Received: 18 November 2020 | Revised: 7 April 2021 | Accepted: 14 April 2021

DOI: 10.1111/mec.15928

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Speciation and secondary contact in a fossorial island endemic, the São Tomé caecilian

Kyle A. O'Connell[1,2,3] | Ivan Prates[1,4] | Lauren A. Scheinberg[5] | Kevin P. Mulder[1,6,7] | Rayna C. Bell[1,5]

[1]Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

[2]Global Genome Initiative, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

[3]Department of Biological Sciences, George Washington University, Washington, DC, USA

[4]Department of Ecology and Evolutionary Biology and Museum of Zoology, University of Michigan, Ann Arbor, MI, USA

[5]Department of Herpetology, California Academy of Sciences, San Francisco, CA, USA

[6]CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal

[7]Center for Conservation Genomics, Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC, USA

Correspondence
Rayna C. Bell and Kyle A. O'Connell, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.
Email: rbell@calacademy.org; kyleaoconnell22@gmail.com

Abstract

A period of isolation in allopatry typically precedes local adaptation and subsequent divergence among lineages. Alternatively, locally adapted phenotypes may arise and persist in the face of gene flow, resulting in strong correlations between ecologically-relevant phenotypic variation and corresponding environmental gradients. Quantifying genetic, ecological, and phenotypic divergence in such lineages can provide insights into the abiotic and biotic mechanisms that structure populations and drive the accumulation of phenotypic and taxonomic diversity. Low-vagility organisms whose distributions span ephemeral geographic barriers present the ideal evolutionary context within which to address these questions. Here, we combine genetic (mtDNA and genome-wide SNPs) and phenotypic data to investigate the divergence history of caecilians (Amphibia: Gymnophiona) endemic to the oceanic island of São Tomé in the Gulf of Guinea archipelago. Consistent with a previous mtDNA study, we find two phenotypically and genetically distinct lineages that occur along a north-to-south axis with extensive admixture in the centre of the island. Demographic modelling supports divergence in allopatry (~300 kya) followed by secondary contact (~95 kya). Consequently, in contrast to a morphological study that interpreted latitudinal phenotypic variation in these caecilians as a cline within a single widespread species, our analyses suggest a history of allopatric lineage divergence and subsequent hybridization that may have blurred species boundaries. We propose that late Pleistocene volcanic activity favoured allopatric divergence between these lineages with local adaptation to climate maintaining a stable hybrid zone in the centre of São Tomé Island. Our study joins a growing number of systems demonstrating lineage divergence on volcanic islands with stark environmental transitions across small geographic distances.

KEYWORDS
amphibian, gene flow, hybridization, in situ diversification, island speciation, *Schistometopum ephele*, *Schistometopum thomense*

 © 2021 John Wiley & Sons Ltd

1 | INTRODUCTION

A period of isolation in allopatry typically precedes local adaptation and subsequent lineage divergence that may ultimately result in speciation (Losos & Ricklefs, 2009; Mayr, 1963). Secondary contact of lineages following transient periods of allopatric divergence can result in lineage fusion or promote reproductive isolation through reinforcement (Choi et al., 2020; Servidio & Noor, 2003). Alternatively, locally adapted phenotypes may arise and persist in the face of gene flow resulting in strong correlations between ecologically-relevant phenotypic variation and corresponding environmental gradients (Thorpe et al., 2015). Quantifying genetic, ecological, and phenotypic variation in these nascent lineages can provide insights into the abiotic and biotic mechanisms that structure populations and ultimately drive the accumulation of species richness and phenotypic diversity. Organisms with low dispersal potential whose distributions span ephemeral geographic barriers present the ideal evolutionary context within which to understand the relative contributions of these evolutionary processes. Here, we investigate the divergence history of the enigmatic and fossorial caecilians (Amphibia: Gymnophiona) endemic to the small, volcanic island of São Tomé.

Physical barriers such as rivers (Vences et al., 2009; Welton et al., 2010), sea level changes (Esselstyn et al., 2009; O'Connell et al., 2018), or volcanic lava flows (Bloor et al., 2008; Brochmann, 1984; Nater et al., 2011) often contribute to allopatric divergence. Landscapes are dynamic, however, and the elimination of such barriers can lead to population expansion, secondary contact, hybridization, and fusing of incipient species, particularly on small oceanic islands (García-Olivares et al., 2017; Garrick et al., 2014; Gow et al., 2006; Grant & Grant, 1996; MacLeod et al., 2015; Roderick et al., 2012; Sardell & Uy, 2016; Taylor et al., 2006). Spatial environmental gradients such as differences in rainfall, temperature, or soil type may further reinforce divergence in allopatry (Losos & Schluter, 2000; Rundle & Nosil, 2005). These environmental transitions can also lead to stable hybrid zones if lineages that meet secondarily are locally adapted (Barton & Hewitt, 1985). Consequently, genetic and phenotypic differentiation along environmental gradients can be difficult to distinguish from isolation by distance (Bradburd et al., 2018; Myers et al., 2019) or allopatric divergence and secondary contact (Portik et al., 2017); however, genomic data paired with demographic modelling approaches can help differentiate among alternative historical scenarios such as divergence in allopatry versus divergence with gene flow (Excoffier et al., 2013; Gutenkunst et al., 2009). Likewise, quantifying ecological divergence of lineages in the early stages of speciation can reveal the roles of environmental adaptation and geographic isolation in promoting population divergence and reproductive isolation (Losos & Schluter, 2000; Marques et al., 2016; Seehausen et al., 2001). Small oceanic islands are a compelling study system for addressing the role of previous isolation versus local and/or ongoing selection in shaping biodiversity because they often exhibit more transient geographic barriers to gene flow coupled with stark environmental transitions across small geographic distances (e.g., Brown et al., 2016; Stenson et al., 2002; Suárez et al., 2014).

Growing evidence suggests that both environmental gradients and a dynamic landscape history shaped species diversification on São Tomé, a volcanic island ~225 km off the coast of West-Central Africa in the Gulf of Guinea archipelago. The island emerged from the sea floor ~13 million years ago (Ma), and despite its small size (~850 km^2), it is topographically complex, with its highest peak at 2024 m (Gillespie & Clague, 2009). Correspondingly, São Tomé exhibits environmental gradients ranging from drier and open habitat in the north to wetter and forested habitat in the south (de Lima et al., 2017; Soares, 2017). Despite the island's long geological history, many studies of taxonomic diversification within São Tomé have inferred that divergence occurred during the Pliocene or Pleistocene (Bell, Drewes, Channing, et al., 2015; Daniels & Klaus, 2018; Stoelting et al., 2014), which coincides with a period of extensive volcanic activity (Barfod & Fitton, 2014). Further, a history of in situ divergence in allopatry followed by secondary contact and hybridization was inferred in São Tomé *Drosophila* (Coyne et al., 2002; Matute & Coyne, 2010) and *Hyperolius* reed frogs (Bell et al., 2015; Bell & Irian, 2019). By contrast, both adaptation along environmental gradients and allopatric divergence have been proposed to explain phenotypic (Nussbaum & Pfrender, 1998) and genetic (Stoelting et al., 2014) variation in the São Tomé Caecilian (*Schistometopum thomense*). Due to their low vagility and strong associations with particular soil types and climates (Gundappa et al., 1981; Jones et al., 2006; Kouete & Blackburn, 2020; Torres-Sánchez et al., 2019), caecilians may provide novel insights into the mechanisms that generate and maintain lineage divergence on small oceanic islands.

Globally, caecilians are distributed throughout the tropics yet are poorly known relative to most vertebrate groups due to their secretive lifestyles and because they are sometimes rare (Heyer et al., 2014; Measey, 2004; Measey et al., 2003). However, *S. thomense* is amenable to study because it is active above and below ground and is abundant across São Tomé, where it occupies diverse habitats from 0 to 1440 m elevation (Measey & Van Dongen, 2006; Nussbaum & Pfrender, 1998; Stoelting et al., 2014). Morphological variation in this species approximately follows a latitudinal cline, with a yellow unflecked morph in the north and a brown, flecked morph in the south (Haft, 1992; Measey & Van Dongen, 2006; Nussbaum & Pfrender, 1998; Stoelting et al., 2014; Taylor, 1965). This morphological variation led to the description of flecked individuals as a separate species, *Schistometopum ephele* (*S. ephele*; Taylor, 1965); however, Nussbaum and Pfrender (1998) interpreted this variation as a phenotypic cline in a single widespread species and synonymized *S. ephele* with *S. thomense*. More recently, Stoelting et al. (2014) detected two distinct mitochondrial haplotype groups that roughly correspond to *S. ephele* and *S. thomense* with a narrow zone of putative admixture in the centre of the island, which they interpreted as evidence of allopatric divergence and secondary contact. In addition, Stoelting et al. (2014) noted that the putative admixture zone coincided with the transition between volcanic flows indicating that volcanism may have played an important role in the

evolutionary history of these lineages. In the present study, we revisit these two alternative hypotheses of demographic history using phenotypic and genetic (mtDNA and genome wide single nucleotide polymorphism [SNP]) data. Specifically, we (i) leverage demographic modelling to test for historical divergence in allopatry versus divergence with continuous gene flow, (ii) quantify the temporal and geographic extent of gene flow in the putative admixture zone, and (iii) contextualize the evolutionary history of the species with respect to environmental gradients and volcanic activity across its range.

2 | MATERIALS AND METHODS

2.1 | Field and museum specimen sampling and colour pattern assessment

For genetic analyses, we included 85 samples from 21 localities across the island (Table S1). Among them, 12 samples were collected by the authors between 2012 and 2016 at three localities including Obo National Park, which had not previously been sampled. Tissue samples (liver) were preserved in 95% ethanol or RNAlater for subsequent DNA extraction and genetic analyses. Additionally, we selected a subset of 73 specimens from the Stoelting et al. (2014) mtDNA study from which to collect nuDNA SNP data (see below). This sampling spans the type locality of *S. thomense* (Bocage 1873), which is only specified to the level of the entire island ("Ile Saint Thomé") but our sampling spans most accessible settlements from the colonial period, and the type locality for *S. ephele* Taylor, 1965 ("Agua Izé, 400–700 m, Ilha São Thomé"), which is probably between Água Izé, a coastal community on the eastern side of the island, and the community of Java at ~600 m that is directly inland of Água Izé (G. Doria, Museo Civico di Storia Naturale "G. Doria", personal communication. Genova; Figure 1). For all samples, we extracted DNA using a DNeasy Blood and Tissue Kit (Qiagen Inc.) and quantified DNA yield using a QUBIT 2.0 Fluorometer (Life Technologies). All specimens are accessioned at the California Academy of Sciences.

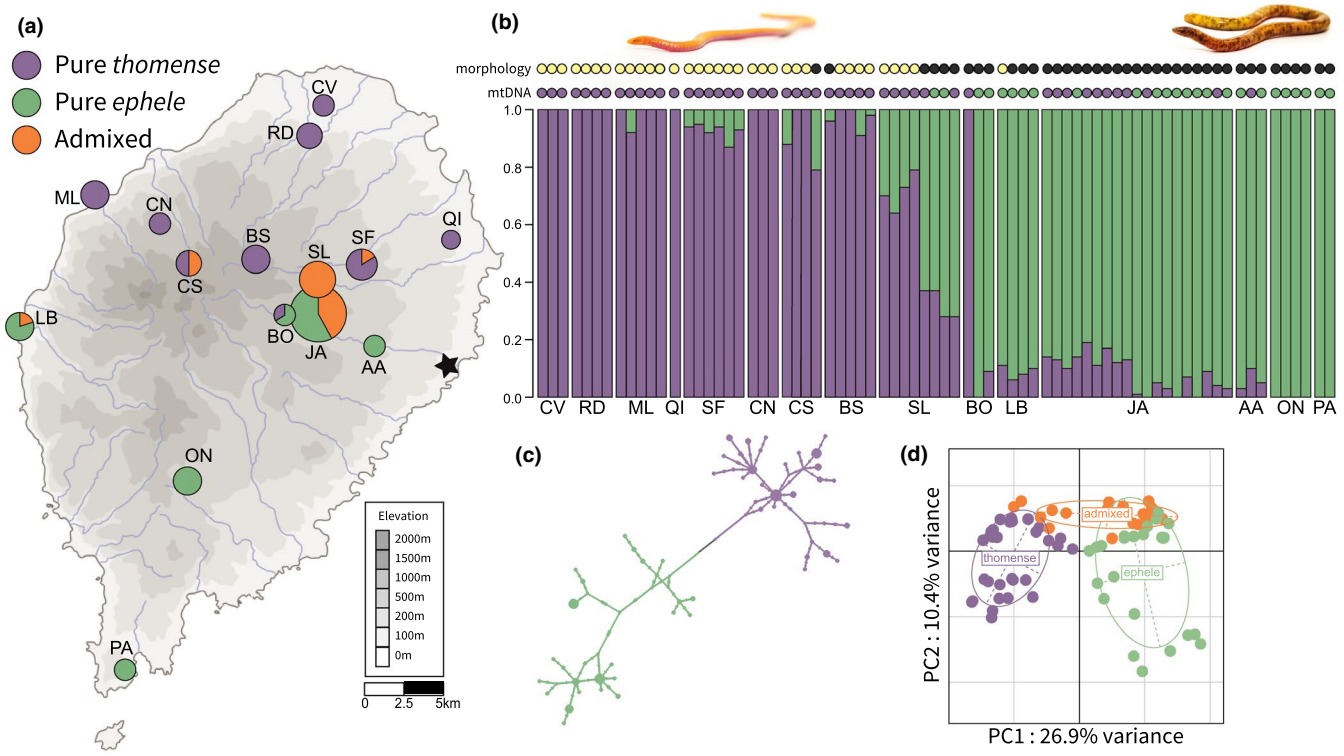


FIGURE 1 *Schistometopum* sampling on São Tomé Island. (a) Map shows distribution of genomic samples with the size of circles proportional to the number of individuals at that site. Individuals with at least 90% ancestry assigned to *Schistometopum thomense* (*S. thomense*) are shown in purple, 90% ancestry assigned to *Schistometopum ephele* (*S. ephele*) in green, and admixed individuals in orange. Site abbreviations are as follows: AA, Anselmo Andrade; BO, Bombaim; BS, Bom Sucesso; CN, Contador Valley North; CS, Contador Valley South; CV, Canavial; JA, Java + Abade; LB, Lemba River; ML, Rio Maria Luisa; ON, Obo National Park; PA, Porto Alegre; QI, Quisinda; RD, Rio d'Ouro; SF, Santa Fe; SL, Santa Luzia. The type locality of *S. ephele* (Água Izé, 400–700 m) is probably between the coastal community of Água Izé (indicated by black star) and Java. (b) Plot of ancestry coefficients estimated with ADMIXTURE version 1.3.0 (Alexander et al. (2009) for $K = 2$. Circles above the plot show the haplotype of each individual from the mitochondrial *ND4* locus, and morphological assignment (yellow, unflecked = yellow; brown, flecked = grey). (c) *ND4* haplotype network for new samples and previously published data (Stoelting et al., 2014) estimated in PopART (Leigh & Bryant, 2015). Twenty-six mutations separate the haplotype groups. (d) Principal component analysis (PCA) of single nucleotide polymorphism (SNP) data with individuals coloured according to their ancestry assignment from (b). Photo credits: A. Stanbridge [Colour figure can be viewed at wileyonlinelibrary.com]

Following previous studies (Nussbaum & Pfrender, 1998; Stoelting et al., 2014), one author (LAS) scored the coloration of all individuals included in the nuclear data set as flecked or unflecked. For the newly collected specimens (n = 12), we compared coloration between photographs in life and the voucher specimens after >3 years of preservation to assess consistency. The remaining individuals were only scored as museum specimens following 13–18 years of preservation. Because colours fade in preservative, we were unable to score individuals with light versus dark brown flecking or light versus dark yellow hue as previous authors have done; the presence or absence of flecking, however, remained prominent in older specimens.

2.2 | Mitochondrial DNA sequencing and haplotype network estimation

To place the 12 newly sampled specimens within the Stoelting et al. (2014) data set, we amplified a partial fragment of the NADH dehydrogenase 4 (*ND4*) gene following their methods. We assembled both reads and edited sequences in GENEIOUS version 11.0 (Kearse et al., 2012) and combined them with sequences (n = 137) generated by Stoelting et al. (2014) downloaded from Genbank (Table S1). We aligned sequences using CLUSTALW version 2.1 (Larkin et al., 2007) and estimated a haplotype network using the TCS algorithm (Clement et al., 2000) implemented in POPART (Leigh & Bryant, 2015).

2.3 | SNP data set collection

We generated double-digest restriction site associated DNA (ddRAD) libraries (Peterson et al., 2012) as described in the Methods S1. Briefly, extractions were digested with the restriction enzymes *SbfI* and *MspI*, and the resulting fragments were tagged with individual barcodes, multiplexed into groups of 11 uniquely barcoded individuals and size selected for fragments between 434 and 538 bp. Barcode groups were PCR amplified, pooled, and sequenced on an Illumina 2500 (SE 150 bp).

Raw data were processed using IPYRAD version 0.7.30 (Eaton & Overcast, 2020). After demultiplexing, we removed seven samples with <200,000 reads and one duplicate sample that was inadvertently sequenced twice. With the remaining 77 samples, we trimmed the first six bp to remove the restriction site, allowing a maximum of five low-quality base calls per read. We followed Ilut et al. (2014) to determine an optimal clustering threshold of 0.96 and up to 16 SNPs per 150 bp locus (at which additional SNPs per locus plateaued). We allowed no barcode mismatches and used the "strict" adapter filtering option, leaving all other parameters as default values. We required each site to be present in at least 70% (55/77) of samples, and dropped three samples missing >90% of loci. To maximize sampling of independent SNP histories, we extracted one SNP per locus, producing a final data set of 6772 SNPs for 74 individuals.

2.4 | Characterizing population structure and the extent of hybridization

Using the 6772 SNP data set we explored genomic structure using principal component analysis (PCA) with the dudi.pca function implemented in ADE4 version 1.7.11 (Dray & Dufour, 2007). To determine the number of genetic demes and degree of admixture among demes we implemented the maximum likelihood approach implemented in ADMIXTURE version 1.3.0 (Alexander et al., 2009) with a range of K values (1–10) and five iterations per K value. Following the recommendation of Linck and Battey (2019), we filtered our data set for minor allele count = 3 using VCFTOOLS version 0.1.15 (Danecek et al., 2011) to produce a data set for ADMIXTURE analyses that contained 3270 SNPs.

To quantify the extent of hybridization between *S. thomense* and *S. ephele*, we used a maximum likelihood approach implemented in the *R* package HIEST version 2.0 (Fitzpatrick, 2012). This method jointly infers the ancestry index (S; the proportion of an individual's alleles descending from alleles in one parental lineage) and interclass heterozygosity (H; the proportion of an individual's loci that have one allele from each ancestral lineage). H values close to one indicate recent hybridization (F1, F2, or backcross generations) and values closer to 0 indicate hybridization in the more distant past. Considering both values together allowed us to quantify the temporal (in generations) and geographic extent of hybridization between lineages. Following developer recommendations to retain ancestry-informative markers, we identified 10 individuals from each lineage with strong concordance between genomic, mitochondrial, and morphological data ($Q \geq 0.9$ or ≤ 0.1 in the ADMIXTURE analysis) and <10% missing data in the 3270 SNP data set. Based on these reference "parental" samples, we estimated allele frequencies for each locus using VCFtools and only retained loci fixed between lineages (parental allele frequencies ≥0.95 or ≤0.05). We removed individuals missing >50% of sites (13 individuals) and loci missing >50% of individuals (one locus) resulting in a final data set of 41 SNPs and 64 individuals. We estimated S and H using the "SANN" method, with 1000 MCMC iterations, a starting grid = 99, and surf = TRUE. HIEST assumes a continuous model of hybridization but also includes a function to compare the fit of the model when classifying each individual as one of the six standard genotype frequency classes (parental, F1, F2 and backcrosses) with that of the continuous model. To differentiate between recent and historical hybridization, we used the function HIclass to estimate likelihoods for early generation hybrids (F1, F2, backcrosses), and HItest to compare likelihoods to those from the continuous model.

2.5 | Testing alternative demographic histories

To test alternative models of diversification history, we used the diffusion approximation method implemented in δaδi (Gutenkunst et al., 2009). We tested 18 historical demographic models from

Portik et al. (2017) including divergence in allopatry versus divergence with continuous gene flow, secondary contact versus contemporary isolation, and instantaneous size change (full range of models shown in Portik et al. (2017); Table S2). We generated a folded two-dimensional site frequency spectrum (2D-SFS) from the VCF format output from ipyrad (https://github.com/isaacovercast/easySFS). To account for missing data among individuals, we downprojected our SNP data set to 25 diploid individuals with 3570 SNPs for *S. thomense* and 25 diploid individuals with 4377 SNPs for *S. ephele*. We also ran the analysis without putative hybrid individuals (>10% admixed) to ensure that these individuals did not bias model selection (*S. thomense*: 19 diploid individuals with 3109 SNPs; *S. ephele*: 15 diploid individuals with 3617 SNPs).

Following Portik et al. (2017) and Barratt et al. (2018), we used modified scripts from dadi_pipeline (https://github.com/dportik/dadi_pipeline) to perform five iterations of each model consisting of four rounds of optimizations with multiple replicates (see below). We used search parameter estimates from the best scoring replicate (highest log-likelihood) to seed searches in the following round. We used the following settings for each round of dadi_pipeline: grid size = 50, 60, 70; replicates = 10, 20, 30, 40; maxiter = 3, 5, 10, 15; fold = 3, 2, 2, 1. We optimized parameters using the Nelder-Mead approach (optimize_log_fmin), and used the optimized parameter sets of each replicate to simulate the 2D-SFS. The log-likelihood of each 2D-SFS was estimated for each model using a multinominal approach, we identified the best-supported model using log-likelihood and AIC, and used the ΔAIC scores to calculate Akaike model weights (ωi). Goodness of fit tests were performed following Barratt et al. (2018) and were based on 250 simulated frequency spectra.

We estimated the divergence time between *S. thomense* and *S. ephele* using the Bayesian coalescent-based program G-PHOCS version 1.3 (Gronau et al., 2011). By incorporating entire loci (as opposed to SNPs), G-PHOCS facilitates the conversion of posterior estimates to years using locus-based mutation rates. Due to computational constraints and developer recommendations, we used 2000 loci and 10 individuals per lineage. To reduce potential biases introduced by admixed individuals or missing data, we sampled individuals with ancestry coefficients corresponding to >95% of the assigned lineage and sequence data for >90% of the loci. The δaδi model with the highest support indicated that divergence occurred in the absence of gene flow (see Results); thus we applied no migration bands in G-PHOCS. We followed Prates et al. (2018) to estimate prior ranges in G-PHOCS(scripts available at https://github.com/ivanprates/2018_Anolis_EcolEvol); we applied a gamma distribution to the θ (genetic diversity) and τ (root age) priors given by shape α = 1 and rate β = 275 (mean = 0.00363). We ensured our distribution encompassed a range of θ values from 0.002 to 0.00568 based on an island-wide N_e estimate of 500,000 individuals (extrapolated from Measey [2006]), which we converted to θ based on the equation $4^*Ne^*\mu$ using upper and lower bounds for mutation rates: 1.42×10^{-9} and 2.14×10^{-9} substitutions per site per year (estimated for two frog genera by Allio et al. [2017]). To improve chain mixing, we applied a 500,000 generation burnin and ran the analysis for 2,000,000 generations sampling every 10,000 generations and checked Markov chain mixing in TRACER version 1.6 (Rambaut et al., 2018). We converted our posterior estimate of the root using the mean of the two amphibian mutation rates from Allio et al. (2017), and a generation time of 2 years (Haft & Franzen, 1996).

2.6 | Environmental variation across sampling sites

To examine whether the two lineages of São Tomé caecilians are ecologically divergent, we assessed associations for admixed and nonadmixed individuals (≥90% assignments) with landscape gradients of climate, topography, land cover, and soil type/age (both associated with periods of underlying volcanic activity). We extracted bioclimatic variables from the WORLDCLIM database (Hijmans et al., 2005) that describe spatial patterns of temperature and precipitation variation. Moreover, we included geomorphological variables that probably impact fossorial organisms: elevation, land cover, and soil type/age (Caldeira & Munhá, 2002; Soares, 2017; Stoelting et al., 2014). Values were extracted from the collection sites of samples (only those for which we generated genomic data) using QGIS version 2.18.15 (available at https://github.com/qgis/QGIS). Due to the logistical difficulty of surveying the south-eastern quadrant of the island, this region remains largely uncharacterised for most variables and no caecilian specimens from this region were available for study. For continuous variables (precipitation, temperature, and elevation) we fitted ANOVAs grouping by *S. thomense*, *S. ephele*, and admixed individuals, and used a Tukey's honest significant differences test to calculate adjusted *p*-values for group mean comparisons.

3 | RESULTS

3.1 | Phenotypic variation in São Tomé caecilians

With few exceptions, flecked and unflecked phenotypes were geographically separated across São Tomé Island, with phenotypic turnover around the latitudinal midpoint of the island (Figures 1 and S1A). Only four out of 21 localities included in this study contained both phenotypes (Bom Sucesso, Contador South, Santa Luzia, and Lemba).

3.2 | Geographic structure and evidence of hybridization

The mtDNA haplotypes from the combined data sets were consistent with the clear northern and southern haplotype groups in Stoelting et al. (2014) and overlap zone in the centre of the island, where both haplotypes were present at four localities (Anselmo Andrade, Bombaim, Java, and Santa Luzia; Figures 1b,c and S1B,C).

A genetic PCA based on the SNP data set identified two clusters that largely corresponded to morphological and mitochondrial

patterns, with the exception of individuals around the putative contact zone (Figure 1a,b). Cross-validation of our ADMIXTURE analysis inferred roughly equal support for K = 2–4 (Figure S2C), with K = 2 splitting individuals into northern and southern groups corresponding to the PCA group assignments and the deepest split in the mitochondrial network. We assigned individuals to *S. thomense* (north) or *S. ephele* (south) based on ancestry coefficients >0.90, and considered those individuals with lower coefficients as admixed for downstream analyses (restricted to Contador South, Java, Lemba, Santa Fe, and Santa Luzia; Figure 1a). Higher K values (K = 3–4) further subdivided *S. thomense* but did not correspond to the fine-scale mtDNA structure recovered by Stoelting et al. (2014; Figure S2).

HIEST analyses based on the set of 41 ancestry-informative SNPs assigned 22 individuals to nonadmixed *S. thomense* (S value ≤ 0.1), 14 individuals as nonadmixed *S. ephele* (S value ≥ 0.9) and 28 individuals as admixed (Figure 2a; Table S1). H values for admixed individuals ranged from 0 to 0.65 (Table S1), consistent with multiple generations of hybridization. Plotting H values relative to latitude indicated that hybridization is restricted to the centre of the island (Figures 2b and S3). In all individuals, hybrid classifications under the continuous model were at least 2 log-likelihood units better than the best classification of early hybrid classes (F1, F2, backcrosses), thus rejecting early hybrid classes in all cases.

3.3 | Demographic history of São Tomé caecilians

Demographic modelling using δaδi based on SNP data from all samples supported a model of divergence in isolation, followed by instantaneous expansion in both lineages and secondary contact with ongoing symmetric migration (Figure 3; Table S2; ΔAIC = 83.5, ωi = 1.0). The second-best model was a three epoch model of divergence in isolation, followed by instantaneous expansion in both lineages and secondary contact with ongoing symmetric migration, followed by isolation in the recent past. When admixed individuals were excluded, this same three epoch model was best-supported (ΔAIC = 10, ωi = 1.0). Because there are no SNP-based mutation rate estimates for caecilians we refrained from converting our unscaled parameters here; however, several inferences can still be made. First, historical effective population size before and after expansion was larger for *S. ephele* than for *S. thomense* and *S. thomense* experienced a greater magnitude change in population size (~5×) relative to *S. ephele* (~2×; Table S2). Second, the relative time between secondary contact and the present (unscaled value of 0.24) was about half that between initial divergence and secondary contact (0.52). Parameter estimates were consistent between the two and three epoch models (Table S2). Goodness of fit tests showed that our empirical values fell slightly outside simulated distributions, indicating a poor fit of the best-supported model to the data (Figure S4). Poor model fit suggests that our cohort of models may be oversimplistic to capture the true evolutionary history of these caecilians (Excoffier et al., 2013). Future studies testing more complex models will benefit from more comprehensive sampling of genomic variation and additional sampling localities in the southern half of São Tomé.

Divergence time estimates using G-PHOCS(based on entire loci with locus-based mutation rates) indicated that initial divergence between *S. thomense* and *S. ephele* occurred ~303.4 kya (280.9–325.8 kya). We used this mean divergence date to convert our scaled time estimates from δaδi to infer that secondary contact occurred ~95.1 kya (88.0–101.1 kya; Figure 3).

3.4 | Environmental variation across sampling sites

We inferred that caecilians occupy a broad environmental space on São Tomé, ranging from habitats receiving between 800 and >1400 mm/year of precipitation (Figure 4). Our sampling indicated that caecilians occur in most soil types/ages on the island (Figure 4), and in all vegetation types (Figure 4). The two lineages segregated strongly in environmental space, particularly along a gradient of

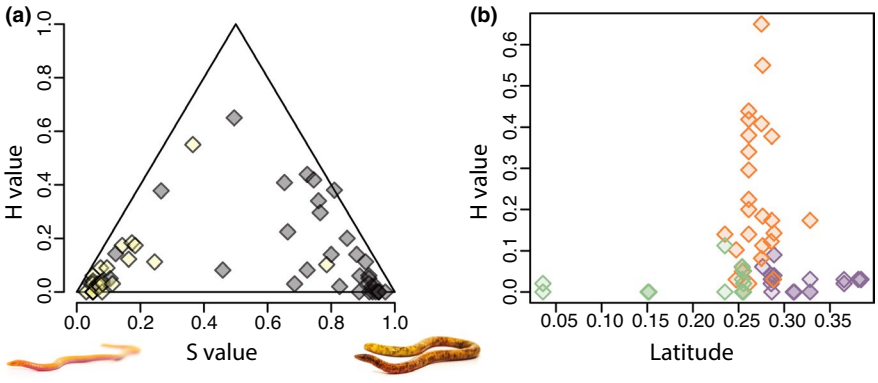
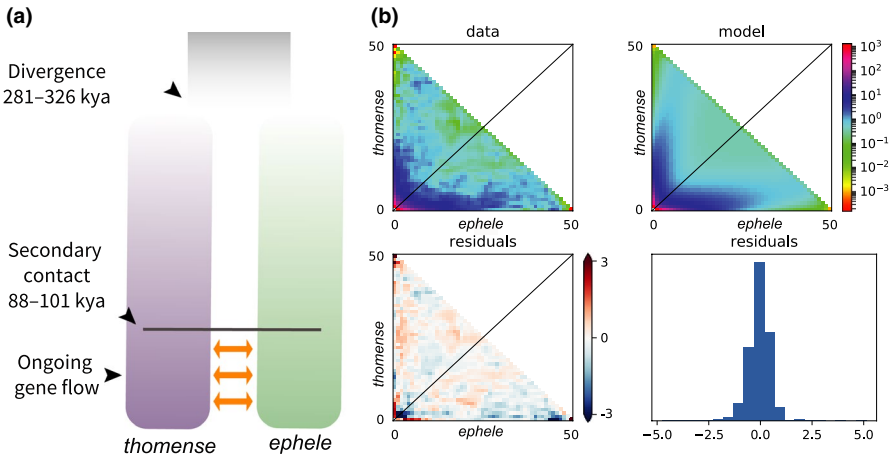


FIGURE 2 Results of the HIEST version 2.0 (Fitzpatrick, 2012) analysis. (a) Joint maximum likelihood estimates of ancestry (S value) and interclass heterozygosity (H value) for *Schistometopum thomense* (*S. thomense*) and *Schistometopum ephele* (*S. ephele*) for 41 diagnostic single nucleotide polymorphisms (SNPs). Individuals are coloured by morphology (yellow, unflecked = yellow; brown, flecked = grey) indicating that most admixed individuals (intermediate S and H values) are flecked. (b) H values plotted against latitude show that admixed individuals are restricted to the centre of the island at the contact zone. Individuals are coloured according to S values (≥0.9 or ≤0.1) [Colour figure can be viewed at wileyonlinelibrary.com]



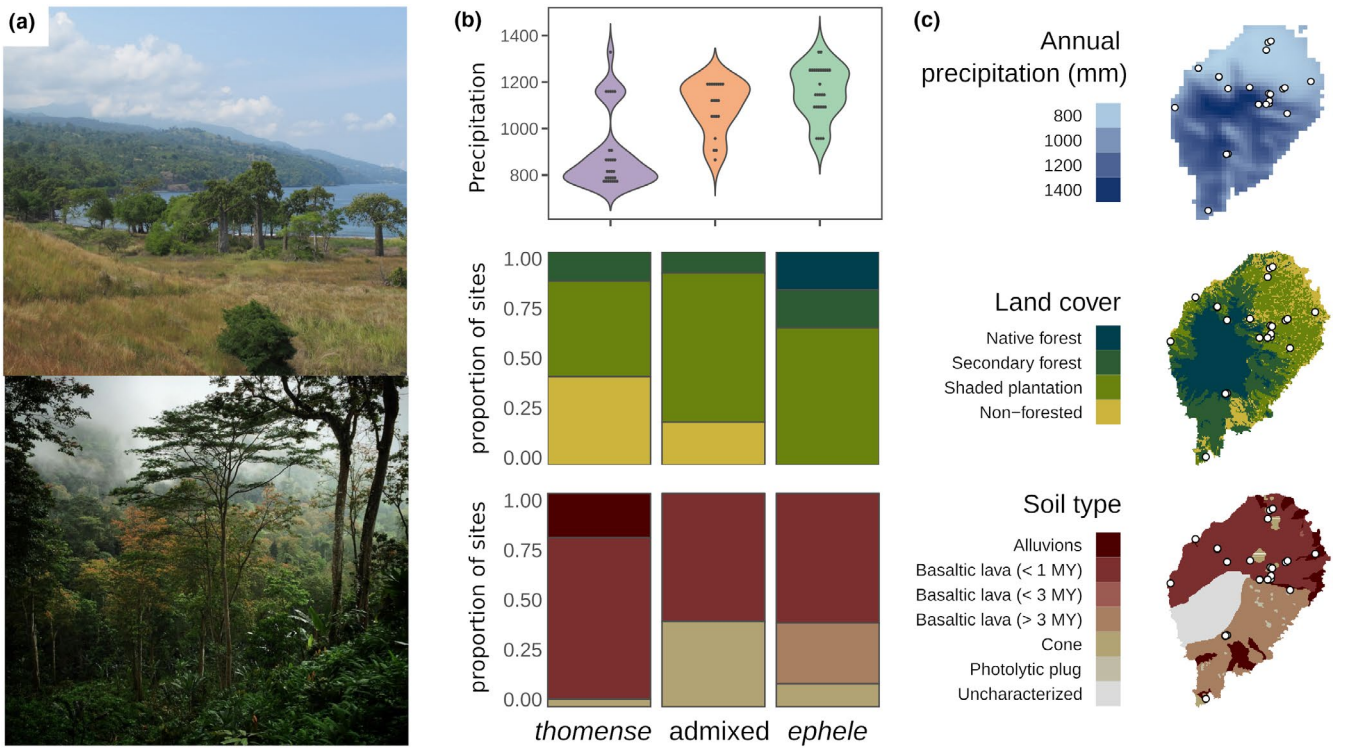


FIGURE 3 Results of demographic modelling (δaδi; Gutenkunst et al., 2009) and demographic parameter estimation (G-PHOCS; Gronau et al., 2011) analyses. (a) Stylized representation of the best supported model from δaδi with parameters superimposed from G-PHOCS. (b) The fit between the best-supported model and the data is shown using the two-dimensional site frequency spectrum (2D-SFS) and plots of the residuals [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 4 Summary of environmental space occupancy analyses. (a) Photos of habitat in representative dry (top) and wet (bottom) regions of São Tomé Island. (b) Violin plot of precipitation values at sites for pure and admixed caecilians (top), bar plots of land cover (middle), and bar plots of soil types and ages (bottom). (c) Annual precipitation (mm) across the island, with drier habitat in the north and wetter habitat in the south (top), land cover across the island, adapted from Soares (2017; middle), and soil types and ages across the island, adapted from Caldeira and Munhá (2002) and Stoelting et al. (2014; bottom). Photo credits: J. Shevock, A. Stanbridge [Colour figure can be viewed at wileyonlinelibrary.com]

precipitation (Figure 4), with *S. thomense* occurring in drier habitats than admixed and *S. ephele* caecilians (adjusted $p < .001$). Although *S. thomense* appear to inhabit a wider range of elevations and temperatures than *S. ephele*, occurrence with respect to these two variables does not differ between the species (Figure S5; adjusted $p > .05$). The distribution of *S. thomense* encompasses the younger basaltic lavas (<1 Ma) that dominate the central and northern half of the island and the contact zone between the lineages roughly coincides with the transition between the younger (<1 Ma) and older (3–8 Ma) basaltic lavas in the centre of the island. *Schistometopum thomense* were also associated with alluvial soils in the northern coastal plain (Figure 4) whereas *S. ephele* were associated with the older basaltic lavas (3–8 Ma) and volcanic cone formations on the southern half of the island. Caecilians occupied nonforested habitats across the island, but *S. thomense* and admixed individuals were primarily found in shaded plantation and nonforested habitats, whereas *S. ephele* were found in native forest, secondary forest, and shaded plantation but not in nonforested habitat (Figure 4).

4 | DISCUSSION

Our genetic and phenotypic data support a history of within-island divergence in allopatry for São Tomé caecilians, followed by secondary contact and hybridization that have blurred lineage boundaries rather than a history of divergence with gene flow along an ecological gradient. The common ancestor of *S. thomense* and *S. ephele* arrived on the island recently, having diverged from its East African sister species *S. gregorii* ~1 Ma (Loader et al., 2007). Correspondingly, using coalescent methods we estimated that subsequent in situ divergence occurred ~300 kya, which is comparable to previous estimates derived from analyses of mitochondrial sequence data (Stoelting et al., 2014). Several other São Tomé organisms with contiguous contemporary distributions have a history of in situ diversification during the Pleistocene, including reed frogs 1.7–0.5 Ma (Bell, Drewes, Channing, et al., 2015), freshwater crabs 1.5–0.5 Ma (Daniels & Klaus, 2018), and fruit flies ~400 kya (Llopart et al., 2002, 2005). These estimates broadly coincide with the most recent period of volcanic eruptive activity on São Tomé from 36 to 860 kya (Barfod & Fitton, 2014). Thus, lineage divergence across co-distributed groups may be associated with catastrophic late Pleistocene volcanic lava flows fragmenting species distributions and interrupting gene flow. These findings are consistent with the hypothesis that volcanic flows are an important but ephemeral mechanism for allopatric divergence in volcanic island systems (Juan et al., 2000), as has been documented in a variety of taxa, including flowering plants (Brochmann, 1984), lizards (Bloor et al., 2008), birds (Milá et al., 2010) and orangutans (Nater et al., 2011).

Besides imposing transient physical barriers to gene flow, volcanic eruptions may also favour divergence through local adaptation when populations become isolated in distinct environments. This hypothesis is consistent with associations of the two caecilian lineages within distinct precipitation regimes and habitats across the island (Figure 4), which may reflect local adaptation to specific soil microhabitats (Torres-Sánchez et al., 2019) as demonstrated in other fossorial vertebrates (Fouquet et al., 2021; Martín et al., 2013). Associations between habitat type and lineage divergence were also reported in reed frogs (Bell & Irian, 2019) and fruit flies (Coyne et al., 2002; Matute & Coyne, 2010) on São Tomé, suggesting that this pattern may be widespread across a variety of organisms on the island, although the specific mechanisms of local adaptation are likely to differ between fossorial versus surface-dwelling taxa. These observations may be somewhat confounded by the strong correlation between geography and environmental variation on São Tomé; however, similar associations have also been documented in organisms from other small volcanic islands including lizards from the Canary Islands (Brown et al., 2017; Gübitz et al., 2005; Pestano & Brown, 1999; Suárez et al., 2014) and birds from Réunion (Gabrielli et al., 2020). Studies of climate-dependent competitive outcomes (e.g., Comeault & Matute, 2021) and functional genomic variation (e.g., Brown et al., 2016) may provide deeper insights as to the relative contributions of geographic barriers and environmental variation to lineage diversification on small (<2500 km^2) oceanic islands.

Secondary contact may be pervasive when allopatric divergence results from ephemeral barriers on small oceanic islands (e.g., Brown et al., 2017). Accordingly, historical demographic analyses inferred that the São Tomé caecilian lineages came into secondary contact ~95 kya and that both lineages have experienced recent population expansion, with a greater magnitude of expansion in *S. thomense* (Figure 3a; Table S2). This difference in expansion is consistent with more extensive and recent volcanic activity across the northern half of the island where *S. thomense* occurs (Barfod & Fitton, 2014). We hypothesize that secondary contact and population size change occurred following the expansion of suitable habitat after the erosion of lava flows, thus facilitating contact between previously separated lineages. Hybrid zones are maintained by selection against hybrid phenotypes, particularly when parental species are locally adapted (Barton & Hewitt, 1985; Kisel & Barraclough, 2010; Mallet & Barton, 1989). Consequently, small islands with distinct habitat transitions may result in particularly narrow hybrid zones (Cooper et al., 2018).

In São Tomé caecilians, the contact zone appears to coincide with the transition between the younger (<1 Ma) and older (3–8 Ma) basaltic lavas in the centre of the island but we did not find a clear association between the parental species or hybrids with our broad classifications of soil type. By contrast, we found significant associations between parental species and hybrids with precipitation (Figure 4b,c) suggesting there may be selection against hybrids in the driest parts of the island resulting in a stable hybrid zone in the centre of the island. In addition, *S. thomense* lack flecking and occur in drier habitats, while *S. ephele* and most hybrid individuals are flecked and occur in wetter habitats indicating there may be habitat-associated selection for divergence in coloration (Lemoine et al., 2019). Divergence in coloration between xeric and mesic habitats across small spatial scales is prevalent in other small island study systems including *Anolis* lizards in the Lesser Antilles (e.g. Lazell, 1972; Muñoz et al., 2013; Thorpe et al., 2015), lizards in the Canary Islands (Brown et al., 1991, 2016; Suárez et al., 2014; Thorpe & Brown, 1989), and Galapagos land snails (Kraemer et al., 2019). Experimental approaches may clarify whether divergent and strong natural selection for locally adapted physiology and/or camouflage underlie correlations between coloration and environment in São Tomé Caecilians.

Differences in mate choice among incipient/recent species can also be an important mechanism for reproductive isolation (Mayr, 1963; Ritchie, 2007) and reinforcement can lead to greater divergence in such traits when hybridization is maladaptive (Butlin, 1987). Courtship behaviour and potential pre- or post-zygotic reproductive barriers in caecilians are very poorly understood, but molecular analyses by Torres-Sánchez et al. (2020) suggest the potential for both sexes to use species-specific peptide pheromones for species recognition and mate choice. Quantifying the peptide pheromone composition of São Tomé caecilians and their hybrids may provide further insights into this signalling modality and its role in speciation. Further, caecilians exhibit internal fertilization via an intromittent organ formed by an eversible portion of the cloaca that varies in shape and ornamentation among species (Gower & Wilkinson,

2002; Wake, 1972). Characterizing variation in phallus morphology among and within lineages of *Schistometopum* on São Tomé and in their East African relatives may clarify whether this structure plays an analogous role in reproductive isolation to the baculum in placental mammals (Ramm, 2007) and hemipenes in squamate reptiles (Klaczko et al., 2015).

5 | CONCLUSIONS

Our study joins a growing number of systems demonstrating speciation at small spatial scales on islands (Bourgeois et al., 2020; Gabrielli et al., 2020; Heaney et al., 2018; Kisel & Barraclough, 2010; Osborne et al., 2020; Savolainen et al., 2006). We propose that transient geographic barriers coupled with local adaption across environmental gradients can contribute to the accumulation of phenotypic and taxonomic diversity. Our integrative morphological and genetic analyses support two discrete lineages corresponding to *S. thomense* and *S. ephele* with a narrow zone of admixture in the centre of São Tomé Island. Demographic modelling supports a history of allopatric divergence in the late Pleistocene followed by secondary contact and hybridization, rather than a scenario of divergence with continuous gene flow. Based on this evolutionary history, we recommend recognizing these lineages as distinct species and remove *S. ephele* Taylor, 1965 from synonymy with *S. thomense* (Bocage 1873).

ACKNOWLEDGEMENTS

For fieldwork on São Tomé we thank the Ministry of Environment (Director General A. de Ceita Carvalho, V. Bonfim, and S. Sousa Pontes) for permission to collect and export specimens for study, STep Up São Tomé (E. N. Seligman, R. dos Santos, and Q. Quade Cabral) and the Omali Lodge for logistical support. We thank L. Esposito, M. A. Jeronimo, R. F. de Lima, L. F. Mendes, B. Simison, and A. Stanbridge for assistance in the field, R. Drewes, R. Stoelting and J. Vindum for collecting many specimens used in this study, and R. Drewes for leading the California Academy of Sciences (CAS) Gulf of Guinea Expeditions. We are grateful to R. Stoelting for generously sharing supporting data from her 2014 study (the volcanic GIS layer and original specimen colour scoring for comparison) and for providing thoughtful feedback on this manuscript. We thank J. Hunt and M. Kweskin at the Laboratories of Analytical Biology at the National Museum of Natural History (NMNH), R. Dikow at the Smithsonian Data Science Laboratory, and A. Lam at the Center for Computational Genomics at CAS for technical support, M. Womack, E. Myers, M. Yuan, R. Schott, and K. de Queiroz for advice in early stages of the project, two high school interns from the NMNH Youth Engagement in Science (YES!) program who helped generate the new mitochondrial sequence data, and M. Fujita, T. Firneno, and J. Maldonado for generously sharing resources from the University of Texas at Arlington. Three reviewers and subject editor Dr. Gillespie provided important insights that improved this manuscript. This work was supported by an NMNH Global Genome Initiative Peter Buck Postdoctoral Fellowship to KAO, an NMNH Peter Buck Postdoctoral Fellowship awarded to IP, a Smithsonian Institution Predoctoral Fellowship to KPM, and the California Academy of Sciences Gulf of Guinea Fund. Most of the laboratory and computer work were conducted in and with the support of the L.A.B. facilities of the National Museum of Natural History (NMNH) and the Smithsonian Institution High Performance Cluster https://doi.org/10.25572/SIHPC.

AUTHOR CONTRIBUTIONS

Kyle A. O'Connell, Lauren A. Scheinberg and Rayna C. Bell conceived of the study. Lauren A. Scheinberg and Rayna C. Bell conducted field sampling. Kyle A. O'Connell collected genetic data and Lauren A. Scheinberg collected morphological data. Kyle A. O'Connell and Ivan Prates implemented analyses with contributions from Kevin P. Mulder and Rayna C. Bell. Kyle A. O'Connell, Rayna C. Bell and Ivan Prates drafted the manuscript with contributions from all authors.

DATA AVAILABILITY STATEMENT

Genomic data have been deposited on the SRA database (SRR12676998–SRR12677081), mtDNA deposited on Genbank (MW290290–MW290301). Input files and scripts for all analyses have been deposited on figshare (https://doi.org/10.25573/data.13085159.v1) and github.com/kyleaoconnell22/sao_tome_caecilians.

ORCID

Kyle A. O'Connell https://orcid.org/0000-0002-0464-9259
Ivan Prates https://orcid.org/0000-0001-6314-8852
Kevin P. Mulder https://orcid.org/0000-0001-6688-8848
Rayna C. Bell https://orcid.org/0000-0002-0123-8833

REFERENCES

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*, 1655–1664. https://doi.org/10.1101/gr.094052.109

Allio, R., Donega, S., Galtier, N., & Nabholz, B. (2017). Large variation in the ratio of mitochondrial to nuclear mutation rate across animals: Implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. *Molecular Biology and Evolution*, *34*, 276–6772. https://doi.org/10.1093/molbev/msx197

Barfod, D. N., & Fitton, J. G. (2014). Pleistocene volcanism on São Tomé, Gulf of Guinea, West Africa. *Quaternary Geochronology*, *21*, 77–89. https://doi.org/10.1016/j.quageo.2012.11.006

Barratt, C. D., Bwong, B. A., Jehle, R., Liedtke, H. C., Nagel, P., Onstein, R. E., Portik, D. M., Streicher, J. W., & Loader, S. P. (2018). Vanishing refuge? Testing the forest refuge hypothesis in coastal East Africa using genome-wide sequence data for seven amphibians. *Molecular Ecology*, *27*, 4289–4308. https://doi.org/10.1111/mec.14862

Barton, N. H., & Hewitt, G. M. (1985). Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, *16*, 113–148. https://doi.org/10.1146/annurev.es.16.110185.000553

Bell, R. C., Drewes, R. C., Channing, A., Gvoždík, V., Kielgast, J., Lötters, S., & Zamudio, K. R. (2015). Overseas dispersal of *Hyperolius* reed frogs from Central Africa to the oceanic islands of São Tomé and Príncipe. *Journal of Biogeography*, *42*, 65–75. https://doi.org/10.1111/jbi.12412

Bell, R. C., Drewes, R. C., & Zamudio, K. R. (2015). Reed frog diversification in the Gulf of Guinea: Overseas dispersal, the progression

rule, and in situ speciation. *Evolution*, *69*, 904–915. https://doi.org/10.1111/evo.12623

Bell, R. C., & Irian, C. G. (2019). Phenotypic and genetic divergence in reed frogs across a mosaic hybrid zone on São Tomé Island. *Biological Journal of the Linnean Society*, *128*, 672–680. https://doi.org/10.1093/biolinnean/blz131

Bloor, P., Kemp, S. J., & Brown, R. P. (2008). Recent volcanism and mitochondrial DNA structuring in the lizard *Gallotia atlantica* from the island of Lanzarote. *Molecular Ecology*, *17*, 854–866. https://doi.org/10.1111/j.1365-294x.2007.03575.x

Bourgeois, Y. X., Bertrand, J. A., Delahaie, B., Holota, H., Thébaud, C., & Milá, B. (2020). Differential divergence in autosomes and sex chromosomes is associated with intra-island diversification at a very small spatial scale in a songbird lineage. *Molecular Ecology*, *29*(6), 1137–1153. https://doi.org/10.1111/mec.15396

Bradburd, G. S., Coop, G. M., & Ralph, P. L. (2018). Inferring continuous and discrete population genetic structure across space. *Genetics*, *210*, 33–52. https://doi.org/10.1534/genetics.118.301333

Brochmann, C. (1984). Hybridization and distribution of *Argyranthemum coronopifolium* (Asteraceae–Anthemideae) in the Canary Islands. *Nordic Journal of Botany*, *4*, 729–736. https://doi.org/10.1111/j.1756-1051.1984.tb02001.x

Brown, R. P., Paterson, S., & Risse, J. (2016). Genomic signatures of historical allopatry and ecological divergence in an island lizard. *Genome Biology & Evolution*, *8*(11), 3618–3626. https://doi.org/10.1093/gbe/evw268

Brown, R. P., Thorpe, R. S., & Báez, M. (1991). Parallel within-island microevolution of lizards on neighbouring islands. *Nature*, *352*(6330), 60–62.

Brown, R. P., Woods, M., & Thorpe, R. S. (2017). Historical volcanism and within-island genetic divergence in the Tenerife skink (*Chalcides viridanus*). *Biological Journal of the Linnean Society*, *122*, 166–175.

Butlin, R. (1987). Speciation by reinforcement. *Trends in Ecology and Evolution*, *2*, 8–13. https://doi.org/10.1016/0169-5347(87)90193-5

Caldeira, R. J., & Munhá, J. M. (2002). Petrology of ultramafic nodules from São Tomé Island, Cameroon volcanic line (oceanic sector). *Journal of African Earth Sciences*, *34*, 231–246. https://doi.org/10.1016/s0899-5362(02)00022-2

Choi, J. Y., Purugganan, M., & Stacy, E. A. (2020). Divergent selection and primary gene flow shape incipient speciation of a riparian tree on Hawaii Island. *Molecular Biology and Evolution*, *37*, 695–710.

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Comeault, A. A., & Matute, D. R. (2021). Temperature-dependent competitive outcomes between the fruit flies *Drosophila santomea* and *Drosophila yakuba*. *The American Naturalist*, *197*(3), 312–323.

Cooper, B. S., Sedghifar, A., Nash, W. T., Comeault, A. A., & Matute, D. R. (2018). A maladaptive combination of traits contributes to the maintenance of a *Drosophila* hybrid zone. *Current Biology*, *28*, 2940–2947.

Coyne, J. A., Kim, S. Y., Chang, A. S., Lachaise, D., & Elwyn, S. (2002). Sexual isolation between two sibling species with overlapping ranges: *Drosophila santomea* and *Drosophila yakuba*. *Evolution*, *56*, 2424–2434.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*, 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Daniels, S. R., & Klaus, S. (2018). Divergent evolutionary origins and biogeographic histories of two freshwater crabs (Brachyura: *Potamonautes*) on the West African conveyer belt islands of São Tomé and Príncipe. *Molecular Phylogenetics and Evolution*, *127*, 119–128. https://doi.org/10.1016/j.ympev.2018.05.016

De lima, R. F., Sampaio, H., Dunn, J. C., Cabinda, G., Fonseca, R., Oquiongo, G., Oquiongo, J., Samba, S., Santana, A., Soares, E., Viegas, L., Ward-francis, A., Costa, L. T., Palmeirim, J. M., & Buchanan, G. M. (2017). Distribution and habitat associations of the critically endangered bird species of São Tomé Island (Gulf of Guinea). *Bird Conservation International*, *27*, 455–469. https://doi.org/10.1017/s0959270916000241

Dray, S., & Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, *22*, 1–20. https://doi.org/10.18637/jss.v022.i04

Eaton, D. A., & Overcast, I. (2020). ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*, *36*, 2592–2594. https://doi.org/10.1093/bioinformatics/btz966

Esselstyn, J. A., Timm, R. M., & Brown, R. M. (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. https://doi.org/10.1111/j.1558-5646.2009.00743.x

Excoffier, L., Dupanloup, I., Huerta-Sánchez, E., Sousa, V. C., & Foll, M. (2013). Robust demographic inference from genomic and SNP data. *PLoS Genetics*, *9*, e1003905. https://doi.org/10.1371/journal.pgen.1003905

Fitzpatrick, B. M. (2012). Estimating ancestry and heterozygosity of hybrids using molecular markers. *BMC Evolutionary Biology*, *12*, 131. https://doi.org/10.1186/1471-2148-12-131

Fouquet, A., Leblanc, K., Framit, M., Réjaud, A., Rodrigues, M. T., Castroviejo-Fisher, S., Peloso, P. L. V., Prates, I., Manzi, S., Suescun, U., Baroni, S., Moraes, L. J. C. L., Recoder, R., Marques-Souza, S., Dal-Vecchio, F., Camacho, A., Guellere, J. M., Rojas-Runjaic, F. J. M., Gagliardi-Urrutia, G., & Fabre, A. C. (2021). Species diversity and biogeography of an ancient frog clade from the Guiana Shield (Anura: Microhylidae: Adelastes, Otophryne, Synapturanus) exhibiting spectacular phenotypic diversification. *Biological Journal of the Linnean Society*, *132*, 233–256.

Gabrielli, M., Nabholz, B., Leroy, T., Milá, B., & Thébaud, C. (2020). Within-island diversification in a passerine bird. *Proceedings of the Royal Society B*, *287*, 20192999. https://doi.org/10.1111/j.1558-5646.2011.01430.x

García-Olivares, V., López, H., Patiño, J., Alvarez, N., Machado, A., Carracedo, J. C., Soler, V., & Emerson, B. C. (2017). Evidence for mega-landslides as drivers of island colonization. *Journal of Biogeography*, *44*(5), 1053–1064.

Garrick, R. C., Benavides, E., Russello, M. A., Hyseni, C., Edwards, D. L., Gibbs, J. P., Tapia, W., Ciofi, C., & Caccone, A. (2014). Lineage fusion in Galápagos giant tortoises. *Molecular Ecology*, *23*, 5276–5290. https://doi.org/10.1111/mec.12919

Gillespie, R. G., & Clague, D. A. (Eds.). (2009). *Encyclopedia of Islands* (No. 2). University of California Press.

Gow, J. L., Peichel, C. L., & Taylor, E. B. (2006). Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. *Molecular Ecology*, *15*, 739–752. https://doi.org/10.1111/j.1365-294x.2006.02825.x

Gower, D. J., & Wilkinson, M. (2002). Phallus morphology in caecilians (Amphibia, Gymnophiona) and its systematic utility. *Bulletin of the Natural History Museum: Zoology Series*, *68*(2), 143–154. https://doi.org/10.1017/s096804700200016x

Grant, P. R., & Grant, B. R. (1996). Speciation and hybridization in island birds. *Philosophical Transactions of the Royal Society of London B*, *351*, 765–772. https://doi.org/10.1515/9781400831920.326

Gronau, I., Hubisz, M. J., Gulko, B., Danko, C. G., & Siepel, A. (2011). Bayesian inference of ancient human demography from individual

genome sequences. *Nature Genetics*, *43*, 1031–1034. https://doi.org/10.1038/ng.937

Gübitz, T., Thorpe, R. S., & Malhotra, A. (2005). The dynamics of genetic and morphological variation on volcanic islands. *Proceedings of the Royal Society B.*, *272*, 751–757. https://doi.org/10.1098/rspb.2004.3018

Gundappa, K. R., Balakrishna, T. A., & Shakuntala, K. (1981). Ecology of *Ichthyophis glutinosus* (Linn.)(Apoda, Amphibia). *Current Science*, *1981*, 480–483.

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics*, *5*, e1000695. https://doi.org/10.1371/journal.pgen.1000695

Haft, J. (1992). Bemerkungen zu den Blindwühlen der Gattung *Schistometopum* von São Tomé (Gymnophiona, Caeciliidae). *Bonner Zoologische Beiträge*, *43*, 477–479.

Haft, J., & Franzen, M. (1996). Freilandbeobachtungen, Verhalten und Nachzucht der São Tomé- Blindwühle *Schistometopum thomense* (Bocage, 1873). *Herpetofauna*, *18*, 5–11.

Heaney, L. R., Kyriazis, C. C., Balete, D. S., Steppan, S. J., & Rickart, E. A. (2018). How small an island? Speciation by endemic mammals (Apomys, Muridae) on an oceanic Philippine island. *Journal of Biogeography*, *45*(7), 1675–1687.

Heyer, R., Donnelly, M. A., Foster, M., & Mcdiarmid, R. (Eds.). (2014). *Measuring and monitoring biological diversity: Standard methods for amphibians*. Smithsonian Institution.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology: A Journal of the Royal Meteorological Society*, *25*, 1965–1978. https://doi.org/10.1002/joc.1276

Ilut, D. C., Nydam, M. L., & Hare, M. P. (2014). Defining loci in restriction-based reduced representation genomic data from nonmodel species: Sources of bias and diagnostics for optimal clustering. *Biomed Research International*, *2014*, 1–9. https://doi.org/10.1155/2014/675158

Jones, D. T., Loader, S. L., & Gower, D. J. (2006). Trophic ecology of East African caecilians (Amphibia: Gymnophiona), and their impact on forest soil invertebrates. *Journal of Zoology*, *269*, 117–126.

Juan, C., Emerson, B. C., Oromí, P., & Hewitt, G. M. (2000). Colonization and diversification: Towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology & Evolution*, *15*, 104–109.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kisel, Y., & Barraclough, T. G. (2010). Speciation has a spatial scale that depends on levels of gene flow. *The American Naturalist*, *175*, 316–334. https://doi.org/10.1086/650369

Klaczko, J., Ingram, T., & Losos, J. (2015). Genitals evolve faster than other traits in *Anolis* lizards. *Journal of Zoology*, *295*, 44–48. https://doi.org/10.1111/jzo.12178

Kouete, M. T., & Blackburn, D. C. (2020). Dietary partitioning in two co-occurring caecilian species (*Geotrypetes seraphini* and *Herpele squalostoma*) in Central Africa. *Integrative Organismal Biology*, *2*, obz035. https://doi.org/10.1093/iob/obz035

Kraemer, A. C., Philip, C. W., Rankin, A. M., & Parent, C. E. (2019). Trade-offs direct the evolution of coloration in Galápagos land snails. *Proceedings of the Royal Society B*, *286*, 20182278. https://doi.org/10.1098/rspb.2018.2278

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, *23*(21), 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Lazell, J. D. (1972). *The anoles (Sauria, Iguanidae) of the lesser Antilles*, Vol. *143*. Harvard University.

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*, 1110–1116. https://doi.org/10.1111/2041-210x.12410

Lemoine, M., Barluenga, M., Lucek, K., Mwaiko, S., Haesler, M., Chapman, L. J., Chapman, C. A., & Seehausen, O. (2019). Recent sympatric speciation involving habitat-associated nuptial colour polymorphism in a crater lake cichlid. *Hydrobiologia*, *832*(1), 297–315.

Linck, E., & Battey, C. J. (2019). Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. *Molecular Ecology Resources*, *19*, 639–647. https://doi.org/10.1111/1755-0998.12995

Llopart, A., Elwyn, S., Lachaise, D., & Coyne, J. A. (2002). Genetics of a difference in pigmentation between *Drosophila yakuba* and *Drosophila santomea*. *Evolution*, *56*, 2262–2277.

Llopart, A., Lachaise, D., & Coyne, J. A. (2005). An anomalous hybrid zone in *Drosophila*. *Evolution*, *59*, 2602–2607. https://doi.org/10.1111/j.0014-3820.2005.tb00972.x

Loader, S. P., Pisani, D., Cotton, J. A., Gower, D. J., Day, J. J., & Wilkinson, M. (2007). Relative time scales reveal multiple origins of parallel disjunct distributions of African caecilian amphibians. *Biology Letters*, *3*, 505–508. https://doi.org/10.1098/rsbl.2007.0266

Losos, J. B., & Ricklefs, R. E. (2009). Adaptation and diversification on islands. *Nature*, *457*, 830–836. https://doi.org/10.1038/nature07893

Losos, J. B., & Schluter, D. (2000). Analysis of an evolutionary species–area relationship. *Nature*, *408*, 847–850. https://doi.org/10.1038/35048558

MacLeod, A., Rodríguez, A., Vences, M., Orozco-terWengel, P., García, C., Trillmich, F., Gentile, G., Caccone, A., Quezada, G., & Steinfartz, S. (2015). Hybridization masks speciation in the evolutionary history of the Galápagos marine iguana. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20150425. https://doi.org/10.1098/rspb.2015.0425

Mallet, J., & Barton, N. (1989). Inference from clines stabilized by frequency-dependent selection. *Genetics*, *122*, 967–976.

Marques, D. A., Lucek, K., Meier, J. I., Mwaiko, S., Wagner, C. E., Excoffier, L., & Seehausen, O. (2016). Genomics of rapid incipient speciation in sympatric threespine stickleback. *PLOS Genetics*, *12*, e1005887. https://doi.org/10.1371/journal.pgen.1005887

Martín, J., López, P., & García, L. V. (2013). Soil characteristics determine microhabitat selection of the fossorial amphisbaenian *Trogonophis wiegmanni*. *Journal of Zoology*, *290*, 265–272. https://doi.org/10.1111/jzo.12033

Matute, D. R., & Coyne, J. A. (2010). Intrinsic reproductive isolation between two sister species of *Drosophila*. *Evolution*, *64*, 903–920. https://doi.org/10.1111/j.1558-5646.2009.00879.x

Mayr, E. (1963). *Animal species and evolution*. Belknap Press.

Measey, G. J. (2004). Are caecilians rare? An east African perspective. *Journal of East African Natural History*, *93*, 1–21.

Measey, G. J. (2006). Surveying biodiversity of soil herpetofauna: Towards a standard quantitative methodology. *European Journal of Soil Biology*, *42*, S103–S110.

Measey, G. J., Gower, D. J., Oommen, O. V., & Wilkinson, M. (2003). Quantitative surveying of endogeic limbless vertebrates—A case study of *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae) in southern India. *Applied Soil Ecology*, *23*, 43–53. https://doi.org/10.1016/s0929-1393(02)00175-0

Measey, G. J., & Van Dongen, S. (2006). Bergmann's rule and the terrestrial caecilian *Schistometopum thomense* (Amphibia: Gymnophiona: Caeciliidae). *Evolutionary Ecology Research*, *8*, 1049–1059. https://doi.org/10.1080/08927014.2004.9522635

Milá, B., Warren, B. H., Heeb, P., & Thébaud, C. (2010). The geographic scale of diversification on islands: Genetic and morphological divergence at a very small spatial scale in the Mascarene grey white-eye (Aves: Zosterops borbonicus). *BMC Evolutionary Biology*, *10*(1), 1–13.

Muñoz, M. M., Crawford, N. G., Mcgreevy, T. J. Jr., Messana, N. J., Tarvin, R. D., Revell, L. J., Zandvliet, R. M., Hopwood, J. M., Mock, E., Schneider, A. L., & Schneider, C. J. (2013). Divergence in coloration and ecological speciation in the *Anolis marmoratus* species complex. *Molecular Ecology*, *22*(10), 2668–2682.

Myers, E. A., Xue, A. T., Gehara, M., Cox, C. L., Davis Rabosky, A. R., Lemos-Espinal, J., Martínez-Gómez, J. E., & Burbrink, F. T. (2019). Environmental heterogeneity and not vicariant biogeographic barriers generate community-wide population structure in desert-adapted snakes. *Molecular Ecology*, *28*, 4535–4548. https://doi.org/10.1111/mec.15182

Nater, A., Nietlisbach, P., Arora, N., van Schaik, C. P., van Noordwijk, M. A., Willems, E. P., Singleton, I., Wich, S. A., Goossens, B., Warren, K. S., Verschoor, E. J., Perwitasari-Farajallah, D., Pamungkas, J., & Krützen, M. (2011). Sex-biased dispersal and volcanic activities shaped phylogeographic patterns of extant orangutans (genus: *Pongo*). *Molecular Biology and Evolution*, *28*, 2275–2288. https://doi.org/10.1093/molbev/msr042

Nussbaum, R. A., & Pfrender, M. E. (1998). Revision of the African Caecilian Genus Schistometopum Parker (Amphibia: Gymnophiona: Caeciliidae). *Miscellaneous Publications of the Museum of Zoology University of Michigan*, *187*, 32. https://doi.org/10.1080/08927014.2004.9522635

O'Connell, K. A., Hamidy, A., Kurniawan, N., Smith, E. N., & Fujita, M. K. (2018). Synchronous diversification of parachuting frogs (Genus *Rhacophorus*) on Sumatra and Java. *Molecular Phylogenetics and Evolution*, *123*, 101–112. https://doi.org/10.1016/j.ympev.2018.02.003

Osborne, O. G., Kafle, T., Brewer, T., Dobreva, M. P., Hutton, I., & Savolainen, V. (2020). Sympatric speciation in mountain roses (*Metrosideros*) on an oceanic island. *Philosophical Transactions of the Royal Society B*, *375*(1806), 20190542.

Pestano, J., & Brown, R. P. (1999). Geographical structuring of mitochondrial DNA in *Chalcides sexlineatus* within the island of Gran Canaria. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*, 805–812. https://doi.org/10.1098/rspb.1999.0709

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*, e37135. https://doi.org/10.1371/journal.pone.0037135

Portik, D. M., Leaché, A. D., Rivera, D., Barej, M. F., Burger, M., Hirschfeld, M., Rödel, M.-O., Blackburn, D. C., & Fujita, M. K. (2017). Evaluating mechanisms of diversification in a Guineo-Congolian tropical forest frog using demographic model selection. *Molecular Ecology*, *26*, 5245–5263. https://doi.org/10.1111/mec.14266

Prates, I., Penna, A., Rodrigues, M. T., & Carnaval, A. C. (2018). Local adaptation in mainland anole lizards: Integrating population history and genome–environment associations. *Ecology and Evolution*, *8*, 11932–11944. https://doi.org/10.1002/ece3.4650

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*, 901–904. https://doi.org/10.1093/sysbio/syy032

Ramm, S. A. (2007). Sexual selection and genital evolution in mammals: A phylogenetic analysis of baculum length. *American Naturalist*, *169*, 360–369. https://doi.org/10.1086/510688

Ritchie, M. G. (2007). Sexual selection and speciation. *Annual Review of Ecology, Evolution, and Systematics*, *38*, 79–102. https://doi.org/10.1146/annurev.ecolsys.38.091206.095733

Roderick, G. K., Croucher, P. J., Vandergast, A. G., & Gillespie, R. G. (2012). Species differentiation on a dynamic landscape: shifts in metapopulation genetic structure using the chronology of the Hawaiian archipelago. *Evolutionary Biology*, *39*(2), 192–206.

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*, 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Sardell, J. M., & Uy, J. A. (2016). Hybridization following recent secondary contact results in asymmetric genotypic and phenotypic introgression between island species of *Myzomela* honeyeaters. *Evolution*, *70*, 257–269. https://doi.org/10.1111/evo.12864

Savolainen, V., Anstett, M. C., Lexer, C., Hutton, I., Clarkson, J. J., Norup, M. V., & Baker, W. J. (2006). Sympatric speciation in palms on an oceanic island. *Nature*, *441*(7090), 210–213.

Seehausen, O., Van Alphen, J. J. M., & Lande, R. (2001). Color polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. *Ecology Letters*, *2*, 367–378. https://doi.org/10.1046/j.1461-0248.1999.00098.x

Servidio, M. R., & Noor, M. A. (2003). The role of reinforcement in speciation: theory and data. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 339–364.

Soares, F. M. (2017). *Modelling the distribution of São Tomé bird species: Ecological determinants and conservation prioritization*. (Unpublished Dissertation) Universidade de Lisboa.

Stenson, A. G., Malhotra, A., & Thorpe, R. S. (2002). Population differentiation and nuclear gene flow in the Dominican anole (*Anolis oculatus*). *Molecular Ecology*, *11*(9), 1679–1688.

Stoelting, R. E., Measey, G. J., & Drewes, R. C. (2014). Population genetics of the São Tomé caecilian (Gymnophiona: Dermophiidae: *Schistometopum thomense*) reveals strong geographic structuring. *PLoS One*, *9*, e104628. https://doi.org/10.1371/journal.pone.0104628

Suárez, N. M., Pestano, J., & Brown, R. P. (2014). Ecological divergence combined with ancient allopatry in lizard populations from a small volcanic island. *Molecular Ecology*, *23*, 4799–4812. https://doi.org/10.1111/mec.12897

Taylor, E. D. (1965). New Asiatic arid African caecilians with redescriptions of certain other species. *University of Kansas Science Bulletin*, *46*, 253–302. https://doi.org/10.5962/bhl.part.20077

Taylor, E. B., Boughman, J. W., Groenenboom, M., Sniatynski, M., Schluter, D., & Gow, J. L. (2006). Speciation in reverse: Morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Molecular Ecology*, *15*, 343–355. https://doi.org/10.1111/j.1365-294x.2005.02794.x

Thorpe, R. S., Barlow, A., Malhotra, A., & Surget-Groba, Y. (2015). Widespread parallel population adaptation to climate variation across a radiation: Implications for adaptation to climate change. *Molecular Ecology*, *24*(5), 1019–1030.

Thorpe, R. S., & Brown, R. P. (1989). Microgeographic variation in the colour pattern of the lizard *Gallotia galloti* within the island of Tenerife: Distribution, pattern and hypothesis testing. *Biological Journal of the Linnean Society*, *38*(4), 303–322.

Torres-Sánchez, M., Gower, D. J., Alvarez-Ponce, D., Creevey, C. J., Wilkinson, M., & San Mauro, D. (2019). What lies beneath? Molecular evolution during the radiation of caecilian amphibians. *BMC Genomics*, *20*(1), 1–13. https://doi.org/10.1186/s12864-019-5694-1

Torres-Sánchez, M., Wilkinson, M., Gower, D. J., Creevey, C. J., & San Mauro, D. (2020). Insights into the skin of caecilian amphibians from gene expression profiles. *BMC Genomics*, *21*, 1–9.

Vences, M., Wollenberg, K. C., Vieites, D. R., & Lees, D. C. (2009). Madagascar as a model region of species diversification. *Trends in Ecology & Evolution*, *24*, 456–465. https://doi.org/10.1016/j.tree.2009.03.011

Wake, M. H. (1972). Evolutionary morphology of the caecilian urogenital system. IV. The cloaca. *Journal of Morphology*, *136*, 353–365. https://doi.org/10.1002/jmor.1051360308

Welton, L. J., Siler, C. D., Bennett, D., Diesmos, A., Duya, M. R., Dugay, R., Rico, E. L. B., Van Weerd, M., & Brown, R. M. (2010). A spectacular new Philippine monitor lizard reveals a hidden biogeographic boundary and a novel flagship species for conservation. *Biology Letters*, *6*, 654–658. https://doi.org/10.1098/rsbl.2010.0119

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: O'Connell KA, Prates I, Scheinberg LA, Mulder KP, Bell RC. Speciation and secondary contact in a fossorial island endemic, the São Tomé caecilian. *Mol Ecol*. 2021;30:2859–2871. https://doi.org/10.1111/mec.15928