Running title: Climate adaptation in tropical island frogs

Survival of climate warming through niche shifts: Evidence from frogs on tropical islands

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Abstract

How will organisms cope when forced into warmer-than-preferred thermal environments? This is a key question facing our ability to monitor and manage biota as average annual temperatures increase, and is of particular concern for range-limited terrestrial species unable to track their preferred climatic envelope. Being ectothermic, desiccation-prone, and often spatially restricted, island-inhabiting tropical amphibians exemplify this scenario. Pre-Anthropocene case studies of how insular amphibian populations responded to the enforced occupation of warmer-than-ancestral habitats may add a valuable, but currently lacking, perspective. We studied a population of frogs from the Seychelles endemic family Sooglossidae which due to historic sea level rise, have been forced to occupy a significantly warmer island (Praslin) than their ancestors and close living relatives. Evidence from thermal activity patterns, bioacoustics, body size distributions, and ancestral state estimations suggest this population shifted its thermal niche in response to restricted opportunities for elevational dispersal. Relative to conspecifics, Praslin sooglossids also have divergent nuclear genotypes and call characters, a finding consistent with adaptation causing speciation in a novel thermal environment. Using an evolutionary perspective, our study reveals that some tropical amphibians have survived episodes of historic warming without the aid of dispersal and therefore may have the capacity to adapt to the currently warming climate. However, two otherwise co-distributed sooglossid species are absent from Praslin, and the deep evolutionary divergence between the frogs on Praslin and their closest extant relatives (~8 million years) may have allowed for gradual thermal adaptation and speciation. Thus, local extinction is still a likely outcome for tropical frogs experiencing warming climates in the absence of dispersal corridors to thermal refugia.

1 Introduction

Species responses to climate change typically result in shifts in distribution, activity pattern, or adaptation to novel climatic conditions (Bradshaw & Holzapfel, 2006; Guerrero, Rosas, Arroyo, & Wiens, 2013; Hoffmann & Sgro, 2011; Parmesan, 2006), but many taxa are considered limited in their ability to respond either behaviourally or physiologically to increases in temperature (Hoffmann & Sgro, 2011; Sinervo et al., 2010). Worryingly, the speed of anthropogenic climate change may prevent many from tracking their preferred climate envelope (Collins & Storfer, 2003; Parmesan, 2006; Parra-Olea, Martinez-Meyer, & de Leon, 2005), with tropical and island species thought to be especially vulnerable (Cang, Wilson, & Wiens, 2016; Jezkova & Wiens, 2016; Foden et al., 2013). Response limitations are most notable in taxa such as amphibians, which are ectothermic, dispersal-limited, and prone to desiccation, leading to significantly lower rates of climatic niche evolution than in endothermic vertebrates (Rolland et al., 2018). Furthermore, and assuming climatic niche conservatism, many studies have predicted that amphibian ranges will fragment and disappear as temperatures warm (Bickford, Howard, Ng, & Sheridan, 2010; Blaustein et al., 2010; Bonetti & Wiens, 2014; Duan, Kong, Huang, Varela, & Ji, 2016). Indeed, populations exposed to less variation in historic temperature regimes may be more sensitive to climate warming (Campbell Grant, Miller, & Muth, 2020; Trisos, Merow, & Pigot, 2020).

Physiological research has suggested that most tropical amphibian species will be forced into the upper levels of their thermal tolerance under global climate projections (von May et al., 2019). However, climate-induced shifts in amphibian distributions are expected to vary geographically (Miller et al., 2018), and some species may have the capacity to respond to warming temperatures via upper thermal tolerance plasticity and variation, i.e. an increased ability to tolerate thermal regimes closer to a species' Critical Thermal Maximum (CT_{max}) (Simon, Ribeiro, & Navas, 2015). Despite the possibility of adaptive capacity, field-based research indicates that Anthropocene range reductions have begun to occur in both tropical and temperate environments. This includes upslope displacement observed in Malagasy frogs (Raxworthy et al., 2008) and reduced range sizes in European and North American amphibians (Winter et al., 2016). Recent climate change is also related to bioacoustic and body size shifts in tropical frogs, leading to potentially deleterious effects on breeding success, resulting in population declines and reduced biomass (Narins & Meenderink, 2014), with subsequent negative implications for food-web dynamics in both terrestrial and aquatic ecosystems (Beard, Vogt, & Kulmatiski, 2002; Whiles et al., 2006).

Variation in thermal environments likely played a prominent role during the pre-Anthropocene diversification and extinction of amphibians (e.g. see Feng et al., 2017; Mors, Reguero, & Vasilyan, 2020; Muzzopappa & Báez, 2009). Elucidating pre-Anthropocene outcomes to warming therefore offers a valuable lens through which to predict responses of extant taxa, yet investigations of this nature are scarce for amphibians, likely due to their scant fossil record and analytical challenges of reconstructing ancestral climate envelopes (Anderson, 2012; Vieites, Nieto-Román, & Wake, 2009). Islands are widely acknowledged as hotspots of both geophysical and biological diversity, and priority systems for conservation. The systems they support are many and varied, and often characterised by relatively small but ecologically significant differences in microclimate and ecology, giving rise to high levels of habitat diversity, and organismal endemicity. Island-endemic amphibian radiations therefore offer a unique opportunity to explore ancient responses to variation in thermal environment, providing key elements for hypothesis testing. First, variation in elevation causes variable thermal landscapes on different islands, offering the ability to test for differences in thermal niche by comparing elevational distributions. Second, islands provide natural replicates for comparing intraspecific traits that may be influenced by temperature. Finally, extinction or exclusion events may be inferred by comparing assemblage structure on different islands, and this can be related to estimated thermal niches and available thermal landscapes.

The frog family Sooglossidae Noble, 1931 are of Gondwanan origin (Biju & Bossuyt, 2003; Feng et al., 2017; Frazão et al., 2015), and endemic to the inner islands of the Seychelles archipelago (being emergent sections of an isolated Gondwanan fragment) and includes four species: *Sooglossus sechellensis* (Boettger, 1896), *Sooglossus thomasetti* (Boulenger 1909), *Sechellophryne gardineri* (Boulenger, 1911) and *Sechellophryne pipilodryas* (Gerlach & Willi, 2002). Known primarily from two islands in the Seychelles—Mahé and Silhouette—in 2009 a population of *So. sechellensis* was discovered on a third island: Praslin (Labisko et al. 2015; Labisko et al. 2019; Taylor et al., 2012; Fig. 1). Praslin (367 m elev.) differs in that it lacks the high-elevation mist-forest habitat found on Mahé (905 m elev.) and Silhouette (740 m elev.). As sooglossids occur primarily in mid- to high-elevation forest habitats on Mahé and Silhouette (Nussbaum, 1984) and no other sooglossid taxa are found on Praslin, persisting in the warmer and drier environmental conditions on Praslin likely required adaptation in *So. sechellensis*.

To test for adaptation, we used an experimental framework based on the principle of severity that is ideal for closely-related organisms (Cox & Logan, 2021). Our overarching hypothesis was that *So. sechellensis* from Praslin have adapted to warmer habitats than other sooglossids, allowing them to persist on an island with limited opportunities for upslope dispersal. We tested this hypothesis across multiple levels of biological organisation using the following four expectations that *So. sechellensis* from

Praslin should: (1) occur at significantly lower elevational distribution, with corresponding warmer climatic conditions, compared to other sooglossids (evidence that they occupy warmer habitats); (2) be active at significantly warmer temperatures than conspecifics (evidence that phenological shifts do not explain any observed differences in distribution); (3) have vocalisations with higher pulse rates and longer call durations than conspecifics (evidence they are calling with warmer body temperatures than conspecifics; Gerhardt & Huber, 2002); (4) have larger body sizes/masses than their conspecifics (evidence they are more robust to temperature-associated desiccation; Levy & Heald, 2015).

To place trait variation among sooglossids in a broader evolutionary context, we also inferred a family-level phylogeny and estimated divergence times. Using elevational distribution as a proxy for climatic niche, we then tested whether *So. sechellensis* on Praslin differed from ancestral sooglossids. Finally, based on the results of our integrative testing framework we compared multi-genomic (mitochondrial and nuclear) and bioacoustic character variation among different island populations of *So. sechellensis* to test if the putative niche shift on Praslin was associated with speciation-level divergence.

2 Methods

2.1 Expectation 1: Locality data, elevation and climate tests

We collected locality data for sooglossid frogs following visual encounter (substrate/leaf-litter searches) and bioacoustic monitoring field surveys between September 2011 and January 2019, and supplemented this with additional data from four sources: (1) data arising from Labisko et al. (2019); (2) data reported by Taylor et al. (2012); (3) data from museum specimens held in the University of Michigan Museum of Zoology, and the University Museum of Zoology, Cambridge (Gerlach & Willi, 2002) collections; and (4) data arising from Senterre et al. (2013a) and Senterre & Kaiser-Bunbury (2014). To infer elevation for cases where only locality data/coordinates were reported, and to standardise elevation data from all previously georeferenced and sampled (waypoint collected) localities, we used the digital elevation model (DEM) database of GPS Visualizer (http://www.gpsvisualizer.com/elevation) which applies the best available DEM (e.g. USGS, NASA) for a given location. For our data, NASA's SRTM3 (90 m resolution) was the best available DEM. All locality data are reported in Appendix S1.

We tested for differences in elevational distribution using Kruskal-Wallis *H* tests, and pairwise Dunn's *post-hoc* tests with Bonferroni correction. We first tested for intraspecific differences among populations of *So. sechellensis* from all three islands: Mahé (N = 237), Silhouette (N = 154), and Praslin (N = 135). Second, we tested for interspecific difference by comparing *So. sechellensis* from Praslin to all other sooglossids including: all other *So. sechellensis* (combined Mahé and Silhouette; N = 391); *So. thomasetti* (combined Mahé and Silhouette; N = 87); *Se. gardineri* (combined Mahé and Silhouette; N = 137); and *Se. pipilodryas* (N = 27). Statistical analyses were performed in PAST v. 3.25 (Hammer, Harper, & Ryan, 2001).

Using sooglossid occurrences we obtained environmental data from the WorldClim (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) and Global Aridity Index (Trabucco & Zomer, 2018) databases, downloaded at 30 arc-seconds (~1 km^2) resolution and projected in QGIS (QGIS Development Team, 2019). We then used these data to (1) derive summary statistics and (2) test for significant differences among island temperature and precipitation bioclim variables for Mahé, Silhouette, and Praslin using Kruskal-Wallis *H* tests, and pairwise Dunn's *post-hoc* tests with Bonferroni correction (Table 1).

2.2 Expectations 2 & 3: Collection and analysis of bioacoustic and local climate data

We collected bioacoustic data from different populations of *So. sechellensis*. Most sooglossid frogs are small and secretive denizens of the forest floor that vocalise from beneath leaf litter, substrate, or from crevices within root systems and boulder piles. They can be heard at any time of day, especially in response to rainfall. However, *So. sechellensis* activity peaks during sunrise and sunset (Edgar, 2003) with field observations suggesting that dusk is the period of greatest activity (Labisko, unpublished data). Between April 2012 and March 2013, we recorded *So. sechellensis* vocalisations across 74 surveys at three locations on Mahé, four on Praslin, and two on Silhouette. Surveys were performed by the same person (J. Labisko) with alternating visits to Mahé and Praslin over the survey period (see Appendix S2: Table S1). We targeted individual sooglossid activity and made uninterrupted recordings spanning ~180 minutes (i.e. ~90 minutes before and after sunset). We recorded calls with a Rycote® pistol-grip mounted Sennheiser® ME66 directional microphone with Rycote® Softee and K6 power unit, connected via Hi-Fi quality digital stereo cable to a Roland® R05 Digital Audio Recorder. An uncompressed sampling rate of 48.0 kHz recorded 16-bit WAV files to a 16 GB SanDisk® SD card.

Measurement of air temperature (T_a) and relative humidity (RH) were recorded every 30 minutes and later matched to individual calls based on timing (see Appendix S2). We used an Omega® PTH-1XA Digital Temperature/Humidity Pen fixed at a height of approximately 1 m above the ground to measure T_a and RH (T_a accuracy: ± 1°C; RH accuracy ±5% at 25°C). To test for differences in calling temperature among *So.*

sechellensis from Mahé, Praslin, and Silhouette, we used a Kruskal-Wallis *H* test, and pairwise Dunn's *post-hoc* tests with Bonferroni correction. We analysed call data using Avisoft-SASLab Lite v. 5.2 (Specht, 2004) and Raven Pro v. 1.4 (Bioacoustics Research Program, 2011) software having first selected vocalisations of best quality—visually strong signals with the least amount of acoustic interference (Köhler et al., 2017). To avoid pseudoreplication only a single call from each survey was used for analysis.

Vocal characters were assessed using default spectrogram settings in Raven (Window: Hann; Discrete Fourier Transform: 256 samples; 3 dB filter bandwidth: 270 Hz). A band limited energy detector was configured to automatically select call variables, thereby minimising manual adjustment of selection parameters. We defined calls, notes, and call/note characters according to Köhler et al. (2017). This included the following note characters: note length (s); number of pulses per note, pulse rate (number of pulses per note divided by note length); and frequency (Hz). Frequency data were further divided into low frequency, high frequency, centre frequency (the frequency dividing the note into two frequency intervals of equal energy), and dominant frequency (the frequency at which max/peak power occurs, also known as the peak frequency).

Advertisement calls of *So. sechellensis* are complex and consist of either a single pulsed primary note (short call) or a primary note followed by one or more secondary notes (long call). We analysed only primary notes as they provided much larger sample sizes, and homology assessment of call characters was unambiguous. To test for differences among primary notes of both short and long call types, we used one-way ANOVAs and *post-hoc* pairwise Bonferroni tests on a subsample of calls using call type (i.e. long or short) as the categorical variable and note characters as dependent variables. Following our data cleaning steps, we performed linear regressions of T_a and

RH against primary note characters (including pulse rate and call duration) using PAST v. 3.25.

As ectotherms, the body temperatures of terrestrial anurans are closely tied to substrate (Gómez-Hoyos, Gil-Fernández, & Escobar-Lasso, 2016; Güizado-Rodriguez & Garcia-Vazquez, 2010; Navas, 1996). To test whether T_a values were a reasonable proxy for substrate temperature (T_s), we collected T_a and T_s data from the immediate vicinity of calling *So. sechellensis* across Mahé, Silhouette, and Praslin during January 2019. Measurement of T_a was recorded as previously described. We recorded T_s using a Traceable® RTD thermometer (accuracy: ± 2°C) with the probe deployed ca. 20 mm into the substrate, alongside a laboratory standard mercury thermometer.

2.3 Expectation 4: Body size comparisons

We collected body size data from live *So. sechellensis* in the field (mass in g, snout–vent length in mm; SVL) following visual encounter surveys (substrate/leaf-litter searches) carried out between September 2011 and May 2013, and from intact museum specimens (SVL only). Live frogs were released immediately after recording morphological data. All measurements were taken by the same person (J. Labisko) and rounded to the nearest 0.1 g or mm (Appendix S1). To determine whether live-recorded and preserved specimen data could be pooled for subsequent analyses (see Deichmann, Boundy, & Williamson, 2009; Lee, 1982), we tested for the effects of preservation on SVL between the two groups.

Sexual dimorphism in body size is observed in all species of the Sooglossidae with females being larger (Nussbaum & Wu, 2007). However, due to uncertainty in verifying sex in the field, and to prevent the sacrifice of live animals or damaging voucher specimens, we did not partition by sex for our analyses. We tested for body size differences among island populations using independent ANOVAs on SVL and body mass data, followed by pairwise Tukey's *post-hoc* tests. During fieldwork, we encountered more subadult/small adult *So. sechellensis* on Mahé than either Praslin or Silhouette. The mean SVL for adult male *So. sechellensis* across islands has been reported as 15 mm (Nussbaum & Wu, 2007), so we undertook a sensitivity analysis by removing all individuals < 15 mm SVL and repeated the ANOVAs for both SVL and mass.

2.4 Phylogeny inference, divergence estimation and ancestral state estimation

To place trait data from sooglossids in an evolutionary context, we used BEAST v. 2.4.8 (Bouckaert et al., 2014), via the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010), to jointly infer a phylogeny and divergence times for our study system. We estimated divergence times using a previously generated dataset (Labisko et al., 2019) that consists of six concatenated genetic markers (16S, cyt-b, POMC, RAG-1, RAG-2, Rho). For divergence estimation purposes we added several outgroups to this dataset (Appendix S2: Table S2). We used four secondary calibration points obtained from Feng et al. (2017): (1) the time to most recent common ancestor (TMRCA) for batrachians at 270 MYA; (2) the TMRCA for cryptobranchid (*Andrias* Tschudi, 1837) and hynobiid (*Batrachuperus* Boulenger, 1878) salamanders at 155 MYA; (3) the TMRCA for Calyptocephallidae Reig, 1960 (*Calyptocephalella* Strand, 1928) and Myobatrachidae Schlegel, 1850 (*Lechriodius* Boulenger, 1882) at 100 MYA; and (4) the TMRCA of Nasikabatrachidae Biju & Bossuyt, 2003 (*Nasikabatrachus* Biju & Bossuyt 2003) and Sooglossidae (ingroup taxa; *Sooglossus* and *Sechellophryne*) at 66 MYA. We used normally distributed priors for calibration points, and a four-model AIC partition scheme selected by PartitionFinder v. 1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012): (1) 16S–cyt-b codon 1 (GTR+I+G); (2) cyt-b codon 2, 1st and 2nd codons for all nuclear loci

(GTR+G); (3) cyt-b 3 codons (TrN+G); and (4) all 3rd codon positions for nuclear locus (HKY+G). We ran 100 million generations with sampling occurring every 5,000 generations. All parameters were checked using Tracer v. 1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) to confirm that effective sample size values exceeded 200. The resultant phylogeny was annotated using TreeAnnotator v. 2.4.8 (Rambaut & Drummond, 2014) and visualised with FigTree v. 1.4.3 (Rambaut, 2016).

To estimate the ancestral niche of sooglossid frogs we used current elevation data (as a proxy for thermal niche) to predict ancestral niches at all branches in the topology. We used the maximum likelihood estimation of ancestral states (Fastanc function) in the phytools v. 0.5.20 package (Revell, 2012) in R v. 3.1.3 (R Core Team, 2017) to generate these values. We pruned the phylogeny used in our divergence estimation analyses to only include members of the Sooglossidae, and also generated a single-gene phylogeny using 16S mtDNA data (which had many more individuals sampled per island and species; N = 204; see Labisko et al. 2019) with raxmlGUI v. 1.3.1 (Silvestro & Michalak, 2011; Stamatakis, 2014) applying default settings with the GTRGAMMA substitution model of nucleotide evolution and 1,000 bootstrap pseudoreplicates.

2.5 Genetic and bioacoustic divergence among island populations of *Sooglossus sechellensis*

Given that adaptive responses to divergent selection often initiate speciation (Nosil, Harmon, & Seehausen, 2009), we tested for evidence of species-level divergence between *So. sechellensis* on Praslin and *So. sechellensis* on Mahé+Silhouette using three lines of evidence commonly used to delimit anuran taxa (mtDNA, nuDNA, and bioacoustics; Streicher, Sadler & Loader, 2020).

To further investigate levels of inter-island divergence, we reanalysed the sequence data from Labisko et al. (2019) and Taylor et al. (2012) via independent principal components analyses of mtDNA and nuDNA using the adegenet v. 1.4.2 package (Jombart, 2008) in R. This method is critical for species delimitation as it allows for independent scrutiny of nuDNA signatures, which may be masked by more variable mtDNA when included in a concatenated matrix for phylogenetic analysis. We also used the ratio of nonsynonymous to synonymous substitutions per site ($d_\mathrm{N}/d_\mathrm{S}$) to test for positive selection in protein-coding genes via DnaSP v. 6.11.01 (Rozas et al., 2017). Additional methods information is presented in Appendix S2.

To assess divergence in call characters (primary+secondary notes), which are typically species-specific (Köhler et al., 2017), we compared the frequency of (1) primary note only and (2) primary note+secondary note calls across *So. sechellensis* on different islands. We also performed linear regressions to compare the number of secondary notes to both $\mathrm{T_a}$ and RH across different island populations using both global (Mahé+Silhouette+Praslin) and subset (Mahé+Silhouette versus Praslin) datasets.

3 Results

3.1 Expectation 1: Lower elevations and a warmer climate

We found that the elevational distribution of *So. sechellensis* was different on each island (H = 319.3, df = 2, P < 0.001), and that frogs from Praslin occurred at lower elevations (mean ± SD = 213 m ± 43 m; median = 198 m, interquartile range [IQR] = 239–180 m) than conspecifics from either Mahé (mean ± SD = 532 m ± 121 m; median = 543 m, IQR = 627–446 m; P < 0.001) or Silhouette (mean ± SD = 450 m ± 74 m; median = 450 m, IQR = 500–398 m; P < 0.001) (Fig. 2a; Appendix S1). Elevational occurrences of *So. sechellensis* also differed between Mahé and Silhouette, with frogs found at lower elevations on Mahé ($P < 0.001$). Comparing the elevational distribution of *So. sechellensis* from Praslin to all other sooglossids revealed that Praslin frogs occur at lower elevations in all instances ($H = 327.8$, df = 4, all $P < 0.001$), including pooled Mahé and Silhouette populations of *So. sechellensis* (median = 500 m, IQR = 572–420 m), *So. thomasseti* (median = 448 m, IQR = 588–381 m), *Se. gardineri* (median = 463 m, IQR = 581–370 m), and the Silhouette endemic *Se. pipilodryas* (median = 389, IQR = 476–309 m) (Fig. 2b).

Analyses of bioclim and Global Aridity Index data for *So. sechellensis* occurrence on Mahé, Silhouette, and Praslin revealed significant differences among islands in all but three instances ($P < 0.001$); the exceptions being bioclim variables: isothermality, temperature seasonality, and precipitation of the driest quarter, between Silhouette and Praslin ($P > 0.05$). *Sooglossus sechellensis* on Praslin occur at localities with higher average temperatures (annually, during the wettest quarter, during the driest quarter, and during the coldest quarter) and maximum temperature of the warmest month (Table 1). Although environmental conditions experienced by *So. sechellensis* on Praslin are warmer and more arid than those on Mahé and Silhouette, comparisons of bioclim precipitation variables do not support the conditions on Praslin as being the driest in all circumstances. For example, precipitation of the wettest month was highest on Praslin (Table 1). Additional results describing conditions on each island are reported in Appendix S2.

3.2 Expectations 2 & 3: Warmer activity and body temperatures

Advertisement calls with corresponding environmental data totalled 844 (Mahé, N = 388; Silhouette, N = 19; Praslin, N = 437) (Appendix S2: Table S1). *Sooglossus sechellensis* from Praslin called at higher T_a (mean ± SD = 25.4°C ± 1.3°C; median =

25.4°C, IQR = 26.1°C–24.2°C; $H = 359.6$, df = 2, $P < 0.001$) than those from Mahé (mean ± SD = 23.8°C ± 0.7°C; median = 23.8°C, IQR = 24.4°C–23.2°C) and Silhouette (mean ± SD = 23.8°C ± 0.5°C; median = 24.2°C, IQR = 24.2°C–23.1°C) (Fig. 3a). We also found that T_a was an appropriate proxy for body temperature as it was positively correlated with T_s ($r = 0.75$, $P < 0.001$) (Appendix S2: Table S3). As such, soil temperatures at time of calling on Praslin (N = 18) were higher than those from a combined dataset of Mahé and Silhouette (N = 14; $P < 0.001$; Appendix S2: Table S3; Fig. S1).

From the 844 *So. sechellensis* advertisement calls, we identified 33 "best quality" calls (*sensu* Köhler et al., 2017), to use for our analyses of quantitative call traits (Mahé, N = 7; Silhouette, N = 4; Praslin, N = 22) (Appendix S2: Table S4). We binned primary note data from Mahé and Silhouette into a single group as initial observations suggested they were similar and subsequent *t*-tests revealed no significant differences among call characters on the two islands (all $P > 0.05$). No differences were detected between the primary notes of short and long call types (Mahé+Silhouette: short, N = 6; long N = 5; $P > 0.05$. Praslin: short, N = 4; long, N = 18; $P > 0.05$); we therefore used primary notes of both call types in subsequent analyses.

Linear regressions between primary note characters and T_a revealed a positive relationship with both note length ($r^2 = 0.21$, $P < 0.01$) and the number of pulses ($r^2 = 0.24$, $P < 0.01$) for *So. sechellensis* from Mahé+Silhouette+Praslin (Fig. 3b; Table 2). The primary note advertisement call made by *So. sechellensis* on Praslin was longer ($P < 0.001$), and contained more pulses ($P < 0.001$) delivered at a lower rate ($P < 0.05$) than their conspecifics from Mahé+Silhouette (Table 2). Praslin frogs show inverse trends to that of Mahé+Silhouette frogs across several vocal traits, one of which (number of secondary notes) was significant ($P < 0.001$).

3.3 Expectation 4: Larger adult body sizes

Snout-vent length data from field caught and museum specimens comprised 324 individuals (Mahé, N = 161; Praslin, N = 51; Silhouette, N = 112), and we found no evidence to indicate that we should differentiate between live vs. preserved frogs in subsequent analyses ($P = 0.098$). There was a significant difference in body size (SVL) for *So. sechellensis* among islands ($F_{(2, 318)} = 10.16$, $P < 0.001$) (Appendix S2: Fig. S2a). We found that *So. sechellensis* on Praslin were larger than conspecifics from both Mahé ($P < 0.001$) and Silhouette ($P < 0.01$) but frogs from Mahé and Silhouette were similar in size ($P = 0.17$). Our sensitivity analysis (removal of individuals < 15 mm SVL) reduced the sample size to 208 (Mahé, N = 92; Praslin, N = 41; Silhouette, N = 75). We again found a significant difference in SVL for *So. sechellensis* among islands ($F_{(2, 213)} = 4.55$, $P < 0.05$) (Appendix S2: Fig. S2b). However, while *So. sechellensis* from Praslin were larger than those from Mahé ($P < 0.05$), they were not larger than those from Silhouette ($P = 0.87$), which were also found to be larger than individuals from Mahé ($P < 0.05$).

Body mass from field caught specimens comprised 142 individuals (Mahé, N = 72; Praslin, N = 49; Silhouette, N = 21). Mass of *So. sechellensis* also differed among islands ($F_{(2, 139)} = 11.33$, $P < 0.001$) (Appendix S2: Fig. S3a). Pairwise tests indicated that Praslin frogs were more massive than conspecifics from both Mahé ($P < 0.01$) and Silhouette ($P < 0.05$) but frogs from Mahé and Silhouette were similar in size ($P = 0.58$). After again removing individuals < 15 mm SVL, our sample size comprised 102 frogs (Mahé, N = 41; Praslin, N = 44; Silhouette, N = 17). A significant difference in body mass was observed for *So. sechellensis* among islands ($F_{(2, 99)} = 3.70$, $P < 0.05$) (Appendix S2: Fig. S3b). However, pairwise *post-hoc* tests did not differentiate between any island population (Mahé–Praslin, $P = 0.053$; Mahé–Silhouette, $P = 0.939$; Silhouette–Praslin, $P = 0.089$), and provided inconsistent results following multiple tests when we adjusted for sample size by randomly selecting 17 frogs each from Mahé and Praslin.

3.4 Phylogeny, divergence dating and ancestral state estimation

Our phylogenetic reconstruction placed the origin of the Sooglossidae (*Sooglossus*+*Sechellophryne*) at 36.6 MYA (95% highest posterior density [HPD] interval 28.7–44.82 MYA), the extant *Sooglossus* taxa at 21.8 MYA (95% HPD 15.2–29.0 MYA), and *Sechellophryne* at 16.3 MYA (95% HPD 10.6–22.2 MYA) (Fig. 4; Appendix S2: Fig. S4). Divergence times and associated 95% HPD intervals for each independent (island) lineage are shown in Table 3. Ancestral state estimation for elevational distribution revealed that the ancestral sooglossid niche was likely comparable to habitat observed at intermediate elevations today (~480 m; Figs. 2 & 4; Appendix S2: Fig. S5).

3.5 Genetic divergence, bioacoustic divergence and speciation

Using multivariate analyses, we found that mtDNA variation clearly differentiated *So. sechellensis* from Praslin, Mahé, and Silhouette (PCs 1 & 2; Fig. 5a) as previously reported (Labisko et al. 2019). However, the combined nuDNA multivariate analysis only differentiated *So. sechellensis* on Praslin from a cluster of individuals from Mahé+Silhouette (PC1; Fig 5b). Thus, across the multi-genomic analysis we observed complete lineage sorting in *So. sechellensis* from Praslin whereas the nuDNA loci sampled from individuals on Mahé and Silhouette was incompletely sorted. We observed no evidence of positive selection on amino acid sequences in the nuclear loci we surveyed, suggesting they have evolved mostly under purifying selection for the retention of ancestral protein sequences (Appendix S2: Table S5).

In addition to primary note differences among calls of *So. sechellensis* on Praslin and their conspecifics from other islands, we observed evidence of bioacoustic divergence in secondary note characters. During our study, we recorded 581 calls with a single primary note (short call) and 411 calls with a primary note accompanied by one or more secondary notes (long call). Mahé and Silhouette frogs vocalised more with short (86% and 87%, respectively) than long (14% and 13%, respectively) calls. In contrast, Praslin frogs vocalised with fewer short (35%) than long (65%) calls. Thus, in addition to longer primary note durations reported above, Praslin frogs vocalised more frequently using secondary notes than their conspecifics on other islands.

Regression analyses on calls with matched environmental condition data (all islands included; N = 844) revealed a significant positive relationship between the number of secondary notes vocalised (range = 0–17, mean = 1.9, SD ± 3.6) and both T_a ($P < 0.001$) and RH ($P < 0.05$) (Table 2). This relationship appears to be driven by the more regular occurrence of secondary notes in *So. sechellensis* from Praslin, as when we repeated the regression analysis using only individuals from Mahé and Silhouette (N = 407; range = 0–8, mean = 0.5, SD ± 1.6), although still positive, the relationships between number of secondary notes and T_a or RH were not significant (both $P > 0.05$). In contrast, when we repeated the regression using only individuals from Praslin (N = 437, range = 0–17, mean = 3.1, SD ± 4.4), we observed significant relationships between the number of secondary notes and both T_a ($P = 0.006$) and RH ($P < 0.001$). However, while the relationship with RH was positive as observed in the global dataset, the relationship with T_a was significantly negative, suggesting that the role of temperature in determining number of secondary notes is unclear (Table 2).

4 Discussion

Using the most complete dataset on the ecology and distribution of the Sooglossidae to date, we found evidence that *So. sechellensis* living on Praslin have adapted to occupy warmer environments than their closest living relatives and ancestors. This interpretation is supported by an integrative testing framework and an evolutionary perspective gained from ancestral state estimation.

4.1 Expectation 1: Elevational distribution and climatic variation

Elevational parapatry between endemic, closely related Seychelles fauna has been reported in skinks (Harris at el. 2015) and caecilians (Maddock, Wilkinson, & Gower, 2018) but the drivers underlying such observations (e.g. thermal gradient) remain untested. Sooglossid frogs are sympatric on Mahé and Silhouette, and it was the elevational distribution of *So. sechellensis* from Praslin which first alerted us to the possibility of thermal adaptation in these frogs. However, there remains a possibility that the distributional patterns we report here are explained not by a shifted thermal niche, but rather by the exclusion of sooglossids on Mahé and Silhouette from lower elevation habitats by human activities. Despite the near-total loss of Seychelles' forest cover following the arrival of human settlers during the latter part of the 18th Century, small pockets of native vegetation persisted in inaccessible mountain regions (Baker, 1877; Kueffer, Beaver, & Mougal, 2013; Wallace, 1892). These scattered refugia undoubtedly provided a source of stock for recolonisation and generation of secondary forest habitats, within which the Sooglossidae are today found. That sooglossids were able to survive in degraded habitat and then recolonise secondary growth forests, is testament to their persistence in the face of considerable historic habitat loss and alteration.

We consider the exclusion of frogs from lower elevation habitat on Mahé and Silhouette due to human disturbance to be an unlikely explanation for the observed distributional patterns for two key reasons: (1) Silhouette is one of the least developed islands in the Seychelles, with a single beachside hotel and ample lowland habitat contiguous with higher-elevation mist forests; (2) although Mahé is more developed than either Praslin or Silhouette, as with Silhouette, there remain many undeveloped low-elevation areas which connect to high-elevation forest. Furthermore, extensive Key Biodiversity Area surveys have been completed across the islands in recent years (see Senterre & Henriette, 2015; Senterre et al., 2013b; Senterre, Henriette, Labrosse, Pinchart, & Senaratne, 2015), which included personnel with Seychelles amphibian expertise (L. Chong-Seng. pers. obs.), yet sooglossids are not established in these lower elevation habitats on Mahé or Silhouette. For these reasons, we believe the most plausible explanation for the observed distributional pattern is that the ancestral niche of sooglossid frogs is cooler habitat, such as that observed at intermediate and higher elevations on Mahé and Silhouette, and predicted by our ancestral state estimations (Fig. 4; Appendix S2: Fig. S5).

4.2 Expectations 2 & 3: Thermal influence on activity patterns and body temperatures

Temperatures at which Praslin-inhabiting *So. sechellensis* vocalised were warmer (Fig. 3a, Appendix S2: Fig. S1), and our regression analyses pairing T_a with call characters implies that their bodies were warmer than vocalising conspecifics on other islands (Fig. 3b, Table 2). However, can we be sure that T_a is a reliable proxy for body temperature, and whether the expected effects of T_a on call characters are supported by previous studies?

Anuran call characters are known to be affected by temperature (Gerhardt & Huber, 2002; Köhler et al., 2017; Ziegler, Arim, & Bozinovic, 2016) and assessments of temperature relationships commonly involve recording of body temperatures. As thermoconformers, the body temperature of small, refugia-using amphibians is closely tied to that of the surrounding microhabitat, and strongly correlates with environmental temperatures at the site of capture (Gómez-Hoyos, Gil-Fernández, & Escobar-Lasso, 2016; Güizado-Rodriguez & Garcia-Vazquez, 2010; Hillman et al., 2009; Navas, 1996), even across elevations (Navas et al., 2013). At such scales, and due to the narrow range within which many terrestrial amphibians operate in order to avoid desiccation (Wells, 2007), the differences between body, substrate, and ambient air temperatures can be limited (Gómez-Hoyos, Gil-Fernández, & Escobar-Lasso, 2016; Güizado-Rodriguez & Garcia-Vazquez, 2010; Resendiz & Luja, 2018). Given the difficulty in attaining and attributing body temperature data to individual frogs, our proxy method of using T_a from the calling sites of individual frogs was the only feasible method for attaining temperature-call data for large sample sizes and statistical rigor. Furthermore, the significant correlation between T_a and T_s supports the comparability of our results with those we would have obtained from directly taking body temperatures.

Our results support *So. sechellensis* from Praslin calling at warmer temperatures than conspecifics from other islands and are also consistent with the timing of the audio recordings we generated during multiple trips and at different times of the year (between April 2012 and March 2013). There were no apparent differences in the timing of vocal activity among Mahé, Silhouette, and Praslin *So*. *sechellensis* populations, all of which were predominantly crepuscular. In other words, instead of coping with increased temperatures through phenological shifts (i.e. more nocturnal activity when temperatures are cooler and humidity is increased), *So. sechellensis* on Praslin have the same activity patterns as their conspecifics on other islands. Interestingly, this occurs despite Praslin having ostensibly more temporal and spatial availability for phenological shifts than Mahé or Silhouette due to the absence of potential sooglossid competitors that are both diurnal (*Se*. *gardineri*, *Se*. *pipilodryas*) and nocturnal (*So*. *thomasseti*).

4.3 Expectation 4: Adult body sizes and thermal adaptation

While intraspecific variation in anuran body size has species-specific factors (Boaratti & Da Silva, 2015), the effects of surface area to volume ratio on evaporative water loss suggests larger anurans are better adapted to warmer and/or drier environments (Farrell & MacMahon, 1969; Heatwole, Torres, Blasini De Austin, & Heatwole, 1969; Levy & Heald, 2015; Nevo, 1973; Olalla-Tárraga, Diniz-Filho, Bastos, & Rodríguez, 2009; Schmid, 1965). We note that while our main analyses support *So. sechellensis* from Praslin having larger body sizes than their conspecifics on other islands, they also have similar upper thresholds of body size (Appendix S2: Figs. S2 & S3). While this indicates that our results were unlikely to have been biased by sampling large females unevenly across islands, it does suggest that our results are explained by smaller adult frogs being less commonly encountered on Praslin (also supported by the lack of significant differences in our sensitivity analyses; Appendix S2: Figs. S2b & S3b). As such, we acknowledge an alternative explanation for our body size results that *So. sechellensis* on Praslin are not larger than their conspecifics, but rather they display more secretive subadult/small adult behaviour which reduces encounter rates. Nonetheless, we note that this alternative scenario remains consistent with adaptation to warmer temperatures, as a plausible explanation for reduced encounter rates for small individuals is the behavioural avoidance of desiccation posed by warmer surface and air temperatures.

4.4 The evolutionary history and isolation of Sooglossidae

The Sooglossidae have a long evolutionary history, having diverged from their closest living relative, the Nasikabatrachidae, some 66 MY (see also Feng et al., 2017; Hime et al., 2021) a timeframe contemporaneous with the separation of microcontinental Seychelles from what became the Indian subcontinent ~63 MA, while on its northward traverse across the Indian Ocean (Chatterjee, Goswami, & Scotese, 2013; Collier et al., 2008). Throughout the Cenozoic, the western Indian Ocean experienced the rise and fall of eustatic sea level (Ali, 2018; Miller et al., 2005), cyclically isolating and reconnecting the Seychelles Inner Islands (Ali, 2018; Rocha, Posada, & Harris, 2013; Ryan et al., 2009). Over the past ~1 MA, global sea levels have fallen more than 60 m below present on at least 14 separate occasions, remaining so for periods of 10,000–80,000 years (based on Miller et al., 2005). Such major drops in sea level would have reconnected all the Seychelles Inner Islands (Ali, 2018; Rocha, Posada, & Harris, 2013; Ryan et al., 2009). The subsequent spread of vegetation, climaxing in the establishment of mature rainforest across reconnected sections of the Seychelles plateau, a region of ~44,000 km^2, could have occurred in just a few thousand years (Liebsch, Marques, & Goldenberg, 2008), providing a series of repeated opportunities for dispersal and gene-flow across an emergent plateau.

Over the last 20,000 years, the Praslin population of *So. sechellensis* appears to have expanded in size, while sooglossid frog populations on other islands have remained constant (Labisko et al., 2019). Global eustacy was ca. 121 m lower than present 10–60 KYA (based on Miller et al., 2005), and assuming other sooglossid taxa were present on an isolated 'Praslin' for any length of time, this does provide support to the hypothesis that *So. sechellensis* expanded their range here while possible competition from other sooglossid taxa decreased, although this is of course difficult to verify. The fact that no other sooglossid frogs occur on Praslin today may be a direct result of naturally forced climatic changes and habitat loss following the end of the last glacial maximum, and its resultant influence on global eustacy (Dutton, Webster, Zwartz, Lambeck, & Wohlfarth, 2015; Labisko et al., 2019; Woodroffe, Long, Milne, Bryant, & Thomas, 2015).

4.5 Evidence for a speciation event on Praslin

Mito-nuclear discordance was previously reported for *So. sechellensis* with mtDNA suggesting a sister relationship between Praslin+Silhouette and Mahé, whereas nuDNA supports a sister relationship between Praslin and Mahé+Silhouette (Labisko et al., 2019). This is largely consistent with the results of our multivariate analyses of these datasets. However, we did not find evidence from nuDNA for the clear separation of individuals from Mahé and Silhouette (Fig. 5b). Regardless, both datasets support the existence of Praslin-specific genetic variants (Fig. 5).

We observed several species-level differences in call characters amongst island populations of *So. sechellensis*. The primary note advertisement calls made by Praslin frogs are longer in duration and contain more pulses delivered at a lower rate than observed in frogs from Mahé and Silhouette (Appendix S2: Table S4), they also had more secondary notes in general. While some of these differences may be explained by T_a at time of calling, global versus island-specific regression analyses of the number of secondary notes versus T_a produced contrasting results, suggesting that more regular use of secondary notes (= longer calls) on Praslin is not purely explained by warmer temperatures.

Considered together, the genomic and bioacoustic differentiation we observed supports a scenario where *So. sechellensis* from Praslin have experienced greater lineage sorting and character displacement than observed on Mahé and Silhouette driven by an isolating thermal niche shift.

4.6 General caveats and future directions

We note several caveats for interpreting our results. First, our sample sizes for Silhouette were not as extensive as those for Praslin and Mahé, and excluding Praslin frogs, we did observe other sooglossids at low elevations (although this was rare; Fig. 2; Appendix S1). Second, we measured a relatively narrow T_a range compared to laboratory-based studies on anuran vocalisations. This narrow range may explain why some call characters were, as expected, significantly and positively associated with T_a when using our global dataset (which spanned the largest range of T_a) but displayed inverse patterns when analysed in island-specific datasets. For example, when analysed alone, *So. sechellensis* from Praslin had unexpectedly negative regression statistics between most call characters and T_a, and in one case (number of secondary notes) the negative relationship was significant (Table 2). Finally, our finding that *So. sechellensis* from Praslin were larger than Mahé conspecifics contrasts with that of Taylor et al. (2012), who reported Mahé frogs as larger overall, albeit from far fewer individuals (N = 12) than we sampled (N = 161).

To enhance our understanding of this system, several relevant questions remain, including: Does adaptive potential for upper-thermal tolerance have a molecular basis amongst the Sooglossidae? If the molecular basis for thermal tolerance is identified, which populations are most at risk from climate change? What are the upper thermal tolerances of species and populations of sooglossid frogs from each island? Do frogs exposed to different island-specific environmental conditions exhibit different behaviour? Does barometric pressure influence calling activity, and does phonotaxic response vary between frogs exposed to conspecific vocalisations from different islands?

5 Conclusions

Niche divergence in amphibians driven by tolerance to warmer or cooler temperatures may be more common than previously expected, with even closely related taxa exhibiting a range of thermal tolerances (Kozak & Wiens, 2007; Navas, 1997; Navas, 2002; von May et al., 2017; von May et al., 2019). This is encouraging as amphibians face an arguably greater threat from rapid increases in global temperature than most other vertebrates (Gunderson, Dillon, Stillman, & Trullas, 2017; Gunderson & Stillman, 2015; von May et al., 2017).

Our study provides an integrative framework of evidence supporting a niche shift to warmer activity temperatures in the species *So. sechellensis*, likely caused by island isolation and an elevational ceiling on Praslin. That no other sooglossids occur on Praslin, or show restricted elevational distributions (Fig. 2), suggests that *So. sechellensis* on Praslin either represent an adaptive event, or they currently survive on an extinction trajectory. The latter is decidedly problematic to verify without prior knowledge of population demography and detailed biogeographic history. However, an adaptive event can be inferred by relating the putative agents of selection (in this case high temperatures) to expected outcomes of those selective pressures via independent hypothesis tests and the principle of severity (Cox & Logan, 2021). While responses to differing precipitation regimes were not as clear, we found that temperature was significantly associated with shifts in active body temperature, call characters, adult body sizes, and species-level divergence, suggesting that *So. sechellensis* have adapted to the warmer conditions on Praslin, providing a rare but positive outlook on the ability of some tropical amphibians to respond to rising global temperatures through adaptation and speciation. However, the deep evolutionary divergence between Praslin frogs and their closest extant relatives (~8 million years) may have allowed for a gradual process of thermal adaptation and speciation. While rapid adaptation to anthropogenic change has been documented in some amphibians (e.g. Freidenburg & Skelly, 2004; Sänen, Laurila, & Merilä, 2003), niche evolution matching projected climate change to the year 2100 may require rates >10,000 times faster than typically observed among vertebrates (Quintero & Wiens, 2013). Thus, local extinction is still a likely outcome for tropical frogs experiencing warming climates in the absence of dispersal corridors to thermal refugia.

Reliably predicting the impact of climate change on amphibians will require detailed information on active body temperatures at the local (microhabitat) scale (Sanabria & Quiroga, 2019). This includes how preferred body temperatures and other traits interact with other drivers of amphibian declines such as disease (Cohen et al., 2017; Murray, Rosauer, McCallum, & Skerratt, 2011; Nowakowski et al., 2016). We therefore recommend continued research attention on this globally important and climate-vulnerable family in order to establish clear goals for effective habitat and conservation management (*sensu* Jones, Watson, Possingham, & Klein, 2016), and to mitigate the cumulative and progressive effects of climate change that are likely to outpace the adaptive potential of most amphibians.

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Author contributions:

J.L., J.J.G., R.A.G., N.B., and J.W.S. conceived the project; J.L., L.C-S., K.S.B., and N.B. undertook fieldwork and contributed to project development; J.L. and J.W.S. performed the analyses and wrote the manuscript to which all authors contributed for the final version.

Data Availability Statement

Raw data are available in Supporting Appendices S1 and S2. All DNA sequences used were previously published and available on NCBI. Sequence alignments and R code used in phylogenetic and multivariate analyses are available via the NHM Data Portal:

https://data.nhm.ac.uk/dataset/data-from-labisko-et-al-sooglossus-adaptation.

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Table 1 Statistical tests of WorldClim (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) bioclimatic variables and Global Aridity Index & Potential Evapo-Transpiration (Trabucco & Zomer, 2018) data for *Sooglossus sechellensis* localities on Mahé (M) (N = 101), Silhouette (S) (N = 44), and Praslin (P) (N = 89). Highest values of group means and significant results in bold. For simplicity, median values and interquartile ranges appropriate for Kruskal-Wallis *H* tests are not shown (climatic variables are generally derived of mean values).

Variable	**Group means**			**Kruskal-Wallis**	**Dunn's *post-hoc* (Bonferroni corrected)**		
	Mahé	Silhouette	Praslin		M–S	M–P	S–P
Annual mean temperature	25.0	25.6	**26.3**	***H* = 162.6, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Mean diurnal range	**5.4**	5.2	5.2	***H* = 77.0, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Isothermality	67.9	**68.4**	68.2	***H* = 40.22, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*P* > 0.05
Temperature seasonality	**80.3**	76.1	76.3	***H* = 112.0, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*P* > 0.05
Maximum temperature of warmest month	28.8	29.3	**29.9**	***H* = 149.3, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Minimum temperature of coldest month	21.0	21.7	**22.2**	***H* = 176.1, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Temperature annual range	**7.9**	7.6	7.7	***H* = 80.9, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Mean temperature of wettest quarter	25.1	25.6	**26.4**	***H* = 154.1, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Mean temperature of driest quarter	24.7	25.3	**26.0**	***H* = 177.3, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Mean temperature of warmest quarter	26.0	26.6	**27.3**	***H* = 157.1, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Mean temperature of coldest quarter	24.0	24.7	**25.3**	***H* = 165.3, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Annual precipitation	**2399.0**	2295.4	2320.5	***H* = 186.8, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Precipitation of wettest month	363.9	356.9	**367.5**	***H* = 138.4, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Precipitation of driest month	**102.2**	95.6	92.2	***H* = 176.1, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Precipitation seasonality	40.3	41.8	**42.4**	***H* = 178.8, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Precipitation of wettest quarter	**948.1**	920.3	934.1	***H* = 196.1, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Precipitation of driest quarter	**358.2**	332.6	334.3	***H* = 165.5, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*P* > 0.05
Precipitation of warmest quarter	**505.9**	482.4	499.3	***H* = 126.5, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Precipitation of coldest quarter	**417.2**	387.7	381.1	***H* = 168.5, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Global Aridity Index	**1.3**	1.3	1.2	***H* = 180.1, df = 2, *P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.01**

Table 2 Linear regressions of *Sooglossus sechellensis* vocal traits against environmental conditions. ‘Total call length’ and ‘number of secondary notes’ are call-centred variables; all other variables are note-centred (based on the primary note). Sample sizes of all variables except ‘number of secondary notes’ reflect the “best quality” calls (*sensu* Köhler et al., 2017) selected for analyses. Sample sizes for ‘number of secondary notes’ (shown in superscript) are derived from all collected call data. Significant results in bold.

Population and trait	**Temperature (T_a)**			**Relative humidity**		
	r	r^2	*P*	*r*	r^2	*P*
Mahé+Silhouette+Praslin (N = 33)						
Total call length	0.1682	0.0283	*P* = 0.349	0.0020	4.E-06	*P* = 0.991
Note length	**0.4537**	**0.2056**	***P* = 0.008**	-0.0291	0.0008	*P* = 0.872
Number of pulses	**0.4923**	**0.2424**	***P* = 0.004**	-0.2202	0.0485	*P* = 0.218
Pulse rate	-0.0538	0.0029	*P* = 0.766	-0.3304	0.1091	*P* = 0.060
Low frequency	0.0054	3.E-05	*P* = 0.976	-0.0165	0.0003	*P* = 0.928
High frequency	0.1561	0.2436	*P* = 0.386	-0.0714	0.0051	*P* = 0.693
Centre frequency	0.0201	0.0004	*P* = 0.912	-0.0572	0.0033	*P* = 0.752
Dominant frequency	0.0443	0.0020	*P* = 0.807	-0.0923	0.0085	*P* = 0.610
Number of secondary notes $^{(N = 844)}$	**0.1496**	**0.0224**	***P* = 1.E-05**	**0.0801**	**0.0054**	***P* = 0.020**
Mahé+Silhouette (N = 11)						
Total call length	0.1711	0.0292	*P* = 0.615	-0.1958	0.0383	*P* = 0.564
Note length	-0.3986	0.1583	*P* = 0.225	0.4723	0.2231	*P* = 0.142
Number of pulses	0.4324	0.1869	*P* = 0.184	-0.5373	0.2887	*P* = 0.088
Pulse rate	0.5996	0.3595	*P* = 0.051	**-0.7471**	**0.5581**	***P* = 0.008**
Low frequency	-0.1011	0.0102	*P* = 0.767	0.2103	0.0442	*P* = 0.535
High frequency	-0.1716	0.0294	*P* = 0.614	-0.0782	0.0061	*P* = 0.819
Centre frequency	-0.1632	0.0266	*P* = 0.632	0.2493	0.0621	*P* = 0.460
Dominant frequency	-0.1794	0.0322	*P* = 0.598	0.0417	0.0017	*P* = 0.903
Number of secondary notes $^{(N = 407)}$	0.0730	0.0053	*P* = 0.142	0.0571	0.0033	*P* = 0.251
Praslin (N = 22)						
Total call length	-0.1177	0.0139	*P* = 0.602	0.1383	0.0191	*P* = 0.540
Note length	0.0102	0.0105	*P* = 0.650	0.0159	0.0003	*P* = 0.944

Number of pulses	0.0691	0.0048	*P* = 0.760	-0.0735	0.0054	*P* = 0.745
Pulse rate	-0.0124	0.0002	*P* = 0.956	-0.1767	0.0312	*P* = 0.432
Low frequency	0.0691	0.0048	*P* = 0.760	-0.1284	0.0165	*P* = 0.569
High frequency	0.1492	0.0223	*P* = 0.507	-0.0552	0.0031	*P* = 0.807
Centre frequency	0.1453	0.0211	*P* = 0.519	-0.1689	0.0290	*P* = 0.452
Dominant frequency	0.1800	0.0324	*P* = 0.423	-0.1584	0.0251	*P* = 0.481
Number of secondary notes $^{(N = 437)}$	**-0.1291**	**0.0157**	***P* = 0.006**	**0.1844**	**0.0340**	***P* = 1.E-04**

Table 3 Divergence times of multi-distributed sooglossid taxa in millions of years. The 95% highest posterior density (HPD) intervals are shown in parentheses.

	Mahé | Silhouette	**Mahé | Praslin+Silhouette**	**Praslin | Silhouette**
Se. gardineri	11.7 (6.2–17.3)	–	–
So. thomasseti	6.1 (3.2–9.6)	–	–
So. sechellensis	–	12.3 (8.0–17.0)	7.9 (4.7–11.6)

Figure legends

Figure 1. Distribution of all known observations of *Sooglossus sechellensis* across three islands of the Seychelles archipelago, and presence/absence of sooglossid taxa (boxed) for each island. Symbols and colours represent sampling localities for *So*. *sechellensis*: Praslin = green circles, Mahé = purple triangles, Silhouette = lilac squares.

Figure 2. Elevation distributions of *Sooglossus sechellensis* and other sooglossids across the Seychelles islands of Praslin, Mahé, and Silhouette. Intraspecific comparisons indicate that *So*. *sechellensis* from Praslin occupy lower elevations than on the other islands (maximum elevations are indicated for each island) (a). Intrafamilial comparisons show that *So*. *sechellensis* from Praslin occupy significantly lower elevations than all other genera and species within the Sooglossidae, with estimated ancestral distributions strongly supporting an ancestral niche of intermediate elevations (b) (see also Fig. 4; Appendix S2: Fig. S5). Colours for independent island populations of *So*. *sechellensis* represent those described in Fig. 1.

Figure 3. The effect of ambient temperature (T_a) on calling behaviour of *Sooglossus sechellensis*. Frogs from Praslin call at significantly higher T_a than conspecifics on other islands (a). This is also reflected by a significant positive relationship between T_a and the number of pulses observed in the primary note of advertisement calls, demonstrating that body temperature at time of calling is influenced by T_a (b). Symbols and colours represent sampling for *So*. *sechellensis*: Praslin = green circles, Mahé = purple triangles, Silhouette = lilac squares.

Figure 4. Maximum likelihood inferred phylogeny for the Sooglossidae based on concatenated mitochondrial and nuclear DNA, with ancestral state estimation. *Sooglossus sechellensis* from Praslin occupy lower elevations than all genera and species within the Sooglossidae, with estimated ancestral distributions strongly supporting an

ancestral niche of intermediate elevations, as indicated by branch colouration: higher elevation = cooler temperatures (dark blue), lower elevations = warmer temperatures (red). Collectively, these results suggest that living at lower elevations has (i) evolved only once during 36 million years of sooglossid evolution, or (ii) that only *So. sechellensis* from Praslin have avoided extinction after occupying low elevation habitats. Filled, coloured symbols adjacent to sample references represent the three island populations of *So*. *sechellensis*: Silhouette = lilac square, Praslin = green circle, Mahé = purple triangle. Open symbols indicate island of origin (as per coloured symbols) for other sooglossid taxa. Node support values are posterior probabilities from time-calibrated Bayesian analyses (see also Appendix S2: Fig. S4, S5).

Figure 5. Principal components analysis of mitochondrial (mtDNA), and nuclear (nuDNA) for *Sooglossus sechellensis* from Praslin, Mahé, and Silhouette, showing the two most explanatory axes. For mtDNA each island population of *Sooglossus sechellensis* is distinct (a). For nuDNA only Praslin Island frogs are differentiated from conspecifics (b). Symbols and colours represent sampling for *So*. *sechellensis*: Praslin = green circles, Mahé = purple triangles, Silhouette = lilac squares.

Supporting information

Appendix S1: Locality information for all observations used in the present study. Includes sample reference and corresponding body size (snout-vent length, mass) data.

Appendix S2: Extended methods and results of climatic data, bioacoustic surveys, and molecular analyses.

Table S1. *Sooglossus sechellensis* advertisement call data, and corresponding ambient air temperature (T_a) and relative humidity (RH) data.

Table S2. GenBank sequence data (and associated citations) used for phylogenetic reconstruction and divergence dating analysis.

Table S3. Substrate (T_s) and ambient temperature (T_a) data recorded across islands during January 2019.

Table S4. Advertisement call traits of the primary note vocalisation of *Sooglossus sechellensis*.

Table S5. Ratio of synonymous and non-synonymous substitutions for nuDNA loci.

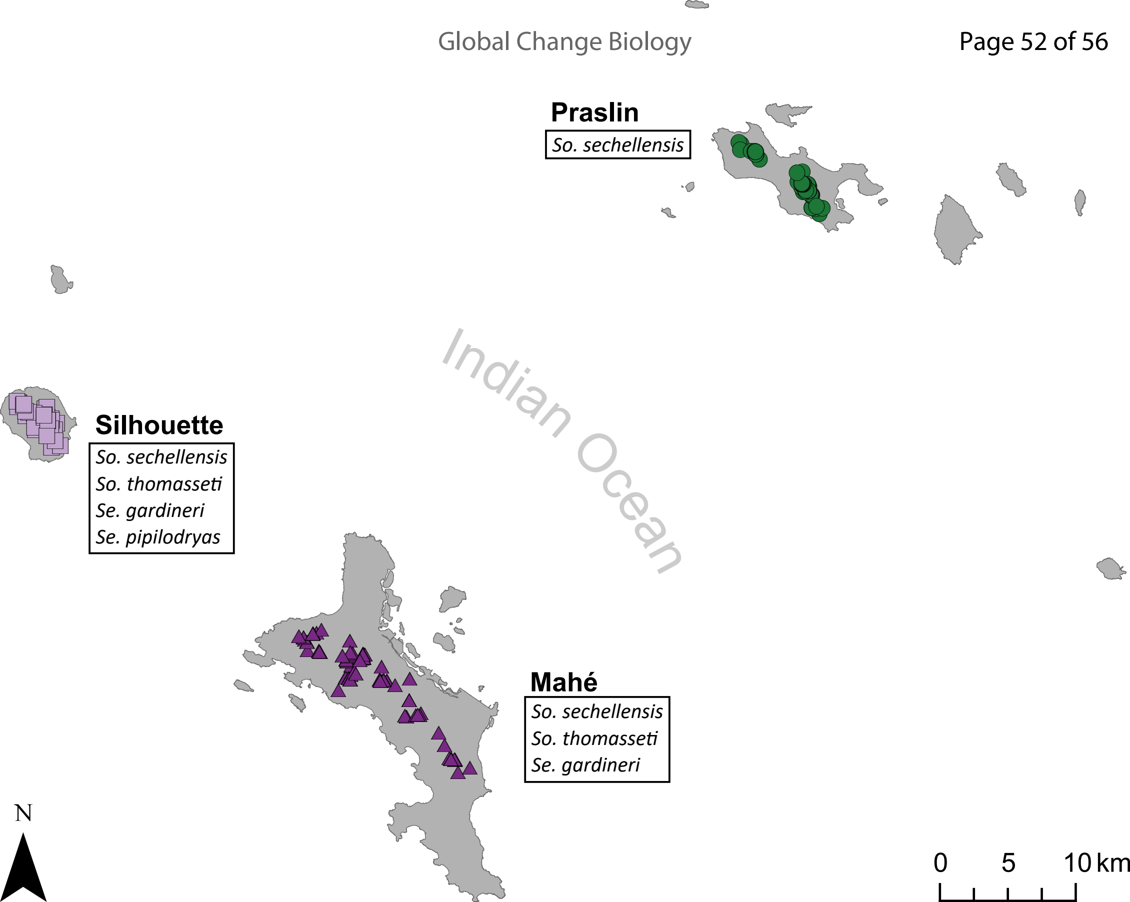
Figure S1. Soil temperatures at time of calling for *Sooglossus sechellensis* from Praslin, Mahé, and Silhouette.

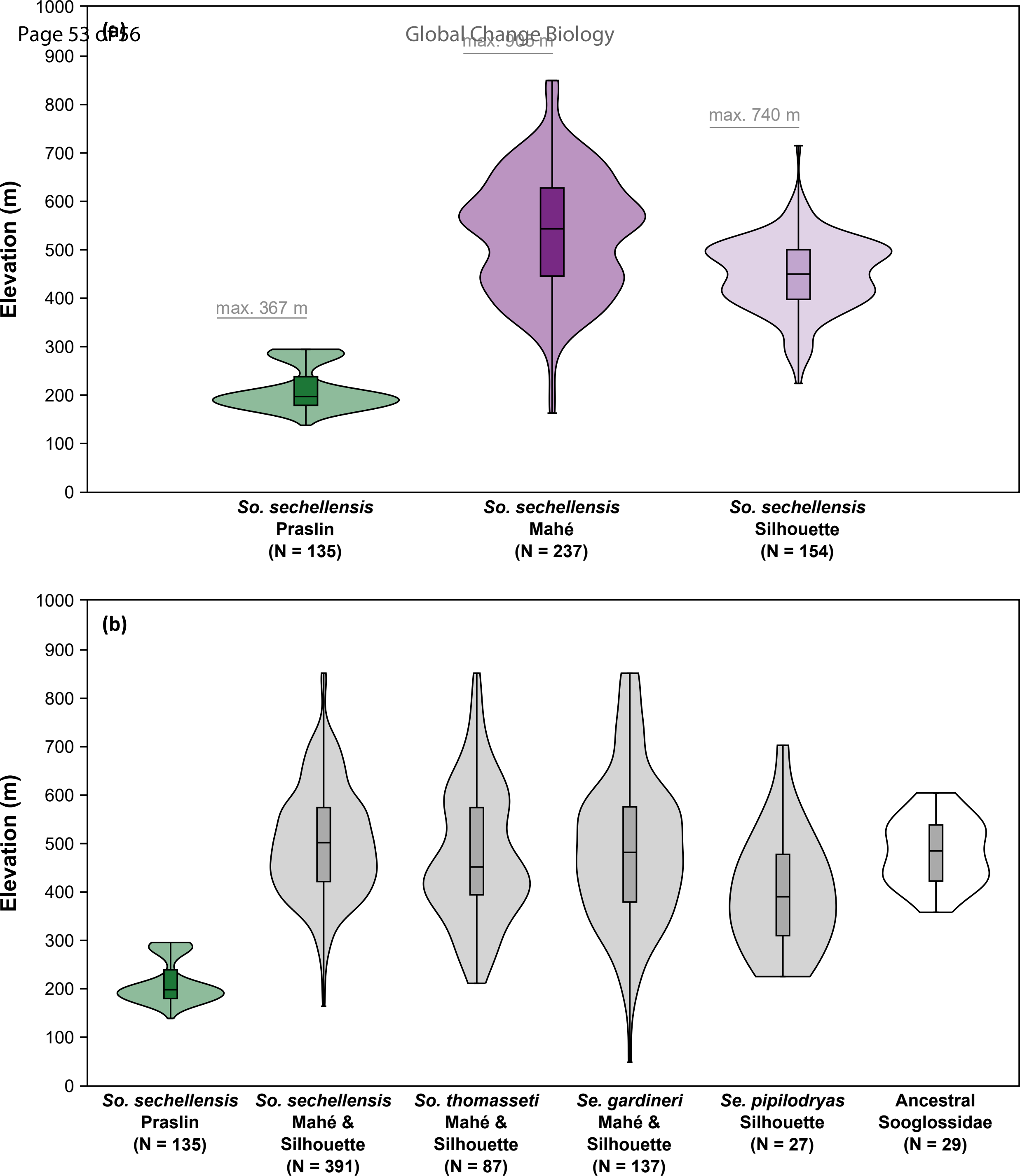
Figure S2. Comparisons of body size (snout-vent length in mm) of *Sooglossus sechellensis* from Praslin, Mahé, and Silhouette.

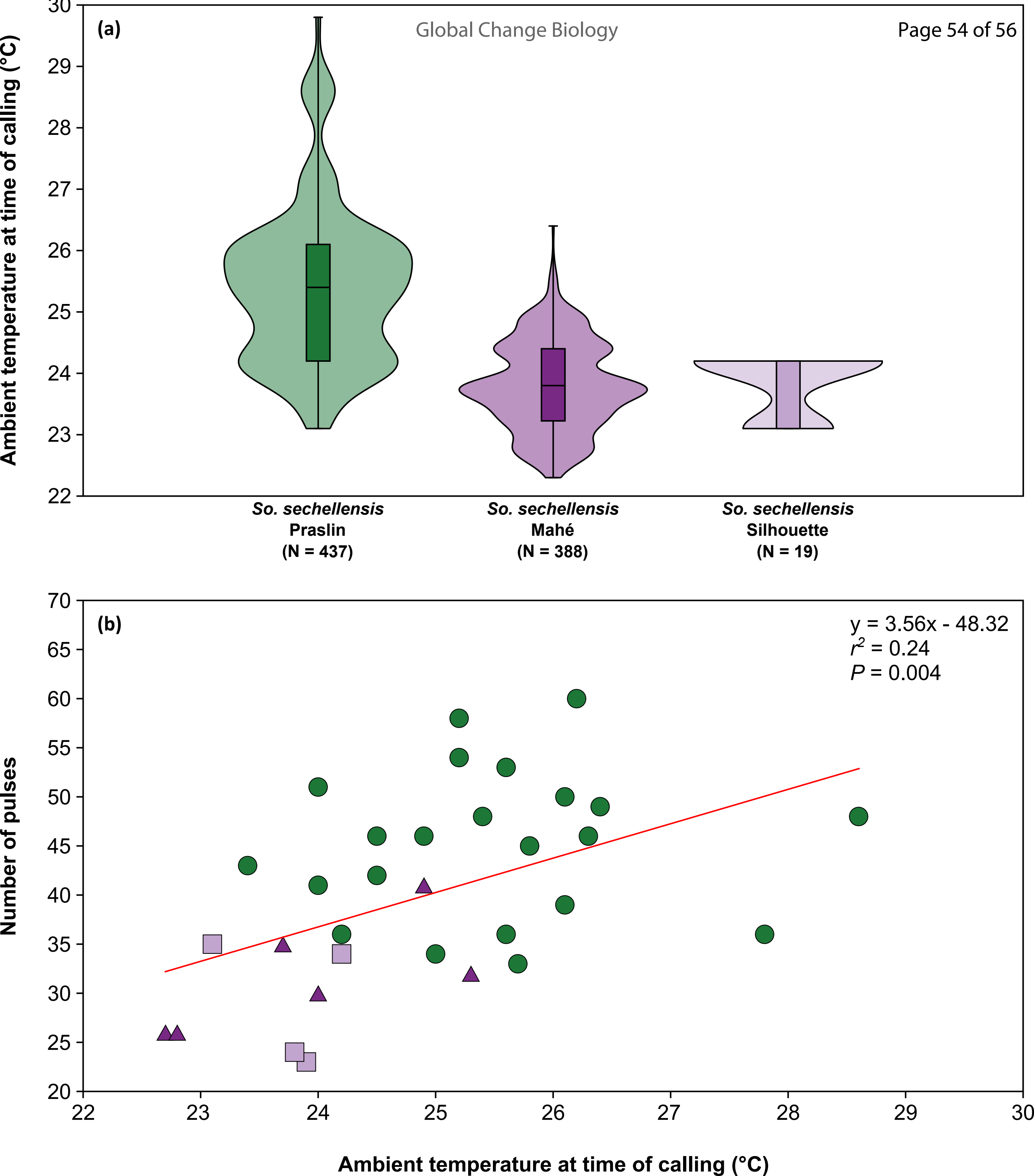
Figure S3. Comparisons of body size (mass in g) of *Sooglossus sechellensis* from Praslin, Mahé, and Silhouette.

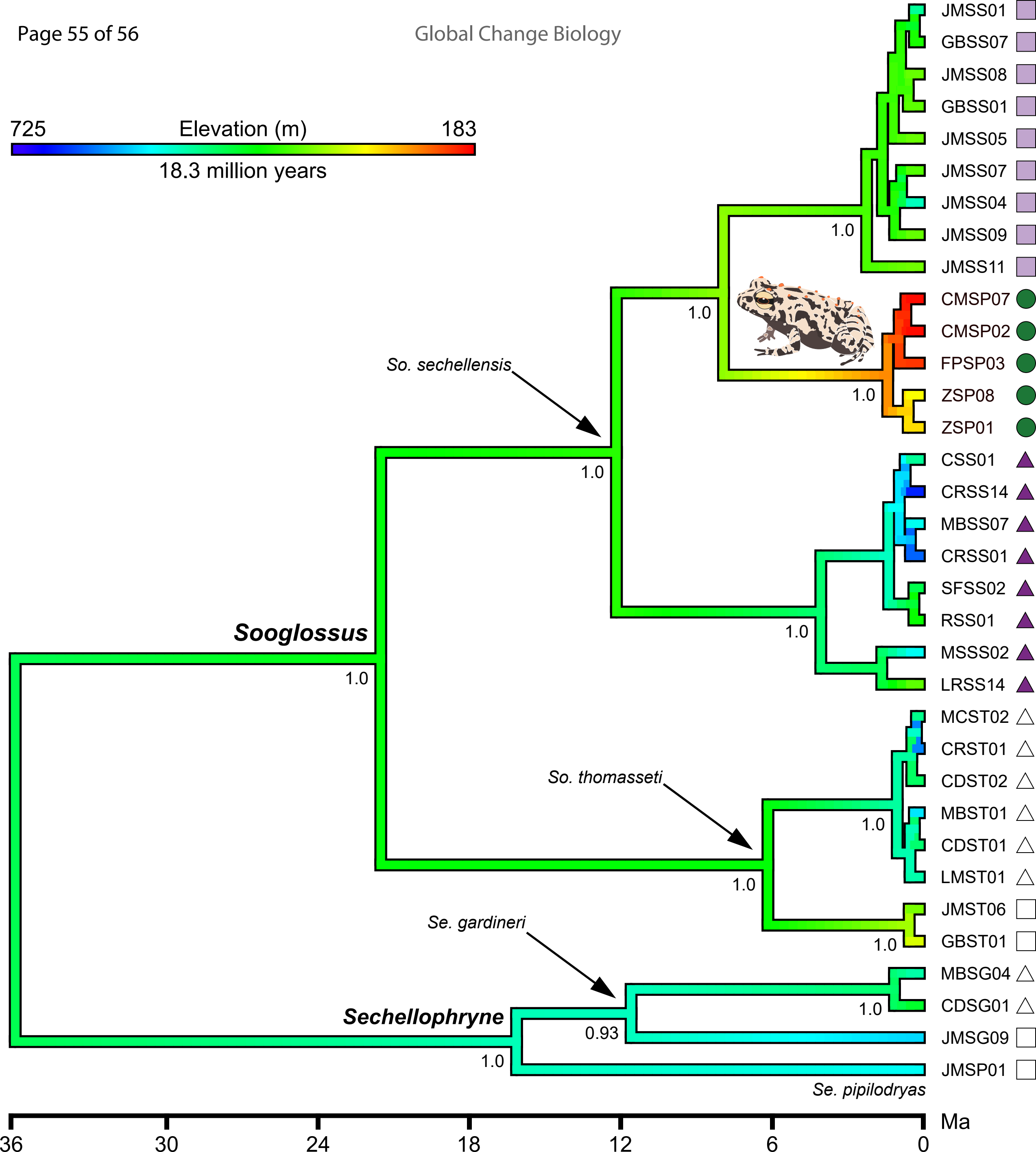
Figure S4. Bayesian inferred phylogenetic relationships and divergence time estimation among the Sooglossidae using concatenated mitochondrial and nuclear DNA.

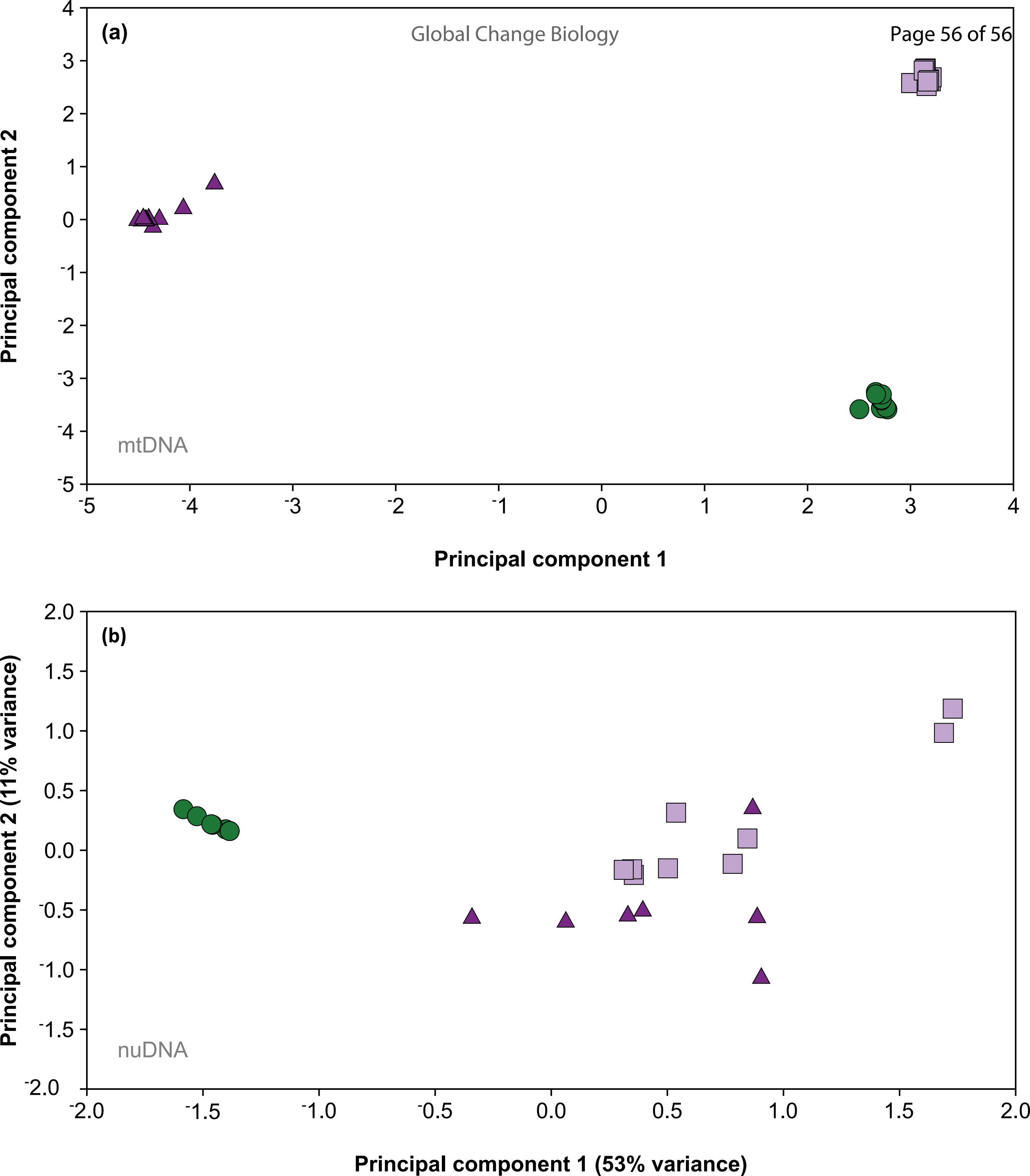
Figure S5. Ancestral state estimation with expanded taxon sampling from a maximum likelihood inferred 16s mtDNA phylogeny for the Sooglossidae.

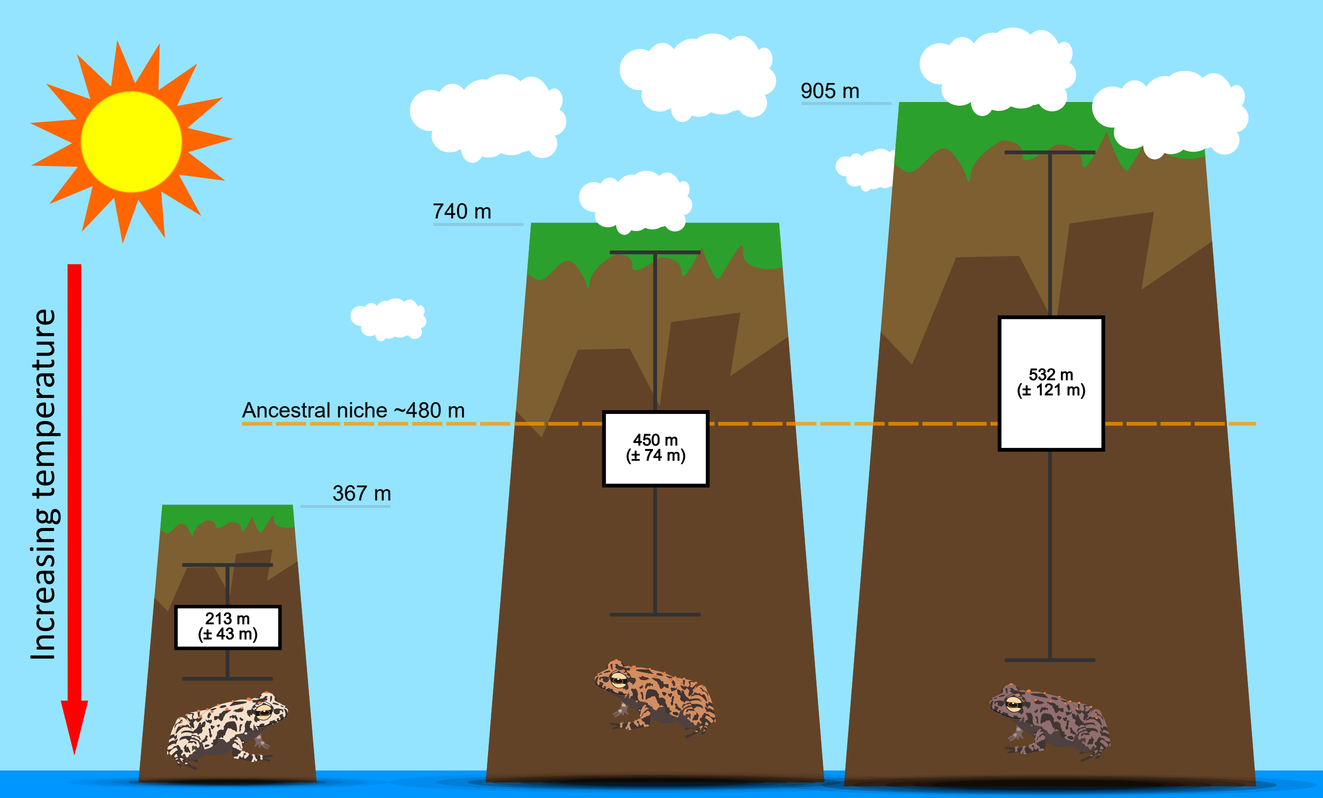

Praslin
So. sechellensis
Indian Ocean
Silhouette
So. sechellensis
So. thomasseti
Se. gardineri
Se. pipilodryas
Mahé
So. sechellensis
So. thomasseti
Se. gardineri
N
0 5 10 km


(a)
Elevation (m)
1000
900
800
700
600
500
400
300
200
100
0
max. 367 m
max. 905 m
max. 740 m
So. sechellensis
Praslin
(N = 135)
So. sechellensis
Mahé
(N = 237)
So. sechellensis
Silhouette
(N = 154)
(b)
Elevation (m)
1000
900
800
700
600
500
400
300
200
100
0
So. sechellensis
Praslin
(N = 135)
So. sechellensis
Mahé &
Silhouette
(N = 391)
So. thomasseti
Mahé &
Silhouette
(N = 87)
Se. gardineri
Mahé &
Silhouette
(N = 137)
Se. pipilodryas
Silhouette
(N = 27)
Ancestral
Sooglossidae
(N = 29)


(a)
Ambient temperature at time of calling (°C)
30
29
28
27
26
25
24
23
22
So. sechellensis
Praslin
(N = 437)
So. sechellensis
Mahé
(N = 388)
So. sechellensis
Silhouette
(N = 19)
(b)
y = 3.56x - 48.32
r^2 = 0.24
P = 0.004
Number of pulses
70
65
60
55
50
45
40
35
30
25
20
22
23
24
25
26
27
28
29
30
Ambient temperature at time of calling (°C)


725
Elevation (m)
183
18.3 million years
JMSS01
GBSS07
JMSS08
GBSS01
JMSS05
JMSS07
JMSS04
JMSS09
JMSS11
CMSP07
CMSP02
FPSP03
ZSP08
ZSP01
CSS01
CRSS14
MBSS07
CRSS01
SFSS02
RSS01
MSSS02
LRSS14
MCST02
CRST01
CDST02
MBST01
CDST01
LMST01
JMST06
GBST01
MBSG04
CDSG01
JMSG09
JMSP01
So. sechellensis
So. thomasseti
Se. gardineri
Se. pipilodryas
Sooglossus
Sechellophryne
1.0
0.93
Ma
36
30
24
18
12
6
0


(a)
mtDNA
Principal component 2
4
3
2
1
0
-1
-2
-3
-4
-5
-5
-4
-3
-2
-1
0
1
2
3
4
Principal component 1
(b)
nuDNA
Principal component 2 (11% variance)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Principal component 1 (53% variance)


Increasing temperature
905 m
740 m
Ancestral niche ~480 m
367 m
213 m
(± 43 m)
450 m
(± 74 m)
532 m
(± 121 m)

How will environmentally sensitive organisms such as ectotherms respond to rising global temperatures? Using an evolutionary perspective, Labisko et al. reveal that some tropical amphibians have survived episodes of historic warming without the aid of dispersal, and therefore may have capacity to adapt to the currently warming climate. Based on an integrative empirical dataset, their study highlights the utility of inferring evolutionary outcomes of paleoclimatic warming events, providing crucial insight into future climate impacts for especially vulnerable organisms.