



Comparative phylogeography of West African amphibians and reptiles

Adam D Leaché,^{1,2} Jamie R. Oaks,³ Caleb Ofori-Boateng,⁴ and Matthew K. Fujita⁵

¹Department of Biology and Burke Museum of Natural History and Culture, University of Washington, Seattle, Washington 98195

²E-mail: leache@uw.edu

³Department of Biological Sciences and Museum of Natural History, Auburn University, Auburn, Alabama 36849

⁴CSIR-Forestry Research Institute of Ghana, Kumasi, Ghana

⁵Department of Biology, Amphibian and Reptile Diversity Research Center, The University of Texas at Arlington, Arlington, Texas 76019

Received October 8, 2019

Accepted January 23, 2020

Comparative phylogeographic studies often support shared divergence times for co-distributed species with similar life histories and habitat specializations. During the late Holocene, West Africa experienced aridification and the turnover of rain forest habitats into savannas. These fragmented rain forests harbor impressive numbers of endemic and threatened species. In this setting, populations of co-distributed rain forest species are expected to have diverged simultaneously, whereas divergence events for species adapted to savanna and forest-edge habitats should be absent or idiosyncratic. We conducted a Bayesian analysis of shared evolutionary events to test models of population divergence for 20 species of anurans (frogs) and squamates (lizards and snakes) that are distributed across the Dahomey Gap, a climate change-induced savanna barrier responsible for fragmenting previously contiguous rain forests of Ghana into two regions: the Togo-Volta Hills and the Southwestern Forests. A model of asynchronous diversification is supported for anurans and squamates, suggesting that drivers of diversification are not specifically related to ecological and life history associations with habitat types. Instead, the wide variability of genetic divergence histories exhibited by these species suggests that biodiversity in this region has been shaped by diversification events that extend beyond the Holocene. Comparisons of the genealogical divergence index, a measure of the genetic divergence between populations due to the combined effects of genetic isolation and gene flow, illustrate that these populations represent a broad sampling of the speciation continuum.

KEY WORDS: Comparative phylogeography, Dahomey Gap, diversification, genealogical divergence index, West Africa.

Identifying the processes that drive the accumulation of tropical rain forest biodiversity is an important goal for understanding global patterns of diversification. The Guinean rain forests of West Africa are a center of biological diversity with spectacular species richness and endemism (Myers et al. 2000; Plana 2004); although the factors responsible for generating this diversity have been studied in detail through a phylogeographic lens for many species (e.g., Leaché and Fujita 2010; Jongsma et al. 2018; Leaché et al. 2017), comparative phylogeographic studies are lacking. In areas with elevated biodiversity levels such as tropical rain forests, comparative phylogeographic analyses can reveal

how the temporal diversity of species composition is shaped by shared environmental and geological history and idiosyncratic responses to recent climate change. Furthermore, simultaneous analysis of multiple and diverse species across a landscape can help disentangle the relative importance of shared ancient events on genetic divergence and speciation.

The Guinean rain forest has experienced dramatic and prolonged oscillations in response to glacial cycles (Hamilton and Taylor 1991; Dupont et al. 2000), which are likely to have caused turbulent population histories in rain forest species. During the late Holocene, the Dahomey Gap, an expanse of dry

forest and savanna, fragmented the continuous belt of Guinean rain forest into its contemporary distribution: the West Africa core, Central African core, and a small residual patch in the Togo-Volta Hills (Booth 1958; Maley 1991; Dupont and Weinelt 1996; Salzmann and Hoelzmann 2005). During wetter periods in the early Holocene, these forest areas were probably continuous and not separated by any savanna barriers (Dupont et al. 2000). These major shifts in the sizes, locations, and fragmentation levels of the Guinean rain forest and savanna biomes create a rich setting to test alternative evolutionary models of rain forest fauna diversification (Moritz et al. 2000).

Amphibian and reptile endemism in sub-Saharan West Africa is high (Penner et al. 2011), and new species are continuing to be discovered and described (e.g., Blackburn et al. 2010; Rödel et al. 2012; Ofori-Boateng et al. 2018). In Ghana, the amphibian and reptile biodiversity has been extensively surveyed, including the southwestern rain forest, Togo-Volta Hills, and the Dahomey Gap (Leaché 2005; Rödel et al. 2005; Leaché et al. 2006). Weak signals of genetic divergence between rain forest populations found in southwestern Ghana and the Togo-Volta Hills are not unexpected, as these regions may have remained isolated since the most recent expansion of the Dahomey Gap approximately 3400 to 4500 years before present (Salzmann and Hoelzmann 2005). However, an emerging phylogeographic pattern observed in this region is for deep genetic divergences among rain forest populations, suggesting more ancient evolutionary origins that predate the most recent expansion of the Dahomey Gap (Barej et al. 2014; Dowell et al. 2016; Jongsma et al. 2018; Leaché et al. 2019a). Determining whether these population divergence events are temporally congruent with recent Holocene climate change or with older biogeographic events requires a comparative study of the genealogical divergence among populations in these communities.

In this study, we evaluate models of shared evolutionary divergence using a comparative phylogeographic approach that evaluates genealogical concordance among codistributed species. Unlike previous methods that use approximate Bayesian computation (ABC) to estimate the probability of shared divergence using single or multiple loci (Hickerson et al. 2007; Oaks 2014), we take a full-likelihood Bayesian approach that estimates population divergences directly from SNP data without estimating gene trees (Bryant et al. 2012; Oaks 2019). Compared to the ABC approach that is sensitive to prior assumptions and biased toward favoring shared divergences, the full-likelihood Bayesian approach can calculate the likelihood of the population history directly from genomic data and produce more accurate and precise estimates of the number and timing of divergence events across pairs of populations (Oaks 2019; Oaks et al. 2019). We also measure the genealogical divergence index (*gdi*) among populations (Jackson et al. 2017; Leaché et al. 2019b) to compare and

contrast genetic divergences among population pairs of different species in a coalescent framework that considers the joint effects of genetic drift and migration. Contrasting *gdi* values among species is a useful approach for evaluating where populations lie on the speciation continuum and thus the importance of shared history in generating species composition in the Guinean rain forests.

Materials and Methods

STUDY SPECIES AND POPULATION SAMPLING

We sampled 20 species, including nine anurans and 11 squamate reptiles, from two forested regions in Ghana that are separated by the Dahomey Gap, southwestern Ghana (SW) and the Togo-Volta Hills (TVH) (Fig. 1A; Table 1; voucher specimen information is provided in Table S1). Five of the anurans (*Ammirana albolabris*, *Arthroleptis poecilonotus*, *Conraua derooi*, *Hyperolius fusciventris*, and *Phrynobatrachus plicatus*) and seven of the squamates (*Cophoscincopus simulans*, *Gonionotophis klingi*, *Hemidactylus fasciatus*/*H. kyaboboensis*, *H. muriceus*, *Lycophidion nigromaculatum*, *Mochlus guineensis*, and *Panaspis togoensis*) are rain forest species. The remaining species are distributed in a combination of degraded forests, forest edges, farmbrush, and savanna habitats. We include one species pair (*H. fasciatus*/*H. kyaboboensis*) to gauge the relative genetic divergences of populations against a recognized species pair (Leaché and Fujita 2010). We define SW and TVH as separate geographic populations for the purposes of testing shared divergence across the Dahomey Gap (Fig. 1A). Our expectation is that rain forest species are more likely to provide evidence for synchronous population divergence in response to the formation of the Dahomey Gap. Shared divergence is not expected for the remaining species, as their ability to tolerate more arid habitats and therefore potentially exchange genes across the Dahomey Gap should produce asynchronous divergence times, and perhaps very shallow population divergences if the populations are in fact exchanging migrants.

GENOMIC DATA COLLECTION

We collected sequence data using the double-digest RADseq protocol (ddRADseq; Peterson et al. 2012). Genomic DNA was isolated from tissue samples using QIAGEN DNeasy extraction kits (QIAGEN Inc., Hilden, Germany). We double-digested 500 ng of genomic DNA for each sample with 20 units each of a rare cutter *SbfI* (restriction site 5'-CCTGCAGG-3') and a common cutter *MspI* (restriction site 5'-CCGG-3') in a single reaction for 8 h at 37°C. Fragments were purified with Sera-Mag Speed-Beads before ligating Illumina adaptors with in-line barcodes. We included an 8-bp unique molecular identifier before the barcode in our adaptors to increase diversity on the Illumina flow

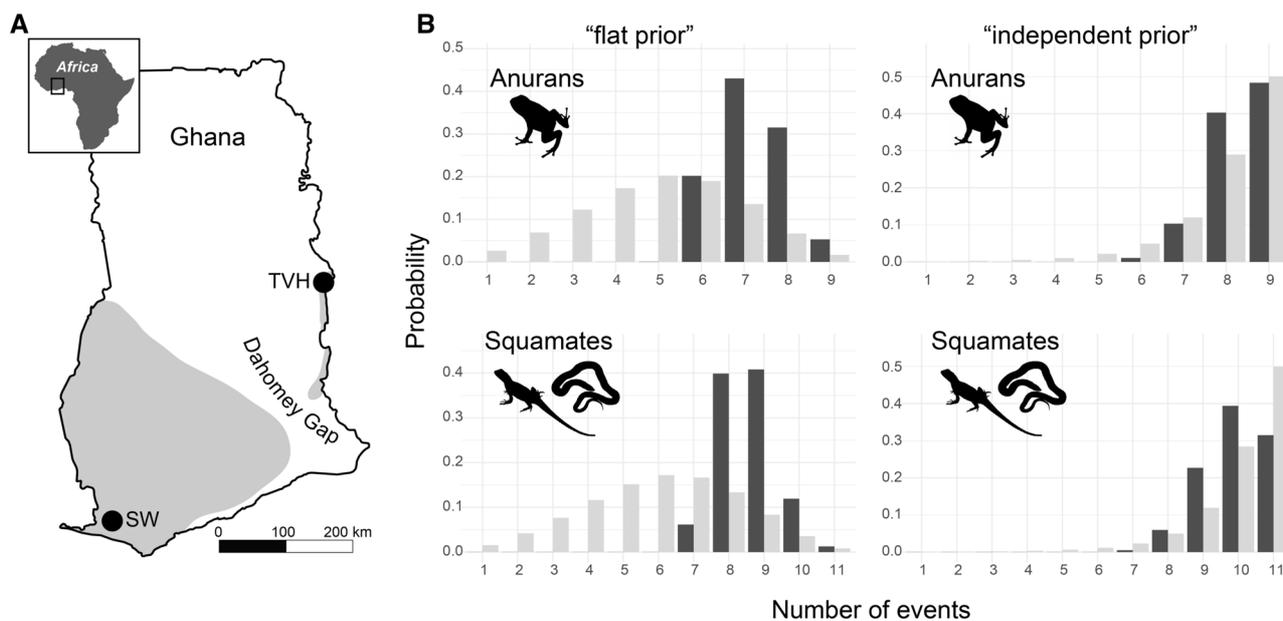


Figure 1. (A) Map of Ghana showing the approximate distributions of rain forest (gray), savanna (white), and the Dahomey Gap. The locations of the two geographic populations included in the comparative phylogeographic analysis, SW (Southwestern Ghana) and TVH (Togo-Volta Hills), are indicated with black dots. (B) Approximate prior and posterior probability distributions for the number of divergence events across population pairs. Prior probabilities are in light gray and posterior probabilities are in dark gray. The “flat prior” spreads the prior probability across all possible unique divergence events, and the “independent prior” places approximately 50% of the prior probability on independent divergence for all species (anurans = 9, squamates = 11).

cell and filter for PCR duplicate reads. The libraries were size-selected (between 415 and 515 bp after accounting for adapter length) on a Blue Pippin Prep size fractionator (Sage Science, Beverly, MA). The final library amplification used proofreading Taq and Illumina’s indexed primers (TruSeq i7 indices). The fragment size distribution and concentration of each pool (5–8 samples per pool; 24 pools total) was determined on an Agilent 2200 TapeStation and qPCR was performed to quantify library concentrations before multiplexing equimolar amounts of each pool for sequencing on one Illumina HiSeq 4000 lane (151 bp, single-end reads) at the QB3 facility at UC Berkeley. The data for *Conraua derooi* were collected with similar protocols on a different sequencing lane (50bp reads) along with other *Conraua* samples as part of a separate study (Fujita et al. unpubl. ms.).

For two species, we used previously collected ddRAD-seq data, including *Agama picticauda* (Krishnan et al. 2019; SRA study accession #SRP108438) and *Hemidactylus fasciatus* (Leaché et al. 2014a; Dryad Digital Repository: <https://doi.org/10.5061/dryad.r55fb>). These data were collected with similar protocols, but with reduced sequencing effort (50bp reads).

We processed raw Illumina reads using the program iPyRAD version 0.6.10 (Eaton 2017). We demultiplexed samples using their unique barcode sequences with no mismatches allowed. Sites with Phred quality scores under 99% (Phred score = 20)

were changed into N characters and reads with ≥ 5 N s were discarded. We assembled data for each species separately to maximize the number of retained loci using a clustering threshold of 90% (within and between samples). Consensus sequences that had low coverage (<6 reads), excessive undetermined or heterozygous sites (>8), or too many haplotypes (>2 for diploids) were discarded. A paralog filter for removing loci with excessive shared heterozygosity among samples was applied (paralog filter = 0.5). We minimized missing data by only retaining loci present for all samples of a species, but this restriction was relaxed to $\geq 75\%$ to obtain more loci for eight species (Table 1). The demultiplexed sequences for all newly collected data (including *Conraua*) are available on the NCBI Sequence Read Archive (PRJNA 531179; Table S1).

To characterize population differentiation due to genetic structure between SW and TVH, we calculated F_{ST} values (Weir and Cockerham 1984) using VCFtools version 0.1.15 (Danecek et al. 2011). We calculated F_{ST} as the mean value across all SNPs assuming two populations (TVH vs. SW).

BAYESIAN ANALYSIS OF SHARED EVOLUTIONARY EVENTS

We used a full-likelihood Bayesian approach, ecoevolity (Oaks 2019; Oaks et al. 2019), to test the simultaneous divergence hypothesis for population pairs distributed across the Dahomey

Table 1. Summary of the ddRADseq data collected for 20 population pairs of tropical anurans and squamate reptiles in Southwestern Ghana (SG) and the Togo-Volta Hills (TVH). Samples sizes are shown as the number of individuals sampled per population (n_{SG} , n_{TVH}). Only loci present for a minimum number of individuals (min. ind.) were used in downstream analyses. Estimates of F_{ST} are averaged across all SNPs.

| Species | Habitat | n_{SG} , n_{TVH} | Min. ind. | Loci | Sites | SNPs | % variable | F_{ST} |
|-----------------------------------|-------------|----------------------|-----------|------|---------|--------|------------|----------|
| Anurans | | | | | | | | |
| <i>Africalus dorsalis</i> | Savanna | 3,3 | 6 | 521 | 67,653 | 2252 | 3.33 | 0.143 |
| <i>Amirana albolabris</i> | Rain forest | 3,6 | 7 | 3797 | 493,435 | 10,038 | 2.03 | 0.256 |
| <i>Arthroleptis poecilonotus</i> | Rain forest | 8,11 | 16 | 363 | 47,014 | 3752 | 7.98 | 0.243 |
| <i>Conraua derooi</i> | Rain forest | 8,7 | 15 | 1954 | 64,297 | 1879 | 2.92 | 0.695 |
| <i>Hyperolius concolor</i> | Savanna | 7,7 | 14 | 170 | 22,098 | 785 | 3.55 | 0.045 |
| <i>H. fusciventris</i> | Rain forest | 4,3 | 5 | 183 | 23,602 | 1526 | 6.47 | 0.507 |
| <i>Leptopelis spiritusnoctis</i> | Savanna | 7,5 | 10 | 815 | 105,764 | 6354 | 6.01 | 0.112 |
| <i>Phrynobatrachus plicatus</i> | Rain forest | 4,2 | 6 | 1524 | 197,746 | 5002 | 2.53 | 0.073 |
| <i>Sclerophrys maculata</i> | Savanna | 4,3 | 7 | 188 | 24,393 | 977 | 4.01 | 0.156 |
| Squamates | | | | | | | | |
| <i>Agama picticauda</i> | Savanna | 12,8 | 15 | 427 | 16,635 | 943 | 5.67 | 0.265 |
| <i>Cophoscincopus simulans</i> | Rain forest | 17,2 | 17 | 2133 | 275,544 | 5493 | 1.99 | 0.331 |
| <i>Gonionotophis klingi</i> | Rain forest | 1,1 | 2 | 2965 | 384,699 | 3749 | 0.97 | – |
| <i>Hemidactylus brookii</i> | Savanna | 2,4 | 6 | 4923 | 639,615 | 11,443 | 1.79 | 0.099 |
| <i>H. fasciatus/kyaboboensis</i> | Rain forest | 4,6 | 10 | 1774 | 69,080 | 2358 | 3.41 | 0.466 |
| <i>H. mabouia</i> | Savanna | 4,4 | 6 | 302 | 39,106 | 2625 | 6.71 | 0.347 |
| <i>H. muriceus</i> | Rain forest | 1,4 | 5 | 1206 | 156,437 | 4184 | 2.67 | 0.010 |
| <i>Lycophidion nigromaculatum</i> | Rain forest | 1,1 | 2 | 4259 | 550,396 | 14,998 | 2.72 | – |
| <i>Mochlus guineensis</i> | Rain forest | 2,3 | 4 | 187 | 24,109 | 632 | 2.62 | 0.000 |
| <i>Panaspis togoensis</i> | Rain forest | 1,3 | 4 | 170 | 22,104 | 331 | 1.50 | 0.129 |
| <i>Trachylepis affinis</i> | Savanna | 2,2 | 4 | 5708 | 740,123 | 14,552 | 1.97 | 0.439 |

Gap. Ecoevolity treats each pair of populations as a species tree with two tips and uses a Dirichlet process prior to estimate the number and timing of divergence events and the assignment of pairs to the events (Oaks 2019). The assumptions of the model include constant population size along each branch, no migration, and that the relative mutation rates are similar among species (Oaks 2019). We suspect that migration is probably relevant for some of the savanna species, and that the consequence of ignoring migration in the coalescent analysis would be a compression of divergence times toward the present (Leaché et al. 2014b). We tested for shared divergence among the anurans (nine population pairs) and squamates (11 population pairs) separately. The Dirichlet process uses a concentration parameter to determine the prior probability for shared divergence events (Oaks 2019). We compared analyses using two assumptions regarding this prior, including an “independent prior” that places approximately 50% of the prior probability on independent divergences for all species and a “flat prior” that attempts to evenly spread the prior probability across all possible numbers of unique divergence events. Details on the specific prior settings are provided in Table S2. For each analysis, we ran four independent analyses for 150,000 generations, sampling every 100th generation. We assessed con-

vergence and mixing by inspecting the potential scale reduction factor and effective sample sizes of the continuous parameters.

CHARACTERIZING THE SPECIATION CONTINUUM

To further characterize the genetic divergence between the 20 population pairs, we used the *gdi*. The *gdi* is a measure of genetic divergence between two populations affected by the combined effects of genetic isolation and gene flow (Jackson et al. 2017), and is calculated as follows:

$$gdi = 1 - e^{-2t/\theta_A}, \quad (1.1)$$

where $2t/\theta_A$ is the divergence time in coalescent units (one coalescent time unit is $2N_A$ generations) and e^{-2t/θ_A} is the probability of deep coalescence. Values for *gdi* can range from 0 to 1, with values near 0 indicating panmictic populations, and values near 1 indicating genetically distinct populations that are candidates for species (Jackson et al. 2017; Leaché et al. 2019b).

We calculated *gdi* values for each population pair using the multispecies coalescent (MSC) model implemented in BPP version 3.4 (Yang and Rannala 2010; Rannala and Yang 2013). The MSC approach provides a measure of uncertainty in population

parameter estimates, and gdi can be calculated using the posterior probability distributions for τ and θ (Leaché et al. 2019b). The posterior probability distributions for τ and θ were estimated using a fixed species tree containing just two populations, TVH and SW (analysis A00; Yang 2015). Diffuse inverse-gamma priors for θ and τ were designated as follows: θ IG(3, 0.02) with mean $0.02/(3 - 1) = 0.01$, and τ IG(3, 0.04) with mean $0.04/(3 - 1) = 0.02$. These priors correspond roughly to an expectation of 1% sequence variation within a population, and 4% sequence variation between populations. To assess convergence, we compared the posterior distributions from four independent runs (MCMC burn-in = 10K, retained samples = 10K, sample interval = 5). We calculated gdi for each species by combining all samples from the posterior distributions using equation (1.1).

Results

GENOMIC DATA

The anuran data provided 170–3797 loci, with the raw sequence variation (% variation) ranging from roughly 2–8% (Table 1). The characteristics of the squamate reptile data were similar, with 170–5708 loci and % variation ranging from 0.97–6.71% (Table 1). Population differentiation due to genetic structure, measured as the mean F_{ST} value across all SNPs, varied widely from 0.045–0.695 in anurans and 0.0–0.466 in squamates (Table 1). Low samples sizes for the two snake species in the study ($n = 2$; *Gonionotophis klingi* and *Lycophidion nigromaculatum*) prevented the calculation of F_{ST} values.

BAYESIAN ANALYSIS OF SHARED EVOLUTIONARY EVENTS

The ecoevolution analyses strongly rejected the hypothesis of shared divergence (number of events = 1) across the Dahomey Gap (Fig. 1). Instead, based on the 95% credible set of models, a minimum of six independent divergence events were supported for anurans and a minimum of seven for squamates (Fig. 1; Tables S3 and S4). Comparisons of analyses using priors that favored independent divergence versus a flat prior had minor influence on the posterior probability distributions for the number of events, and analyses using either prior provided strong support against shared divergence among all population pairs (Fig. 1; Tables S3 and S4).

Strong support for shared divergence was found in two cases for anurans (Fig. 2), including *Afrivalus dorsalis* + *Sclerophrys maculata* (inhabitants of degraded forest habitats) and *Arthrolepis poecilonotus* + *Conraua derooi* (inhabitants of rain forest, but *Arthrolepis* is also found in clearings and moist degraded forests). Within squamates (Fig. 2), one shared divergence included three lizards that are found throughout savanna and for-

est edge habitats: *Agama picticauda*, *Hemidactylus mabouia*, and *Trachylepis affinis*. A second shared divergence in squamates included a rain forest snake (*Gonionotophis klingi*) and rain forest lizard (*Panaspis togoensis*). Reducing the size of the dataset to a maximum of 200 loci per species produced qualitatively similar results for divergence values, but with less precision (Fig. S1).

CHARACTERIZING THE SPECIATION CONTINUUM

The MSC analyses of the 20 population pairs provided a wide range of gdi values ranging from <0.2 to nearly 1.0 (Fig. 3). Five anurans were consistent with a single species model ($gdi < 0.2$): *Amnirana albolabris*, *Hyperolius concolor*, *Leptopelis spiritusnoctis*, *Phrynobatrachus calcaratus*, and *Sclerophrys maculata*; three population pairs were supported as potential candidate species ($gdi > 0.7$): *Arthrolepis poecilonotus*, *Conraua derooi*, and *Hyperolius fusciventris* (Fig. 3). For squamate reptiles, five were supported as potential candidate species ($gdi > 0.7$), and the remaining had intermediate or low gdi values (Fig. 3). The populations supported as potential candidate species included *Cophoscincopus simulans*, *Hemidactylus fasciatus/H. kayboensis*, *Lycophidion nigromaculatus*, *Panaspis togoensis*, and *Trachylepis affinis* (Fig. 3). Reducing the size of the datasets to a maximum of two samples per species typically caused an increase in gdi values, which likely reflects the decreased estimates of θ that result from sampling only two alleles per population (Fig. S2). We also report a positive correlation between gdi and F_{ST} values (Fig. S3).

Discussion

Several factors are thought to be responsible for generating the high levels of diversity observed in tropical Africa, including vicariance through isolation in forest refugia, river barriers, and local adaptation along ecotones (Moritz et al. 2000; Plana 2004). Phylogeographic studies of single species have provided support for each of these models (Portik et al. 2017; Charles et al. 2018; Leaché et al. 2019a), although comparative studies that test for shared divergences among species across important biogeographic areas in this region have not been conducted. Our comparative study of 20 species separated by the Dahomey Gap, one of the largest and most formidable biogeographic barriers in West Africa, rejects a history of shared divergence (Fig. 1). Instead, the comparative phylogeographic results provide strong evidence for independent divergence events among species, suggesting that the spatiotemporal dynamics of population divergence play a crucial role in the diversification of communities in this region. The loose association of the anuran and squamate communities with the Dahomey Gap suggests that the influence of this barrier on population divergences was intermittent and incomplete, and

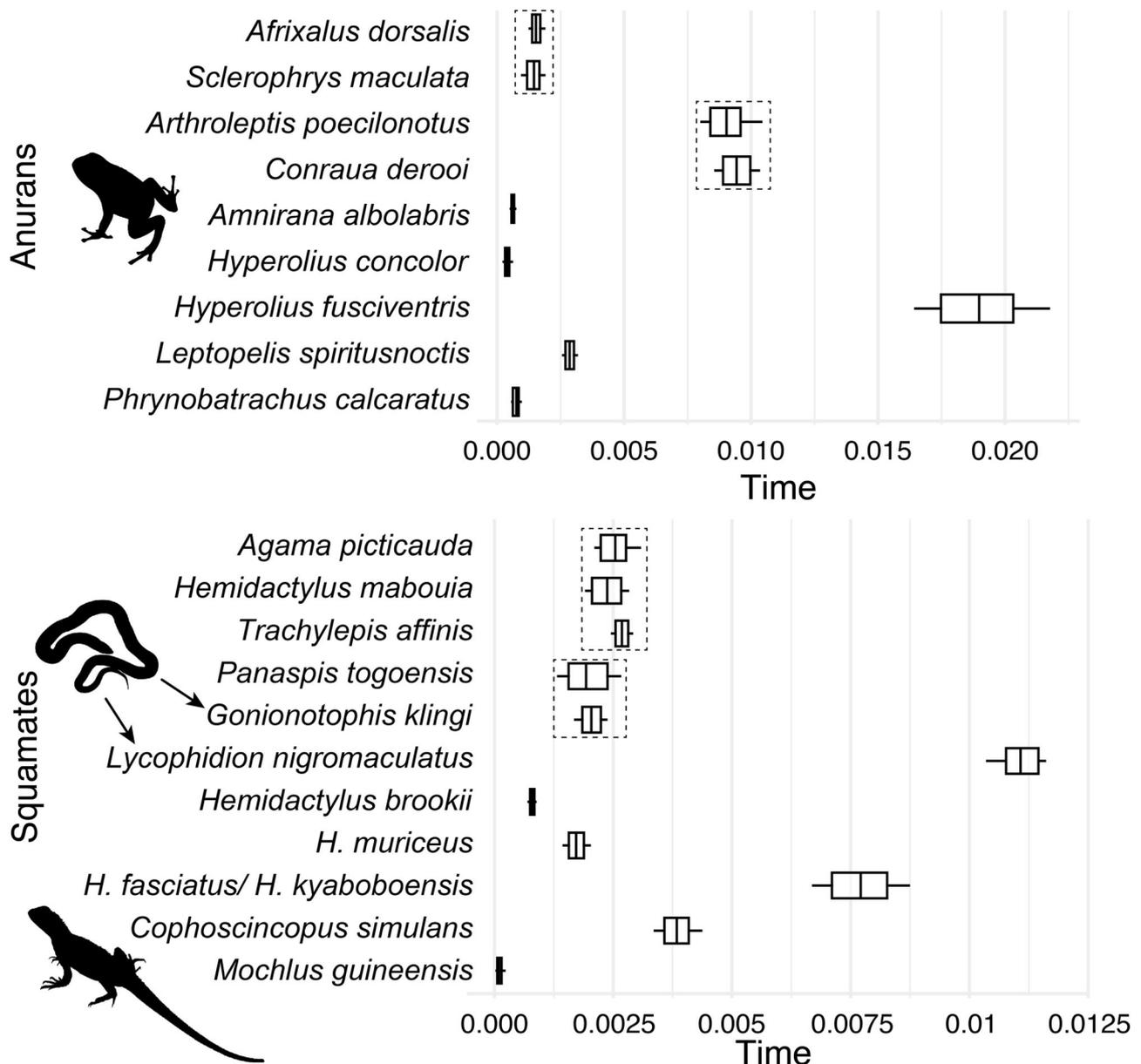


Figure 2. Posterior distributions (box and whisker plots) of divergence times (expressed as sequence divergence) for each population pair, separated by anurans and squamates. Species that show evidence for shared divergence are grouped in boxes with dashed lines.

would therefore be enlightened by further studies of population demographic histories.

It is reasonable to assume that codistributed species with similar life history strategies or habitat associations might experience a shared response to extrinsic diversification events. This is because phenotypes are typically the targets of selection, and they therefore play an important role in determining local adaptation and diversification (Zamudio et al. 2016). We found mixed evidence for shared divergence among species with similar life history strategies and ecological associations. Four examples of shared divergence were supported by our comparative phylogeographic analyses (Fig. 2); two involved rain forest species and two

included species inhabiting savanna and forest edges. However, for the two instances of codivergence in anurans, the species are terrestrial (*Sclerophrys maculata*) versus arboreal (*Afrixalus dorsalis*), or aquatic (*Conraua derooi*) versus terrestrial (*Arthroleptis poecilonotus*). One example of codivergence in squamates includes two rain forest species that are both terrestrial leaf-litter specialists (*Gonionotophis klingi* and *Panaspis togoensis*), whereas the other example includes three savanna species that are either terrestrial (*Trachylepis affinis*) or arboreal (*Agama picticauda* and *Hemidactylus mabouia*). Despite these examples of shared divergence that each involve a few species, the majority of divergences occurred independently (Fig. 1). A limitation of the

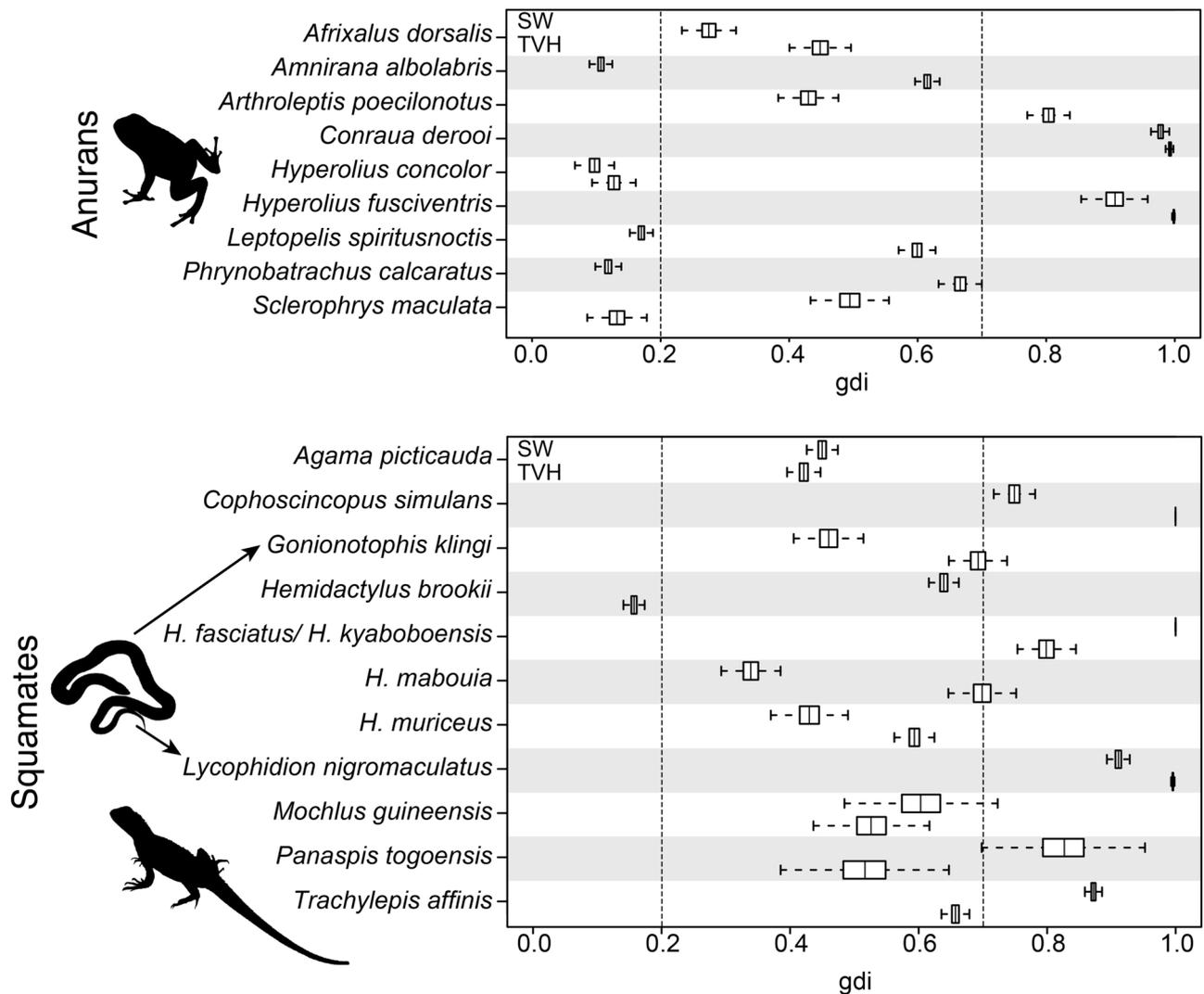


Figure 3. The 20 population pairs of anurans and squamate reptiles are distributed across the speciation continuum, although none are strictly panmictic ($gdi = 0$). Box and whisker plots represent posterior probability distributions of gdi values calculated from BPP analyses. Two gdi values are shown for each species (SW population above, TVH population below). The dashed vertical bars indicate gdi thresholds; $gdi < 0.2$ suggests a single species, $gdi > 0.7$ supports distinct species, and gdi values between 0.2 and 0.7 represent ambiguous species status.

ecoevolvy model is that it does not include migration, but even if there were repeated episodes of migration in the past, the method still allows us to test when each pair stopped sharing genes most recently.

A major assumption of the comparative phylogeographic analysis is that all species share the same substitution rate. Differences in substitution rates among species could result in differences in the estimated divergence times and a general lack of evidence for shared divergence (Oaks 2019). Restricting comparative phylogeographic analyses to closely related species, or species contained in the same genus, is one way of reducing potential problems with the substitution rate assumption, as close relatives should have similar rates (Oaks et al. 2019). Our anal-

yses of anurans and squamates each contained multiple species from the same genus, and in each case the species diverged independently (Fig. 2). This suggests that substitution rate variation may not be driving differences in divergence times. For example, the anurans *Hyperolius concolor* and *H. fusciventris* diverged at opposite ends of the spectrum (Fig. 2). In squamates, we included four *Hemidactylus* pairs, none of which were supported as codiverging (Fig. 2). These two outcomes alleviate some concerns about our simplifying assumption of equal rates of substitution among species.

The overall picture from this comparative phylogeographic investigation is that species are evolving idiosyncratically in these communities. The comparative species delimitation

approach using the *gdi* heuristic raises an important question concerning the equivalence of the populations that were compared; specifically, do some of these populations represent distinct species, and more generally, where do these populations lie on the path to speciation? Assuming that these 20 population-pairs are equivalent units of analysis is questionable given that their population divergence histories represent a broad spectrum of the speciation continuum (Fig. 3). Although some of the populations are only weakly differentiated ($gdi < 0.2$), others have high levels of genealogical divergence ($gdi > 0.7$) that could be indicative of separate evolutionary lineages (Fig. 3). One of the pairs included in this study, *Hemidactylus fasciatus* + *H. kyaboboensis*, provides a frame of reference for contrasting *gdi* levels against independent evolutionary lineages, and several of the sampled populations have *gdi* values that are equivalent with these lineages (e.g., *Conraua* and *Hyperolius fusciventris*; Fig. 3). It is possible that some of these population pairs are more accurately described as separate species given their *gdi* measurements, but additional types of data and a broader phylogeographic perspective should be considered before making taxonomic changes.

AUTHOR CONTRIBUTION

ADL, MKF, and COB conducted fieldwork; ADL and MKF designed the study and collected the data; ADL and JRO analyzed the data; all authors wrote the manuscript.

ACKNOWLEDGMENTS

This work was funded by grants from the National Science Foundation to ADL (NSF DEB-1456098), MKF (NSF DEB-1457232), and JRO (NSF DEB-1656004). We thank three anonymous referees and Jessica Light for constructive comments.

DATA ARCHIVING

Demultiplexed ddRADseq data are available at the NCBI Sequence Read Archive (PRJNA 531179). Processed data files are available on Dryad (<https://doi.org/10.5061/dryad.xpnvx0kbq>).

LITERATURE CITED

- 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.
- Blackburn, D. C., V. Gvozdik, and A. D. Leaché. 2010. A new squeaker frog (Arthroleptidae: *Arthroleptis*) from the mountains of Cameroon and Nigeria. *Herpetologica* 66:335–348.
- Booth, A. H. 1958. The Niger, the Volta and the Dahomey Gaps as geographic barriers. *Evolution* 12:48–62.
- Bryant, D., R. Bouckaert, J. Felsenstein, N. A. Rosenberg, and A. Roychoudhury. 2012. Inferring species trees directly from biallelic genetic markers: bypassing gene trees in a full coalescent analysis. *Mol. Biol. Evol.* 29:1917–1932.
- Charles, K. L., R. C. Bell, D. C. Blackburn, M. Burger, M. K. Fujita, V. Gvozdik, G. F. Jongsma, M. T. Kouete, A. D. Leaché, and D. M. Portik. 2018. Sky, sea, and forest islands: diversification in the African leaf-folding frog *Afraxalus paradorsalis* (Anura: Hyperoliidae) of the Lower Guineo-Congolian rainforest. *J. Biogeogr.* 45:1781–1794.
- Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, et al. 2011. The variant call format and VCFtools. *Bioinformatics* 27:2156–2158.
- Dupont, L. M., and M. Weinelt. 1996. Vegetation history of the savanna corridor between the Guinean and the Congolian rain forest during the last 150,000 years. *Veg. Hist. Archaeobot.* 5:273–292.
- Dupont, L. M., S. Jahns, F. Marret, and S. Ning. 2000. Vegetation change in equatorial West Africa: time-slices for the last 150 ka. *Palaeogeogr. Palaeoecol.* 155:95–122.
- Dowell, S. A., D. M. Portik, V. de Buffrénil, I. Ineich, E. Greenbaum, S.-O. Kolokotronis, and E. R. Hekkala. 2016. Molecular data from contemporary and historical collections reveal a complex story of cryptic diversification in the *Varanus* (*Polydaedalus*) *niloticus* species group. *Mol. Phylogenet. Evol.* 94:591–604.
- Eaton, D. 2017. ipyrad version 0.7.13. Available at <http://ipyrad.readthedocs.io>.
- Hamilton, A. C., and D. Taylor. 1991. History of climate and forests in tropical Africa during the last 8 million years. *Clim. Change* 19:65–78.
- Hickerson, M. J., E. Stahl, and N. Takebayashi. 2007. msBayes: a flexible pipeline for comparative phylogeographic inference using approximate Bayesian computation (ABC). *BMC Bioinformatics* 8:268.
- Jackson N., B. Carstens, A. Morales, and B. C. O'Meara. 2017. Species delimitation with gene flow. *Syst. Biol.* 66:799–812.
- Jongsma, G. F. M., M. F. Barej, C. D. Barratt, M. Burger, W. Conradie, R. Ernst, E. Greenbaum, M. Hirschfeld, A. D. Leaché, J. Penner, et al. 2018. Diversity and biogeography of frogs in the genus *Ammirana* (Anura: Ranidae) across sub-Saharan Africa. *Mol. Phylogenet. Evol.* 120:274–285.
- Krishnan, S., M. K. Fujita, C. Ofori-Boateng, and A. D. Leaché. 2019. Geographic variation in West African *Agama picticauda*: insights from genetics, morphology, and ecology. *Afr. J. Herpetol.* 68:33–49.
- Leaché, A. D. 2005. Results of a herpetological survey in Ghana and a new country record. *Herpetol. Rev.* 36:16–19.
- Leaché, A. D. and M. K. Fujita. 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proc. R. Soc. B* 277:3071–3077.
- Leaché, A. D., M.-O. Rödel, C. W. Linkem, R. E. Diaz, A. Hillers, and M. K. Fujita. 2006. Biodiversity in a forest island: reptiles and amphibians of the West African Togo Hills. *Amphib. Reptile Conserv.* 41:22–45.
- Leaché, A. D., M. K. Fujita, V. N. Minin, and R. R. Bouckaert. 2014a. Species delimitation using genome-wide SNP data. *Syst. Biol.* 63:534–542.
- Leaché, A. D., R. B. Harris, B. Rannala, and Z. Yang. 2014b. The influence of gene flow on species tree estimation: a simulation study. *Syst. Biol.* 63:17–30.
- Leaché, A. D., J. A. Grummer, M. Miller, S. Krishnan, M. K. Fujita, W. Böhme, A. Schmitz, M. LeBreton, I. Ineich, L. Chirio, et al. 2017. Bayesian inference of species diffusion in the West African *Agama agama* species group (Reptilia, Agamidae). *Syst. Biodivers.* 15:192–203.
- Leaché, A. D., D. M. Portik, D. Rivera, M.-O. Rödel, J. Penner, V. Gvozdik, R. C. Bell, E. Greenbaum, C. Ofori-Boateng, M. Burger, et al. 2019a. Exploring rain forest diversification using demographic model testing in the African foam-nest treefrog (*Chiromantis rufescens*). *J. Biogeogr.* 46:2706–2721.
- Leaché, A. D., T. Zhu, B. Rannala, and Z. Yang. 2019b. The spectre of too many species. *Syst. Biol.* 68:168–181.
- Maley, J. 1991. The African rain forest vegetation and palaeoenvironments during late Quaternary. *Clim. Change* 19:79–98.

- Moritz, C., J. Patton, C. Schneider, and T. B. Smith. 2000. Diversification of rainforest faunas: an integrated molecular approach. *Ann. Rev. Ecol. Syst.* 31:533–563.
- Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. Da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853–858.
- Oaks, J. R. 2014. An improved approximate-Bayesian model-choice method for estimating shared evolutionary history. *BMC Evol. Biol.* 14:150.
- Oaks, J. R. 2019. Full Bayesian comparative phylogeography from genomic data. *Syst. Biol.* 68:371–395.
- Oaks, J. R., C. D. Siler, and R. M. Brown. 2019. The comparative biogeography of geckos challenges predictions from a paradigm of climate-driven vicariant diversification across an island archipelago. *Evolution* 73:1151–1167.
- Ofori-Boateng, C., A. D. Leaché, B. Obeng-Kankam, N. G. Kouamé, A. Hillers, and M.-O. Rödel. 2018. A new species of Puddle Frog, genus *Phrynobatrachus* (Amphibia: Anura: Phrynobatrachidae) from Ghana. *Zootaxa* 4374:565–578.
- Penner, J., M. Wegmann, A. Hillers, M. Schmidt, and M.-O. Rödel. 2011. A hotspot revisited—a biogeographical analysis of West African amphibians. *Divers. Distrib.* 17:1077–1088.
- Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher, and H. E. Hoekstra. 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One* 7:e37135.
- Plana, V. 2004. Mechanisms and tempo of evolution in the African Guineo-Congolian rainforest. *Philos. Trans. R. Soc. B Biol. Sci.* 359:1585–1594.
- Portik, D. M., A. D. Leaché, D. Rivera, M. F. Barej, M. Burger, M. Hirschfeld, M.-O. Rödel, D. C. Blackburn, and M. K. Fujita. 2017. Evaluating mechanisms of diversification in a Guineo-Congolian forest frog using demographic model selection. *Mol. Ecol.* 26:5245–5263.
- Rannala B., and Z. Yang. 2013. Improved reversible jump algorithms for Bayesian species delimitation. *Genetics* 194:245–253.
- Rödel, M.-O., M. Gil, A. C. Agyei, A. D. Leaché, R. E. Diaz, M. K. Fujita, and R. Ernst, 2005. The amphibians of the forested parts of south-western Ghana. *Salamandra* 41:107–127.
- Rödel, M.-O., M. F. Barej, A. Hillers, A. D. Leaché, et al. 2012. The genus *Astylosternus* in the Upper Guinea rainforests, West Africa, with the description of a new species (Amphibia: Anura: Arthroleptidae). *Zootaxa* 3245:1–29.
- Salzmann, U. and P. Hoelzmann. 2005. The Dahomey Gap: an abrupt climatically induced rain forest fragmentation in West Africa during the late Holocene. *The Holocene* 15:190–199.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370.
- Yang Z. 2015. The BPP program for species tree estimation and species delimitation. *Curr. Zool.* 61:854–865.
- Yang Z., and B. Rannala. 2010. Bayesian species delimitation using multilocus sequence data. *Proc. Natl. Acad. Sci. USA*, 107:9264–9269.
- Zamudio, K. R., R. C. Bell, and N. A. Mason. 2016. Phenotypes in phylogeography: species' traits, environmental variation, and vertebrate diversification. *Proc. Natl. Acad. Sci. USA*, 113:8041–8048.

Associate Editor: J. Light
Handling Editor: M. R. Servedio

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Posterior distributions (box and whisker plots) of divergence times (expressed as sequence divergence) for each population pair, separated by anurans and squamates.

Figure S2. Comparison of *gdi* value for the 20 population pairs of anurans and squamate reptiles using different numbers of samples.

Figure S3. Correlation between *F_{ST}* and *gdi* values (mean of 15 the posterior distribution) for anurans and squamate reptiles in each population (SW and TVH).

Table S1. Specimens of anurans and squamate reptiles included in the study.

Table S2. Prior settings for ecoevolution analyses.

Table S3. Comparative phylogeographic results for anurans using ecoevolution.

Table S4. Comparative phylogeographic results for squamates using ecoevolution.