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Historical Biogeography of the *Leptodactylus fuscus* Group (Anura, Leptodactylidae): Identification of Ancestral Areas and Events that Modeled their Distribution

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The objective of the present study was to reconstruct the biogeographic history of the monophyletic group *Leptodactylus fuscus*. We carried out two complementary historical biogeographic approaches: one estimates the ancestral areas with the statistical dispersion and vicariance method (S-DIVA). The other detects disjoint distributions among sister groups, which provides information about barriers that separate populations through a spatial analysis of vicariance (VIP method). For that, we used a database of species presence records and a topology of a phylogenetic cladogram, both obtained from updated published data that incorporates the current phylogenetic, taxonomic and distributional arrangements for the group. For the analysis of ancestral areas, the following areas of the *L. fuscus* group distribution were used: the Carribean, Chacoan, Parana, Amazonian and North American in Pacific subregions. The optimal reconstruction obtained with S-DIVA showed five vicariance events, two extinctions and 50 dispersals. The spatial analysis of vicariance revealed 19 disjointed sibling nodes and two distributions on nodes removed in the consensus tree. The results suggest that the ancestor of the *Leptodactylus fuscus* group occupied large areas within the Amazon and Chacoan subregions. Due to several dispersal events, the ancestor distribution range may have expanded to the Caribbean subregion. This expansion could have occurred during wetter periods, when forests were more extensive, which would have allowed the invasion of open habitats within humid forest systems. It is important to note that ecological factors and marine transgressions that occurred during the Miocene could have had a great influence on the current distribution of the group.

Key words: Ancestor, Biogeographic methods, Miocene, Vicariance, Distribution.

BACKGROUND

The reconstruction of biological diversity distribution patterns, and the identification of the processes that have shaped these, are essential to understanding why species are distributed where we find them today, and how they have been assembled over time (Sanmartín 2012). Historical biogeography

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addresses these topics through methods that allow us to infer the distribution area of the ancestor of a given monophyletic group, and the series of events such as vicariance, dispersion and extinction through time (Bremer 1992), which are the first steps to exploring the biogeographic history of a region.

Several biogeographic studies targeting vertebrates of the Neotropical region have been conducted (*e.g.*, South Florida, South Mexico, West Indies, Central America and much of South America, Amorim and Pires 1996; Cabrera and Willink 1973). For example, for the lizard genus *Leposoma* (Squamata: Gymnophthalmidae), the phylogeny, biogeography and divergence times were studied (Pellegrino et al. 2011); in Neotropical birds (Tanagers), the phylogeny and biogeography were assessed through dispersal-vicariance analysis (DIVA method, Sedano and Burns 2010). Furthermore, areas of endemism of anurans, lizards, continental turtles, and primates were evaluated using parsimony analysis of endemicity (PAE) (Ron 2000; Ippi and Flores 2001). In Tropical Centro and South America the historical biogeography and diversification of the Allocentroleniae clade (Centrolenidae + Allophrynidae) was studied through statistical dispersion and vicariance analysis (S-DIVA) (Castroviejo-Fisher et al. 2014). Other biogeographical studies performed for amphibians of South America were carried out by Santos et al. (2009) through maximum-likelihood inference of geographic range evolution, DIVA, and Bayesian analysis of ancestral areas. Likewise, the molecular phylogeny and biogeography of the subfamily of anurans Phyzelaphryninae (Fouquet et al. 2012) and the systematics and biogeography of *Adenomera* genus (Fouquet et al. 2014) were evaluated. Also, the reconstruction of ancestral states of the genus *Leptodactylus* of the West Indies was studied using the Bayesian method (Hedges and Heinicke 2007).

Anurans have proven to be a good target group for studies on biogeographic analysis and species diversification because they show limited dispersal capacities and strong habitat dependence (Zeisset and Beebee 2008). The genus *Leptodactylus* belongs to the family Leptodactylidae (Fitzinger 1826) and includes 83 species of predominantly Neotropical lowland frogs (Frost 2022). *Leptodactylus* comprises four species groups: *L. fuscus*, *L. melanonotus*, *L. latrans*, and *L. pentadactylus* (Heyer 1969; de Sá et al. 2014). Among these, the *L. fuscus* group, with 34 recognized species, is the most diverse. The monophyly of this group has been recently corroborated on the basis of behavioral, molecular and morphological characters (Ponssa 2008; de Sá et al. 2014), making it a good candidate for reconstructing their biogeographic history. However, the biogeographical hypotheses proposed for the *L. fuscus* group are so far descriptive, without an explicit methodology that supports them (Heyer 1978 1982). Based on the systematics of the group, the species distribution and a vegetation map, Heyer (1978) proposed a hypothetical semi-fossorial ancestor of open, xeric vegetation, from which the group would have expanded to open regions of humid forests within the Neotropical region. Subsequently, Heyer (1982) postulated vicariance hypotheses for the genus based on refuges and on the Andes orogenesis.

In a given phylogeny, taxon history methods are used to explain the distribution of a particular taxon in a geological context. For example, quantitative biogeographic methods such as a) the Vicariance Spatial Analysis implemented in the Vicariance Inference Program (VIP) (Arias et al. 2011), use distribution and phylogenetic data to identify disjunctions and geographical barriers between sister species distributions or b) DIVA Dispersion-Vicariance Analysis (Ronquist 1997) and its S-DIVA extension (Yu et al. 2010), uses predefined areas to estimate the ancestral area in each node under a parsimony assumption. Ancestral area methods have been criticized, mainly because they have used a strictly dispersalist approach (Ebach 1999; Morrone 2002) or due to biases related to a higher probability of more plesiomorphic areas being part of the ancestral area (Santos 2007). The Dispersal-Vicariance Analysis has also been criticized for its bias towards an all-vicariance explanation (Díaz Gómez 2009) and for its inability to model extinction and range expansions (Kodandaramaiah 2010). However, ancestral area methods are widely used to infer the history of a given taxon. DIVA particularly remains very popular in the literature (Kodandaramaiah 2010), which facilitates comparisons among analyzes for the same study area and/or other taxonomic groups, as long as their limitations are considered. On the other hand, the estimation of vicariance barriers through the VIP method is complementary to the determination of ancestral areas, since the first uses observed distribution records as input data while the latter uses predefined areas. This is an adequate alternative to solving the problems derived from the allocation of predefined areas, such as cases of widely distributed taxa and overlapping terminal ranges (Hovenkamp 1997 2002). In the present work, the geographical distribution patterns of the *L. fuscus* group were analyzed and new biogeographical hypotheses are proposed for them. To achieve this, a spatial analysis of dispersion and vicariance was performed to identify possible ancestral areas and biogeographic/historical events that likely modeled the distribution of the group. This is the first bogeographic analysis for this group, whose monophyly was previously corroborated, using an explicit and quantitative methodology. This study can help identify events that could have affected the distribution of other types of organisms present in the area.

MATERIALS AND METHODS

Distribution of the group

The *Leptodactylus fuscus* group is distributed throughout much of the Neotropical region (a few species have colonized the southern Neartic region) from Texas to Argentina. The group occurs on both sides of the Andes Mountains in northern South America, but only at the east in the southern region (de Sá et al. 2014). In addition, it inhabits the Margarita Islands, Trinidad and Tobago (Heyer and Reid 2003). The group is found below 2000 m asl in a wide diversity of environments such as open habitats, rainforests, dry forests, grasslands, savannas and human-modified landscapes such as agricultural and cattle farming lands (Ab'Sáber 1977; Joly et al. 1999; de Sá et al. 2014; Medina et al. 2016). It can also colonize riverbanks and recently modified habitats in forested regions (Wynn and Heyer 2001).

Database of geographical records

We obtained a database of 5411 presence points for 36 species, 32 of which were species from the *L. fuscus* group, in addition to *L.* cf. *mystaceus* (*sensu* Alves Da Silva et al. 2020), and three of which belonged to each of the remaining external groups, *i.e.*, gr. *L. latrans*, *L. melanonotus* and *L. pentadactylus* (Table S1). We used a published database of herpetological collection records, scientific articles, and free online databases (Medina et al. 2020). The published database included geographical records in which the specimen identities were corroborated by their external morphology in FML, MACN, MLP, MCN, MNRJ, MZUSP and CFBH (Brazil) and IIBP and MNHNP (Paraguay) (abbreviations of museum names follow Sabaj Pérez 2010) herpetological collections; and personal collections of María Laura Ponssa (L) and Julián Lescano (JL) (Argentina). Also, Medina et al. (2020) include records with no revision, such as the LGE-IBS herpetological collections from Argentina, QCAZ from Ecuador, the published database available at http://www.chalk.richmond.edu/Leptodactylus/maps.html, GBIF (Global Biodiversity Information Facility), Species Link (http://splink.cria.org.br/) and several scientific articles (see Medina et al. 2020 for details on the database construction). For the present work we added geographical records from the Natural Science Museum of Universidad Nacional de Salta (MCN) collection, whose specimens were revised by MC. We also updated the new taxonomic arrangements of gr. *L. fuscus*, more specifically the redefinition of *L. mystacinus*, *L. mystaceus* and the new species *L. apepyta*, *L. barrioi*, *L. kilombo*, *L.* cf. *mystaceus* and *L. watu* (Schneider et al. 2019; Alves Da Silva et al. 2020). In the case of records with no geographic coordinates, we geo-referenced their location using Google Earth, gazetteers (Ornithological Gazetteers, Paynter and Traylor 1991; Fallingrain, www.fallingrain.com), and Wikimapia (http://wikimapia.org).

Phylogenetic hypothesis

We worked with the phylogenetic hypothesis proposed by de Sá et al. (2014) based on morphological, molecular and behavioral characters, which includes 80% of the genus species. The location in the cladogram of species not included in de Sá et al. (2014), such as *L. cupreus* and *L. apepyta*, were assigned according to Schneider et al. (2019); *L. barrioi*, *L. kilombo*, *L. watu* and *L. spixi* followed Alves Da Silva et al. (2020), *L. caatingae* and *L. oreomantis* were excluded from the analysis because they have not been included in phylogenetic studies so far. For that, we built a meta-tree based on the general topology of de Sá et al (2014), Schneider et al. (2019) and Alves Da Silva et al. (2020).

In the de Sá et al. (2014) phylogeny, *L. mystaceus* and *L. fuscus* were not recovered as monophyletic groups, suggesting the existence of cryptic species. For *L. mystaceus*, two lineages are recognized: one corresponding to a sample from Pará (*L. mystaceus* 1), and the other two samples from Sergipe and San Pablo (*L. mystaceus* 2, 3). For *L. fuscus*, two lineages are recognized as well (*L. fuscus* 1, 2, 3, 5 and *L. fuscus* 6, 7, 8, 9). However, the database of presence records for both lineages of each species elucidates continuous or overlapped records between them. To tackle this paraphyly, for *L. fuscus* we follow recently molecular analysis carried out by Schneider et al. (2019), who found a single clade, in which the *L. fuscus* terminals 1–2 and 4–7 have relatively low genetic distances (excluding sequences 3, 8, and 9 due to low quality or missing data). This clade is located as the sister taxon to the clade formed by *L. longirostris*, *L. poecilochilus* and *L. fragilis*. For *L. mystaceus* we follow Alves Da Silva et al. (2020), who redefine *L. mystaceus* through molecular analyses, and describe the new species *L. barrioi*, *L. kilombo*, *L.* cf. *mystaceus* and *L. watu. Leptodactylus bolivianus*, *L. melanonotus* and *L. pentadactylus* were considered external groups belonging to the groups *L. latrans*, *L. melanonotus*, and *L. pentadactylus*, respectively.

Analysis of ancestral areas

For the analysis of ancestral areas, we assigned predefined areas to the taxa. The assigned areas were chosen based on Morrone (2006) biogeographic regionalization for Latin America and the Caribbean. As the *L. fuscus* group is widely distributed, we chose biogeographic sub-regions, because in this way the number of areas is limited and in turn covers large geographical areas. Within the distribution of the *L. fuscus* group there are provinces that were not assigned to any sub-regions, such as the Region of Mexican and South American transitions (Fig. 1). These areas were not included in the analysis, but that did not affect the results because on the one hand none of the *L. fuscus* group species are exclusive to these transitional areas. On the other hand, these areas show a steep altitude gradient, thus they are not representative of the distribution of the *L. fuscus* group species, which occur mainly in lowlands. Records located towards the margin of the distribution, outside the limits of the Morrone (2006) regionalization, were excluded. This is the case of the *L. fragilis* Texas records, which represent less than 8% of its records (*i.e.*, their exclusion would not affect the results of the analysis; results including these records are shown in Fig. S1). The area assigned to each taxon was obtained by selecting the sub-regions that contained species presence points with the DIVA-GIS program (Version 7.5.0.0). The selected sub-regions were: (A) Caribbean: includes southern Mexico, Central America, the Antilles and northwest South America; (B) Chacoan: includes northern and central Argentina, southern Bolivia, western and central Paraguay, Uruguay, and central and northeastern Brazil; (C) Parana: extends from northeastern Argentina, eastern Paraguay, and southeastern Brazil; (D) Amazonian: the largest Neotropical region, it includes Brazil, the Guianas, Venezuela, Colombia, Ecuador, Peru, Bolivia, Paraguay and Argentina; and (E) North American Pacific (Nearctic Region): includes North and central Mexico (Fig. 1).

The ancestral areas were determined using a

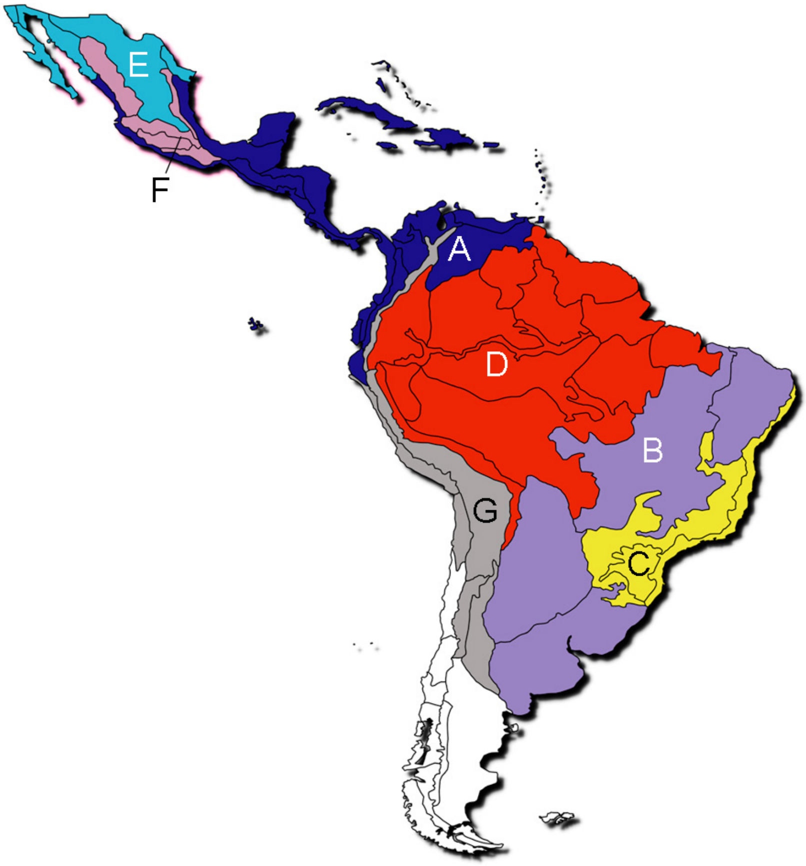

Fig. 1. *Leptodactylus fuscus* group species distribution area. Biogeographical subregions and provinces proposed by Morrone (2006) are shown in different colors. A: Caribbean subregion; B: Chacoan subregion; C: Parana subregion; D: Amazonian subregion; E: North American Pacific subregion; F: Mexican transition zone region; G: South American transition zone region.

dispersion and vicariance analysis (DIVA) through the optimization of a three-dimensional cost matrix built from phylogenetic relationships and distributional data of one or more taxa that inhabit the same areas. The costs assigned to the matrix varied according to the biogeographical events; *e.g.*, for vicariance (speciation through the breakup of a wide distribution in two groups of mutually exclusive areas) and sympatry (speciation within a single area), the assigned cost was 0; dispersal and extinction had a cost of 1 per unit of area added to or removed from the original distribution. The optimal reconstructions are those that require the fewest number of dispersal events (Ronquist 1996 1997). For this study, we used the program S-DIVA, a Statistical Dispersal Vicariance Analysis (Yu et al. 2010) implemented in the RASP software (Yu et al. 2013). This program evaluates the statistical support for each reconstruction, where the frequency of an ancestral range in a node is averaged in all the trees. The number of maximum areas allowed at the nodes was restricted to three. The analysis was performed using the following parameters by default: hold = 32,767, bound = 32,767, Keep = 65,536. Here we show only the results of the most likely states.

Spatial analysis of vicariance

Vicariance events between sister species, and hypothetical barriers that affected the distribution of the group were inferred by applying a spatial analysis of vicariance implemented in VIP (Vicariance Inference Program, Arias 2010), which uses punctual presence records, thus avoiding the assignment of predefined areas to a taxa. VIP is sensitive to two parameters that can be modified by the user: cell size and cost of removing nodes. If cell size is too large, the number of disjunctions may be underestimated since many species distributions that are actually disjunct will appear to occupy the same cell on the grid. On the contrary, if cell size is too small, the number of disjunctions can be overestimated, since some localities will appear in different cells despite being very close in space (Arias et al. 2011). VIP uses distributions at internal nodes as statements similar to those used in taxonomic revisions to refer to the distribution of higher taxa, which are not necessarily based on the ancestral area concept. Instead, VIP based in the Hovenkamp method (1997 2001) emphasizes disjunctions that correspond to speciation (Arias 2011). However, the current distribution of a taxon may mask potentially disjunct distributions of sister nodes (*e.g.*, after dispersal), erasing evidence of them. In that case, when distributions are less than ideal, ignoring the distribution of the problematic node(s) may allow us to consider splits that are basal to the node of interest as disjunct distributions (Page 1994a b; implicit in Brooks 1990). Thus, VIP implements an optimality criterion, through the "cost of distribution removal", which seeks the best compromise between the maximum possible disjunct sister nodes and the minimum number of distribution eliminations. As an example, if distribution removal had a cost of 0, the result would be the maximum number of possible nodes explained by allopatry, while if removal had an infinite cost, the result would be the maximum number of possible nodes explained without removal, similar to "assumption 0." In a dichotomic tree, if removals were set to a cost of 1.0, then the results would be equal (in terms of cost and reconstruction) to set the cost as 0.0 (Arias 2011). Thus, it is recommended to set the cost of distributional removal with a value higher than 1. Therefore, we explored the results with four cell sizes (0.1, 0.5, 2.0, 3.0) and three cost values (0.5, 1.8, 3.0), assigning a maximum fill value of two, an overlap up to 15% because most species are widely distributed in space, and the "partial elimination" option was not allowed. The search was established at 5000 iterations, keeping 20 reconstructions per iteration. Barriers were spatially represented with Voronoi lines (De Berg et al. 2008).

RESULTS

Analysis of Ancestral Areas

The analysis of ancestral areas revealed that the biogeographic history of the group comprised dispersion, extinction, and vicariance events. The optimal reconstruction obtained resulted in five vicariant events, two extinction events, and 50 dispersal events (Fig. 2).

An early vicariance event took place at node 71, fragmenting the ancestral distribution giving rise to two nodes: node 38, which included the ancestor of *L. bolivianus*, *L.melanonotus* and *L. pentadactylus*, and node 70, which included the ancestor of the *L. fuscus* group, whose ancestral area was formed by BD (Chacoan/Amazonian). From the latter, a vicariance event that fragmented the ancestral area took place, giving rise to two ancestral areas of nodes 69 and 68: the former was formed by B (Chacoan), which included the ancestor of *L. syphax* and *L. laticeps*; while the ancestral area of node 68 was formed by D (Amazonian), where the ancestor of the remaining group species was distributed. From node 68, a dispersal event towards area A (Caribbean) took place, followed by speciation, giving rise to the ancestral areas of *L. labrosus*-*L. ventrimaculatus* in area D (Amazonian) (node 67); and of the remaining group species in AD (Caribbean/

Amazonian) areas (node 66). From node 66 there was a dispersal event towards area B (Chacoan) followed by speciation, giving rise to nodes 65, whose ancestral area was formed by D (Amazonian) and included the ancestor of the *L. mystacinus* complex; and to node 61, formed by the ABD areas (Caribbean/Chacoan/Amazonian).

Within the clade that comprises the *L. mystacinus* complex, we observed a vicariance event in node 63 formed by the CD (Parana/Amazonian) areas, which resulted in the ancestral area of *L. cupreus* formed by C (Parana); and the ancestral area of node 62 formed by D (Amazonian). The latter included the ancestor of *L. apepyta* and *L. mystacinus*.

In node 61 (ABD areas), there was an extinction event in area B (Chacoan) followed by speciation, which gave rise to *L. albilabris*, distributed in area A (Caribbean); and to node 60, formed by the AD areas (Caribbean/Amazonian; ancestral area of the remaining group the species). Then, a dispersion event followed by speciation took place towards area B (Chacoan). This event gave rise to two nodes: node 59, formed by ABD (Caribbean/Chacoan/Amazonian), which included the ancestor of the *L. fuscus*, *L. fragilis*, *L. longirostris* and *L. poecilochilus* group; and node 56, formed by the BD areas (Chacoan/Amazonian).

Node 49 was formed by the CD areas (Parana/Amazonian), from which was a dispersal event towards

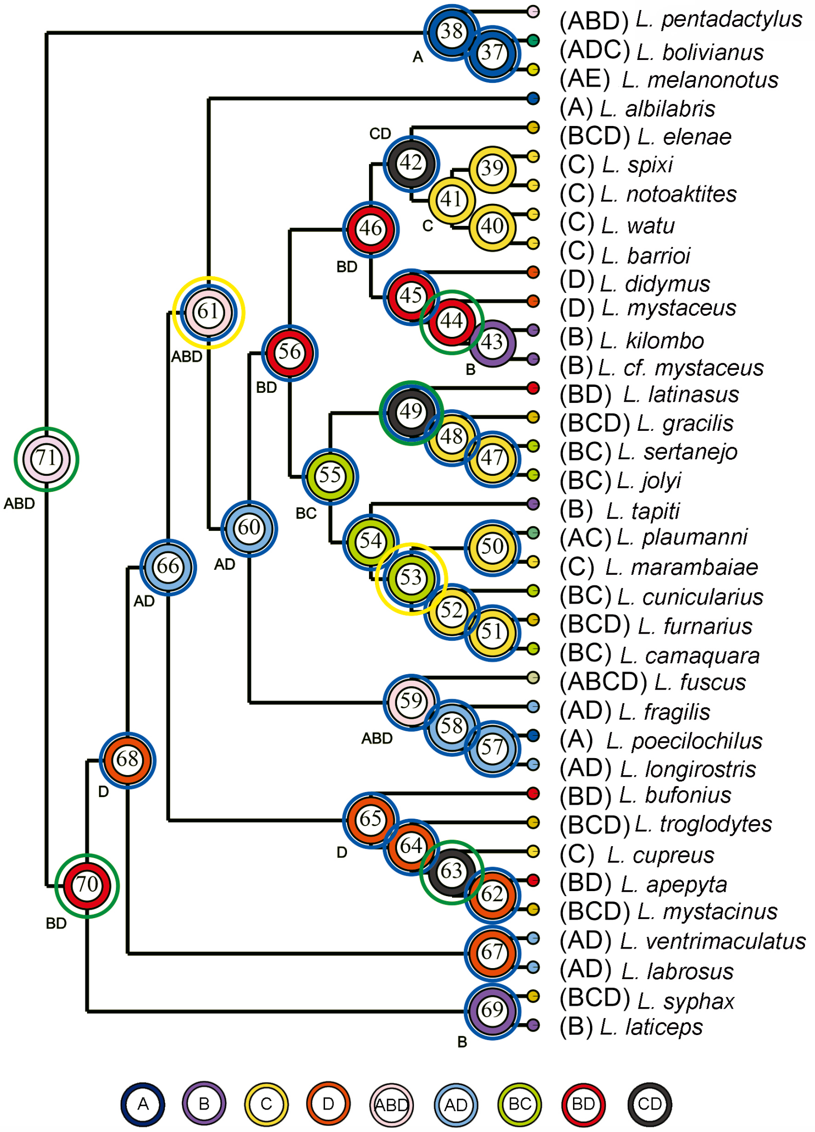

Fig. 2. Ancestral areas of *Leptodactylus fuscus* group. Nodes show the most probable state. The letters and coarse colored circles indicate the ancestral areas of each node. Green, blue and yellow thin circles show vicariance, dispersions and extinctions events, respectively. A: Caribbean subregion; B: Chacoan subregion; C: Parana subregion; D: Amazonian subregion; E: North American Pacific subregion.

area B (Chacoan) followed by a vicariance event fragmented the ancestral distribution. This gave rise to the ancestral area of *L. latinasus*, formed by BD areas (Chacoan/Amazonian); and to node 48, formed by area C (Parana), which included the ancestor of *L. jolyi*, *L. sertanejo* and *L. gracilis*.

Within the *L. mystaceus* complex, we highlighted node 44, which was formed by the BD areas (Chacoan/Amazonian). Then, a vicariance event fragmented the area giving rise to the ancestral area of *L. mystaceus*, which was distributed in area D (Amazonian); and to the ancestral area B (Chacoan) of node 43. The latter included the ancestor of *L. kilombo* and *L.* cf. *mystaceus*.

Spatial analysis of vicariance

The VIP analysis with a cell size of 0.5° × 0.5° and a cost of removal of 1.8 led to six memory reconstructions, each with 16 disjunction nodes, and the consensus resulted in a tree with 19 disjunction nodes and two removed nodes (Fig. 3A). As expected, higher cell size values (2.0° and 3.0° of cells side) produced few disjunctions with a large number of nodes removed. On the other hand, lower cell size values (0.1° of cells side) led to similar results to 0.5° cell side. Regarding the cost of distribution removal, for a higher value (3.0) a lower number of disjunctions with cero removed nodes were recovered. This is probably a blurring effect of the disjunction event resulting of subsequent dispersions. On the contrary, lower values of cost of distribution removal (0.5) produced even fewer disjointed nodes, but with a high number of eliminated nodes, being artificial results (see Table S2–3 to compare VIP performance in each case).

A basal vicariance event was found at node 71, whose barrier was postulated to occur between latitudes 19° and 2°S approximately. This disjointed *L. bolivianus*, *L. melanonotus* and *L. pentadactylus* (node 38) to the north, and the ancestor of the *L. fuscus* group to the south (Fig. 3B). A vicariance event took place in node 68, whose barrier was located approximately between longitudes 72° and 61°W. It crossed a large part of the Amazonian and Caribbean sub-regions, and produced the separation of two clades: to the west, the species of node 67 (*L. labrosus* and *L. ventrimaculatus*), and to the east the rest of the species of node 66 (Fig. 3C).

Another hypothetical barrier was obtained in node 55, which crossed three sub-regions (Parana, Chacoan and Amazonian), and which was located approximately between longitudes 50° and 63°W. The species included in node 54 (*L. tapiti*, *L. plaumanni*, *L. camaquara*, *L. furnarius* and *L. cunicularius*), were distributed towards the northeast of the barrier. The species included in node 49 (*L. latinasus* species complex) were located towards the southwest, removing the vicariant node 48 (*L. jolyi*, *L. sertanejo* and *L. gracilis*) whose species were widely distributed in the area (Fig. 3D). Node 44 exhibited a vicariant event, whose barrier was located approximately between longitudes 58° and 49°W, crossing the Chacoan and Amazonian sub-regions. The species *L. kilombo* and *L.* cf. *mystaceus* were distributed towards the east in the biogeographic provinces of Cerrado and Caatinga, while *L. mystaceus* was located towards the west in the Amazonian sub-region (Fig. 4A).

Node 64 presented a vicariance event whose barrier was between 48° and 39°W, crossing the Amazonian, Chacoan and Parana sub-regions. The species included in node 63 were distributed towards the southwest of the barrier, while *L. troglodytes* was distributed towards the east. This was obtained by eliminating the distribution of *L. cupreus* (Fig. 4B).

DISCUSSION

This study constitutes the first reconstruction of the biogeographical history of the *Leptodactylus fuscus* group with quantitative and explicit methods. We postulated hypotheses on the ancestral areas, the events that modeled the historical distribution, the biogeographical barriers that might have led to the diversification of the group, and consequently the geographical distribution patterns of the species.

Heyer (1978) proposed one of the studies that has contributed the most to the biogeography of the group, since it describes possible zoogeographic patterns. Based on its distribution, he hypothesizes a widely distributed ancestor in the Neotropical region and identifies some species associated with dry forests, with more plesiomorphic characters, such as *L. ventrimaculatus*, *L. labrosus*, *L. bufonius* and *L. troglodytes*. To explain the distribution of the ancestor in the Amazon, which was adapted to xeric environments, he suggested the presence of extensive dry corridors existing until the Miocene, when a lowland sector in the Andean chain in formation was still present towards the area where Ecuador is currently located (Solbrig 1976). This would have allowed the ancestor of the group to spread out in this region. After the Andes rose, more humid periods would have eliminated these Amazon corridors, causing the ancestor group to be eliminated from the Amazon basin. Our hypothesis of ancestral area does not concur with Heyer (1978), because the reconstruction with S-DIVA proposed a basal node formed by the ABD sub-regions (Caribbean/Chacoan/Amazonian), which

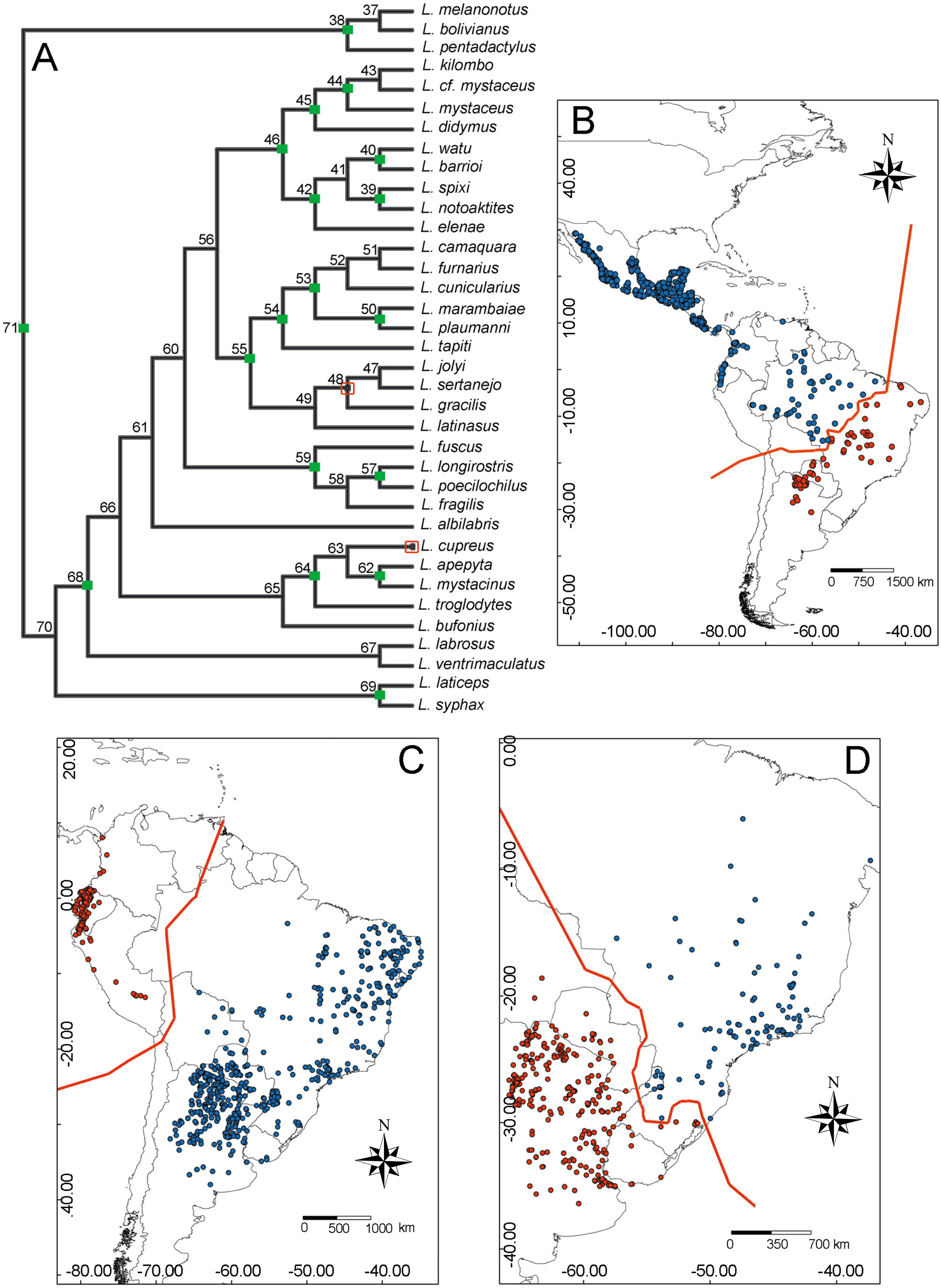

Fig. 3. Spatial analysis of vicariance. A: Tree of the *Leptodactylus fuscus* group showing a consensus reconstruction of historical biogeography by vicariance inference. Green squares show disjunction; red empty squares show the nodes ignored by the program. B–D: Hypothetical barrier at vicariant node 71 (B), 68 (C) and 55 (D); red and blue dots show disjunct sister clades.

included the ancestral areas of the external group and of the *L. fuscus* group. The fragmented distribution would have given rise to the ancestor of the *L. fuscus* group, distributed towards the south of the barrier, occupying the BD sub-regions (Chacoan and Amazonian).

Following the biogeographic history of the group, several dispersal events that allowed the ancestor's range to expand would have occurred. These took place initially towards the north, occupying large areas that encompassed the Chacoan, Amazonian, and Caribbean sub-regions (node 61). This expansion could have occurred during wetter periods, when forests were more extensive, which would have allowed the invasion of open habitats within rainforest systems (Heyer 1978). Hedges and Heinicke (2007) indicate a possible dispersal event for *Leptodactylus albilabris*, from South America to Puerto Rico, Hispaniola and St. Croix islands. The lowland distribution of this species and the fact that most of the Puerto Rico bank was a continuous land area during the last ice age suggest that populations likely diverged in the late Pleistocene. Furthermore, in some cases dispersal may have occurred on flotsams after storms, although human introductions cannot be ruled out (Hedges and Heinicke 2007). The analysis with S-DIVA showed a dispersal event (node 61) towards the Caribbean sub-region, which might have given rise to the distribution of *L. albilabris*, in agreement with the hypothesis of Hedges and Heinicke (2007).

Regarding node 68, we recovered a vicariant event in VIP, which explains the disjunctive distribution of *L. ventrimaculatus* and *L. labrosus* towards the western Andes, and of the remaining species of the group to the east. This event is not recognized in the results obtained with S-DIVA, which indicate a dispersal event, rather than a vicariance event. The so called "tethys" sleeve, the marine transgressions that occurred during Miocene and covered great areas between the Andean Mountain Range and the foundations of rocky beds in Guyana and Brazil (Webb 1995; Ortiz-Jaureguizar and Cladera 2006) are hypothesized to explain this disjunctive distribution. Similar distribution patterns to those obtained by this study were observed among the spider species of the genus *Cyriocosmus*, *C. leetzi* and *C. elegans* (Ferretti et al. 2012).

Heyer (1982) proposed a series of models to explain the distribution patterns of certain species of the genus *Leptodactylus*. One of these models is the "ecological determinism," which states that ecological factors are the main determinant of species distributions, regardless of historical distribution changes that could have occurred through dispersion or vicariance. This hypothesis could explain the vicariance event that we found in VIP, whose barrier separated two biogeographic sub-regions: the Chacoan sub-region (where species included in node 49 are distributed) and the Parana sub-region (node 54) (Fig. 3D). This would be an example of dynamic vicariance, in which climate changes gradually displace a biotic component and the environment has greater importance as a niche

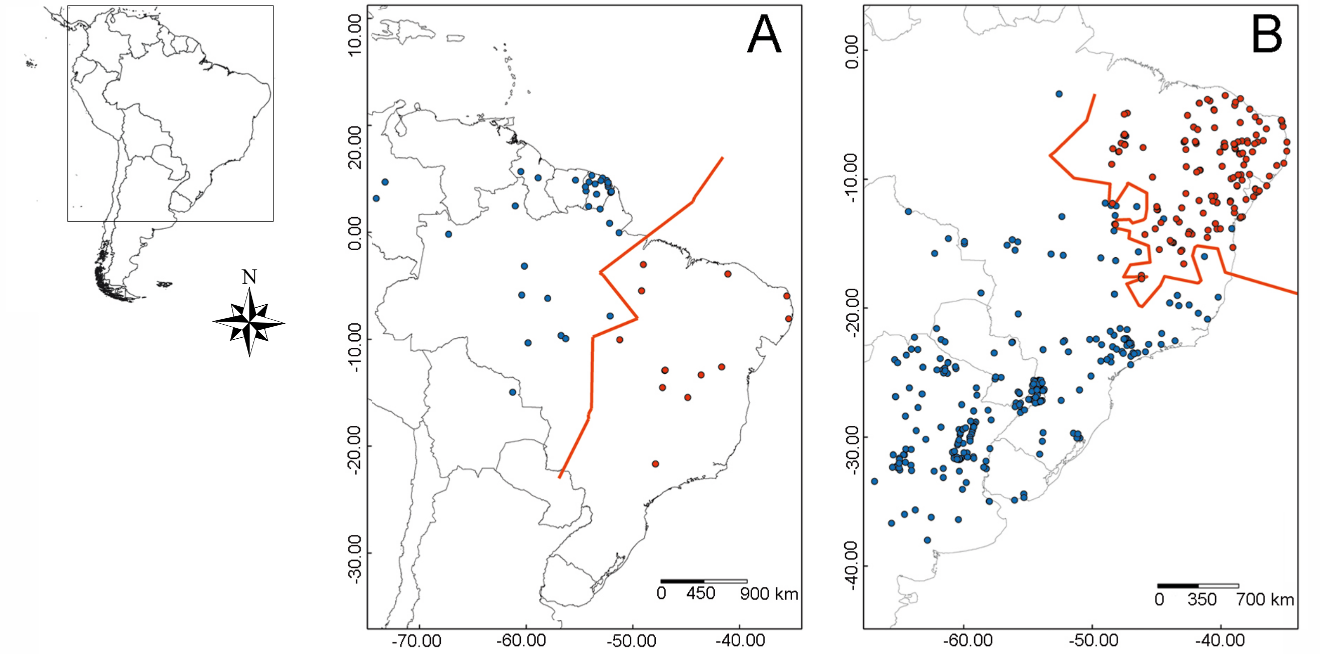

Fig. 4. Spatial analysis of vicariance. A-B: Hypothetical barrier at vicariant node 44 (A) and 64 (B); red and blue dots show disjunct sister clades.

modulator of the resulting species. The vicariant events of node 44 recognized in VIP and S-DIVA are also an example of how ecological factors, climate and soil conditions, among others, could have fragmented the ancestor distribution of the species included in that node, which resulted in the disjunctive distribution of *L. mystaceus* in the Amazonian sub-region, and *L.* cf. *mystaceus* and *L. kilombo* in the Chacoan subregion. These findings agree with the phylogenetic relationships raised by Alves Da Silva et al. (2020), who recovered *L. mystaceus* and *L.* cf. *mystaceus* as different evolutionary lineages with allopatric distribution because acoustic data showed a clear distinction between them despite their cryptic morphology (Fig. 4A). During the tertiary period it has been hypothesized that the development of the Chaco xeric sub-region (including the provinces of Caatinga, Cerrado, Chaco and Pampa) or diagonal of open formations (Vanzolini 1963), divided the ancient Amazonian-Paranaense forest into the current Amazonian and Parana sub-regions (Morrone and Coscarón 1998; Morrone 2000). This could explain the disjunctive distribution of the species included within node 44, where the environment would have acted as a niche modeler. On the other hand, Ab'Saber (1977) stated that during a major dry episode in South America (equivalent to the Wisconsin-Wiirm Ice Age) an expansion of semi-arid vegetation on the continent occurred. According to this view, vegetation similar to that of the Caatinga could have surrounded a central area of closed vegetation in central Brazil, and both seasonal formations might have extended towards the Amazonian region, while the tropical rainforests suffered contraction. The slow return of humid climate during the last 12,000 years might have favored the re-expansion of rainforests together with soil leaching and acidification in several areas, such as the Cerrado region, thus fragmenting the semi-arid vegetation typical of base-rich soils (Ratter et al. 1988). The fragmented distribution patterns obtained in node 64 in VIP (Fig. 4B), could illustrate this hypothesis. Furthermore, similar distribution patterns have been found in Prado and Gibbs' work (1993) regarding several species of plants (*Anadenanthera colubrina*, *Amburana cearensis*, *Enterolobium contortisiliquum*), where soil type and chemical characteristics, such as acidity, could have played an important role in the distribution of the species.

It is important to highlight that most of the patterns resulting from different methodologies do not match, neither in the events that occur in the nodes nor in the resulting species the distributions. The fact that we used large, predefined areas in the analysis of ancestral areas with S-DIVA could have led to an underestimation of several disjunctive distributions, and to a greater amount of dispersal events compared to vicariant events, even though this method should favor explanations through vicariant events (Díaz Gómez 2011). As this program does not allow operating with more than 15 area units, it compelled us to choose large areas to meet this requirement. Another disadvantage of this program is that the root node tends to include all areas occupied by the terminals, favoring explanations by vicariance thus slightly modifying the final results. To solve this problem, we decided to add external groups to the analysis based on Ronquist's (1997) suggestion, which would cause the root node to change thus restricting the number of areas. Despite having added external groups, the ancestral area was not modified, and only an area where one species (*L. melanonotus*) was distributed was added (North American Pacific sub-region). Because Ronquist (1997) proposed to limit the number of ancestral areas as another solution, we limited the number of areas to three. However, it is important to clarify that there are no criteria for choosing the number of areas to restrict.

CONCLUSIONS

The present study suggests that a vicariant barrier may exist based on the congruent distribution patterns of different taxa. However, determining which type of barrier it might be is difficult, due to the constantly changing scenarios and the uncertain timing of these events (Bush et al. 2007). In this study we focused on significant vicariant events that occurred at basal nodes, and some necessary movements that should have occurred in the group. We proposed hypotheses of the historical events and biogeographical barriers that seem to have given rise to the current distribution of the *L. fuscus* group, in which the vicariant processes are the most relevant ones for the diversification of the group. The use of the fossil record to calibrate the molecular clock and thus estimate divergence time between species is crucial to support these hypotheses. To analyze node age and replacement rates, future work is needed. The fossil record proposed for *Leptodactylus* by Gómez et al. (2013) from the Chapadmalalan (early Pliocene) of the South American Pampas; a Bayesian MCMC (Markov Chain Monte Carlo) approach (Drummond and Rambaut 2007) could highlight this issue. Nevertheless, this is the first contribution to the historical biogeography of the *L. fuscus* group based on quantitative methodology, a complete sample of species identified to date, and available phylogeny.

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Competing interests: MC, RGM, MLP and JMDG declare that they have no conflict of interest.

Availability of data and materials: Geographical records in decimal degrees are available in supplemental material table S1.

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Supplementary materials

Table S1. Geographical records of the *Leptodactylus* species used in the analysis of ancestral areas and in the spatial analysis of vicariance. The table indicates the species name, longitude and latitude in decimal degrees, bibliographic reference from the database where the record was obtained, and additional comments of specimens revised by the authors for this work. (download)

Table S2. Comparative results of the spatial analysis of vicariance with the Vicariance Inference Program (VIP) setting different cell size values. Number of disjunct and removed nodes recovered in the consensus tree at each run are shown. *For each run, the cost of distribution removal was fixed to 1.8, the overlap up to 15%, and the “partial elimination” option was not allowed. (download)

Table S3. Comparative results of the spatial analysis of vicariance with the Vicariance Inference Program (VIP) setting different values of cost of distribution removal. Number of disjunct nodes and removed nodes recovered in consensus tree at each run are shown. *For each run the cell size was fixed to 0.5° × 0.5°, the overlap up to 15%, and the “partial elimination” option was not allowed. (download)

Fig. S1. Species distribution area and ancestral areas of *Leptodactylus fuscus* group including distributional data of Texas. A: Biogeographical subregions and provinces are shown in different colors. Caribbean subregion (A); Chacoan subregion (B); Parana subregion (C); Amazonian subregion (D); North American Pacific subregion (E); Tamaulipan province from Alleghany subregion of the Neartic Region (F), Mexican transition zone region (G); South American transition zone region (H). A, B, C, D, E, G, H are established based on the regionalization proposed by Morrone (2006), F is established from the regionalization proposed by Escalante et al. (2021). B: Nodes show the most likely state. The letters and coarse colored circles indicate the ancestral areas of each node. Green, blue and yellow thin circles show vicariance, dispersions and extinctions events, respectively. (download)