Diversification of the terrestrial frog genus *Anomaloglossus* (Anura, Aromobatidae) in the Guiana Shield proceeded from highlands to lowlands, with successive loss and reacquisition of endotrophy

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ABSTRACT

Two main landscapes emerge from the Guiana Shield: the highlands to the west called the Pantepui region and the Amazonian lowlands to the east, both harbouring numerous endemic species. With 32 currently recognized species, the genus *Anomaloglossus* stands out among Neotropical frogs as one that diversified only within the Guiana Shield both in the highlands and the lowlands. We present a time-calibrated phylogeny obtained by using combined mitogenomic and nuclear DNA, which suggests that the genus originates from Pantepui where extant lineages started diversifying around 21 Ma, and subsequently (ca. 17 Ma) dispersed during the Miocene Climatic Optimum to the lowlands of the eastern Guiana Shield where the ability to produce endotrophic tadpoles evolved. Further diversification within the lowlands in the *A. stepheni* group notably led to an evolutionary reversal toward exotrophy in one species group during the late Miocene, followed by reacquisition of endotrophy during the Pleistocene. These successive shifts of reproductive mode seem to have accompanied climatic oscillations. Long dry periods might have triggered evolution of exotrophy, whereas wetter climates favoured endotrophic forms, enabling colonization of terrestrial habitats distant from water. Acquisition, loss, and reacquisition of endotrophy makes *Anomaloglossus* unique among frogs and may largely explain the current species diversity. The micro evolutionary processes involved in these rapid shifts of reproductive mode remain to be revealed.

1. Introduction

The Neotropics harbour the highest species diversity in many groups of animals and plants, especially in Amazonia (Antonelli and Sanmartín, 2011; Jenkins et al., 2013; Myers et al., 2000). The origin and buildup of Amazonian diversity has puzzled biologists for almost two centuries (Bates, 1863; Humboldt, 1820; Wallace, 1852), and our current understanding of the underlying processes remains limited (but see Silva et al., 2019). Most studies of evolutionary diversification in Amazonia have examined faunal exchanges with adjacent biomes (Antonelli et al., 2018), and in situ processes on relatively recent time frames (Pliocene-Pleistocene) (Ribas et al., 2009; Smith et al., 2014). The few studies that have investigated diversification within Amazonia over longer and older periods identified a key role of climate-mediated landscape changes as well as the transformation of enormous drainages linked to the Andean orogeny as important drivers of vicariance for building the unique richness of species in this region (Albert et al., 2021; Cracraft et al., 2020; Elias et al., 2009; Réjaud et al., 2020).

The Guiana Shield (GS) lies in the northern part of South America. In contrast with the rest of Amazonia, this area has likely been geologically and hydrologically relatively stable throughout the Cenozoic (Hoorn et al., 2010; Lujan and Armbruster, 2011). Therefore, the Andean uplift and associated hydrological transformations probably have had little direct influence on the diversification of groups distributed across the

Guiana Shield (but see Kok, 2013). While this geological region has long been considered one of the four main biogeographic 'districts' of Amazonia (Wallace, 1852), it comprises two distinct biogeographic regions. First, the highland formations of the western GS, known as the Pantepui region, are faunistically distinct from Amazonia (Kok, 2013; Vilhena and Antonelli, 2015). By contrast, the lowlands of the eastern GS, bounded by the lower Amazon and Negro rivers to the south and Pantepui to the west, belong to a unique Amazonian bioregion based on species composition of birds (Naka, 2011) and frogs (Godinho and da Silva, 2018; Vacher et al., 2020) communities. The numerous lineages that are endemic to these two GS regions indicate that, even though not of the magnitude of what is found in the Andes, significant levels of in situ diversification have happened, due to isolation of the GS lowlands from the rest of Amazonia by rivers and savannas, and elevation gradient for the GS highlands. This is particularly obvious when considering endemic highland fauna and flora of the Pantepui region (Berry and Riina, 2005; Désamoré et al., 2010; Kok et al., 2018, 2017, 2015, 2012; Pinheiro et al., 2019; Rull, 2005; Salerno et al., 2012). Conversely, the GS lowlands are less isolated and as a result most of their lineages show close affinities with Amazonian relatives, and only a few groups such as the frogs *Adelophryne* (Fouquet et al., 2012a) and *Synapturanus* (Anura) (Fouquet et al., 2021), the Gymnophiona *Microcaecilia* (Correia et al., 2018) and *Rhinatrema* (Maciel et al., 2018) or the fish *Harttiella* (Covain et al., 2016) have unambiguously diversified within this region. Spatio-temporal congruence in phylogeographic patterns across frog species in lowland lineages suggests that climatic changes during late Pliocene and Pleistocene played an important role in explaining this diversification (Fouquet et al., 2012b). Dispersal events between the western highlands and eastern lowlands of the GS appear to be rare, and when they have been reported by earlier studies, patterns of early diversification in the lowlands and subsequent dispersal towards highlands have been found, e.g. in the plant family Rapateaceae (Givnish et al., 2000) or the other way around in frogs of the genus *Otophryne* (Fouquet et al., 2021), and *Stefania* (Kok et al., 2017).

A fascinating example of GS endemic group is the frog genus *Anomaloglossus*. With 32 currently described species (Frost, 2021) and at least five undescribed species (Fouquet et al., 2020, 2019b, 2018; Vacher et al., 2017) distributed both in the highlands and lowlands, this group underwent a series of major diversification events within the region. Vacher et al. (2017) hypothesized a highland origin for the group with subsequent dispersal to the lowlands, but this scenario was poorly supported by phylogenetic analyses. Moreover, the genus harbours a striking diversity in life-history traits, particularly among the lowland species that either display (1) exotrophic and phoretic tadpoles, (2) endotrophic and phoretic tadpoles, or (3) endotrophic and nidicolous tadpoles (Fouquet et al., 2019b; Vacher et al., 2017). Exotrophic tadpoles display a fully functional mouth and feed on nutrients found in the environment, whereas endotrophic tadpoles do not possess a functional mouth and develop only on vitelline reserves (see Fouquet et al., 2019b). Exotrophic tadpoles are necessarily transported by the male parent to water bodies (phoretic) to fulfill their development and as such are found near streams (as in all Pantepui species and some species of the *A. stepheni* group). Endotrophic tadpoles can either be nidicolous (complete their development in terrestrial nests; as in some species of the *A. stepheni* group) and found away from water bodies; or phoretic (being transported by the male parent and completing their development on its back; as in all species of the *A. degranvillei* group and in *A. apiau*) and are then tightly associated with streams. Previous results suggest that endotrophy may have evolved several times during the evolutionary history of the group (Vacher et al., 2017), and it is generally admitted that complex parental investment such as brooding, and "viviparity" are very unlikely to undergo reversals in amphibians (Furness and Capellini, 2019). Therefore, the fact that this genus has diversified extensively both in the highlands and the lowlands and that this diversification may be linked with the loss and acquisition of endotrophy is rather intriguing.

Herein we tested the hypotheses that *Anomaloglossus* originated from Pantepui and subsequently dispersed to the lowlands, and that endotrophy was acquired several times as previously suggested (Vacher et al., 2017). We discuss whether putative range expansion could be related to historical environmental change and how the evolution of larval development may have been associated with diversification of the genus in the lowlands. To address these questions, we (1) investigated the phylogenetic relationships within *Anomaloglossus* using complete mitogenomes and seven nuDNA loci, (2) inferred the ancestral states of tadpole developmental mode, and (3) explored the historical biogeography of this group.

2. Material and methods

2.1. Molecular data

We obtained data for 21 out of the 32 described species and for five undescribed species of *Anomaloglossus* (Fouquet et al., 2019b; Kok et al., 2012; Vacher et al., 2017). Additionally, we included three more terminals representing diverging lineages within *Anomaloglossus baeobatrachus*, *A. surinamensis* and *A.* sp. 1 (Fouquet et al., 2019b; Vacher et al., 2017). Therefore, the ingroup (*Anomaloglossus*) was represented by 29 terminals in total. Eleven nominal species of *Anomaloglossus* were missing. However, all of them occur in the Pantepui region, are geographically close to species included in our dataset and are certainly closely related to species included in our study (Kok et al., 2012, also see Suppl. Mat. S5). We also included 11 outgroups representing major Dendrobatoidea lineages. We extracted DNA from liver tissue of 28 samples using the Wizard Genomic extraction protocol (Promega; Madison, WI, USA) (Table S1). For *Anomaloglossus*, we obtained 11 new mitogenomes using a shotgun approach with an Illumina sequencer (Illumina, USA) and 14 mitogenomes from GenBank. Four remaining *Anomaloglossus* terminals, for which we could not obtain material, were completed with available mtDNA loci from GenBank. The final matrix totaled 40 terminals. Mitogenomes were assembled de novo using the python-based organelle assembler Org.Asm (Coissac, 2016) and using a mapping in Geneious v.9 (Besnard et al., 2014). The mitochondrial genomes were annotated using MITOS web annotator (Bernt et al., 2013), and when needed, these annotations were corrected in Geneious v.9.

Additionally, we amplified and sequenced three protein-coding nuclear loci (*tyrosinase - TYR*; *proopiomelanocortin C -POMC*; and *recombination activating gene exon 1 -RAG1*). For *TYR*, we used tyrE dendro5 and tyrE dendro primers (Fouquet et al., 2012b), for *POMC* we used POMC-1 and POMC-2 primers (Wiens et al., 2005), and for *RAG1* we used MARTFL1 (Hoegg et al., 2004) and RAG1-AD2R (Fouquet et al., 2014) for the first fragment and RAG1-810F and RAG1-1240R (Fouquet et al., 2014) for the second fragment. PCR were conducted in a final volume of 25 µl each containing 2 µl of DNA template, 14.36 µl Mq water, 5 µl of 10 × PCR Buffer, 1.25 µl of each primer, 1.67 µl of MgCL2, 0.5 µl of dNTPs, and 0.22 µl of GoTaq (Promega, Madison, Wisconsin, USA). The PCR conditions were as follows: 8 cycles of denaturation (45 s at 94 °C), annealing (60 s at 46 °C), and elongation (90 s at 72°), followed by 22 cycles of denaturation (45 s at 94 °C), annealing (60 s at 50 °C), and elongation (90 s at 72 °C). We completed these datasets by adding available sequences from GenBank (Grant et al., 2017; Vacher et al., 2017). We also retrieved the 28S locus using the same approach used to assemble the mitogenomes (shotgun). Finally, we added three more nuDNA loci (*RHO*, *H3F3C*, *SIAH1*) available in GenBank for 11 *Anomaloglossus* terminals and the outgroups. Even though 18 *Anomaloglossus* terminals are lacking information for these three nuclear fragments (see Suppl. Table 1), representatives of the main lineages within the genus are represented, and most of the outgroups are well covered, which helped to improve time estimates for deeper relationships. Also, *TYR*, *POMC*, *RAG1* fragments cover most of *Anomaloglossus* species (Suppl. Table 1) and provide well-supported relationships (Suppl. Fig. 5).

Novel sequences were deposited in GenBank and are listed in

Table S1. Nucleotide sequences were aligned using MAFFT v.7 (Katoh and Standley, 2013) using default parameters (gap opening penalty = 1.53; gap extension penalty = 0.123; progressive method = FFT-NS-2) for the mitogenomes and the seven nuDNA fragments. We discarded the tRNA genes and the D-loop from the mitogenomes, thus keeping the 12S-16S and the protein-coding sequences (CDS) totaling 14073 bp. Finally, we concatenated all the loci (mitogenomes, 28S, TYR, POMC, RAG1, RHO, H3F3C, SIAH1) using the program FasConcat v.1.0 (Kück and Meusemann, 2010) totaling a 18,445 bp matrix.

2.2. Phylogenetic analysis

We conducted maximum likelihood (ML) analyses using different data sets to evaluate potential incongruence (1) for the eight concatenated loci; and (2) for each locus separately ML analyses were performed with RAxML v.8.2.4 using the GTR+Γ model. Support of nodes was obtained from 1000 nonparametric bootstrap replicates using the fast bootstrapping algorithm. All the ML gene and concatenated trees that we recovered were totally congruent, with a single exception: the position of *A. saramaka* using *SIAH1* only, which is moderately supported as nested within the Pantepui clade (Suppl. Fig. 5). Therefore, we conducted further analyses combining all the loci.

We selected the best-fit partition scheme and model of evolution for each partition using PartitionFinder V2.1.1 (Lanfear et al., 2012), according to the Bayesian Information Criterion (BIC) using the greedy scheme and linked branch length. We predefined 14 blocks, one for rRNA genes (12S and 16S), one for each codon position of concatenated mtDNA CDS regions, and one for each nuDNA CDS regions. This analysis identified seven partitions (1: 12-16S; 2: mtDNApos1, 3: mtDNApos2, 4: mtDNApos3, 5: *POMC* + *RAG1*, 6: *TYR* + *RHO* + *SIAH1*, 7: *H3F3C* + *28S*).

2.3. Time-calibrated phylogeny

Divergence times were estimated using BEAST v2.5.2 (Bouckaert et al., 2014) with a birth–death tree prior to account for extinction. We parameterized unlinked substitution models and unlinked clock models as suggested by the PartitionFinder analysis. Divergence-time estimation was implemented using an uncorrelated relaxed lognormal clock model of the distribution of rates among branches for each partition (Drummond et al., 2006). We relied on two secondary calibrations (priors set as uniform distributions) based on an extensive nuclear genomic dataset of world amphibians (220 loci 291 kb) (Hime et al., 2021) and used the most recent common ancestors (MRCA) of (1) Dendrobatoidea (between 33.9 Ma and 42.2 Ma HPDs); and (2) Aromobatidae (between 26.4 Ma and 34.4 Ma HPDs). We set two Markov chain Monte Carlo (MCMC) runs of 200 million iterations each, recording every 10,000th iteration and using the first 10 % of iterations as burn-in. We combined the log and the tree files and the resulting posterior samples of trees of the two independent runs using Log-Combiner 2.5 (Bouckaert et al., 2014) and checked convergence of model parameters via time-series plots. Chain mixing was considered adequate when parameters achieved an effective sample size above 500 (obtained for all parameters). We extracted a maximum clade credibility tree (based on the 36,002 resulting trees) using Tree annotator 2.5 (Bouckaert et al., 2014).

2.4. Biogeographic reconstruction

In order to estimate the ancestral range for *Anomaloglossus* species, we defined nine biogeographic regions that encompass the current distribution ranges of the different *Anomaloglossus* lineages and related Aromobatidae that were included in the phylogenetic tree: Atlantic Forest (A), Andes (B), Amazonia excludint the GS (C), Pantepui region (D), western GS (E), southern GS (F), eastern GS (G), these last three areas being the ones recovered from Vacher et al. (2020) based on frog

species composition. We assigned all the Dendrobatidae to "Andes" given that the origin of the family has been inferred to be within this range (Santos et al., 2009). Within Aromobatidae we assigned *Allobates femoralis* to "Amazonia" since this terminal represents a clade that started to diversify in the western part of this region (Réjaud et al., 2020).

We tested three types of models according to biogeographic inference they would favour (1) DIVA, a likelihood version of the parsimony-based dispersal-vicariance analysis (Ronquist, 1997), which favours diversification by vicariance, (2) BAYAREA, a likelihood version of the Bayesian inference of historical biogeography for discrete areas (Landis et al., 2013), which favours in situ diversification, and (3) the Dispersal Extinction Cladogenesis model (DEC) of Lagrange (Ree et al., 2005) which equally favours vicariance and in situ diversification.

Model parameters and ancestral areas were reconstructed using the optimx routine in the package *BioGeoBEARS* (Matzke, 2013a) implemented in R (R Development Core Team, 2016) for six biogeographical models: DEC and DEC+j, with an additional "j" parameter accounting for founder cladogenesis (Matzke, 2013b); DIVA and DIVA+j; BAYAREA and BAYAREA+j.

The best fit of the models was assessed by comparing weighted Akaike's Information Criterion (AIC) scores (Matzke, 2013a).

2.5. Ancestral trait reconstruction

We used a parsimony character mapping approach in Mesquite 3.70 (Maddison and Maddison, 2021) to infer the ancestral states of tadpole development: (1) exotrophic tadpoles vs. (2) endotrophic tadpoles. The references to character attribution are provided in Table S4. When it was not possible to observe the phenotype of the tadpole, we inferred it through parental traits, as we observed that exotrophic tadpoles are associated with adults that live along small streams, and endotrophic tadpoles are associated with adults living in terra firme habitats (Fouquet et al., 2019a, Vacher et al., 2017).

3. Results

3.1. Time-calibrated phylogeny

All the nodes of the inferred tree are strongly supported (0.95–1.0 posterior probability) except for two moderately supported ones (0.90–0.95) (Fig. 1). Within Aromobatidae, *Anomaloglossus* represents a strongly supported clade with a crown age estimated at 21.4 Ma (18.6–24.4 95 % HPDs). *Anomaloglossus tamacuarensis*, a species from the southern Pantepui region (Fig. 1), is strongly supported as the sister group of all other *Anomaloglossus*. In addition to this early diverging lineage, three strongly supported clades emerge within *Anomaloglossus*: a Pantepui group, which includes all species found in Pantepui; the "*degranvillei*" group, with five species that all occur in the eastern GS; the "*stepheni*" group, which contains 12 species of the lowlands of the eastern and the Southern GS. The split between the Pantepui group and the lowlands clade formed by the "*degranvillei*" and "*stepheni*" groups is estimated to date back to the early Miocene, at 17.9 Ma (15.9–20.1). The "*degranvillei*" and the "*stepheni*" groups also diverged from one another in the early Miocene (16.5 Ma, 14.7–18.7). The "*degranvillei*" group comprises two major clades that diverged some 8.8 Ma (7.6–10.2), one formed by *Anomaloglossus degranvillei*, *A. blanci*, and *A. dewynteri*, which are currently known to occur only in French Guiana, and another formed by two lineages currently assigned to *A. surinamensis*. The estimate of the crown age of the "*stepheni*" group is 14.4 Ma (12.7–16.3). The two earliest divergences within the "*stepheni*" group are of *A. stepheni* and *A. apiau*, two endotrophic species occurring in the southern GS. The other species of the *stepheni* group occur in the eastern GS and are exotrophic except for two lineages assigned to *Anomaloglossus baeobatrachus*, which emerged during the Pleistocene.
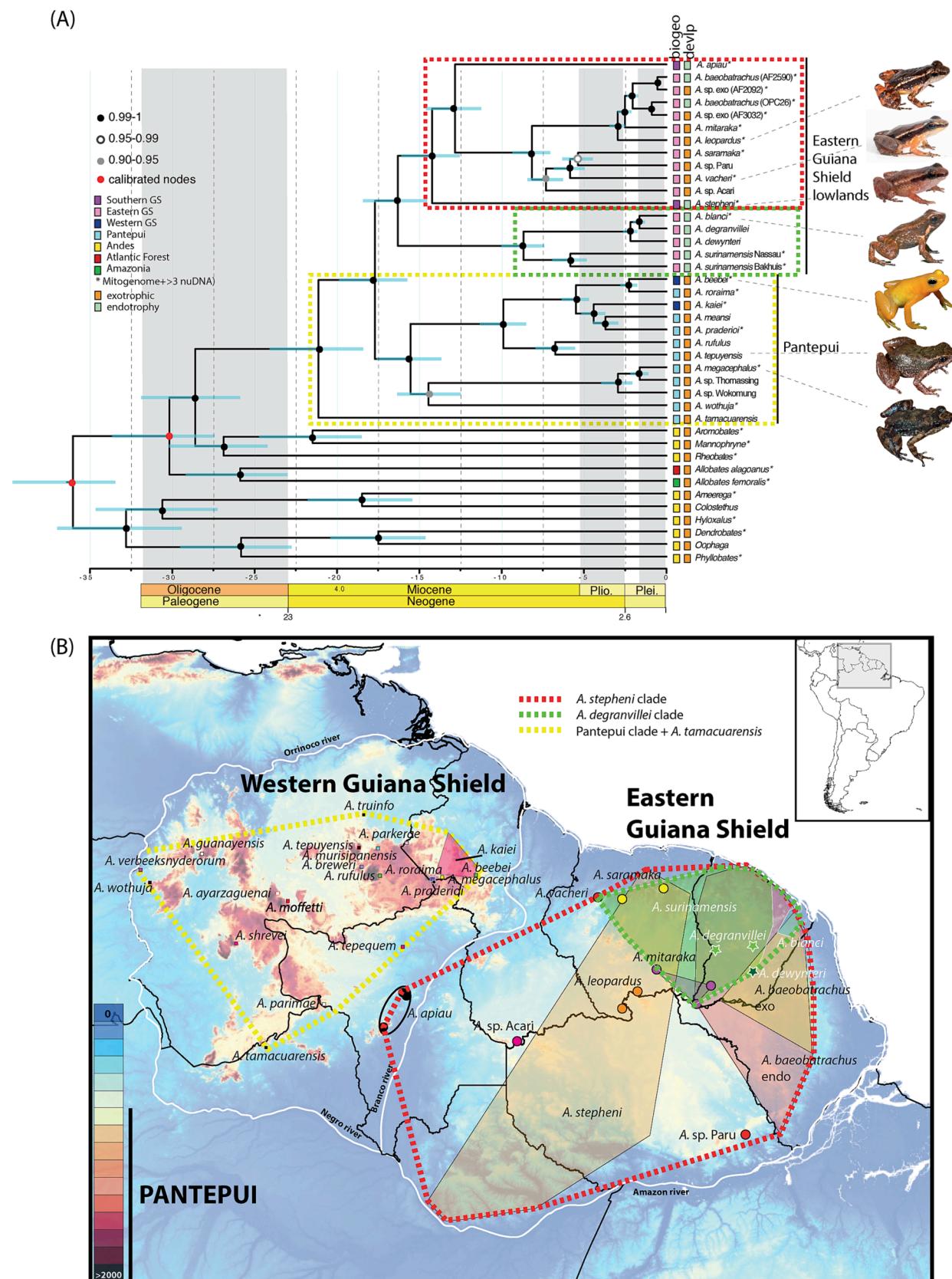
Fig. 1. (A) Time-calibrated phylogeny of *Anomaloglossus* inferred in BEAST 2 based on concatenated mitogenomes and seven nuclear DNA fragments (28S, *TYR*, *POMC, RAG1, RHO, H3F3C, SIAH1*), totaling 18,445 bp. Branch support is expressed as Bayesian posterior probability. Completion matrix is provided in Table S1. (B) Map of the eastern and western Guiana Shield (white lines) with the distribution of all the *Anomaloglossus* species that are circumscribed within dotted convex polygons depicting major lineages. The range of the species with many occurrence records are illustrated with filled-coloured polygons. These results show that diversification started during the early Miocene (21 Ma), and the genus is composed of four major clades, two distributed in the highlands, and two in the lowlands.

3.2. Biogeography and evolution of reproductive mode

The best models including the "j" parameter are DEC+j and DIVA+j, which had similar likelihoods and very similar results and obtained higher likelihoods than the models without the "j" parameter (Table S3). Amongst the models not including the "j" parameter, the best one is the DIVA model. Hereafter, we thus describe the results from the DIVA and DIVA+j models.

The MRCA of *Anomaloglossus* and its sister group (*Aromobates*, *Rheobates*, *Mannophryne*) was likely located in the Andes according to the DIVA+j model (this state is ambiguous with the DIVA model), thus implying an initial dispersal/vicariance ca. 30 and 21 Ma between the Guiana Shield and the northern Andes (Fig. 2). The initial diversification within *Anomaloglossus*, ca. 21 Ma, is located within Pantepui by all the models including the "j" parameter" (but remains ambiguous with the DIVA model). Similarly, all the models including the "j" parameter supported an eastward dispersal from Pantepui to the GS lowlands ca. 16.5 Ma, but where exactly the lowland clade originated in the Guiana

Shield remains ambiguous according to the DIVA model. This dispersal event during the Miocene climatic optimum is concomitant with the transition to endotrophy according to the ancestral state analysis (Fig. 2). Subsequently, during the last 10 Ma, the "*degranvillei*" group diversified within the eastern GS. The history of the "*stepheni*" group is more complex since it seems to have started to diversify in the southern GS and subsequently dispersed to the eastern GS between 8 and 13 Ma according to the DEC+J model (ambiguous according to the DIVA model). This last putative dispersal was concomitant with a reversal from endotrophy to exotrophy according to the ancestral state reconstruction and coincided with a gradual cooling period in the late Miocene (Fig. 2). Like that of the "*degranvillei*" group, subsequent diversification of the "*stepheni*" group (eight species) occurred within the eastern GS during the last 10 Ma. Finally, during the Pleistocene, endotrophy evolved again in the *Anomaloglossus baeobatrachus* complex (Fouquet et al., 2019a).
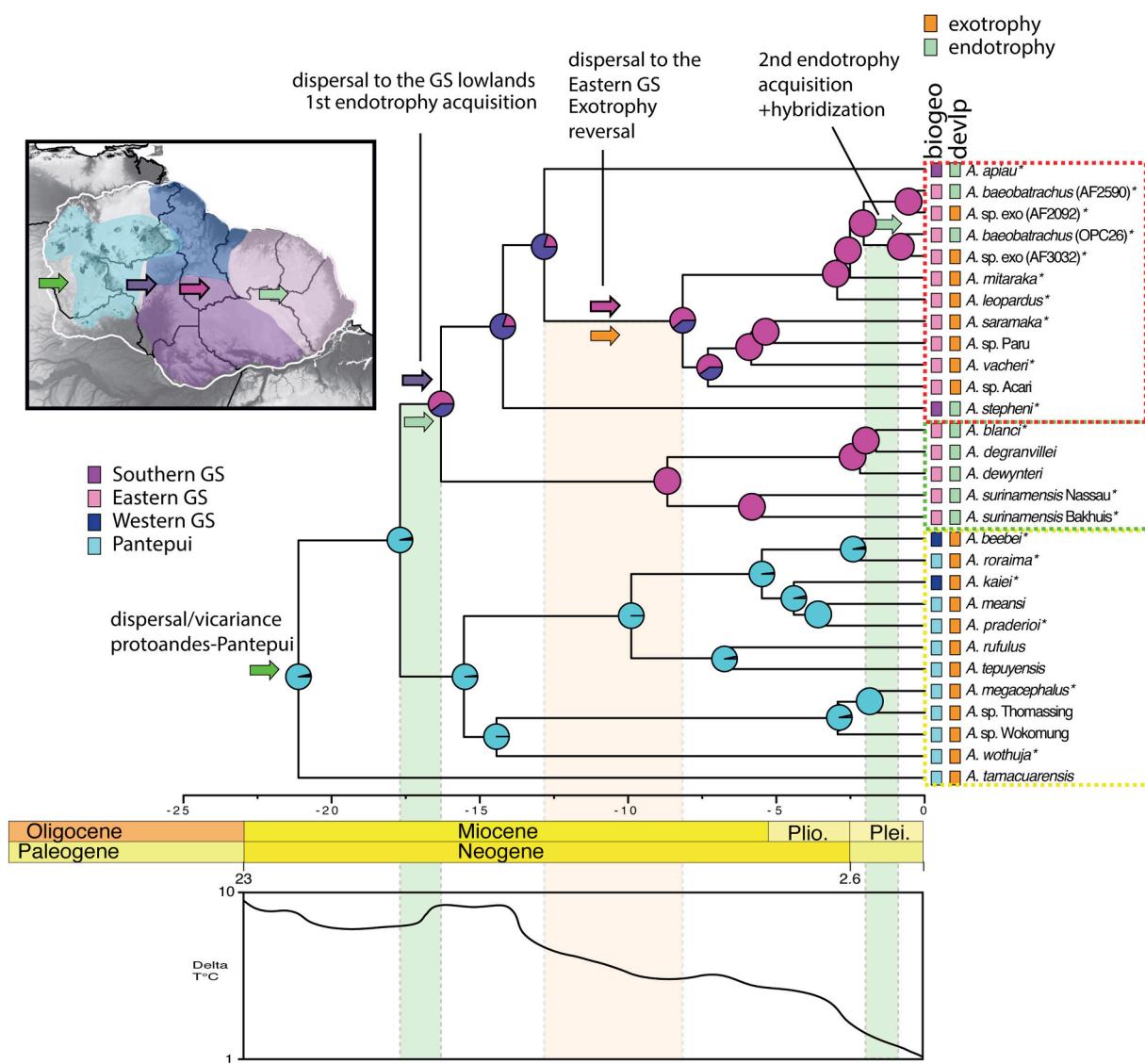
Fig. 2. Maximum clade credibility chronogram inferred in BEAST 2 based on mitogenomic and nuclear DNA, and ancestral areas for *Anomaloglossus* inferred in BioGeoBEARS under the DIVA+J model (results of the DEC model are available in Supporting Fig. 2b). Coloured circles on the tips of the tree indicate the geographical distribution. Pie charts on nodes show the proportion of most likely ancestral areas. Colours of node pie charts correspond to the geographical areas shown in the map. The plot below the geological time scale represents temperature fluctuations through time. These results show an origin of the genus in the highlands of the GS in the early Miocene, then a dispersal to the lowlands of the GS that coincided with the evolution of endotrophy, and a further dispersal to the eastern lowlands of the GS that coincided with a reversal towards exotrophy during the mid-Miocene.

4. Discussion

4.1. Biogeography of Anomaloglossus

The biogeographic history of *Anomaloglossus* provides a striking example of diversification within the Guiana Shield since, to our knowledge, no other vertebrate lineage has diversified to that extent in Pantepui and eastern lowlands throughout the Neogene. This frog genus thus exemplifies the important role that the Guiana Shield may have played in the diversification of the Neotropical biota. South America has little drifted latitudinally over the past 100 Ma, its northern part remaining mainly tropical throughout that period (Hammond, 2005). Moreover, the region remained relatively geologically stable throughout the Cenozoic, unlike the predominantly sedimentary and geodynamic western part of the continent, in particular Western Amazonia (Bicudo et al., 2020; Hoorn et al., 2010). Relative climatic and geologic long-term stability as well as altitudinal gradients may explain why the Guiana Shield highlands permitted the persistence of so many lineages throughout the Neogene (Fouquet et al., 2024; Kok et al., 2018, 2017) and acted as a source of diversity for the surrounding areas for many animal and plant groups, including *Anomaloglossus*. In amphibians, other compelling examples of lineages for which Pantepui acted as a center of diversification from the Early Neogene onwards and that are also distributed in the eastern Guiana Shield have been found in Otophryninae (Fouquet et al., 2021) and *Adelophryne* (Fouquet et al., 2012a). This could also be the case of the frog clade Cophomantini (Pinheiro et al., 2019), the caecilians *Microcaecilia* and *Rhinatrema-Epicrionops* (San Mauro et al., 2014), and plant families such as Bromeliaceae and Rapateaceae (Givnish et al., 2011).

Despite the relative stability of the Guiana Shield over the Cenozoic, climatic conditions have nevertheless fluctuated (Colinvaux et al., 2000; Mayle et al., 2004; Pennington et al., 2000) and have been identified as the cause of Pleistocene speciation in the GS lowlands (Fouquet et al., 2012b; Kok et al., 2018, 2017; Noonan and Gaucher, 2005). The multiple patches of savanna and different types of forests scattered throughout the GS are the product of these historical changes (Gond et al., 2011), and the region is still characterized by a pronounced climatic heterogeneity, with a dry corridor between southern Guyana and the state of Pará while the easternmost part of the shield receives more rainfall (Mayle & Power, 2008). All currently known exotrophic species of *Anomaloglossus* are associated with fresh-water streams that occur in mountains or hilly landscapes. Consequently, they all display relatively restricted ranges both in Pantepui and in the GS lowlands. Similarly, the species of the "*degranvillei*" group and *A. apiau* carry endotrophic tadpoles until metamorphosis but are also associated with streams and are all narrow-range endemics (Fouquet et al., 2018; Vacher et al., 2017). Conversely, *Anomaloglossus stepheni* and *A. baeobatrachus* are both endotrophic and nidicolous and are widely distributed in comparison (Fouquet et al., 2019b). Therefore, the breeding habitat seems to represent an important constraint on the distribution range of the *Anomaloglossus* species, an observation in line with the association between parental care and terrestriality, which is found in anurans in general. Nevertheless, endotrophy with nidicoly is expected to be a successful strategy only in relatively climatically stable areas, i.e., with prolonged rainy periods, allowing a longer parental care, which is required when both embryonic and entire larval development are terrestrial (Vagi et al., 2019). Therefore, we assume that past fluctuating climatic conditions have been crucial in shaping the ranges of these distinct life-history strategies.

4.2. Evolution of larval development

Historical temperature changes are paradigmatic in biotic diversification, in particular for amphibians (Rolland and Condamine, 2019), and it has been likely the case for *Anomaloglossus*. Diversification within *Anomaloglossus* appears to have involved long-term persistence in relatively isolated mountain ranges, since the lowland lineages have derived recently (around 16 Ma) from Pantepui lineages. Interestingly, this dispersal out of the highlands towards the lowlands is concomitant with the evolution of endotrophy in the genus (Fig. 2) during a warm climatic period, the Miocene Climatic Optimum (Böhme, 2003; Hoorn et al., 2022). This new reproductive mode (endotrophic tadpoles) may have allowed more independence from aquatic habitats, and the ancestors of lowland *Anomaloglossus* may have benefited from prevailing warm/wet climatic conditions and expanded their range throughout the terra firme forests of the eastern GS. Conversely, this warm period may have restricted populations or species with exotrophic tadpoles, associated with cold-water streams, to higher elevational ranges in Pantepui. Early Miocene diversification across Pantepui has been similarly documented in *Oreophrynella* (Kok et al., 2018) and *Stefania* (Kok et al., 2017), which could also be linked with upward isolation in relation to colder climates.

Within the lowlands, at a relatively small spatial scale, we detected a clear biogeographic signal, with two major clades occupying distinct areas, one circumscribed to the eastern GS ("*degranvillei*" group) and one that apparently originated in the Southern GS ("*stepheni*" group) and secondarily dispersed to the eastern GS. This last event, which occurred between 13 and 8 Ma, coincided with a cooling period of the Miocene (Hoorn et al., 2010) and was accompanied by a putative reversal from endotrophy to exotrophy (Fig. 2). During this period, *terra firme* lowland forests may have been fragmented, or at least precipitation regimes may have been disturbed, causing species' ranges to become disjunct (Pons and De Franceschi, 2007), thus providing ideal conditions for allopatric speciation to occur. As endotrophy probably requires a high level of atmospheric humidity to prevent the larvae from drying during terrestrial development, drier conditions would have favoured the exotrophic phenotype as it is likely less dependent on atmospheric humidity over the endotrophic form and increased adaptation to stream-associated microhabitats that have more stable hygrometric conditions than *terra firme* (Vacher et al., 2017).

Perhaps the most intriguing shift in reproductive mode within the genus corresponds to a secondary acquisition of endotrophy during the Pleistocene in the *Anomaloglossus baeobatrachus* complex. The existence of two distinct phenotypes within this shallow clade has been the focus of an extensive genomic study (Fouquet et al., 2019a), and our time-calibrated tree does not include relationships among the closely related species of this complex, which underwent past introgressions. The Pleistocene has been a period of rapid fluctuations of temperatures and possibly rainfall in Amazonia (Rull, 2008). Dry-cold conditions were likely unfavorable for endotrophic populations, which may require stable wet conditions to breed in the leaf litter on the hilly landscape of the Guiana Shield. Interestingly, the distribution of the endotrophic species suggests that this life-history strategy originated in the eastern-most part of the Guiana Shield, where the conditions are today the wettest and were possibly more stable during dry-cold conditions of the Pleistocene. Moreover, the current warm and wet climatic cycle may have favoured the endotrophic species, which are currently much more numerous than the exotrophic species. Conversely, the interior of the Guiana Shield, where the exotrophic species seems to originate, is significantly drier. Today, the exotrophic species are rare and found only in small patches isolated along slow-flowing streams. This putative series of events highlights how strikingly rapid (ca. 1 Ma) evolutionary changes of reproductive mode can be in this group of frogs.

Endotrophy has evolved at least 16 times independently in anurans (Gomez-Mestre et al., 2012). However, reversals from endotrophy to exotrophy are very rare in frogs and have only been previously characterized in Hemiphractidae (Castroviejo-Fisher et al., 2015), *Adenomera* (Fouquet et al., 2014), and *Anomaloglossus* (herein), as well as once in Caudata (Chippindale et al., 2004), and once in caecilians (San Mauro et al., 2014). This is consistent with the common view that complex parental investment such as brooding and endotrophy is very unlikely to undergo reversals (Furness and Capellini, 2019; Gomez-Mestre et al.,

2012).

To our knowledge, *Anomaloglossus* represents the first case of successive acquisition, loss, and reacquisition of endotrophy in amphibians. As mentioned earlier, the evolution of endotrophy in itself is frequent in anuran and this strategy has sometimes evolved recently (in the last 10–5 Ma) and several times independently as in *Allobates* (Réjaud et al., 2020), *Chiasmocleis* (Peloso et al., 2014), and *Adenomera* (Fouquet et al., 2014). Identifying the specific genes underlying the transition to endotrophy and understanding their evolutionary history deserve further investigation and even though still highly speculative, the apparent versatility of endotrophic evolution may have been facilitated by a simple genic system (Sun et al., 2020), possibly involving thyroid hormones (Callery and Elinson, 2000).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2023.108008.

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