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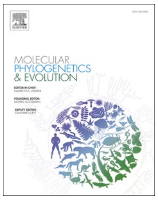

The phylogenetic position of ridley's worm lizard reveals the complex biogeographic history of New World insular amphisbaenids

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ABSTRACT

The archipelago of Fernando de Noronha (FN) is located in the Equatorial South Atlantic Ocean, at 375 km off the northeastern coast of Brazil. Its endemic vertebrate land fauna is restricted to only six species, and three main hypotheses have been proposed to explain their presence in the archipelago. These hypotheses suggest FN had alternative biogeographic connections with: 1) the West Indies; 2) the South America mainland; or 3) Africa. Here, we evaluate for the first time the phylogenetic position of *Amphisbaena ridleyi* within the diversity of Amphisbaenia, and we infer the biogeographic processes that explain its presence in FN and its relationship with amphisbaenids from the West Indies. We analyzed a comprehensive multilocus dataset for Amphisbaenidae using maximum likelihood and time-calibrated Bayesian phylogenetic approaches. Based on our time-calibrated tree, we tested different biogeographic scenarios through historical biogeographic analyses. Our phylogenetic results for the high-level relationships of Amphisbaenia can be parenthetically summarized as (Rhineuridae, (Blanidae, (Bipedidae, (Cadeidae, (Trogonophidae, (Amphisbaenidae))))). Nine highly supported groups of species were recovered among the mainland South American amphisbaenids (SAA), whereas two phylogenetically distant groups of species were inferred for the West Indies: 1) WIC01, an Oligocene lineage present in Cuba and Hispaniola, which is the sister group of all other SAA groups; and 2) WIC02, a Miocene lineage that is restricted to southern Hispaniola and is closely related to *Am. ridleyi*. We estimated two events of transatlantic dispersal of amphisbaenians from Africa to West Indies: the dispersal of Cadeidae during the Middle Eocene, and the dispersal of the ancestor of *Amphisbaena* during the transition Eocene/Oligocene. These events were likely affected by the North Equatorial and South Equatorial currents, respectively, which have been flowing westwards since the Paleocene. The ancestral cladogenesis of *Amphisbaena* during the Late Oligocene is likely related to overwater dispersal events, or alternatively can be associated with the fragmentation of GAARlandia, when WIC01 was isolated in the West Indies, while the remaining groups of *Amphisbaena* diversified throughout the South American continent. During the Late Miocene, the ancestor of WIC02 dispersed from northern South America to the West Indies, while *Am. ridleyi* dispersed from the same region to FN. The overwater dispersal of WIC02 was driven by the North Brazilian Current and the dispersal of *Am. ridleyi* was likely influenced by the periodic shifts in direction and strength estimated for the North Equatorial Counter-Current during the Late Miocene.

1. Introduction

Fernando de Noronha (FN) is a small volcanic archipelago situated in the equatorial South Atlantic Ocean, at 375 km off the northeastern coast of Brazil. Although its geographic isolation, the biogeographic origin of its endemic fauna has already offered some unique insights about the processes that shaped the neotropical biota (e.g., Carleton and Olson, 1999; Pereira and Schrago, 2017). From the beginning of the

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16th century to the first decades of the 20th century, the largest island of FN (around 16.4 km^2) was a common oceanic stopover, constantly visited by European sailors and explorers because of its strategic position in the south equatorial region (e.g., Charles Darwin visited FN in 1832, during the Beagle voyage). Although the continuous afflux of explorers has provided primal accounts about the biodiversity of FN, it was only in the last decades of the 19th century that the biogeographic history of the archipelago started to be appropriately evaluated through the efforts of the British naturalist H. N. Ridley (Ridley, 1888, 1890).

Ridley visited FN in 1887 and he was the first to analyze and properly collect the "lizard with two heads", which was known for over 350 years since the discovery of the archipelago by Amerigo Vespucci in 1503 (Branner, 1888). Ridley correctly identified the lizard as an endemic amphisbaenid and briefly stated it would be "allied not to Brazilian but to West-Indian forms" (Ridley, 1888). He shipped several specimens to Europe, where they were analyzed by G. A. Boulenger, who described *Amphisbaena ridleyi* Boulenger, 1890 in honor of the British naturalist.

The relationship between *Am. ridleyi* and the West-Indian amphisbaenids was generally accepted and unquestioned during the earliest decades of the 20th century (e.g., Mertens, 1934; Scharff, 1912). This hypothesis of relationship was only challenged in 1963 when Carl Gans redescribed *Am. ridleyi* (Gans, 1963). Gans suggested that the affinity proposed by Ridley and Boulenger was mistaken because they had based their conclusions on a poor taxon sampling of amphisbaenids from South America (SA). Since then, the phylogenetic affinity and biogeographic origin of *Am. ridleyi* have been considered a mystery (Laguna et al., 2010; Pregill, 1984).

After Ridley's primal publications (Ridley, 1888, 1890), the biogeographic history of FN has been the subject of several studies (e.g., Joyeux et al., 2001; Olson, 1977; Neves et al., 2016; Pereira and Schrago, 2017). Analyses focused on sea organisms have confirmed the biogeographic link between FN and the West Indies (WI) (e.g., reef fishes, Floeter et al., 2007; decapod crustaceans, Tavares et al., 2017; shore mollusks, Vermeij et al., 2010; and benthonic flora, Villaça et al., 2006), although studies on the FN small endemic land fauna have resulted in a very diverse set of alternative biogeographic hypotheses.

One of the most accepted alternative hypotheses derives from the description of *Noronhomys vespuccii* Carleton and Olson, 1999, a large and currently extinct species of oryzomyine (Cricetidae: Sigmodontinae), which represents the only known mammal endemic from FN. Carleton and Olson (1999) showed *No. vespucci* is closely related to *Ludomys* and *Holochilus*, two extant South American genera of semiaquatic rodents. The authors expanded their hypothesis by suggesting that a semiaquatic ancestor drifted to FN on floating logs during the late Pliocene or early Pleistocene, and since then the species adapted to a terrestrial lifestyle.

Similar to *No. vespuccii*, the endemic bird fauna of FN partially supports the hypothesis of a close connection between the archipelago and the mainland SA. Besides the well-known diversity of vagrant bird (Whittaker et al., 2019), only three species of land birds are endemic to FN (Olson, 1981): a vireo (*Vireo gracilirostris*), a flycatcher (*Elaenia ridleyana*), and an extinct undescribed flightless rail (family Rallidae). Although no phylogenetic study has included any of these three species, the ornithological literature has been consistent in considering the morphological similarity between *Vi. gracilirostris* and *El. ridleyana* with its generic counterpart distributed in mainland Brazil (Mejías et al., 2020; Olson, 1994; Rheindt et al., 2008) as evidence supporting their close relationship. On the other hand, the extinct undescribed flightless rail is supposed to belong to a different and undescribed genus, not found in Brazil, and possibly associated with the Inaccessible Island rail, *Laterallus podarces* (Stervander et al., 2019), from the Tristan Archipelago in the central South Atlantic Ocean (Olson, 1977).

Besides *Am. ridleyi*, the only other endemic squamate from FN is the scincid lizard *Trachylepis atlantica* (Schmidt, 1945) (previously included in the genus *Mabuya*). Molecular phylogenetic analyses have suggested an African origin for this endemic skink, which would have diverged during the Early Miocene (around 17 Mya) (Mausfeld et al., 2002; Pereira and Schrago, 2017; Whiting et al. 2006). To account for the conflict between the estimated time of divergence of *Tr. atlantica* from its African ancestors, and the estimated age of formation of FN (around 12.4 Mya; Lopes et al., 2014), Pereira and Schrago (2017) suggested a stepping-stone process of dispersal through paleo islands—very similar to that proposed by Olson (1977) to explain the origin of the FN endemic flyless rail.

Amphisbaena ridleyi shares several morphological traits with continental South American congeners—such as unfused head scales and pleurodont dentition—although its short tail is distinct, reducing sharply and then progressively posteriorly to the cloaca and continuing as a smooth cone to the tip (Gans, 1963). This unique tail shape represents a similar condition also shared by the African genus *Trogonophis*. Moreover, the highly specialized cranial morphology of *Am. ridleyi* (Pregill, 1984) has precluded any definitive conclusion about its affinity-based exclusively on osteological evidence (Gans, 1963; Pregill, 1984).

Considering the scenarios described above, three main biogeographical hypotheses for the origin of *Am. ridleyi* can be outlined: 1) the ancestor of *Am. ridleyi* is related to West Indian forms (hypothesis derived from Ridley's original comments, also supported by studies on sea organisms and plants); 2) the ancestor of *Am. ridleyi* dispersed directly from the Brazilian coast (Gan's hypothesis also supported by the extinct rodent and two endemic land-birds); and 3) the presence of *Am. ridleyi* in FN is the result of dispersal from Africa on a route that included central Atlantic islands (supported by the molecular studies on the endemic skink and the extinct flightless rail).

Here, we evaluate for the first time the phylogenetic position of *Am. ridleyi* within Amphisbaenia, aiming to bring additional evidence about the processes that resulted in the colonization of FN by land vertebrates, and assessing its connection with the biogeographic history of Africa, South America, and the WI. We based our study on a multilocus molecular dataset used to estimate a time-calibrated tree, and we implemented historical biogeographic model-based analyses to test different scenarios. Additionally, we provide a comprehensive evaluation of the phylogenetic relationships within Amphisbaenidae, and we reassessed the biogeographic history of the group, commenting on the impacts of oceanic currents affecting overwater dispersion during the Eocene, Oligocene and Miocene in the Equatorial Atlantic.

2. Materials and methods

2.1. Taxon sampling and DNA sequencing

We collected three individuals of *Amphisbaena ridleyi* (field numbers MTR 11966–11968) under rocks in the main island of FN (3°51′21.2″ S and 32°26′31.5″ W, IBAMA permit number 02010.000240/2007-03) during the period of 20th to 22nd June 2007. For these individuals, we amplified and sequenced for the first time three mitochondrial–12S ribosomal RNA (*12S*), 16S ribosomal RNA (*16S*), and NADH dehydrogenase subunit 2 (*nd2*); and three nuclear gene fragments–Brain-derived neurotrophic factor (*bdnf*), Recombinant activating gene 1 (*rag1*), and Oocyte maturation factor (*c-mos*). We also generated sequences for other amphisbaenids to fill sampling gaps for these genes, aiming to assemble a molecular matrix avoiding non-random missing data (Sanderson et al., 2010). The list of sequenced samples can be seen in Table S1.1 (Appendix A in Supplementary Material) and the sequences are deposited in GenBank (accession OK523339-OK523371).

DNA was extracted from fresh liver preserved in 95% ethanol using high salt precipitation method (Bruford, et al., 1992). PCRs were performed using standard protocols developed by Kearney and Stuart (2004) and Mott and Vieites (2009), with adjustments to increase amplification efficiency of amplification. All sequences were quality checked, and when necessary, edited manually. The consensus of both strands was generated using Geneious 7.1.8 (Kearse et al., 2012).

We also included sequences from GenBank for six families of amphisbaenids, as followed: Rhineuridae (one species), Bipedidae (three species), Blanidae (three species), Cadeidae (one species), Trogonophidae (three species), and Amphisbaenidae (68 species) (see complete list of terminals in Table S1.1). We root our tree using sequences of *Lacerta agilis*, following the hypothesis of Lacertibaenia (Vidal and Hedges, 2005) in which Lacertidae is the sister group of Amphisbaenia (Burbrink et al., 2020).

2.2. Alignment and phylogenetic inference

Sequences were aligned using MAFFT 1.3.6 (Katoh and Standley, 2013) as implemented in Geneious. We used default parameters for gap opening and extension. All protein-coding genes were visually checked using Geneious to verify if they follow the correct reading frame. We used PartitionFinder 2 (Lanfear et al., 2016) to choose the combined sets of partitioning schemes and models of molecular evolution. The concatenated matrix was divided into 14 partitions (each coding genes were partitioned by codon positions and each rRNA was analyzed as a separate partition) and we performed a search using the *greedy* option. We allowed the program to select using Akaike Information Criterion with correction (AICc) only the GTR-based models of molecular evolution (GTR and GTR + G) implemented in RAxML 8.2.3 (Stamatakis, 2014). We did not allow PartitionFinder to select models with correction for the proportion of invariant sites (P-Invar), as suggested by Alexander Stamatakis in RAxML's manual (https://cme.h-its.org/exelixis/resource/download/NewManual.pdf), to avoid correlation between values of alpha and P-Invar.

We performed a maximum likelihood (ML) analysis using RAxML 8.2.3 using the algorithm that conducts a rapid bootstrap analysis and searches for best scoring ML tree in the same run (option *-f a*). We run 1000 bootstrap replicates, and the best-scoring ML tree was estimated 200 times using as starting tree each 5th bootstrap tree. To assess the robustness of our ML tree topology, we evaluated the following three alternative approaches to provide internal branch supports: Felsentein bootstrap proportions (FBP, Felsentein, 1985), Shimodaira-Hasegawa-Like test (SHL, Anisimova and Gascuel, 2006; Guindon et al., 2010), and transfer bootstrap expectation metrics (TBE, Lemoine et al. 2018). Although FBP has been the predominant method for assessing branch support in systematics (Lemoine et al. 2018), some studies have suggested its usefulness can be limited by several factors (Sanderson, 1995; Soltis and Soltis, 2003; Susko, 2009), for example, be misleading at shorter branches (Wiens et al., 2008; Pyron et al., 2011), suffer from the "star-tree paradox" (Steel and Matsen, 2007; Susko, 2008), or be strongly sensitive to unstable rouge taxa (Wilkinson, 1994; Lemoine et al. 2018). FBP provides for each inner branch a summarized result of the proportion of trees that recovered the specific branch based on a set of replicates of random data resampling. Therefore, the method can be strongly affected by the number and type of sites/characters that support the branches (Harshman, 1994; Soltis and Soltis, 2003).

Differently than FBP, SHL is a modification of the standard likelihood-ratio test, and it is supposed to be less sensible to shorter branches because it would not be affected by the relative number/type of sites supporting short inner branches (which frequently produce low values of FBP). In brief, for each specific internal branch, SHL compares the ML value of the best ML tree to the ML values of the suboptimal trees provided by the nearest-neighbor interchange (NNI) rearrangements of that specific internal branch (Guindon et al., 2010). On the other hand, TBE was proposed as an improved version of FBP, in which the effect of rogue taxa can be quantified by a transfer index that represents the number of taxa that must be transferred to equate a branch of the best ML tree with its closest branch in a non-parametric bootstrap tree (Lemoine et al. 2018). The TBE value is then recursively calculated for all branches and average among all bootstrap trees.

We estimated FBP using the 1000 bootstrap replicates implemented in our main RAxML analysis and, based on the resulting bootstrap trees, we calculated TBE using RAxML-NG (Kozlov et al., 2019). The SHL was estimated as implemented in RAxML (option–f J) for each branch of the tree. We considered values greater than 70% as indicating highly supported branches for FBP and TBE, while for SHL we considered highly supported only branches with values greater than 80%.

Besides the concatenated analysis (super matrix) we also generated using RAxML independent gene trees for each locus (mitochondrial DNA, *bdnf*, *c-mos*, and *rag1*). These gene trees were used to estimate a species tree using ASTRAL v.5.7.8 (Zhang et al., 2018). Additionally, we generated a species tree based on nuclear gene trees only, and an ML analysis of concatenated nuclear loci to compare the phylogenetic signal among loci and the influence of the mitochondrial and nuclear data on both approaches. We also computed Shimodaira-Hasegawa test to estimate the significance of the log likelihood difference between our best ML tree and specific alternative topologies for Amphisbaenidae (e.g., Longrich et al., 2015). Shimodaira-Hasegawa tests were carried out as implemented in RAxML.

2.3. Divergence time estimation

We used the Bayesian approach implemented in BEAST v2.6.3 (Bouckaert et al., 2019) to estimate divergence times among lineages of Amphisbaenia. For this analysis, we set the data partitions per sequenced gene fragment (six partitions), and we linked the clock and site models among the nuclear loci and the rRNA genes to avoid over-parameterization and improve convergence (Rannala, 2002). We ran BEAST using the bModelTest plugin (Bouckaert and Drummond, 2017) to estimate the site models, allowing the program to average overall models. The bModelTest plugin switches between substitution models during the MCMC analysis, inferring and marginalizing site models, thus averaging out uncertainty over the models. The mutation rate was estimated for all partitions, and the set of models was defined as 'all-reversible'. We used the relaxed Bayesian molecular clock method (Drummond et al., 2006) with uncorrelated log-normal rates among branches (Drummond et al., 2006), assuming a Birth-and-Death process prior for the speciation model. We ran two independent runs (different seeds) of 5×10^8 generations, sampling the parameters and trees every 5×10^4 generations. Four calibration points were implemented using monophyletic constraints, they are described in Appendix B (Supplementary Material). We used log-normal priors with an off-set fixed to the age of the fossil and default mean and standard deviation values for all calibration points. We included additional topological constraints based on the ML tree aiming to speed up the Bayesian analysis by reducing the tree topology space (xml file available at https://doi.org/10.6084/m9.figshare.14939241).

We used Tracer v1.6.1 (Rambaut et al., 2014) to check for trace convergence and values of ESS (effective sample size), and LogCombiner v2.4.7 (Bouckaert et al., 2019) to perform the burn-in and subsample the tree files to 10,000 trees. TreeAnnotator v2.4.7 (Bouckaert et al., 2019) was used to summarize the tree distribution and the estimated parameters. All analyses using BEAST were performed online at the CIPRES Science Gateway (Miller et al., 2010).

2.4. Biogeographic analysis

To encompass the global and local biogeographic patterns of Amphisbaenia, we assigned each terminal distribution to one or three (maximum) of the following nine biogeographic regions adapted from Holt et al. (2013) and Batista et al. (2020): Afrotropical (AF), Antilles (AT), Nearctic (NA), Central Panamanian (PA), Amazon Forest (AM; including all South American Equatorial Rain Forests), Atlantic Forest (AF), Open Diagonal (OD, including all South American open formations), Fernando de Noronha (FN), and Mediterranean-Saharo-Arabian (ME). The regions PA, AM, AF, and OD derived from the split of the Panamanian and Neotropical regions from Holt et al. (2013), and we based the limits among these subregions on Olson et al. (2001).

We performed historical biogeographic analyses using the R package

BioGeoBEARS (Matzke, 2013a, 2013b). We implemented the following three biogeographic models: DIVALIKE (a likelihood version of dispersal-vicariance DIVA; Ronquist, 1997), DEC (Dispersal-Extinction-Cladogenesis, Ree et al., 2005), and BAYAREALIKE (a likelihood version of BayArea model; Landis et al., 2013). For each of these biogeographical models, we also implemented models allowing the estimation of a parameter describing founder-event jump dispersal (+j), totaling six different model implementations. We compared all models using AICc (Akaike Information Criterion with correction), aiming to choose the best fitting model for our dataset. These six models were combined with three different time stratified scenarios. The first scenario, NTS (No Time Strata), was set defining no time stratum and represents our null biogeographic hypothesis. Our second scenario, TSDM (Time Stratified/Dispersal Matrix), included seven strata, for which seven different matrix of dispersal multipliers were implemented. In the third scenario, TSDMA (Time Stratified/Dispersal Matrix/Allowed Areas), besides including seven time-strata and seven dispersal multipliers matrices, we also included a matrix indicating the allowed areas for each time stratum. The time periods set for the time stratified analyses can be seen in Appendix B. The dispersal multipliers were set as 1.0 (very probable) for land adjacent regions; 0.5 (probable) for water separated regions distant less than 1000 km; 0.1 (not probable) for regions distant over 1000 km; and 0.0001 (not allowed) for area not available for dispersal (e.g., submerged islands). To account for areas that were not present in some of the defined time periods (e.g., Central Panamanian, Antilles, and FN), we did not allow the estimation of the correspondent area-states during these periods. All files and scripts used to test the models and scenarios in BioGeoBEARS are available at https://doi.org/10.6084/m9.figshare.14939298.

3. Results

3.1. Phylogenetic relationships

Our final concatenated alignment comprises 4813 base pairs (1024 bp for *12S*, 536 bp for *16S*, 765 for *nd2*, 691 for *bdnf*, 1251 for *rag-1*, and 584 for *c-mos*) and it is available at https://doi.org/10.6084/m9.figshare.14939340. The selected partition scheme includes 10 partitions, and the partition set can be seen in Table S1.2.

Concerning the high-level affinities within Amphisbaenia, the resulting tree topology of our main ML analysis (Figures 1 and S1 presented in Appendix C) was similar to those presented by previous studies (Gauthier et al., 2012; Longrich et al., 2015; Measey and Tolley, 2013; Mott and Vieites, 2009; Vidal et al., 2008). All presently recognized families of Amphisbaenia were recovered as monophyletic in our analyses (Fig. 1). The extant monophyletic families of Amphisbaenia and their support values (percentages of FBP/SHL/TBE in parenthesis) are as follows: Bipedidae (100/100/100), Blanidae (100/100/100), Trogonophidae (100/100/100), and Amphisbaenidae (98/94/100). Monophyly of Cadeidae was not tested because we only sampled one species for this family.

Blanidae and Bipedidae were retrieved as successive sister groups of Amphisbaenoidea, a highly supported clade (86/93/98) formed by Cadeidae, Trogonophidae, and Amphisbaenidae. The basal relationship among these lineages received low values of bootstrap in our analysis. The Cuban family Cadeidae was positioned as the sister group of a highly supported clade (100/100/100) formed by families Trogonophidae and Amphisbaenidae (Fig. 1). The relationship among Blanidae, Bipedidae, and Amphisbaenoidea was not well corroborated by all support metrics in our phylogenetic analyses. Blanidae and Bipedidae were recovered as successive sister groups of Amphisbaenoidea in the ML and Bayesian analyses (see below).

Within Amphisbaenidae, the African genera *Zygaspis*, *Cynisca,* and *Chirindia* formed a monophyletic group, however with low bootstrap values (Figure S1). In our analyses, *Chirindia* and *Cynisca* clustered together in a highly supported clade (93/93/97), having *Zygaspis* as their sister group. The other three African genera of Amphisbaenidae, *Geocalamus, Monopeltis*, and *Dalophia,* clustered together with low bootstrap values (Figure S1) and were recovered as a sister group of a monophyletic *Amphisbaena* (95/95/99, *sensu* Mott and Vieites, 2009).

Similar to previous findings (e.g., Mott and Vieites, 2009; Longrich et al., 2015; Teixeira et al., 2019), the genus *Amphisbaena* is non-monophyletic if the genera *Leposternon*, *Bronia*, *Cercolophia,* and *Aulura* are considered as valid. Here, we are following the taxonomic proposal of Mott and Vieites (2009), considering all South American and West-Indian species of amphisbaenids as included into *Amphisbaena*. The group of West-Indian species of *Amphisbaena* was recovered as non-monophyletic (Figures 1 and S1), indicating the presence of two very divergent phylogroups: WIC01 (68/96/89), composed by *Am. manni*, *Am. xera*, *Am. caeca*, *Am. fenestrata, Am. schmidti*, *Am. bakeri, Am. barbouri*, *Am. carlgansi,* and *Am. cubana*; and WIC02 (72/100/86) formed by the species *Am. leali*, *Am. innocens,* and *Am. hyporissor* from the southern Hispaniola Island. Clade WIC01 was recovered as the sister group of all other species of *Amphisbaena*, while WIC02 was positioned in a highly supported clade (98/71/100), as the sister group of *Am. ridleyi* (Fig. 1).

In general, the basal relationship among the South American species of *Amphisbaena* received low values of bootstrap in our tree topology. However, several highly supported clades can be identified, forming the following group of species: G01 (100/80/100) *Am. talisiae*, *Am. silvestri*, and *Am. anaemariae*; G02 (100/58/100) formed by *Am. leeseri*, *Am. kingii*, *Am. augustifrons*, *Am. darwini*, and *Am. munoai*; G03 (100/80/100), including *Am. polystega*, *Am. infraorbitale*, and *Am. microcephala*; G04 (100%) composed by *Am. cuiabana* and *Am. hastata*; G05 (100/96/100) formed by *Am. bahiana*, *Am. longinqua*, *Am. mongoyo*, and *Am. uroxena*; G06 (100/94/100), comprising *Am. roberti*, *Am. caiari*, *Am. mitchelli*, and *Am. miringoera*; G07 (98/98/99) composed by *Am. ignatiana*, *Am. arenaria*, *Am. kraoh*, *Am. saxosa*, and *Am. carli*; G08 (100/100/100), including *Am. vermicularis*, *Am. pretrei*, *Am. leucocephala*, *Am. alba*, *Am. bolivica*, and *Am. camura* (groups G07 and G08 were recovered as sister groups supported by 100/99/100 percentage values); and G09 (99/90/99) formed by *Am. mertensii* and *Am. cunhai*. *Amphisbaena brasiliana* and *Am. fuliginosa* were retrieved as the sister group of the clade formed by *Am. ridleyi* and WIC02, although supported by low values of bootstrap (Fig. 1).

The gene tree analyses recovered similar topologies, corroborating most of high-level relationships within Amphisbaenia and the species groups of South American amphisbaenids. The gene trees are presented in Appendix C (Figures S2-S5) and some contrasting aspects of their topologies are discussed below. Similarly, the topology of the ML tree based on the concatenated nuclear loci (Figure S6) was most similar to our main concatenated ML result. On the other hand, the resulting topology of the species tree analysis (Figure S7) suggested a different relationship among some groups of species within Amphisbaenidae. Instead of presenting WIC01 as the sister group of all other South American amphisbaenids—like in the ML tree based on the concatenated matrix—our species tree analysis retrieved WIC01 as belonging to a clade formed by G01, G02, G03, G04 and G05, which is the sister group of a clade composed by all other South American amphisbaenids, except *Am. anomala*.

3.2. Divergence time estimation

The topology of the Bayesian calibrated tree (Fig. 2) was very similar to the topology of our ML tree (Fig. 1). The analysis indicates that the TMRCA (Time of the Most Recent Common Ancestor) of all Amphisbaenia dates to the Middle Cretaceous (about 88 Mya), while the TMRCA of the Amphisbaeniformes (Bipedidae, Blanidae, Cadeidae, Trogonophidae, and Amphisbaenidae) date to the Early Paleocene (around 66 Mya). Within Amphisbaeniformes, all main clades diverged during the Middle Eocene to Middle Oligocene, between approximately 57 to 38 Mya.

The divergence between Amphisbaenoidea (Cadeidae,

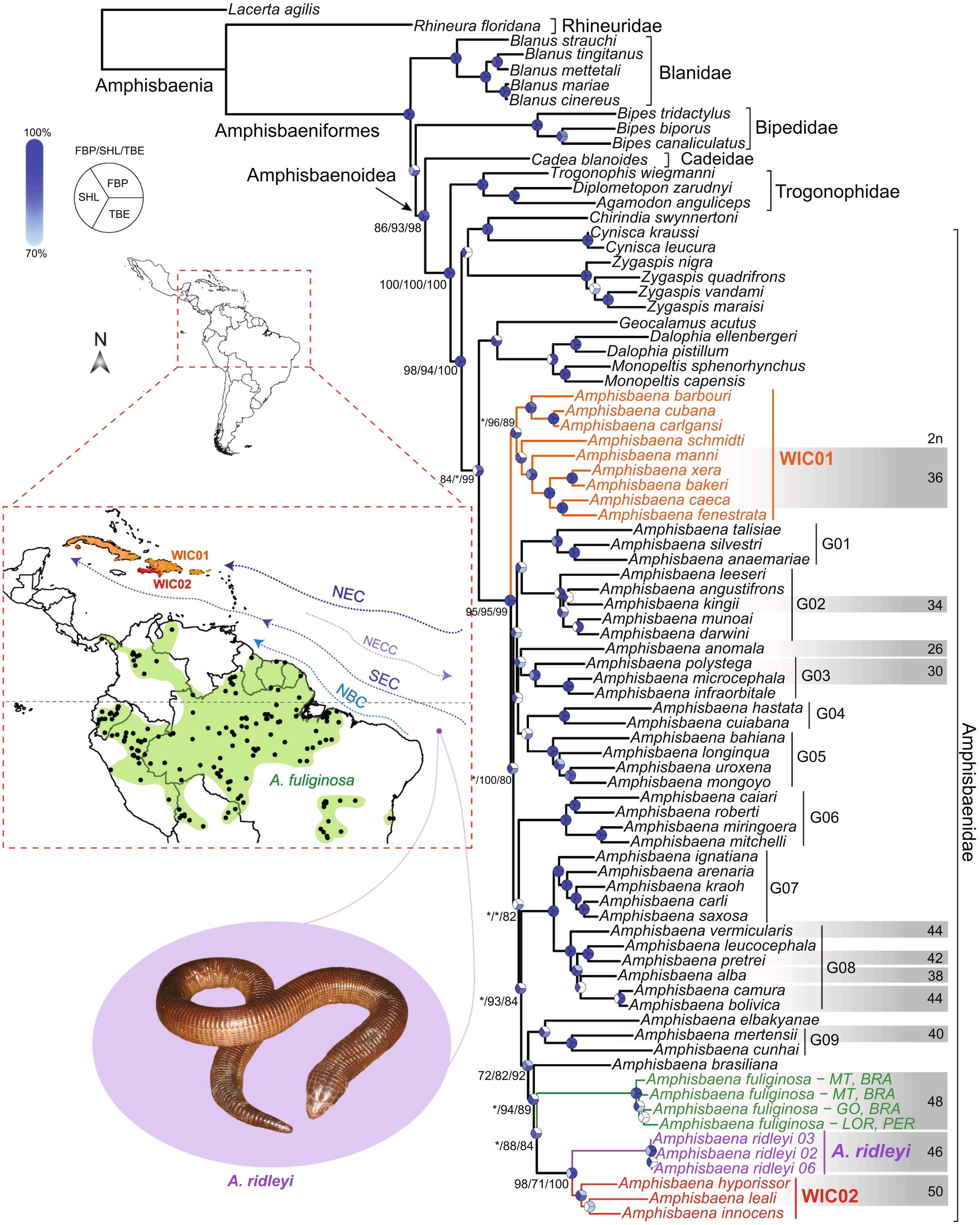

Fig. 1. Maximum likelihood phylogenetic tree of Amphisbaenia. Numbers on branches, near nodes represent values for Felsentein bootstrap proportions (FBP), Shimodaira-Hasegawa-Like test (SHL), and transfer bootstrap expectation metric (TBE). Colored pies on nodes illustrate, based on a blue gradient, the values obtained for all support metrics. Asterisks near nodes and white pie slices indicate support lower than 70%). NEC, North Equatorial Current; SEC, South Equatorial Current; NECC, North Equatorial Contra-Current; NBC, North Brazilian Current. G01 to G09, indicate the mainland South American groups of species identified for *Amphisbaena*. WIC01 and WIC02, indicate the species groups of *Amphisbaena* identified for the West Indies. MT and GO, Brazilian (BRA) states of Mato Grosso and Goiás, respectively. LOR, department of Lorena in Peru (PER). Values inside gray boxes indicate the karyotype number (2n) estimate for species of *Amphisbaena* (see text for references). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

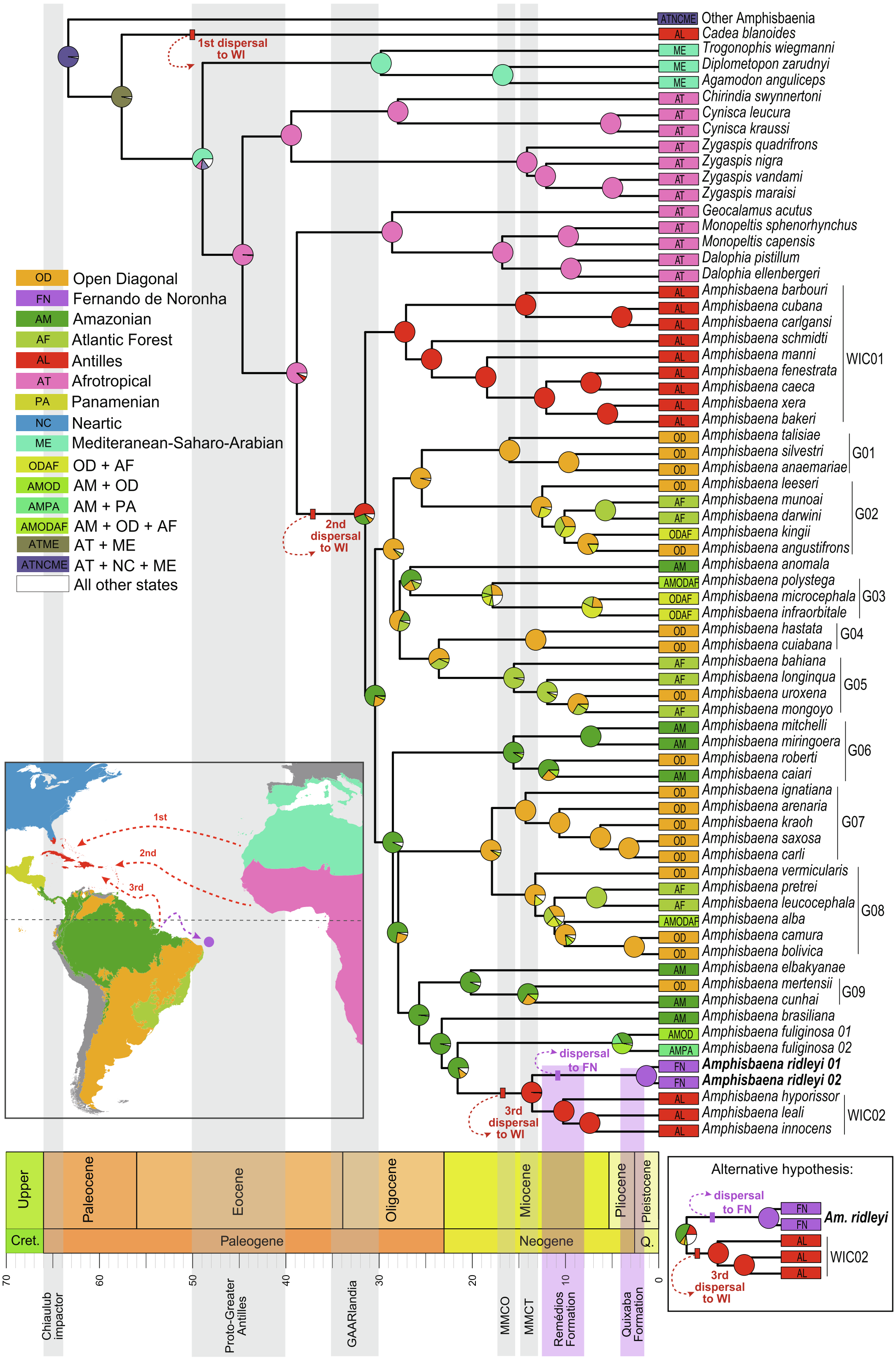

Fig. 2. Calibrated Bayesian phylogenetic tree of amphisbaenoids and historical biogeographic ancestral regions reconstruction based on the DEC + J model and TSDM scenario (see text for details). Pie charts represent the probabilities of ancestral ranges at the respective nodes. G01 to G09, indicate the mainland South American groups of species identified for *Amphisbaena*. WIC01 and WIC02, indicate the species groups of *Amphisbaena* identified for the West Indies. MMCO, Mid Miocene Climatic Optimum; MMCT, Mid Miocene Climatic Transition. Alternative biogeographic hypothesis for *Amphisbaena ridleyi* and WIC02 based on the DEC + J model and TSDM scenario considering the direction and strength of Equatorial Atlantic currents (see text for details).

Trogonophidae, and Amphisbaenidae) and Bipedidae/Blanidae occurred during the Middle Paleocene (about 60 Mya), while the divergence between Trogonophidae and Amphisbaenidae occurred in the Middle Eocene (around 44 Mya). Amphisbaenidae started diversifying during the Middle Eocene (about 42 Mya), and the cladogenic event that split the African and Neotropical radiations of Amphisbaenidae was estimated to have occurred after the Late Eocene (approximately 38 Mya).

Our estimates suggest that WIC01 diverged from all other species of *Amphisbaena* just after the transition between the Early and Late Oligocene (about 31 Mya). The basal divergence among the species groups of *Amphisbaena* occurred between the Late Oligocene and the Early Miocene (from 30 to 17 Mya). The clade composed by *Am. ridleyi* and WIC02 diverged from the mainland lineage of *Am. fuliginosa* during the Early Miocene (about 21 Mya), while the TMRCA between *Am. ridleyi* and WIC02 was estimated at approximately 13 Mya, during the Middle Miocene.

3.3. Biogeographic analysis

Our historical biogeographic analyses indicated that "Dispersal-Extinction-Cladogenesis", including the "jumping dispersal" parameter (DEC + J), is the best model for all tested scenarios (Table S1.3). When the "jumping dispersal" parameter is disregarded, the best model is "Dispersal-Extinction-Cladogenesis" (DEC) for all scenarios.

The main differences among NTS, TSDM and TSDMA were restricted to ancestral region estimated for the MRCA of Amphisbaeniformes and the MRCA of Amphisbaenoidea. The distinct reconstructions for these scenarios, under DEC + J and DEC models, can be seen in Appendix C. Among the simplest (NTS) and the most constrained (TSDMA) scenario, we chose to describe the reconstructions based on TSDM, but we will also comment below specific differences among them.

The most probable region for the ancestor of crown-Amphisbaenia was estimated as Afrotropical (AT) + Nearctic (NC) + Mediterranean-Saharo-Arabian (ME) (AT + NC + ME), which suffered a subsequent vicariant event related to the split between *Rhineura* and the Amphisbaeniformes (Fig S8). A transoceanic dispersal was estimated for the ancestral of Bipedidae and the most probable region for the ancestor of Amphisbaenoidea was estimated as AT + ME. The ancestor of Cadeidae underwent the first dispersal event from Africa to the West Indies (AL), while the common ancestor of Trogonophidae and Amphisbaenidae dispersed from ME to AT, and from AT, the ancestor of the Neotropical amphisbaenids dispersed to South America.

The Antilles (AL) is the most probable region estimated for the ancestor of *Amphisbaena*, suggesting a second dispersal from Africa to the WI. The WIC01 represents the lineage that maintained the original distribution restricted to AL, whereas Amazonian (AM) is the most probable region estimated for the ancestor of all other South American species of Amphisbaenia, indicating a dispersal to AM from AL (but see discussion for alternative hypothesis). AM was frequently estimated as the most probable region for most of the basal ancestors within the diversity of South American *Amphisbaena*, suggesting several dispersals from AM to the Open Diagonal (OD). The oldest of these dispersal events is related to the MRCA of species groups G01, G03, G04 and G05. Within this clade, a subsequent dispersal from OD to Atlantic Forest (AF) occurred for groups G03 and G05. A more recent dispersal from AM to OD was estimated for the MRCA of groups G08 and G07, with a subsequent dispersal from OD to AF within group G08.

Our analysis indicates AL as the hypothetical region for the common ancestor of *Am. ridleyi* and the WIC02 (Fig. 2), suggesting a third dispersal to AL—this time from the Amazonian region—with a subsequent dispersion from AL to FN (but see discussion for alternative hypothesis).

4. Discussion

4.1. Reassessing the biogeography of Amphisbaenia

With few exceptions, our phylogenetic result corroborates previous studies regarding the relationship and deep divergence among the high-level groups of Amphisbaenia (Longrich et al., 2015; Vidal et al., 2008). These groups are highly contrasting considering their diversity and distribution. Over 200 species are currently included in Amphisbaenia (Uetz et al., 2021), which is classified into the following six families (Kearney, 2003; Vidal et al., 2008): 1) Rhineuridae, which includes a single extant species distributed in the southeastern USA (central Florida and in Lanier county, Georgia); 2) Bipedidae, with three species, is endemic to Pacific regions of Mexico; 3) Blanidae, which comprises seven species and represents the only family with a European distribution, but also occurs in Morocco, Syria, and Lebanon; 4) Cadeidae, represented by only two species restricted to Cuba; 5) Trogonophidae, with six species, is distributed in the Arabic Peninsula—including Socotra Island—north and northeastern Africa; and 6) Amphisbaenidae, which includes about 184 species distributed in disjunct regions of Africa, SA, and the WI.

Earlier studies have suggested that the biogeographic pattern of amphisbaenians resulted from vicariant events related to processes of landmass fragmentation (Gans, 1990; Hembree, 2006; Kearney, 2003). However, more recent analyses have supported an alternative scenario dominated by transoceanic dispersal—supposedly events of rafting on mats of soil and vegetation—instead of pure vicariant processes (Longrich et al., 2015; Vidal et al., 2008). Although our analyses have not been designed to evaluate the origin of the main lineages of Amphisbaenia, our results mostly concur with Vidal et al. (2008) and indicate ancestral events of vicariance during the Late Cretaceous and Early Paleogene, followed by more recent dispersal events (Figure S8).

Amphisbaenoidea diverged within Africa, and Cadeidae dispersed from there to the WI during Middle Eocene (1st transatlantic dispersal). Also, within Africa, Trogonophidae and Amphisbaenidae diverged during the Middle Eocene, and the ancestor of *Amphisbaena* dispersed to the WI during the Eocene/Oligocene transition (2nd transatlantic dispersal).

The direction of these two overwater dispersal events concurs with the direction of the North Equatorial Current (NEC) and South Equatorial Current (SEC) that flow from northern and central Africa to the WI and South America/WI, respectively (Amemou et al., 2020; Stramma and England, 1999). NEC is a superficial current, mainly driven by the Coriolis force, having its direction established since the Paleocene (Haq, 1981), or maybe even earlier, during the Upper Cretaceous (Donnadieu et al., 2016; Uenzelmann-Neben et al., 2017). It has been assumed that during parts of the Paleocene NEC reached as far as the Pacific Ocean through the Caribbean Sea Gateway, since the WI and the Panamanian isthmus were not present to block its passage (Haq, 1981; Hedges, 2001). We suppose that during Middle Eocene, when the African and South American plates were considerably closer than today, NEC was the driven force responsible for the dispersal of the ancestor of Cadeidae from northern Africa to the WI.

On the other hand, SEC has a more complex circulation system (Luko et al., 2021) and a historical record of cyclical shifts in direction and strength (Mix and Morey, 1996; Flores et al., 2000). SEC can be generally classified as been composed by three main branches: the northern branch (flowing ~ 2°N), the central branch (~5°S), and the southern branch (~15°S; Stramma and England, 1999). The SEC central and southern branches flow from Africa to the South American coast, southern to the Equator, while the SEC northern branch flows directly through the Equatorial Atlantic region, reaching the Guiana Current, Caribbean Sea, and the West Indies (Lumpkin and Garzoli, 2005). Based on our biogeographic scenario, we infer that the ancestor of *Amphisbaena* likely dispersed from central Africa to the WI by drifting through the SEC northern branch during the Eocene/Oligocene transition.

4.2. The West Indies colonization and the diversification of Amphisbaena in the South American continent

Although based on similar molecular datasets, our ML analysis does not support a monophyletic group of West Indian amphisbaenids, as suggested by Longrich et al. (2015). In their broader study, they included morphology and molecular evidence to estimate the position of the fossil diversity of Amphisbaenia, shedding light on the initial processes of diversification within the group. Our study, on the other hand, had a more specific goal, and it was mainly designed to evaluate the phylogenetic relationship among South American amphisbaenids. When considering only Amphisbaenidae, our molecular matrix is composed of 68 terminals, comprising 20 species more than the molecular matrix used by Longrich et al. (2015). Moreover, our gene sampling provided a more extensive overlap between both groups of species from the WI (WIC01 and WIC02) and the continental diversity of amphisbaenids (Table S1). In their molecular matrix, 16S is the only gene that provides proper sequence overlap between WIC01, WIC02 and continental amphisbaenids, while in our molecular matrix, all genes present at least some level of overlap among all these groups of species.

Based on these differences in sampling design, we presume that our study provides a more comprehensive test of the phylogenetic relationship within Amphisbaenidae, and we sustain that our tree topology fit better in the general set of results generated by our phylogenetic analyses. For example, WIC01 and WIC02 did not cluster together in any resulting gene tree topologies, as well as they did not group together in our species tree analysis (Appendix C), corroborating our main ML result. Similarly, the Shimodaira-Hasegawa test between our ML tree and the constrained tree mirroring the tree topology presented by Longrich et al. (2015) indicates a significant log-likelihood difference (-63.078852). This difference suggests that Longrich et al.'s topology is significantly worse than ours—based on our own molecular dataset—thus supporting the position of WIC01 and WIC02 as indicated in our ML tree.

Although we consider that our results provide a new and accurate perspective about the biogeographic links between the WI and the South American continent, we acknowledge that the non-monophyly of the West-Indian amphisbaenids represent and unexpected outcome. Therefore, we evaluated the impact of a monophyletic group composed by WIC01, WIC02 and *Am. ridleyi* on our biogeographic analysis. To test this alternative hypothesis, we implemented the TSDMA scenario in BioGeoBEARS based on the constrained tree used to test Longrich et al.'s topology (input files and scripts available at https://doi.org/10.6084/m9.figshare.14939298). The result of this alternative analysis suggests that the ancestral of *Amphisbaena* dispersed from Africa to the Equatorial rain forests of SA during the Middle Eocene, and from the mainland of SA to the WI in the transition between Eocene and Oligocene (Appendix C). Based on this scenario the colonization of FN by *Am. ridleyi* occurred through a dispersal event from the WI, which happened during the transition between Early and Middle Miocene (Appendix C). If this alternative scenario is supported by further studies, the discussion provide below about the colonization of the WI needs to be revised, but as we stated before, our set of results seem to give support to a non-monophyletic group of West-Indian amphisbaenids (Fig. 1; Appendix C).

Since the seminal biogeographic studies from late 19th and early 20th centuries (e.g., Wallace, 1876; 1911; Barbour, 1915; Matthew, 1915; Bond, 1934), the origin of the diverse fauna of land vertebrates present in the WI has been tentatively explained by distinct hypotheses. Hedges (1996, 2001, 2006) provided comprehensive descriptive reviews about the main hypotheses of Caribbean biogeography. More recently, Rodriguez-Silva and Schlupp (2021) presented an extensive analysis of published studies concerning the biogeography of terrestrial and aquatic habitats in the WI. Based on their review, we can summarize the principal biogeographic scenarios in the following three hypotheses: (1) vicariance through plate-tectonism, which suggests a direct land contact between the Proto-Antilles and continental North and South America (e.g., Rosen, 1985); (2) overwater dispersal that supports transoceanic rafting following the North Equatorial, North Brazilian, and/or Guiana currents (e.g., Hedges, 1996, 2001, 2006); and (3) vicariance through the fragmentation of GAARlandia, which is based on the supposed existence of a currently submerged land bridge connection between the Greater Antilles and SA (e.g., Alonso et al., 2012).

The results of our biogeographic analysis partially corroborate the hypothesis of overwater dispersal by suggesting two pre-Miocene transatlantic dispersals of amphisbaenians from Africa to the WI. The earliest of these two events represents the dispersal of Cadeidae during the Middle Eocene (about 50 Mya). Although this hypothesis has also been suggested by Vidal et al. (2008) and Longrich et al. (2015), we estimated that this dispersal occurred 10 million years before previously supposed by these studies. During this period, the magmatic activity was ceasing in the Caribbean region, and the WI were represented by a series of evanescent islands forming the Proto-Greater Antilles (Iturralde-Vinent and MacPhee, 1999; Pindell and Erikson, 1994). Based on our biogeographic scenario, the dispersal of Cadeidae may constitute one of the earliest and longstanding events of colonization regarding the West-Indian fauna of land vertebrates.

Considering our biogeographic scenario for the WI, the second event of pre-Miocene transatlantic dispersal represents the rafting of the ancestor of *Amphisbaena* during the Late Eocene. This hypothesis differs from Vidal et al. (2008) and Longrich et al. (2015) because our result does not support a direct dispersal of amphisbaenids from Africa to South America (SA), neither a subsequent dispersal of *Amphisbaena* from SA to the WI during the transition Eocene/Oligocene. Instead, our results suggest the opposite; the ancestor of *Amphisbaena* first dispersed from Africa to the WI, and from there, *Amphisbaena* dispersed to SA during the transition Eocene/Oligocene. However, the geomorphology of the WI during this period are supposed to be very distinct from their current formation (Cornée et al., 2021).

It has been proposed that the Greater Antilles and the Aves Ridge formed a somewhat continuous land bridge with SA from 35 to 30 Mya (Helcombe and Edgar, 1990; Woods, 1990), known as the GAARLandia (Greater Antilles Aves Ridge; Iturralde-Vinent, 1998; Iturralde-Vinent and MacPhee, 1999). Although subject to some criticisms (Ali, 2012; Hedges, 2006), the GAARlandia land bridge has been the preferred hypothesis in several historical biogeographic studies (e.g., Alonso et al., 2012; Chamberland et al., 2018; Říčan et al., 2013; Tong et al., 2019) and even in recent geomorphological analyses (Cornée et al., 2021; Garrocq et al., 2021; Philippon et al., 2020) (a more detailed description of recent studies concerning GAARlandia can be found in Appendix B).

Recently, Ali and Hedges (2021) provided a thorough reanalysis of an updated compilation of land-vertebrate studies of the West Indian colonization (35 clades). Although most of the evaluated clades in their analysis have intervals of colonization that can corroborate the GAARlandia hypothesis (divergence time around 34 Mya), they frequently range from 30 to 50 Mya, which makes difficult to support, beyond doubts, the vicariant hypothesis of colonization by vicariance. Ali and Hedges (2021) also questioned some recent palaeogeographic studies that have been used to support GAARlandia (Cornée et al., 2021; Garrocq et al., 2021; Philippon et al., 2020). They properly highlighted that some of these same studies (e.g., Garrocq et al., 2021), together with regional bathymetric data of the Caribbean Sea, have suggested that the southern and central regions of the Aves Ridge were submerged when they were supposed to be exposed to support the GAARlandia hypothesis (Ali and Hedges, 2021).

Although the best model (DEC + J) suggests an overwater dispersal as the main biogeographic process in the colonization of the WI and the South American continent, some of our alternative results seem to support GAARlandia as the probable ancestral region colonized by amphisbaenids that dispersed from Africa around 40–30 Mya. In our analysis, the second-best model (DEC) in all tested scenarios (NTS, TSDM, and TSDMA) supports this hypothesis, and indicates a region composed of the WI (AL) and SA (AM and/or OD) as the ancestral region for the MRCA of *Amphisbaena* (Appendix C). Based on these alternative results, GAARlandia could be the probable region from where *Amphisbaena* initially diversified, extending its distribution throughout the WI and SA during the Late Oligocene. Therefore, instead of explaining the colonization of the WI and the mainland SA only by dispersal events, this alternative scenario includes a vicariant process related to the fragmentation of an ancestral region composed of the WI and SA. Because the existence of GAARlandia is still contentious, we argue that other approaches should be used to test this hypothesis—considering amphisbaenids as a model. The intraspecific variation of West Indian amphisbaenids was never assessed to date, and we suppose that such a study would provide inferences of ancestral events of population size fluctuation that could bring new evidence to this debate.

After the initial colonization of South America, the mainland lineage of *Amphisbaena* dispersed throughout the continent. Based on our biogeographic results, the Equatorial rain forests (AM) represent the ancestral region from where most of the continental diversity of amphisbaenids has derived. However, this result should be interpreted with caution, since the basal cladogenic events among the continental diversity of *Amphisbaena*—which sustained the state optimization of AM as the ancestral region— received weak support in our phylogenetic analyses. If this scenario shows to be correct, at least two main events of dispersal happened from AM to the South American Open Areas (OD). These events have occurred at different times, the first one during the Late Oligocene (~28 Mya), and the second during the Early Miocene (~17 Mya). Around the Oligocene, the rise of the Andes changed the main Amazonian drainage basins, generating the aquatic environments known as "Pebas system" about the Early Miocene (Hoorn et al., 2010). This aquatic system was subsequently altered during the Middle Miocene, forming large parts of the Amazon River basin. These geomorphological events have been suggested as the main drivers of organismic diversification in Amazonia during the transition Paleogene/Neogene and throughout the Early and Middle Miocene (Hoorn et al., 2010). We suppose these events, associated with the transition between wet and dry climates during the Miocene (Kurschner et al., 2008; Super et al., 2020), could be responsible for shaping the distribution of amphisbaenids in forested and open areas of SA. However, because our study was not designed to evaluate all aspects of the biogeography history of the mainland diversity of *Amphisbaena*, these scenarios need to be tested based on a statistical analysis including an increased sampling of continental species for the genus.

4.3. *The Miocene dispersal of amphisbaenids from South America to the West Indies*

Our results show the existence of two genetically distinct phylogroups of amphisbaenids in the WI. The first phylogroup, WIC01, represents an Eocene lineage, directly descendent from the initial split of the original dispersion of amphisbaenids from Africa to the New World during the Late Eocene (see discussion above, Fig. 2). The second phylogroup, WIC02, is more recent, having its origin estimated between the Early and Middle Miocene, probably derived from an Amazonian ancestor (Fig. 2). The phylogroup WIC02 is sister to *Am. ridleyi*, suggesting that the ancestor of the endemic amphisbaenid from FN is closely related to amphisbaenids from the WI.

As commented before, although unexpected, the existence of two West-Indian phylogroups—as well as the close relationship among WIC02, *Am. ridleyi* and the Amazonian amphisbaenids—seems to be supported by independent evidence. The reported number of chromosomes for several species of *Amphisbaena* (reviewed in Falcione and Hernando, 2010; Laguna et al., 2010) indicates a substantial difference between species belonging to WIC01 from those included in WIC02 (Fig. 1). All species from WIC01 present $2n = 36$, while the species allocated in WIC02 have $2n = 50$. Moreover, the clade formed by WIC02 ($2n = 50$), *Am. ridleyi* ($2n = 46$) and *Am. fuliginosa* ($2n = 48$) represents the group with the largest number of chromosomes within Amphisbaenia. Although acknowledging that raw comparison of the number of chromosomes *per se* cannot be unmistakably interpreted as a conclusive signal of phylogenetic relationship, we suppose that such shared large number of chromosomes can be considered as an independent corroboration of the close relationship among WIC02, *Am. ridleyi* and *Am. fuliginosa* (a list of other species we expect to belong to this group can be found in Appendix B).

We estimate that WIC02 was originated through a dispersal event from northern SA to the WI during the Early Miocene, a period with intense climate fluctuations and changes in the sea level (Miller et al., 2020). Between 17 and 15 Mya global temperatures were approximately 10–15 °C higher than today, characterizing the Middle Miocene Climate Optimum (MMCO) (Methner et al., 2020). During this period, the climate was more humid, and the forested areas likely covered a large portion of SA. Just after MMCO, the global temperatures dropped abruptly and in less than three million years achieved global average 6–8 °C cooler than before. This period spanned from 15 to 12 Mya and is known as Middle Miocene Climate Transition (MMCT; Flower and Kennett, 1994) or Middle Miocene Disruption (Zachos et al., 2001). During the last stages of MMCT the eustatic sea-level dropped more than 30 m, and vast areas currently submersed in the Caribbean Sea and on the South American continental platform were emersed (Miller et al., 2020). Additionally, the Pebas system that flooded the western Amazon region during the Early Miocene (Figueiredo et al., 2009; Jaramillo et al., 2017) was drained eastwards in the Middle Miocene, affected by the Andean uplift, increasing the strength and sedimentation rates of the Amazon River (Hoorn et al., 2010; Shepard et al., 2010), and probably facilitating rafting events from the Amazon Forest to the Equatorial Atlantic seas.

We suppose that the ancestral of WIC02 was probably distributed across the northeastern Amazon Forest (e.g., similar distribution than *Am. fuliginosa*; Fig. 1), and during the MMCT, this hypothetical ancestor drifted on logs or floating mats of soil and vegetation through the Amazon River, achieving the Atlantic Ocean. From there, the ancestor of WIC02 drifted northwest, following the North Brazilian Current (Fig. 1), achieving the southern portion of the Hispaniola Island (see below our comments on a model-based evaluation of this biogeographic hypothesis).

4.4. *The colonization of Fernando de Noronha by Amphisbaena ridleyi*

Although our results strongly support a close relationship between *Am. ridleyi* and the phylogroup WIC02, thus corroborating the hypothesis proposed by Ridley and Boulenger (Ridley, 1888; Boulenger, 1890), the TMRCA between *Am. ridleyi* and WIC02 is older than the estimated date for the formation of the FN. The oldest exposed rocks in FN derived from magmatic events dated from the transition between Middle to Late Miocene, around 12 Mya (Almeida, 2002; Cordani, 1970; Lopes et al., 2014). Two distinct hypotheses have been proposed to explain the formation of FN, one suggests magmatic events originated along the Fernando de Noronha fracture zone (Almeida, 1955; reviewed in Almeida, 2002) and another that supposes the existence of an oceanic hotspot near the archipelago (Duncan, 1981; Mizusaki et al., 2002) (a more complete description of these main hypotheses can be found in Appendix B).

These two hypotheses present underlying differences, nevertheless, they imply a similar pattern of submerged seamounts and tablemounts (guyots) in the Equatorial South Atlantic Ocean. These submerged seamounts form the Fernando de Noronha Ridge (FNR), which presents a west to east order of decreasing age. Based on the summit depths of these seamounts in the FNR (Nautical Chart 10, available at www.marinha.mil.br) and the estimated sea-level changes during the Miocene (Miller et al., 2020), we suppose these seamounts could represent a putative series of paleo islands that would have allowed a stepping-stone process of biogeographic dispersal from mainland SA to FN. Hypotheses of paleo islands have been proposed to explain the colonization of other volcanic islands (e.g., Mairal et al., 2015; Zaher et al., 2018) and a more detailed description of such hypotheses can be found in Appendix B. The existence of paleo islands, permitting a stepping-stone process of oceanic dispersal along the FNR, can equate the estimated date of divergence between *Am. ridleyi* and WIC02 (about 13.6 Mya) with the estimated age of formation of FN (around 12.4 Mya). However, we acknowledge that the origin of *Am. ridleyi*, and its sister group relationship with WIC02, as suggested in our biogeographic analysis, is unexpected and challenging based on previously available evidence.

Although several studies have supported a biogeographic relationship between FN and the WI, all these hypotheses assume dispersal events following the direction of the superficial oceanic currents that flow westward, principally the North Brazilian Current (NBC; Fig. 1; Renner, 2004; Schott et al., 1998). The estimated dispersal of *Am. ridleyi* from the WI to FN contradicts the direction and influence of these main oceanic currents in the Equatorial Atlantic Ocean. Idiosyncratic ad hoc events, like tsunamis or hurricanes, have been frequently suggested to account for contraflow dispersal events (e.g., Carlton et al., 2017; Dewald and Pike, 2014). However, aiming to test the influence of NBC on the dispersal of *Am. ridleyi* to FN and WIC02 to the WI, we implemented two slightly modified sub-scenarios of the TSDMA scenario in BioGeoBEARS (input files and scripts available at https://doi.org/10.6084/m9.figshare.14939298). In the first sub-scenario, we set the dispersal events from WI to FN as 10 times less probable than dispersal events from FN to WI; and in the second we reduced 10 times the dispersal probabilities for any dispersal event between WI to FN. In both analyses, the resulting ancestral region for the MRCA of WIC02 and *Am. ridleyi* changed to AM (these results can be found in Appendix C).

This alternative hypothesis—in which the amphisbaenids of both regions, WI and FN, have an Amazonian origin—is consistent with the influence of the NBC and the Atlantic Northern Equatorial Countercurrent (NECC; Fig. 1; Katz, 1993; Mélice and Arnault, 2017). The NECC is an eastward countercurrent that frequently flows around 3°N (about 300 km width), and it is characterized by its extreme seasonality (Carton and Katz, 1990). The NECC has a recorded history of indirect influence of El Niño, when NECC became notably stronger (Katz, 1993). The seasonality of NECC can affect the whole dynamic of ocean circulation on the Equatorial Atlantic Ocean. Simulations have shown that in some scenarios, a system can be formed that links NECC with the Inter-Tropical Convergence Zone (ITCZ) and the Amazon and Orinoco River runoffs (Arnault et al., 2021), which is affected by NECC retroflection and rings of propagation, generating tropical instability waves (Mélice and Arnault, 2017).

Considering our alternative biogeographic hypothesis that takes the Equatorial currents into account, we suppose that around the Late Miocene, the ancestor of *Am. ridleyi* would have rafted on logs or floating mats of soil and vegetation through the Amazon River, achieving the Late Miocene shallow waters of the Brazilian Platform. From there, the ancestor of *Am. ridleyi* would have reached the ITCZ during the influence of El Niño and consequently the NECC that favored it dispersal to FN, or more probably to some paleo-island on the FNR. The estimated changes in direction and strength of NECC during the Late Miocene indirectly support this hypothesis, by suggesting that the countercurrents were stronger and achieved lower latitudes (Herold et al., 2012). It was already proposed that the fluctuation in strength and direction of the NECC during some periods in the Late Miocene shifted southward the position of the ITCZ, and the influence of NECC was so prominent that affect the African coastal currents in the Gulf of Guinea (Norris, 1998).

By combining the dynamic of NBC and NECC during the Miocene in the scenario concerning the dispersal of WIC02 and *Am. ridleyi*, we highlight the biogeographic importance of seasonal and periodic changes in the direction and strength of the currents of the Equatorial Atlantic Ocean. However, these hypotheses should be validated through analyses focused on other insular organisms or using a comparative biogeographic approach.

4.5. The risk of extinction of Amphisbaena ridleyi in Fernando de Noronha

Although we did not assess a large sample of *Amphisbaena ridleyi*, the three sequenced individuals were collected in different areas of FN, and they show a perturbing shallow genetic diversity. In fact, we identified only three SNPs (single nucleotide polymorphism) when considering all six sequenced genes for these individuals.

Fernando de Noronha is located on a volcanic edifice about 4,000 m high from the ocean bed, presenting a wide submerse erosive platform with over 60 km of diameter (Almeida, 2002). This huge volcanic edifice was formed during two main volcanic events (Fig. 2): the initial magmatic process known as Remédios Formation, in which the rocks are dated between 12.3 and 8 Mya; and the Quixaba Formation with rocks dated between 4.2 and 1.5 Mya (Lopes and Ulbrich, 2015). Our biogeographic scenario suggests that *Am. ridleyi* was present in FN just after the establishment of the archipelago, and thus the magmatic eruptions of the Quixaba Formation likely impacted the species viability. We suppose the low genetic diversity of *Am. ridleyi* represents a signal of a population bottleneck associated with the volcanic events happened during the Early Pliocene.

Although FN represents a Brazilian protected area, the main island is presently dominated by vines and shrubs, since most of the original vegetation was almost cleared out during the 19th century. The archipelago is currently occupied by approximately 3,000 residents, which are concentrated on the main island (Almeida, 2002). Since the 90 s the tourism has increased more than 900% and the impacts on the remaining patches of natural vegetation and the continuous alteration of soil features are the main threats to the endemic land fauna. Moreover, invasive species, including rats, mice, and feral cats, have been a serious problem for the islands' native fauna (Johnson, 1989).

Based on the small area of distribution, low genetic variability, and the increasing impact on natural ecosystems of FN, we suggest that *Am. ridleyi* represents a threatened species. Following the criteria of IUCN (International Union for Conservation of Nature), we suggest that *Am. ridleyi* fits in the category CR B1a,b [iii], as a "critically endangered species". By recommending the inclusion of the endemic amphisbaenid of FN in the list of endangered species, we aim to encourage Brazilian authorities to assume their role and fulfill their duties as protectors of Brazilian biodiversity. The conservation of *Am. ridleyi*, besides representing the protection of another threatened endemic species from Brazil, also represent the preservation of an intriguing part of the long evolutionary history of amphisbaenians.

Uncited references

CRediT authorship contribution statement

Roberta Graboski: Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Felipe G. Grazziotin:** Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Tamí Mott:** Conceptualization, Data curation, Investigation, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Miguel Trefaut Rodrigues:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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

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