispersal ability would be important for successful, continent-wide colonisation. With an area of approximately 30 million km^2, the potential carrying capacity of Africa dictated by the species-area relationship alone (MacArthur and Wilson 1967; Lomolino 2000) is exceedingly high and it seems plausible that a saturation point of resources, causing a diversity dependent decline in diversification, may never be reached by toads, or at least has not been reached yet, as has been speculated for African catfish (Day et al. 2013).

The dynamic geographic changes that impact the history of life are likely to produce highly complex conditions for diversification of lineages under the assumptions of an EO model. For example, the successive rising of new islands in Southeast Asia is thought to have produced repeated EO for shrews to diversify, contra to expectations sustaining a constant diversification rate, instead of reaching the expected diversity-dependent slowdown (Esselstyn et al. 2009). Since the Oligocene, Africa has experienced a fluctuating climate (Richards 1973; Flenley 1979; Livingstone 1993; Parmentier et al. 2007) and one can imagine that the resulting expansion and contraction of habitats and species ranges (Nakazawa and Peterson 2015) could equally have resulted in a pattern of repeated regional opportunity, concealing any singular continent-wide EO signal. Geographic range expansions into the Andes for example has been attributed to promoting repeated bursts of diversification within continent-wide radiations of legumes (Drummond et al. 2012b) and hummingbirds (McGuire et al. 2014). In line with the notion of continuous opportunity, the slow decline of competing lineages (as seen in Quental and Marshall 2013) may have similarly presented bufonids with gradual niche-filling opportunities over its entire history, not just immediately after its colonization of Africa.

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

Caveats

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

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

Conclusion

Bufonids are renowned as one of the few amphibian radiations that have achieved a near global distribution, with peaks in diversification rates during dispersal periods to new continents facilitated by the evolution of the 'toad-like' phenotype. Yet despite impressive present-day diversity, upon arriving in Africa, lineage and trait diversification rates appear to have been constant over time, showing no early burst signal as might be expected under an EO model. Based on the findings presented here and recent studies in other groups, we conclude that constant-rate and trait diversification might be the more pervasive model for continent-scale radiations in general. The constant overall rate might be due to more complex geographic and climatic histories of continents coupled with lineage specific traits, such as those promoting habitat generalism, which might buffer against rapid EO-driven diversification. Limitations of current methods to detect early burst signals for old radiations and a depauperate fossil record makes an adequate evaluation of these factors difficult at this point, but our initial investigations into the role habitat generalism may have in buffering speciation is encouraging for further investigations into understanding how habitat tolerance affects large scale colonization success and diversification rates.

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References

Abascal, F., R. Zardoya, and M. J. Telford. 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. Nucleic Acids Res. 38:1–7.

Alhajeri, B. H., J. J. Schenk, and S. J. Steppan. 2015. Ecomorphological diversification following continental colonization in muroid rodents (Rodentia: Muroidea). Biol. J. Linn. Soc. 117:463–481.

Andreone, F., A. Channing, R. C. Drewes, J. Gerlach, F. Glaw, K. M. Howell, M. Largen, S. P. Loader, S. Lötters, L. R. Minter, M. Pickersgill, C. Raxworthy, M.-O. Rödel, A. Schiøtz, D. Vallan, and M. Vences. 2008. Amphibians of the Afrotropical realm. Pp. 53–64 *in* Threatened Amphibians of the World. Lynx Edicions, Barcelona.

Arbogast, B. S., S. V. Drovetski, R. L. Curry, P. T. Boag, G. Seutin, P. R. Grant, B. R. Grant, and D. J. Anderson. 2006. The origin and diversification of Galapagos mockingbirds. Evolution 60:370–382.

Barej, M. F., A. Schmitz, R. Günther, S. P. Loader, K. Mahlow, and M.-O. Rödel. 2014. The first endemic West African vertebrate family - a new anuran family highlighting the uniqueness of the Upper Guinean biodiversity hotspot. Front. Zool. 11:8.

Barker, F. K., K. J. Burns, J. Klicka, S. M. Lanyon, and I. J. Lovette. 2013. Going to extremes: contrasting rates of diversification in a recent radiation of New World passerine birds. Syst. Biol. 62:298–320.

Baur, B. and J. Bengtsson, J. 1987. Colonizing ability in land snails on Baltic uplift archipelagos. J. Biogeogr. 14:329–341.

Blackburn, D. C., E. M. Roberts and N. J. Stevens. 2015. The earliest record of the endemic African frog family Ptychadenidae from the Oligocene Nsungwe Formation of Tanzania. J. Vertebr. Paleontol. 35:e907174–6.

Blair, W. F. 1972. Evolution in the Genus *Bufo*. 1st ed. University of Texas Press, Texas.

Brock, C. D., Harmon, L. J. and M. E. Alfaro. 2011. Testing for temporal variation in diversification rates when sampling is incomplete and nonrandom. Syst. Biol. 60:410–419.

Burbrink, F. T., and R. A. Pyron. 2009. How does ecological opportunity influence rates of speciation, extinction, and morphological diversification in new world ratsnakes (tribe Lampropeltini)? Evolution 64:934–943.

Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. Am. Nat. 164:683–695.

Cannatella, D. C., and R. O. de Sá. 1993. *Xenopus laevis* as a model organism. Syst. Biol. 42:476–507.

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17:540–552.

Claramunt, S., E. P. Derryberry, J. V. Remsen, and R. T. Brumfield. 2012a. High dispersal ability inhibits speciation in a continental radiation of passerine birds. P. Roy. Soc. B-Biol. Sci. 279:1567–1574.

Claramunt, S., E. P. Derryberry, R. T. Brumfield, and J. V. Remsen Jr. 2012b. Ecological opportunity and diversification in a continental radiation of birds: climbing adaptations and cladogenesis in the Furnariidae. Am. Nat. 179:649–666.

Clarke, B. T. 2001. Towards a natural classification of African toads (Anura, Bufonidae): Past progress and future prospects. Afr. J. Herpetol. 50:19–30.

Cusimano, N., and S. S. Renner. 2010. Slowdowns in diversification rates from real phylogenies may not be real. Syst. Biol. 59:458–464.

Davis, M. P., P. E. Midford, and W. Maddison. 2013. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. BMC Evol. Biol. 13:38.

Davis, W. B. 1938. Relation of size of pocket gophers to soil and altitude. J. Mammal. 19:338–342.

Day, J. J., C. R. Peart, K. J. Brown, J. P. Friel, R. Bills, and T. Moritz. 2013. Continental diversification of an African catfish radiation (Mochokidae: *Synodontis*). Syst. Biol. 62:351–365.

Dennis, R. L. H., P. B. Hardy and L. Dapporto. 2012. Nestedness in island faunas: novel insights into island biogeography through butterfly community profiles of colonization ability and migration capacity. J. Biogeogr. 39:1412–1426.

Derryberry, E. P., S. Claramunt, G. Derryberry, R. T. Chesser, J. Cracraft, A. Aleixo, J. Pérez-Emán, J. V. Remsen Jr, and R. T. Brumfield. 2011. Lineage diversification and morphological evolution in a large-scale continental radiation: the neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution 65:2973–2986.

Dobzhansky, T. 1950. Evolution in the tropics. Am. Sci. 38:209–221.

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012a. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29:1969–1973.

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4:e88.

Drummond, C. S., R. J. Eastwood, S. T. S. Miotto, and C. E. Hughes. 2012b. Multiple continental radiations and correlates of diversification in *Lupinus* (Leguminosae): testing for key innovation with incomplete taxon sampling. Syst. Biol. 61:443–460.

Duellman, W. E. 1993. Amphibians in Africa and South America: evolutionary history and ecological comparisons. Pp. 200–243 *in* P. Goldblatt, ed. Biological relationships between

Africa and South America. Yale University Press, New Haven and London.

Duellman, W. E. 1999. Patterns of distribution of amphibians: a global perspective. JHU Press, Baltimore.

Duellman, W. E., and L. Trueb. 1994. Biology of amphibians. Johns Hopkins University Press, Baltimore.

Esselstyn, J. A., R. M. Timm, and R. M. Brown. 2009. Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews. Evolution 63:2595–2610.

Estes, R., and R. J. Wassersug. 1963. A Miocene toad from Colombia, South America. Breviora. 193:1–13.

Felsenstein, J. 1973. Maximum-likelihood estimation of evolutionary trees from continuous characters. Am. J. Hum. Genet. 25:471–492.

FitzJohn, R. G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Flenley, J. 1979. The equatorial rain forest: a geological history. Butterworths, London.

Fritz, S. A., K. A. Jønsson, J. Fjeldså, and C. Rahbek. 2011. Diversification and biogeographic patterns in four island radiations of passerine birds. Evolution 66:179–190.

Frost, D. R. 2016. Amphibian Species of the World: an Online Reference. Version 6.0. Electronic Database accessible at http://researchamnhorg/herpetology/amphibia/indexhtml. American Museum of Natural History, New York, USA. (last accessed 25 February 2016).

Frost, D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. O. De Sa, A. Channing, M. Wilkinson, and S. C. Donnellan. 2006. The amphibian tree of life. B. Am. Mus. Nat. Hist. 297:1–291.

Gardner, J.D. and J.-C. Rage. 2016. The fossil record of lissamphibians from Africa, Madagascar, and the Arabian Plate. Palaeobio. Palaeoenv. 2016:1–52.

Gernhard, T. 2008. The conditioned reconstructed process. J. Theor. Biol. 253:769–778.

Grant, P. R. 1999. Ecology and evolution of Darwin's finches. pp 512. Princeton University Press, Princeton, NJ.

Graybeal, A. 1997. Phylogenetic relationships of bufonid frogs and tests of alternate macroevolutionary hypotheses characterizing their radiation. Zool. J. Linn. Soc. Lond. 119:297–338.

Greenbaum, E., and C. Kusamba. 2012. Conservation implications following the rediscovery of four frog species from the Itombwe Natural Reserve, eastern Democratic Republic of the Congo. Herpetol. Rev. 43:253–259.

Harmon, L. J., and S. Harrison. 2015. Species diversity is dynamic and unbounded at local and continental scales*. Am. Nat. 185:584–593.

Harmon, L. J., J. A. Schulte, A. Larson, and J. B. Losos. 2003. Tempo and mode of evolutionary radiation in iguanian lizards. Science 301:961–964.

Harmon, L. J., J. B. Losos, T. Jonathan Davies, R. G. Gillespie, J. L. Gittleman, W. Bryan Jennings, K. H. Kozak, M. A. McPeek, F. Moreno-Roark, T. J. Near, A. Purvis, R. E. Ricklefs, D. Schluter, J. A. Schulte II, O. Seehausen, B. L. Sidlauskas, O. Torres-Carvajal, J. T. Weir, and A. O. Mooers. 2010. Early bursts of body size and shape evolution are rare in comparative data. Evolution 64:2385–2396.

Harmon, L., J. Melville, A. Larson, and J. B. Losos. 2008a, The role of geography and ecological opportunity in the diversification of day geckos (*Phelsuma*). Syst. Biol. 57:562–573.

Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008b. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129–131.

Hernández Fernández, M., and E. S. Vrba. 2005. Macroevolutionary processes and biomic specialization: testing the resource-use hypothesis. Evol. Ecol. 19:199–219.

Hughes, C., and R. Eastwood. 2006. Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. P. Natl. Acad. Sci. USA 103:10334–10339.

Hunter, J. P., and J. Jernvall. 1995. The hypocone as a key innovation in mammalian evolution. P. Natl. Acad. Sci. USA 92:10718–10722.

IUCN SSC Amphibian Specialist Group 2013. n.d. *Laurentophryne parkeri*. in IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. www.iucnredlist.org. (last accessed on 6 January 2014).

Jønsson, K. A., P.-H. Fabre, S. A. Fritz, R. S. Etienne, R. E. Ricklefs, T. B. Jørgensen, J. Fjeldså, C. Rahbek, P. G. P. Ericson, F. Woog, E. Pasquet, and M. Irestedt. 2012. Ecological and evolutionary determinants for the adaptive radiation of the Madagascan vangas. P. Natl. Acad. Sci. USA 109:6620–6625.

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30:772–780.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Kisel, Y., L. McInnes, N. H. Toomey, and C. D. L. Orme. 2011. How diversification rates and diversity limits combine to create large-scale species-area relationships. Philos. T. Roy. Soc. B 366:2514–2525.

Kozak, K. H., and J. J. Wiens. 2006. Does niche conservatism promote speciation? A case study in North American salamanders. Evolution 60:2604–2621.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29:1695–1701.

Laurent, R. F. 1950. Diagnoses préliminaires de treize batraciens nouveaux d'Afrique centrale. Rev.

Zool. Bot. Afr. 44:1–18.

Liedtke, H. C. 2014. The evolution of terrestrial breeding in African amphibians (PhD Thesis). University of Basel.

Liedtke, H. C., H. Müller, J. Hafner, P. Nagel, and S. P. Loader. 2014. Interspecific patterns for egg and clutch sizes of African Bufonidae (Amphibia: Anura). Zool. Anz. 253:309–315.

Liow, L.H., T. B. Quental and C. R. Marshall. 2010. When can decreasing diversification rates be detected with molecular phylogenies and the fossil record? Syst. Biol. 59:646–659.

Livingstone, D. A. 1993. Evolution of African climate. Pp. 456–472 *in* P. Goldblatt, ed. Biological relationships between Africa and South America. Yale University Press, New Haven and London.

Lomolino, M. V. 2000. Ecology's most general, yet protean pattern: the species-area relationship. J. Biogeogr. 27:17–26.

Losos, J. B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. Am. Nat. 175:623–639.

Losos, J. B., and D. L. Mahler. 2010. Adaptive Radiation: the Interaction of Ecological Opportunity, Adaptation, and Speciation. Pp. 381–420 *in* M. Bell, D. J. Futuyma, W. F. Eanes, and J. S. Levinton, eds. Evolution Since Darwin: The First 150 Years. Sinauer, Sunderland, MA.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, NJ.

Mahler, D. L., L. J. Revell, R. E. Glor, and J. B. Losos. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. Evolution 64:2731–2745.

Martín, C., M. A. Alonso-Zarazaga, and B. Sanchiz. 2012. Nomenclatural notes on living and fossil amphibians. Graellsia 68:159–180.

McGuire, J. A., C. C. Witt, J. V. Remsen Jr, A. Corl, D. L. Rabosky, D. L. Altshuler, and R. Dudley.

2014. Molecular phylogenetics and the diversification of hummingbirds. Curr. Biol. 24:910–916.

McKenna, D. D., A. S. Sequeira, A. E. Marvaldi, and B. D. Farrell. 2009. Temporal lags and overlap in the diversification of weevils and flowering plants. P. Natl. Acad. Sci. USA 106:7083–7088.

Measey, G. J., and K. A. Tolley. 2009. Investigating the cause of the disjunct distribution of *Amietophrynus pantherinus*, the Endangered South African western leopard toad. Conserv. Genet. 12:61–70.

Mooers, A. O., and S. B. Heard. 1997. Inferring evolutionary process from phylogenetic tree shape. Q. Rev. Biol. 72:31–54.

Morin, P. J., S. P. Lawler, and E. A. Johnson. 1988. Competition between aquatic insects and vertebrates: interaction strength and higher order interactions. Ecology 69:1401–1409.

Morlon, H., E. Lewitus, F. L, Condamine, M. Manceau, J. Clavel and J. Drury. 2016. RPANDA: an R package for macroevolutionary analyses on phylogenetic trees. Method. Ecol. Evol. doi:10.1111/2041-210X.12526.

Morlon, H., T. L. Parsons, and J. B. Plotkin. 2011. Reconciling molecular phylogenies with the fossil record. P. Natl. Acad. Sci. USA 108:16327–16332.

Moyle, R. G., C. E. Filardi, C. E. Smith and J. Diamond. 2009. Explosive Pleistocene diversification and hemispheric expansion of a "great speciator". P. Natl. Acad. Sci. USA 106:1863–1868.

Müller, H., H. C. Liedtke, M. Menegon, J. Beck, L. Ballesteros-Mejia, P. Nagel and S. P. Loader. 2013. Forests as promoters of terrestrial life-history strategies in East African amphibians. Biol. Lett. 9:20121146–20121146.

Nakazawa, Y., and A. T. Peterson. 2015. Effects of climate history and environmental grain on species' distributions in Africa and South America. Biotropica 47:292–299.

Nee, S., A. O. Mooers, and P. H. Harvey. 1992. Tempo and mode of evolution revealed from molecular phylogenies. P. Natl. Acad. Sci. USA 89:8322–8326.

Onstein, R. E., R. J. Carter, Y. Xing, J. E. Richardson, and H. P. Linder. 2015. Do Mediterranean-type ecosystems have a common history?-Insights from the Buckthorn family (Rhamnaceae). Evolution 69:756–771.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20:289–290.

Parmentier, I., Y. Malhi, B. Senterre, R. J. Whittaker, A. T. D. N, A. Alonso, M. P. B. Balinga, A. Bakayoko, F. Bongers, C. Chatelain, J. A. Comiskey, R. Cortay, M.-N. D. Kamdem, J.-L. Doucet, L. Gautier, W. D. Hawthorne, Y. A. Issembe, F. N. Kouamé, L. A. Kouka, M. E. Leal, J. Lejoly, S. L. Lewis, L. Nusbaumer, M. P. E. Parren, K. S. H. Peh, O. L. Phillips, D. Shell, B. Sonké, M. S. M. Sosef, T. C. H. Sunderland, J. Stropp, H. Ter Steege, M. D. Swaine, M. G. P. Tchouto, B. S. Van Gemerden, J. L. C. H. Van Valkenburg, and A. H. Wöll. 2007. The odd man out? Might climate explain the lower tree α-diversity of african rain forests relative to amazonian rain forests? J. Ecol. 95:1058–1071.

Peters, R. H. 1986. The ecological implications of body size. Cambridge University Press, Cambridge.

Phillimore, A. B., R. P. Freckleton, C.D. L. Orme and I. P. F. Owens. 2006. Ecology predicts large☐scale patterns of phylogenetic diversification in birds. Am. Nat. 168:220–229.

Pinto, G., D. L. Mahler, L. J. Harmon, and J. B. Losos. 2008. Testing the island effect in adaptive radiation: rates and patterns of morphological diversification in Caribbean and mainland *Anolis* lizards. P. Roy. Soc. B-Biol. Sci. 275:2749–2757.

Pons, J., T. G. Barraclough, J. Gomez-Zurita, A. Cardoso, D. Duran, S. Hazell, S. Kamoun, W. Sumlin and A. Vogler. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55:595–609.

Portik, D.M. and T. J. Papenfuss. 2015. Historical biogeography resolves the origins of endemic

Arabian toad lineages (Anura: Bufonidae): Evidence for ancient vicariance and dispersal events with the Horn of Africa and South Asia. BMC Evol. Biol. 15:1–19.

Poynton, J. C. 1997. On *Bufo nyikae* Loveridge and the *B. lonnbergi* complex of East African highlands (Anura: Bufonidae). Afr. J. Herpetol. 46:98–102.

Poynton, J. C., and A. J. L. Lambiris. 1998. On *Bufo pantherinus* A. Smith, 1828 (Anura: Bufonidae), the leopard toad of the southwestern Cape, South Africa, with the designation of a neotype. Afr. J. Herpetol. 47:3–12.

Poynton, J. C., and D. G. Broadley. 1988. Amphibia Zambesiaca 4. Bufonidae. Ann. Natal Mus. 29:447–490.

Pramuk, J. B., T. Robertson, J. W. Sites, and B. P. Noonan. 2008. Around the world in 10 million years: biogeography of the nearly cosmopolitan true toads (Anura: Bufonidae). Global Ecol. Biogeogr. 17:72–83.

Price, J.P. and W. L. Wagner. 2004. Speciation in Hawaiian angiosperm lineages: cause, consequence, and mode. Evolution 58:2185–17.

Price, S. L., S. Powell, D. J. C. Kronauer, L. A. P. Tran, N. E. Pierce, and R. K. Wayne. 2014a. Renewed diversification is associated with new ecological opportunity in the Neotropical turtle ants. J. Evolution. Biol. 27:242–258.

Price, T. D., D. M. Hooper, C. D. Buchanan, U. S. Johansson, D. T. Tietze, P. Alström, U. Olsson, M. Ghosh-Harihar, F. Ishtiaq, S. K. Gupta, J. Martens, B. Harr, P. Singh, and D. Mohan. 2014b. Niche filling slows the diversification of Himalayan songbirds. Nature 509:222–225.

Pybus, O. G., and P. H. Harvey. 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. P. Roy. Soc. B-Biol. Sci. 267:2267–2272.

Pyron, R. A., and J. J. Wiens. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Mol. Phylogenet. Evol. 61:543–583.

Quental, T.B. and C.R. Marshall. 2013. How the Red Queen drives terrestrial mammals to extinction. Science, 341: 290–292.

R core team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna , Austria, http://www.R-project.org/.

Rabosky, D.L. 2006. LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. Evol. Bioinform. 2:247–250.

Rabosky, D. L. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS ONE 9:e89543.

Rabosky, D. L. 2009a. Ecological limits and diversification rate: alternative paradigms to explain the variation in species richness among clades and regions. Ecol. Lett. 12:735–743.

Rabosky, D. L. 2009b. Extinction rates should not be estimated from molecular phylogenies. Evolution 64:1816–1824.

Rabosky, D. L., and A. H. Hurlbert. 2015. Species richness at continental scales is dominated by ecological limits*. Am. Nat. 185:572–583.

Rabosky, D. L., and E. E. Goldberg. 2015. Model inadequacy and mistaken inferences of trait-dependent speciation. Syst. Biol. 64:340–355.

Rabosky, D. L., and I. J. Lovette. 2008a. Density-dependent diversification in North American wood warblers. P. Roy. Soc. B-Biol. Sci. 275:2363–2371.

Rabosky, D. L., and I. J. Lovette. 2008b. Explosive evolutionary radiations: decreasing speciation or increasing extinction through time? Evolution 62:1866–1875.

Rabosky, D. L., F. Santini, J. Eastman, S. A. Smith, B. Sidlauskas, J. Chang, and M. E. Alfaro. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat. Communications 4:1–8.

Rabosky, D. L., M. Grundler, C. Anderson, J. J. Shi, J. W. Brown, H. Huang, and J. G. Larson. 2014. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees.

Methods Ecol. Evol. 5:701–707.

Rage, J.-C., and Z. Roček. 2003. Evolution of anuran assemblages in the Tertiary and Quaternary of Europe, in the context of palaeoclimate and palaeogeography. Amphibia-Reptilia 24:133–167.

Rambaut, A., and A. J. Drummond. 2012a. LogCombiner v1.7.5. Computer program and documentation distributed by the author. Available from http://beast.bio.ed.ac.uk/LogCombiner (last accessed on 26 August 2013).

Rambaut, A., and A. J. Drummond. 2012b. TreeAnnotator v1.7.5. Computer program and documentation distributed by the author.http://beast.bio.ed.ac.uk/TreeAnnotator. (Accessed on 26 August 2013).

Raven, P. H., and D. I. Axelrod. 1974. Angiosperm biogeography and past continental movements. Ann. Miss. Bot. Gard. 61:539–673.

Räsänen, K., F. Söderman, A. Laurila, and J. Merilä. 2008. Geographic variation in maternal investment: acidity affects egg size and fecundity in *Rana arvalis*. Ecology 89:2553–2562.

Reid, N. M. 2014. bGMYC: A Bayesian MCMC implementation of the general mixed Yule-coalescent model for species delimitation. R package version 1.0.2.

Reid, N. M., and B. C. Carstens. 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BMC Evol. Biol. 12:196.

Richards, P. W. 1973. Africa, the "odd man out." Pp. 21–26 *in* B. J. Meggers, S. A. Edwards, and W. D. Duckworth, eds. Tropical forest ecosystems in Africa and South America: a comparative review. Smithsonian Institution Press, Washington.

Robichaux, R. H., G. D. Carr, M. Liebman, and R. W. Pearcy. 1990. Adaptive radiation of the hawaiian Silversword alliance (Compositae-Madiinae): ecological, morphological, and physiological diversity. Ann. Miss. Bot. Gard. 77:64–72.

Rödel, M.-O. 2000. Herpetofauna of West Africa, Vol. I: Amphibians of the West African savanna.

Edition Chimaira, Frankfurt am Main

Rödel, M.-O., and W. R. Branch. 2003. Herpetological survey of the Haute Dodo and Cavally forests, western Ivory Coast, Part I: Amphibians. Salamandra 38:213–232.

Roelants, K., D. J. Gower, M. Wilkinson, S. P. Loader, S. D. Biju, K. Guillaume, and L. Moriau. 2007. Global patterns of diversification in the history of modern amphibians. P. Natl. Acad. Sci. USA 104:887–892.

Roff, D. A. 2002. Life history evolution. Sinauer Associates, Incorporated, Sunderland, MA.

Rowe, K. C., K. P. Aplin, P. R. Baverstock, and C. Moritz. 2011. Recent and rapid speciation with limited morphological disparity in the genus *Rattus*. Syst. Biol. 60:188–203.

Ruta, M., K. D. Angielczyk, J. Fröbisch, and M. J. Benton. 2013. Decoupling of morphological disparity and taxic diversity during the adaptive radiation of anomodont therapsids. P. Roy. Soc. B-Biol. Sci. 280:20131071.

Salisbury, C. L., N. Seddon, C. R. Cooney and J. A. Tobias. 2012. The latitudinal gradient in dispersal constraints: ecological specialisation drives diversification in tropical birds. Ecol. Lett. 15:847–855.

Schenk, J. J., K. C. Rowe, and S. J. Steppan. 2013. Ecological opportunity and incumbency in the diversification of repeated continental colonizations by muroid rodents. Syst. Biol. 62:837–864.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford Series in Ecology and Evolution, Oxford.

Schweizer, M., S. T. Hertwig, and O. Seehausen. 2014. Diversity versus disparity and the role of ecological opportunity in a continental bird radiation. J. Biogeogr. 41:1301–1312.

Sepkoski, J. J. 1998. Rates of speciation in the fossil record. Philos. T. Roy. Soc. B 353:315–326.

Simpson, G. G. 1953. The Major Features of Evolution. Columbia University Press, New York.

Slater, G. J., S. A. Price, F. Santini, and M. E. Alfaro. 2010. Diversity versus disparity and the radiation of modern cetaceans. P. Roy. Soc. B-Biol. Sci. 277:3097–3104.

Slingsby, J. A., M. N. Britton, and G. A. Verboom. 2014. Ecology limits the diversity of the Cape flora: phylogenetics and diversification of the genus *Tetraria*. Mol. Phylogenet. Evol. 72:61–70.

Steelman, J. T., and P. D. Danley. 2003. The stages of vertebrate evolutionary radiation. Trends Ecol. Evol. 18:126–131.

Steppan, S. J., C. Zawadzki, and L. R. Heaney. 2003. Molecular phylogeny of the endemic Philippine rodent *Apomys* (Muridae) and the dynamics of diversification in an oceanic archipelago. Biol. J. Linn. Soc. 80:699–715.

Tandy, M., and R. Keith. 1972. *Bufo* of Africa. Pp. 119–170 *in* W. F. Blair, ed. Evolution in the Genus *Bufo*. University of Texas Press, Texas.

Tihen, J. A. 1962. A review of New World fossil bufonids. Am. Midl. Nat. 1–50.

Tihen, J. A. 1951. Anuran remains from the Miocene of Florida, with the description of a new species of *Bufo*. Copeia 1951:230–235.

Tolley, K. A., A. L. De Villers, M. I. Cherry, and G. J. Measey. 2010. Isolation and high genetic diversity in dwarf mountain toads (*Capensibufo*) from South Africa. Biol. J. Linn. Soc. 100:822–834.

Van Bocxlaer, I., K. Roelants, S. D. Biju, J. Nagaraju, and F. Bossuyt. 2006. Late Cretaceous vicariance in Gondwanan Amphibians. PLoS ONE 1:e74.

Van Bocxlaer, I., S. P. Loader, K. Roelants, S. D. Biju, M. Menegon, and F. Bossuyt. 2010. Gradual adaptation toward a range-expansion phenotype initiated the global radiation of toads. Science 327:679–682.

Vences, M., D. R. Vieites, F. Glaw, H. Brinkmann, J. Kosuch, M. Veith, and A. Meyer. 2003. Multiple overseas dispersal in amphibians. P. Roy. Soc. B-Biol. Sci. 270:2435–2442.

Von Rintelen, K., M. Glaubrecht, C. D. Schubart, A. Wessel and T. Von Rintelen, von. 2010. Adaptive radiation and ecological diversification of Sulawesi's ancient lake shrimps. Evolution, 64:3287–3299.

Whittaker, R. J., and J. M. Fernandez-Palacios. 2007. Island biogeography: ecology, evolution, and conservation. Oxford University Press, Oxford.

Wilgenbusch, J. C., D. L. Warren, and D. L. Swofford. 2004. AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. http://ceb.csit.fsu.edu/awty (last accessed on 26 August 2013).

Yoder, A. D., E. Clancey, S. des Roches, J. M. Eastman, A. H. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, J. Robertson, B. A. J. Sarver, J. J. Schenk, S. F. Spear, and L. J. Harmon. 2010. Ecological opportunity and the origin of adaptive radiations. J. Evolution. Biol. 23:1581–1596.

Figure legends

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

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

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

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

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

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

Supporting information

Supporting Information S1: Extended version of Methods section

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

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

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

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

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

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

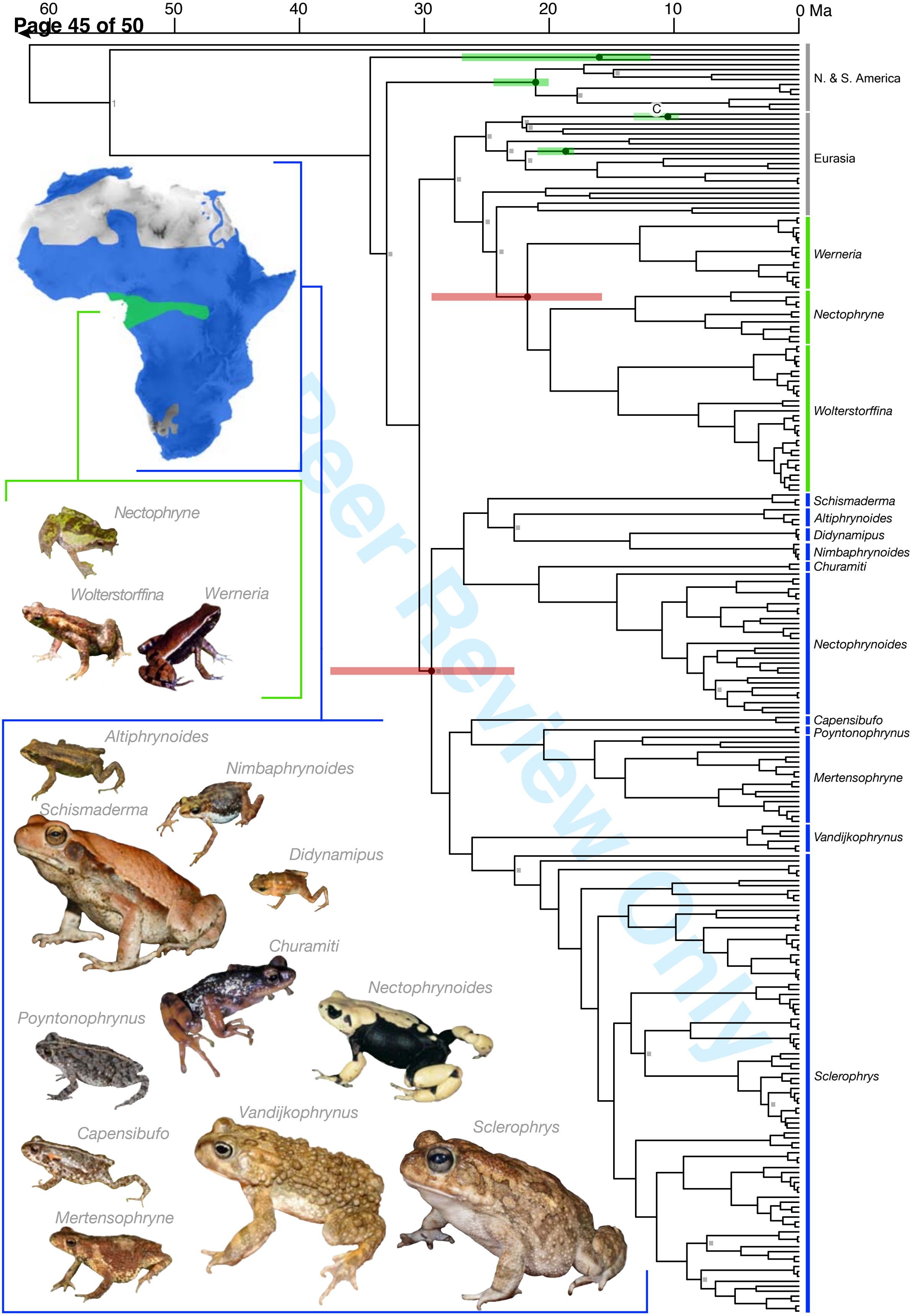
Supporting Information S8. Investigating the effects of bGMYC oversplitting.

Table 1. Summary statistic of diversification models fitted to the branching times of the a) species delimited bGMYC tree and b) CRS tree. The models tested are pure-birth (PB) and birth-death (BD) with constant rates, PB with an exponential speciation rate (PBλexp), BD with a constant speciation rate and exponential extinction rates (BDλcst-μexp), BD with an exponential speciation rate and constant extinction rate (BDλexp-μcst) and BD with both exponential speciation and extinction rates (BDλexp-μexp). Parameters refer to the estimated rates at the tips and the corresponding time-variation parameter.

Model	λ Parameters	μ Parameters	LH	AICc	ΔAICc	Aw
A) bGMYC tree						
Constant Rate Models						
PB	0.137		-298.691	599.421	-	0.501
BD	0.137	<0.001	-298.691	601.503	2.081	0.177
Variable Rate Models						
PBλexp	0.136; 0.002		-298.685	601.491	2.069	0.178
BDλcst-μexp	0.137	<0.001; -0.218	-298.691	603.626	4.205	0.061
BDλexp-μcst	0.136; 0.002	<0.001	-298.685	603.614	4.193	0.062
BDλexp-μexp	0.135; 0.002	<0.001; -0.056	-298.685	605.782	6.360	0.021
B) CRS tree						
Constant Rate Models						
PB	0.121		-191.348	384.765	-	0.452
BD	0.121	<0.001	-191.348	386.907	2.142	0.155
Variable Rate Models						
PBλexp	0.106; 0.015		-191.008	386.226	1.460	0.218
BDλcst-μexp	0.146	378.188; -2225.174	-190.891	388.210	3.445	0.081
BDλexp-μcst	0.106; 0.015	<0.001	-191.008	388.444	3.679	0.072
BDλexp-μexp	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 (z0), 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	ΔAIC	AW
a) Body size						
BM	constant	z0=1.692	13.392	-22.785	9.666	0.008
OU	constant	z0=1.697; α=0.034	15.339	-24.677	7.774	0.019
λ	constant	z0=1.689; λ=0.808	19.226	-32.451	-	0.950
EB	variable	z0=1.692; a=-1e-6	13.392	-20.785	11.666	0.003
LIN	variable	z0=1.698; b=0.217	14.745	-23.490	8.961	0.011
δ	variable	z0=1.702; δ=1.849	14.635	-23.271	9.180	0.010
b) Clutch size						
BM	constant	z0=5.160e-11	-18.984	41.969	-	0.328
OU	constant	z0=0.005; α=0.007	-18.908	43.817	1.848	0.130
λ	constant	z0=-0.001; λ=0.965	-18.635	43.270	1.301	0.171
EB	variable	z0=-3.169e-7; a=-1e-6	-18.984	43.969	2.000	0.121
LIN	variable	z0=0.004; b=0.015	-18.92	43.840	1.871	0.129
δ	variable	z0=0.003; δ=1.079	-18.97	43.940	1.971	0.122
c) Egg size						
BM	constant	z0=-1.707e-11	37.909	-71.818	1.785	0.115
OU	constant	z0=-0.013; α=0.041	39.588	-73.175	0.428	0.226
λ	constant	z0=-0.001; λ=0.8	39.801	-73.603	-	0.280
EB	variable	z0=-1.180e-7; a=-1e-6	37.909	-69.818	3.785	0.042
LIN	variable	z0=-0.013; b=98.913	39.271	-72.542	1.061	0.165
δ	variable	z0=-0.016; δ=2.254	39.31	-72.620	0.983	0.171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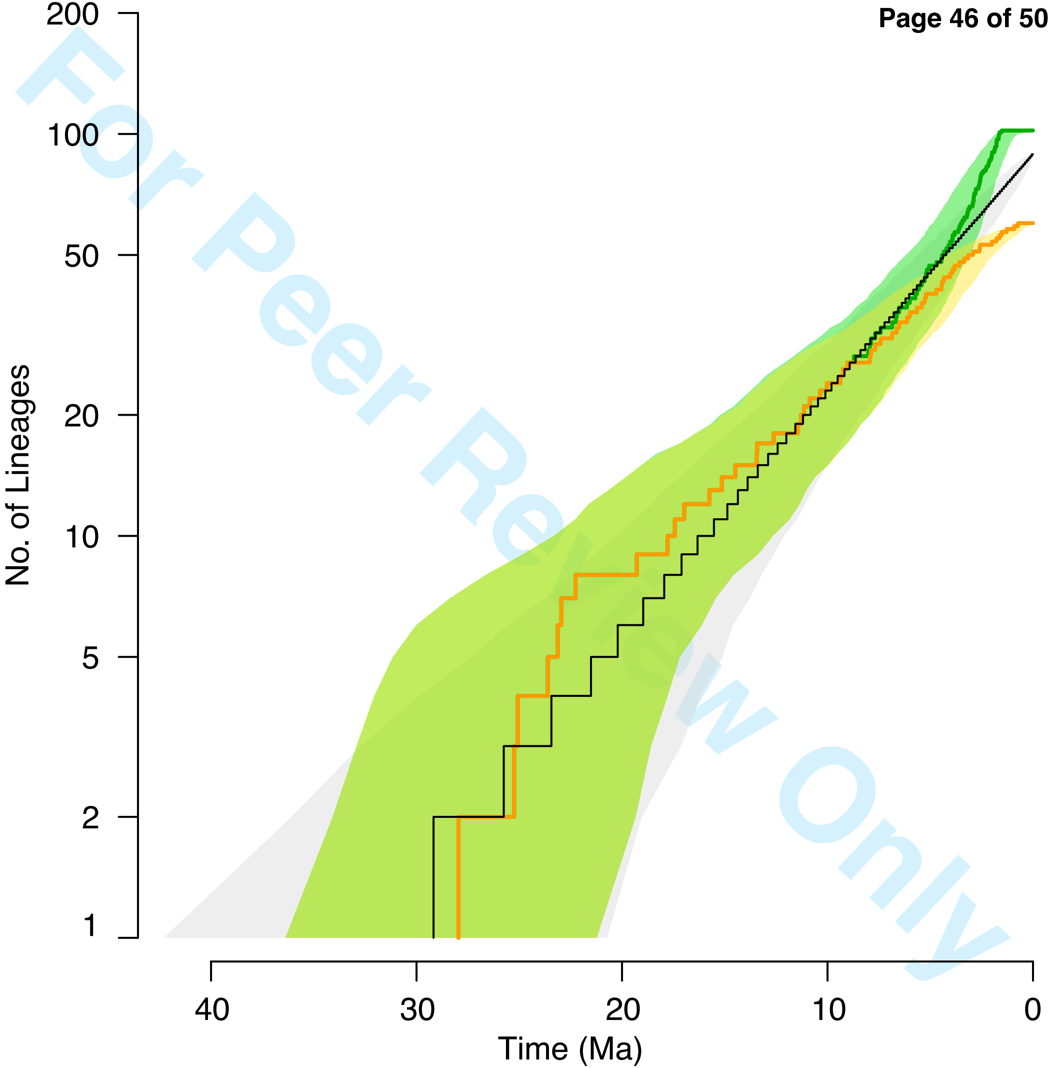
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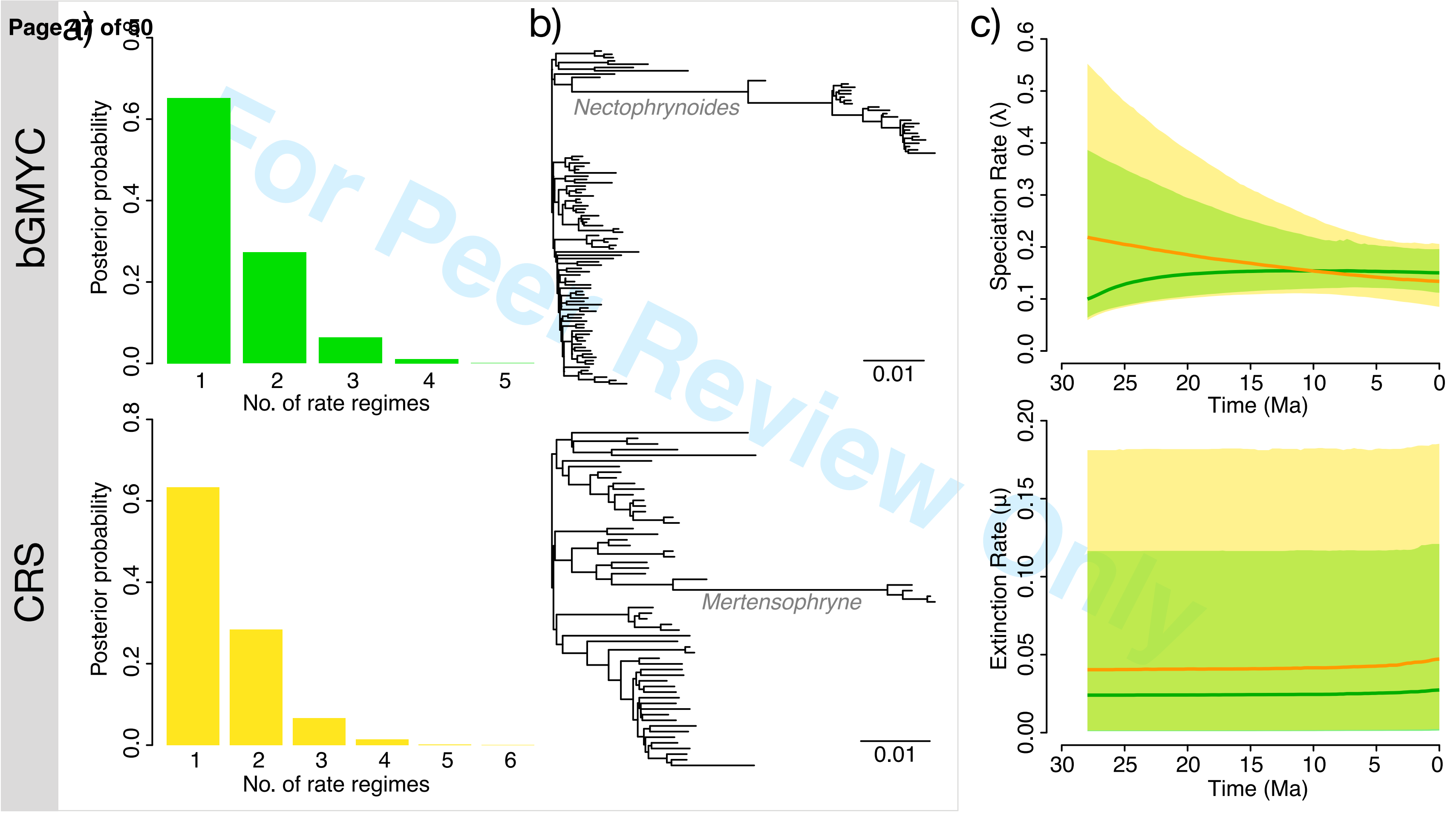
a)
b)
c)
bGMYC
CRS
Posterior probability
0.0
0.2
0.4
0.6
0.8
1
2
3
4
5
6
No. of rate regimes
Nectophrynoides
Mertensophryne
0.01
Speciation Rate (λ)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Extinction Rate (μ)
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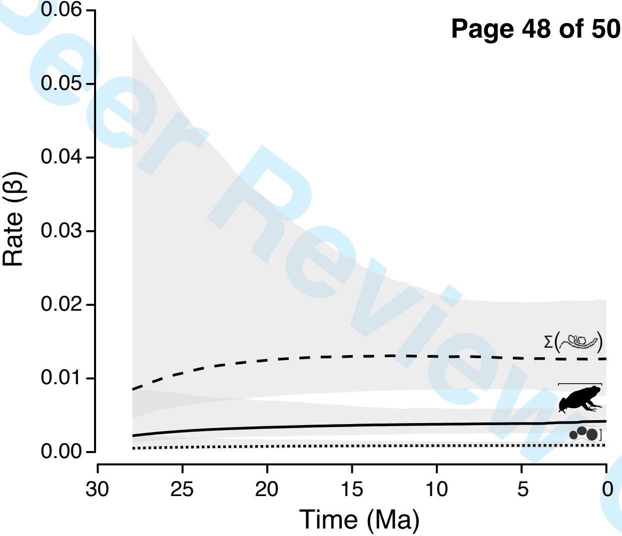
Time (Ma)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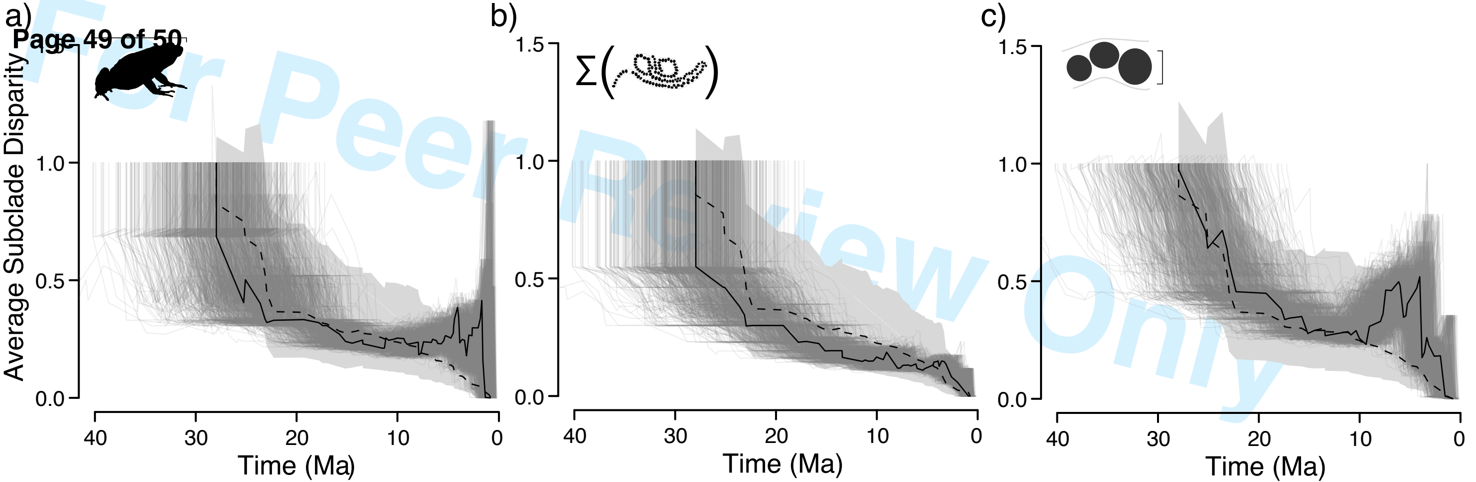
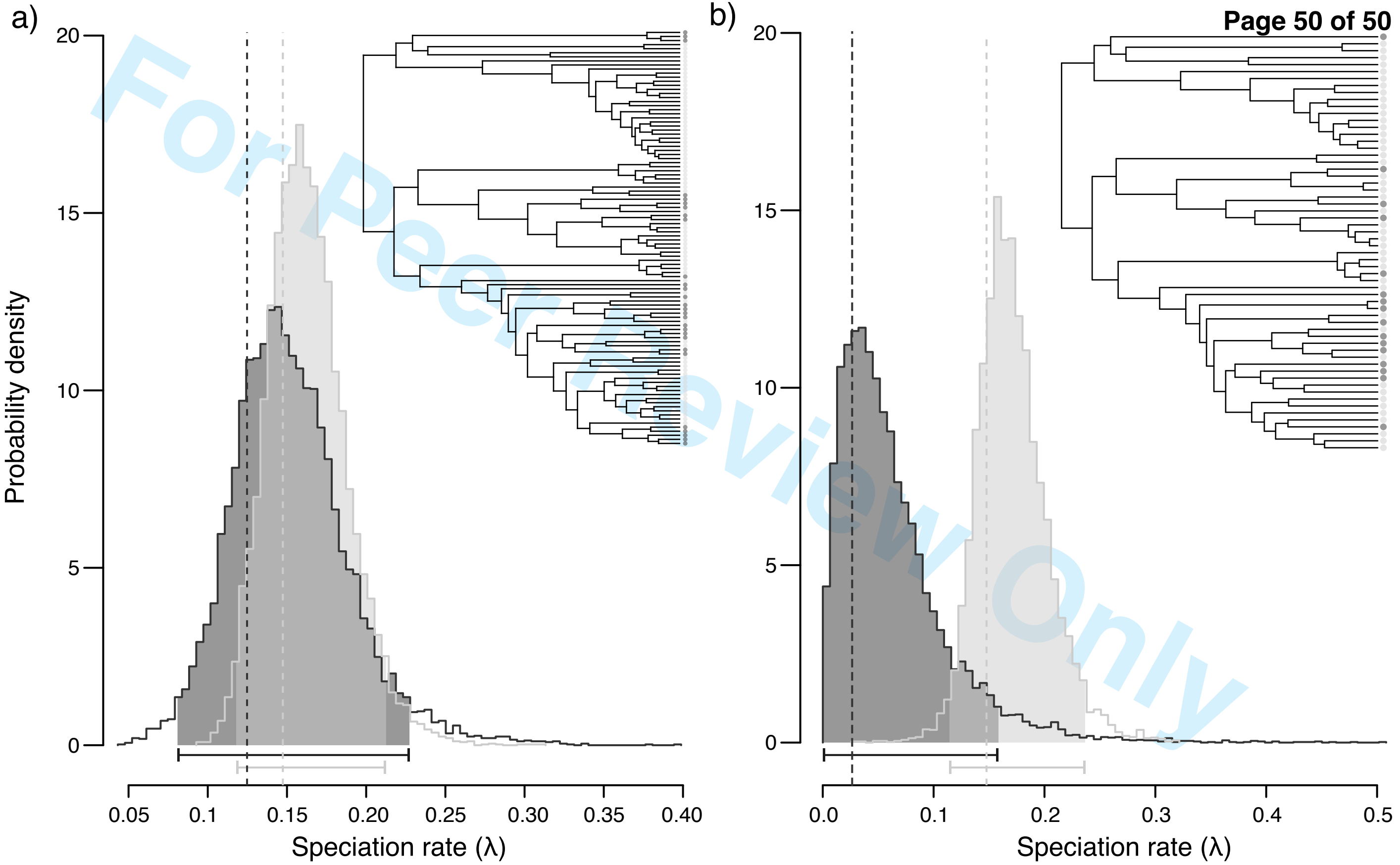
25
20
15
10
5
0
Σ( )


a)
b)
c)
Average Subclade Disparity
1.5
1.0
0.5
0.0
40
30
20
10
0
Time (Ma)
Time (Ma)
Time (Ma)


a)
Probability density
20
15
10
5
0
0.05
0.1
0.15
0.20
0.25
0.30
0.35
0.40
Speciation rate (λ)
b)
20
15
10
5
0
0.0
0.1
0.2
0.3
0.4
0.5
Speciation rate (λ)