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RESEARCH ARTICLE

Phylogeography and potential distribution of *Sturnira lilium* and *S. giannae* (Chiroptera: Phyllostomidae) with range extension for *S. giannae* in the Cerrado and Pantanal biomes

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ABSTRACT. *Sturnira*, known as the yellow-shouldered bat, has a wide geographical distribution and encompasses 24 distinct species. Within Phyllostomidae, *Sturnira* is the most diverse, with four species in Brazil: *S. lilium*, *S. magna*, *S. tildae*, and *S. giannae*. *Sturnira* species occur on the eastern slopes of the Andes and adjacent Amazonian lowlands, spanning from Colombia to northern Bolivia, the Brazilian Amazon, the southern lowlands of Venezuela, and the Guianas. In the present study the following were investigated: the phylogenetic relationships of *Sturnira*, employing the mitochondrial cytochrome b gene as a marker; the phylogeography of *S. lilium* and *S. giannae*, aiming to elucidate their geographical boundaries and phylogenetic positions; the morphology of *S. lilium* and *S. giannae*, and their potential distributions. The results indicate that there are two major clades within *Sturnira*, one including *S. lilium*, *S. parvidens*, and *S. bakeri* as the sister group of *S. giannae*, *S. luisi*, and *S. paulsoni*, and another clade with the remaining species. Morphological analyses showed that the diagnostic characteristics previously advanced for *S. lilium* and *S. giannae* overlap. Our findings expanded the known distribution of *S. giannae*, and show an area where *S. lilium* and *S. giannae* overlap in distribution. This area spans from the south of the state of Mato Grosso to the south of the state of Maranhão; the two species are sympatric in northeast Brazil and syntopic in the Pantanal. The comprehensive species distribution model suggested that the sympatry between *S. lilium* and *S. giannae* is notably larger than documented here.

KEY WORDS. Cryptic species; cytochrome b gene; habitat suitability; sympatry; syntopic.

INTRODUCTION

Bats of the family Phyllostomidae are one of the most extensively studied groups within the order Chiroptera. This heightened scientific interest stems from their remarkable species richness, coupled with their diverse morphology and ecology (Freeman 2000, Hedrick and Dumont 2018). Phylogenetic relationships within Phyllostomidae have proven challenging to resolve solely through morphological characters, due to the rapid diversification and multiple homoplasies (Velazco 2005, Datzmann et al. 2010, Velazco and Patterson 2013, 2019). Consequently, molecular data, such as those presented by Holanda et al. (2012) and Rojas et al. (2016), play a crucial role in illuminating the evolutionary history of phyllostomid bats. However, many interspecific and intraspecific relationships remain to be investigated (Dávalos and Jansa 2004, Dias et al. 2017). As a result, our understanding of the diversity within this group continues to be notably deficient.

Morphologically cryptic species pose a challenge when accessing species' diversity, because speciation is not always associated with clear morphological differences or allopatric distribution (Bickford et al. 2007, Lajus et al. 2015). DNA sequence analyses have uncovered cryptic species, and in many

instances, ecological characterizations typically confirm the distinct species status (Hoffmann and Baker 2001, Jacobs and Barclay 2009, Lim et al. 2017, 2020). Species complexes are often found after the detection of considerable genetic distance values among the populations of a species (Hoffman and Baker 2001, Campbell et al. 2004), and molecular approaches such as phylogeography and species delimitation tests can aid in resolving taxonomic issues (Campbell et al. 2004, Turmelle et al. 2011, Coraman et al. 2013).

Sturnira Gray, 1842 (Stenodermatinae), commonly known as yellow-shouldered bats, have a wide geographic distribution, spanning from Mexico to the Antilles through South America, reaching Northern Argentina (Velazco and Patterson 2014, 2019). Currently, *Sturnira* comprises 24 recognized species, being the most species-rich genus in Phyllostomidae (Velazco and Patterson 2014, 2019). In Brazil, four species have been documented: *S. lilium* (É. Geoffroy, 1810), *S. magna* de la Torre, 1966, *S. tildae* de la Torre, 1959, and *S. giannae* Velazco and Patterson, 2019 (Garbino et al. 2020).

Phylogenetic data (Velazco and Patterson 2013) and morphological analyses (Velazco and Patterson 2019) have facilitated the description of *S. giannae*, formerly considered part of the *S. lilium* complex (Velazco and Patterson 2019). After these analyses the distribution of *S. lilium* was restricted to portions of the Brazilian shield in Brazil, Bolivia, Paraguay, Uruguay, and Argentina (Velazco and Patterson 2014), and the distribution of *S. giannae* was restricted to Ecuador, Peru, northern Bolivia, the Brazilian states of Amazonas and Pará, French Guiana, Suriname, Guyana, Venezuela, and Trinidad & Tobago (Velazco and Patterson 2019). Notably, *S. giannae* is found predominantly in areas of Amazonian Forest.

Based on the environmental characteristics of the documented range of a species, such as climate, it is possible to infer the specie's potential distribution, and the design of long-term conservation efforts (Debata et al. 2019, da Silva et al. 2021). In this context, employing Species Distribution Modeling (SDM) becomes pivotal, providing robust predictions for decision-making. SDM serves to identify critical areas, or hotspots, areas suitable for the translocation of individuals, and the management of invasive species (Elith and Leathwick 2009, Marco-Júnior and Siqueira 2009, Cordeiro et al. 2018). This tool is particularly useful when dealing with limited information about the geographic distribution of species, especially species that are difficult to detect or are locally rare (Elith and Leathwick 2009, Marco-Júnior and Siqueira 2009).

We uncovered evidence that the distribution of *S. giannae* and *S. lilium* overlaps. In areas where the two occur sympatrically, there are diagnostic characters that allow their identification. In light of this scenario, the aim of this study was to investigate the distribution of *S. giannae*, particularly in the Pantanal and Cerrado biomes, and to explore its phylogenetic relationships within congeners. Additionally, we assessed the taxonomic status of populations of *S. lilium* and *S. giannae* through the analysis of morphology and the cytochrome b gene.

MATERIAL AND METHODS

The present study included specimens of *Sturnira* collected during fieldwork, which were handled following the national guidelines and provisions of 'Instituto Chico Mendes de Conservação da Biodiversidade' (ICMBio, license number 60058). Collecting was also approved by the 'Comissão de Ética no Uso de Animais' (CEUA, process 01200.001568/2013-87) from the Universidade Federal do Rio de Janeiro. Each site was sampled for six hours, beginning at sunset. Specimens were caught with mist nets installed on trails and clearings in the vegetation. The nets were inspected every 15 minutes. The captured specimens were pre-identified in the field (Gardner 2007, Reis et al. 2017).

The animals were euthanized with barbiturate (60 mg/kg) administered intraperitoneally along with lidocaine at a concentration of 10 mg/kg, as recommended by CONCEA (2013). Formaldehyde (10%) was injected to fix the specimens that were preserved in fluid (70% alcohol). Collected specimens were deposited in the Mammal Collection of the Museu Nacional (UFRJ), Rio de Janeiro, Brazil (Table S1).

Information on museum and collector acronyms cited in this study are: American Museum of Natural History (AMNH), New York, USA; Carnegie Museum of Natural History (CM), Pittsburgh, USA; Cleveland Museum of Natural History (CMNH), Ohio, USA; Field Museum of Natural History (FMNH), Chicago, USA; Museum of Natural Science, Louisiana State University (LSUMZ), Baton Rouge, USA; Museu Nacional, Universidade Federal do Rio de Janeiro (MN), Rio de Janeiro, Brazil; Museu Paraense Emílio Goeldi (MPEG), Belém, Brazil; Museu de História Natural do Ceará Prof. Dias da Rocha (MCHN-MAM), Ceará, Brazil; Museum of Southwestern Biology (MSB), University of New Mexico, Albuquerque, Mexico; Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (MUSM), Lima, Peru; Museum of Vertebrate Zoology (MVZ), University of California, Berkeley, USA; Royal Ontario Museum (ROM), Toronto, Canada; National Museum of Natural History (USNM), Smithsonian Institution, Washington, USA; Museum of Texas Tech University (TTU), Lubbock, Texas, USA;

Laboratory for Rabies Diagnosis at the Pasteur Institute of São Paulo (SP), Brazil; Laboratory of Biology and Parasitology of Wild Reservoir Mammals (LBCE), Fiocruz, Rio de Janeiro, Brazil; and catalog number of TJ McCarthy (TJM) and BD Patterson (BDP).

Study area

Nineteen individuals were collected in Brazil, 18 in the Pantanal and one in the Cerrado domain. The individuals from the Pantanal were collected at the Santa Lucia ranch, municipality of Barão de Melgaço (16°53'38.25"S; 55°54'24.98"W), state of Mato Grosso. This region comprises a mosaic of open fields and forest formations with varying levels of anthropization resulting from livestock-grazed areas. The individual collected in the Cerrado was found at the edge of a forested quartzite canyon (9°22'49.80"S; 46°14'37.57"W) in the municipality of Alto Parnaíba, state of Maranhão. The area is dominated by "Campo Sujo" (dirty field), where there are scattered trees and shrubs, and a large proportion of grassland (Oliveira and Marquis 2002), occasionally with small rocky outcrops of limited extent in the sampled area.

Morphological analysis

Adults of *S. lilium* (14) and *S. giannae* (5) were examined morphologically (Table S1). A digital caliper with a precision of 0.01 mm was used to make the measurements. Cranial-dental measurements are delimited in Fig. 1 following Velazco and Patterson (2014) and were: Greatest length of skull (GLS), Condylo-incisive length (CIL), Condylo-canine length (CCL), Postorbital breadth (PB), Braincase breadth (BB), Mastoid breadth (MB), Zygomatic breadth (ZB), Maxillary toothrow length (MTRL), Width at M2 (M2-M2), Dentary length (DENL), Mandibular toothrow length (MANDL). The external measures include forearm length, hair length at the posterior edge of the uropatagium, dorsal hair length between shoulders, and ventral fur length.

Isolation and amplification of the mitochondrial DNA and sequencing

DNA isolation was performed following the phenol-chloroform protocol (Sambrook and Russel 2001) from liver or muscle samples preserved in absolute alcohol. DNA concentration was checked with spectrophotometry (NanoDrop), and its quality was assessed on 0.8% agarose gels. Amplification of the mitochondrial gene cytochrome b (mt-Cytb) was performed by polymerase chain reaction (PCR). The reaction was prepared for a total of 25.0 µL, consisting of DNA (100 to 200 ng), dNTPs (25 mM/mL), primers

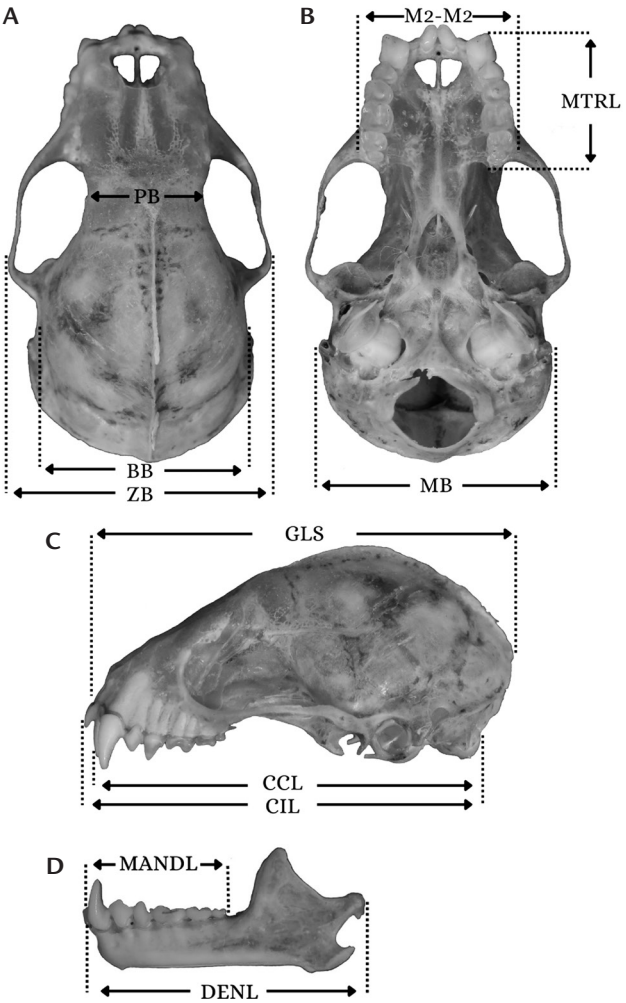

Figure 1. Skull of *Sturnira lilium* (MN 82216) showing the (A) dorsal view, (B) ventral view, (C) lateral view, and (D) the mandible in lateral view, with the measurements used in this study.

(10 pmol), 10x amplification buffer, 100 nM MgSO4, and one unit of Taq Platinum (Invitrogen) or GoTaq (Promega) polymerase, and executed on a thermal cycler (Eppendorf model Mastercycler Gradient, MJ Research PTC-100® or PTC-200 Peltier Thermal Cycler). A fragment containing the complete mt-Cytb sequence, with 1,140 base pairs (bp), was amplified using the Rhino F and Rhino R primer pair (Corrêa 2016). Amplification conditions consisted of denaturation at 95 °C for 2 min, followed by 35 cycles at 95 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 2 min, with a final extension at 72 °C for 8 min. Gene nomenclature follows the Gene Nomenclature Committee at the European Bioinformatics Institute (HUGO).

An aliquot of 3 µL of amplified DNA was added to 1 µL of GelRed (Invitrogen) and 3 µL of 1 Kb size marker (Invitrogen), and subjected to 1.5% agarose gel electrophoresis in 1x NaOH buffer to verify the size and quality of the amplified product. The purification of the amplified DNA, to remove unamplified fragments and excess reagents, such as primers or free nucleotides in solution, was performed with two protocols, using the PureLink – Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen) and Polyethylene Glycol (PEG). An aliquot of 3 µL of the purified product, mixed with 1 µL of GelRed, was subjected to 1.5% agarose gel electrophoresis to verify the result of the procedure. The agarose gels of the amplified and purified product were analyzed on a UV transilluminator. Image recording was performed by photo documentation. Samples with good amplification and purification quality were selected for the sequencing step.

Phylogenetic analysis

A total of 173 *Sturnira* sequences were analyzed, 25 of which were sequenced in the present study, and 148 were obtained from the GenBank. The specimens originating these sequences were from various locations in Mexico, Central America, and South America (Table S1). For population and phylogenetic molecular analyses, mt-Cytb sequences, spanning 1,140 bp, were aligned using MEGA 11 (Tamura et al. 2021). Sequences from the GenBank of *Carollia manu* Pacheco, Solari & Velazco, 2004 (KC753898), *Lionycteris spurrelli* Thomas, 1913 (AF423096), *Rhinophylla pumilio* Peters, 1865 (AF187029), and *Vampyressa bidens* (Dobson, 1878) (FJ154181) were used as outgroups based on previous studies (Velazco and Patterson 2013).

Phylogenetic relationships and the Bayesian molecular dating tree estimations were inferred using BEAST2 (Bouckaert et al. 2014). For this analysis, an uncorrelated, relaxed lognormal clock was employed (Trifinopoulos et al. 2016). The GTR+GAMMA+I nucleotide substitution evolution model was selected based on the Akaike information criterion test (Akaike information criterion [AIC]) using MrMoldeTest (Trifinopoulos et al. 2016). This model counts general time with unequal rates and unequal base frequency (Tavaré 1986), and rate heterogeneity between sites invariant plus discrete Gamma model (Gu et al. 1995). The calibration points used were the same as those used by Velazco and Patterson (2013), following the normal distribution of priors. Calibration point A (Lonchophyllinae and Carolliinae + Glyphonycterinae + Rhinophyllinae + Stenodermatinae – 18.9–25.1 Ma) was given M = 0.46 and S = 0.7. Calibration point B (Carolliinae + Glyphonycterinae and Rhinophyllinae + Stenodermatinae – 17.3–23.3 Ma) was given M = 0.01 and S = 0.91. Calibration point C (Rhinophyllinae and Stenodermatinae – 14.9–20.6 Ma) was given M = 0.01 and S = 0.9. The last calibration point D (*Sturnira* and Stenodermatinae – 12.8–18.5 Ma), with M = 0.01 and S = 0.78. The tree was calculated under the Birth/Death speciation model, which assumes that at any point in time, each lineage can undergo speciation at a rate of λ or become extinct at a rate of μ (Heath 2015). The Markov Chain Monte Carlo (MCMC) analysis was run for 120 million generations, stored every 10,000 generations. The effective sample size (ESS) values were considered when they were above 200. The maximum credibility tree of the clade was constructed using Tree Annotator v.2.6.2 (Bouckaert et al. 2014), discarding the initial 10% of data as burn-in. The tree was summarized and edited using FigTree v.1.4.0 (Rambaut and Drummond 2018).

Estimates of genetic distance and diversity

Haplotypes (H) were characterized in the DNA sequence polymorphism 6 program (DNAsp 6, Rozas et al. 2017), not considering sites with gaps or missing data. Estimates of genetic distance between sequences were analyzed in MEGA 11 (Tamura et al. 2021) using the Kimura 2-parameter model, with standard error estimates obtained by bootstrap (1,000 replicas). All ambiguous positions, which correspond to "missing data" or "gaps," were removed for each pair of sequences using the "pairwise deletion" option (K2p, Kimura 1980).

Genetic diversity indices were estimated for *S. giannae* and *S. lilium*, including the number of segregating sites (S), the average number of pairwise differences, nucleotide diversity (π), haplotype diversity (Hd), and standard deviation (sd). To test for past population expansion, we used two widely used statistical tests in demographic event analyses: Tajima's D (Tajima 1989) and Fu's FS test (Fu 1997). All analyses were performed using the ARLEQUIN software (Excoffier and Lischer 2010). Haplotype diversity represents the probability that two randomly sampled alleles are different, while nucleotide diversity is defined as the average number of nucleotide differences per site compared between DNA sequence pairs (Nei 1987).

Species distribution modeling

For the species distribution modeling (SDM) analyses, the locations of the specimens collected in this study and those provided by the LBCE were used. Additionally, we

incorporated the localities of *S. lilium* from the collection of mammals of the Museu Nacional (UFRJ) and Museu de História Natural do Ceará Prof. Dias da Rocha (MHNCE). The identification of museum specimens was confirmed by us. The literature records for the areas where *S. giannae* and *S. lilium* are sympatric were not used because they are questionable. In total, we gathered 73 occurrences, 53 for *S. giannae* and 20 for *S. lilium* (Appendix 1).

We defined South America as the background extent for SDMs and used Maxent 3.4.4 (https://biodiversityinformatics.amnh.org/open_source/maxent/). Maxent utilizes species presence data derived from georeferenced occurrence points and environmental variables to model potential distribution (Phillips et al. 2006). All SDM analyses were performed in the R environment (R Core Team 2021), using the 'raster' package (Hijmans et al. 2015), 'dismo' package (Hijmans et al. 2017), and the 'rJava' package (Urbanek et al. 2021). The following parameters were used for all models: logistic output format (habitat suitability on a scale of 0 to 1), 80% of records used as training and 20% for testing data, a maximum of 500 iterations, 10 replicates, and 10,000 background points (Phillips et al. 2006). The threshold used for suitable habitat distribution was defined by the Minimum Training Presence (MTP) threshold overlaid on the model training area (Pearson et al. 2007).

We modeled the potential distribution (i.e., habitat suitability) of the species by initially considering the 19 bioclimatic variables available in WorldClim 2.1 (Fick and Hijmans 2017), representing current conditions (years 1970–2000). We extract the values of the predictors at the point locations using the 'extract' function of the 'raster' package (Hijmans and Elith 2017). From this set of variables, we applied a correlation threshold of 0.7 for selection using the variance inflation factor (VIF) to identify those with low collinearity. The selection procedures were implemented in the R software using the 'usdm' package (usdm 2.1-7, Naimi et al. 2014). The variables selected for the habitat models were Mean Diurnal Range (Mean of monthly (max temp – min temp) (BIO2), Isothermality (BIO3), Mean Temperature of Wettest Quarter (BIO8), Precipitation of Wettest Month (BIO13), Precipitation of Driest Month (BIO14), Precipitation Seasonality (BIO15), Precipitation of Warmest Quarter (BIO18), and Precipitation of Coldest Quarter (BIO19). The models were projected with a spatial resolution of 2.5 arc-minutes (~ 4.5 km; Hijmans et al. 2005, Fick and Hijmans 2017). The proportional contributions (%) of each environmental variable to each model were analyzed through jackknife tests (Phillips et al. 2006).

To estimate the predictive accuracy of potential habitat models, we used the Area Under the Curve (AUC) metrics, based on the area under the Receiver Operating Characteristic (ROC) curve (Swets 1988, Elith et al. 2006). AUC evaluates the efficiency of model predictions in discriminating between locations where observations are present or the species not being recorded; it is one of the most widely used independent evaluators of the model's discriminatory power threshold. AUC values above 0.9 indicate high model performance, while values close to 0.5 indicate models equivalent to or worse than random (Peterson et al. 2011). For comparative purposes, the resulting images of each model (with continuous values from 0 to 1) were reclassified into four classes of environmental suitability: unsuitable (UNS, 0 – value MTP), moderate suitability (MOS, value MTP – 0.50), high suitability (HIS, 0.50–0.75) and very high suitability (VHS, 0.75–1.00). We plotted a final map with the occurrences and the final distribution model in ArcGIS.

RESULTS

The external and cranial measurements of *S. lilium* and *S. giannae* show little or no differences in their maximum, minimum, and average values, most likely due to the small number of specimens analyzed (Table 1). The length of the forearm of the male of *S. giannae* varied extensively, increasing the overlap of the forearm length of males and females. The clinoid processes were not observed in *S. giannae* (n = 4). The anterior process of the glenoid fossa, although well-developed in three specimens, was absent in one individual. Both *S. lilium* (Fig. 2D) and *S. giannae* (Fig. 2E) exhibit upper incisive with simple cusps (unicuspid). In *S. giannae*, the shape of incisive teeth can be simple or bicuspid (Fig. 2F). The metaconid shape of the lower first molar (m1) is variable in both species. Additionally, the metaconid shape of the lower second molar (m2) is variable in both species (Fig. 2D, E). In *S. lilium*, the metaconids and entoconids of the third lower molar (m3) are separated by a deep notch (Fig. 2D), whereas in *S. giannae*, they are either separated by a shallow notch or not separated at all (Fig. 2E, F).

We sequenced the complete mt-Cytb gene of 25 specimens, 18 *S. lilium*, five *S. giannae* and two *S. tildae* (Table S1). A total of 134 haplotypes in 173 complete mt-Cytb sequences (1,140 base pairs) of *Sturnira* were obtained, covering all described species. In the Bayesian inference (BI) tree, the values of posterior probability (pp) were significant (pp > 0.8), showing high support for the main clades and most of the subclades. The BI (Fig. 3) presented two major clades

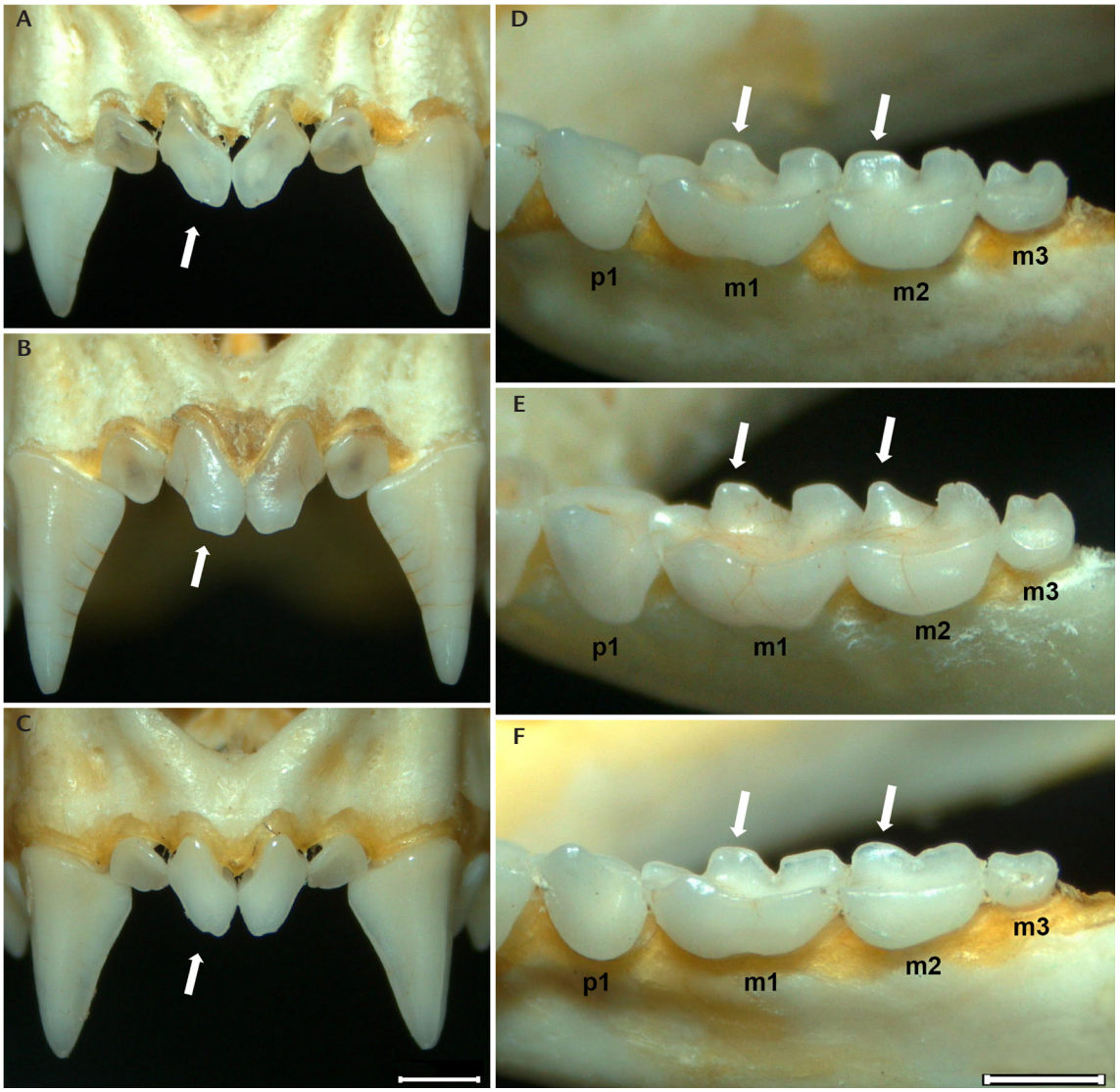

Figure 2. Detail of the teeth in *S. lilium* (A and D – MN 82228) and *S. giannae* (B and E – MN 84766, C and F – MN 82217). The shape of the upper internal incisor unicuspid in *S. lilium* (A) and unicuspid or bicuspid in *S. giannae* (respectively B, C). Metaconid of first inferior molar (m1) wide mesodistally and short cervico-occlusal (D, E, and F). Metaconid of second inferior molar (m2) large mesodistally in *S. lilium* (D) and in *S. giannae* (F), or short mesodistally and long cervico-occlusally (E) in *S. giannae*. Scale bars: 1 mm.

within the genus *Sturnira* (pp = 0.99), one divided into two subclades (pp = 0.99) composed by *S. luisi* + *S. paulsoni* as the sister group of *S. giannae* (pp = 0.99), and another subclade (pp = 0.99) composed by *S. lilium* as the sister group to the clade containing *S. parvidens* + *S. bakeri* (pp = 0.99). The second clade (pp = 0.89) was divided into two subclades, one containing *S. magna* as the sister group to *S. bogotensis* + *S. erythromos*, without support, and *S. mordax* as the sister group to *S. tildae* (pp = 0.99), and another subclade composed by *S. oporaphilum* as the sister group to *S. ludovici* (pp = 0.94), and *S. burtonlimi* as the sister group of *S. hondurensis* (pp = 0.99). All species with more than one haplotype were recovered as being monophyletic. A phylogenetic tree with individually identified sequenced specimens from the states of Mato Grosso and Maranhão is represented in Supplementary Material (Fig. S1).

The greatest interspecific genetic distance estimate was between *S. magna* and *S. bakeri* (9.69%), and the smallest was between *S. giannae* and *S. luisi* (1.64%), while *S. giannae* and *S. lilium* showed an intermediate value (5.85%). The genetic distance estimates between *S. tildae* and *S. lilium* or *S. giannae* were 7.81 and 6.79%, respectively. The greatest intraspecific genetic distance estimate was in *S. hondurensis* (1.72%, n = 6), and the smallest was in *S. mordax* (0.25%, n = 3) (Table 2). The values of genetic diversity indicated less variability within *S. lilium*, with haplotype and nucleotide diversity values smaller than those observed in *S. giannae* (Table 3). Tajima's D values were negative and significant (p) for populations of *S. giannae* and *S. lilium*, which can indicate an abundance of nucleotide variation and recent population expansion (Table 3). The results of the Fu's Fs test, based on haplotype distribution, also showed negative and significant (p) values for both populations (Table 3) reinforcing the evidence for a population expansion.

Current potential distribution models were consistent in part with the known range of the species (Fig. 4), con-

Table 1. Skull-dental (mm) and weight (g) measurements of *S. lilium* and *S. giannae*, with minimum (Min) and maximum (Max) values.

Character	*S. lilium* (7 females)			*S. lilium* (7 males)			*S. giannae* (5 males)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Greatest length of the skull	22.17	21.42	22.77	22.68	22.41	23.38	22.41	21.79	23.26
Condylo-incisive length	20.30	20.09	20.77	20.76	20.55	21.08	20.59	19.84	21.59
Condylo-canine length	19.45	19.15	19.91	19.85	19.69	20.19	19.60	18.97	20.57
Postorbital breadth	5.86	5.68	6.04	6.10	5.87	6.37	6.06	5.78	6.25
Cranial breadth	10.36	9.90	10.73	10.63	10.34	11.17	10.38	9.69	10.78
Mastoid breadth	11.88	11.55	12.23	12.27	11.74	12.57	12.24	11.94	12.75
Zygomatic breadth	13.41	12.24	14.09	13.88	13.62	14.60	13.53	13.01	14.47
Maxillary tooth row length	6.47	6.42	6.54	6.64	6.49	6.93	6.58	6.24	6.87
Width at M2	8.06	7.89	8.27	8.19	7.97	8.52	8.06	7.88	8.32
Dental length	14.62	14.37	14.86	14.98	14.61	15.39	14.85	14.53	15.35
Mandibular tooth row length	7.31	7.13	7.72	7.54	7.10	7.90	7.44	7.16	7.81
Forearm length	42.69	41.32	44.22	42.96	41.43	44.82	41.80	38.89	44.83
Weight	19.57	18.00	22.50	21.32	19.00	26.00	20.70	17.00	24.00
Hair length the posterior edge of uropatagium	5.19	4.06	6.36	5.03	3.84	5.90	5.33	4.85	6.50
Dorsal hair length between shoulders	8.06	7.11	8.69	7.63	7.08	8.97	6.52	5.49	7.27
Ventral fur length	4.87	4.11	5.52	4.58	3.95	5.16	4.39	4.13	4.65

Table 2. Genetic distance between *Sturnira* species estimates with the Kimura 2-parameter model using the cytochrome b gene. In bold, highlight the intraspecific distances. Values between parentheses are the number of haplotypes per species.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*S. lilium* (24)	**0.47**													
2	*S. giannae* (35)	5.85	**0.73**												
3	*S. tildae* (13)	7.81	6.79	**0.53**											
4	*S. paulsoni* (1)	5.93	1.78	7.51											
5	*S. bakeri* (3)	6.85	5.81	9.00	6.34	**1.17**									
6	*S. luisi* (10)	6.00	1.64	7.32	1.69	6.06	**0.96**								
7	*S. parvidens* (18)	5.90	4.65	8.08	5.18	3.20	5.20	**0.70**							
8	*S. oporaphilum* (7)	7.48	6.84	8.35	7.24	8.18	7.69	7.89	**1.28**						
9	*S. burtonlimi* (1)	8.14	6.70	8.03	7.02	8.71	7.39	7.82	4.51						
10	*S. mordax* (3)	6.90	5.69	5.63	6.31	8.03	6.54	7.43	5.88	4.67	**0.25**				
11	*S. magna* (4)	8.48	7.95	8.29	8.17	9.69	8.28	9.60	8.30	7.10	6.20	**0.27**			
12	*S. ludovici* (2)	7.49	6.57	7.36	6.94	7.61	7.01	7.48	4.71	5.13	5.14	6.96	**0.37**		
13	*S. hondurensis* (6)	7.94	7.22	9.20	7.88	8.91	8.24	8.09	5.83	4.39	6.91	8.28	5.79	**1.72**	
14	*S. erythromos* (6)	7.08	6.87	6.91	7.38	7.73	7.26	7.59	7.65	6.86	5.80	6.79	6.89	8.40	**0.68**
15	*S. bogotensis* (1)	7.57	6.00	6.75	6.74	8.91	6.74	7.90	7.17	7.03	5.64	6.07	5.56	8.15	5.80

Table 3. Genetic diversity of *S. giannae* and *S. lilium* performed with the cytochrome b gene discriminating number of sequences (n), haplotypes (h), segregate sites (S), haplotype diversity (Hd), nucleotide diversity (π), Tajima's D (D), and Fu's Fs (Fs) tests. Standard deviation (sd) and p-values ($p \leq 0.05$).

Species	n	h	S	Hd/sd	π/sd	D	p	Fs	p
S. giannae	38	35	19	0.994/ 0.008	0.071/ 0.037	-1.764	0.022	-24.333	0
S. lilium	28	24	10	0.989/ 0.012	0.031/ 0.018	-2.205	0.001	-21.183	0

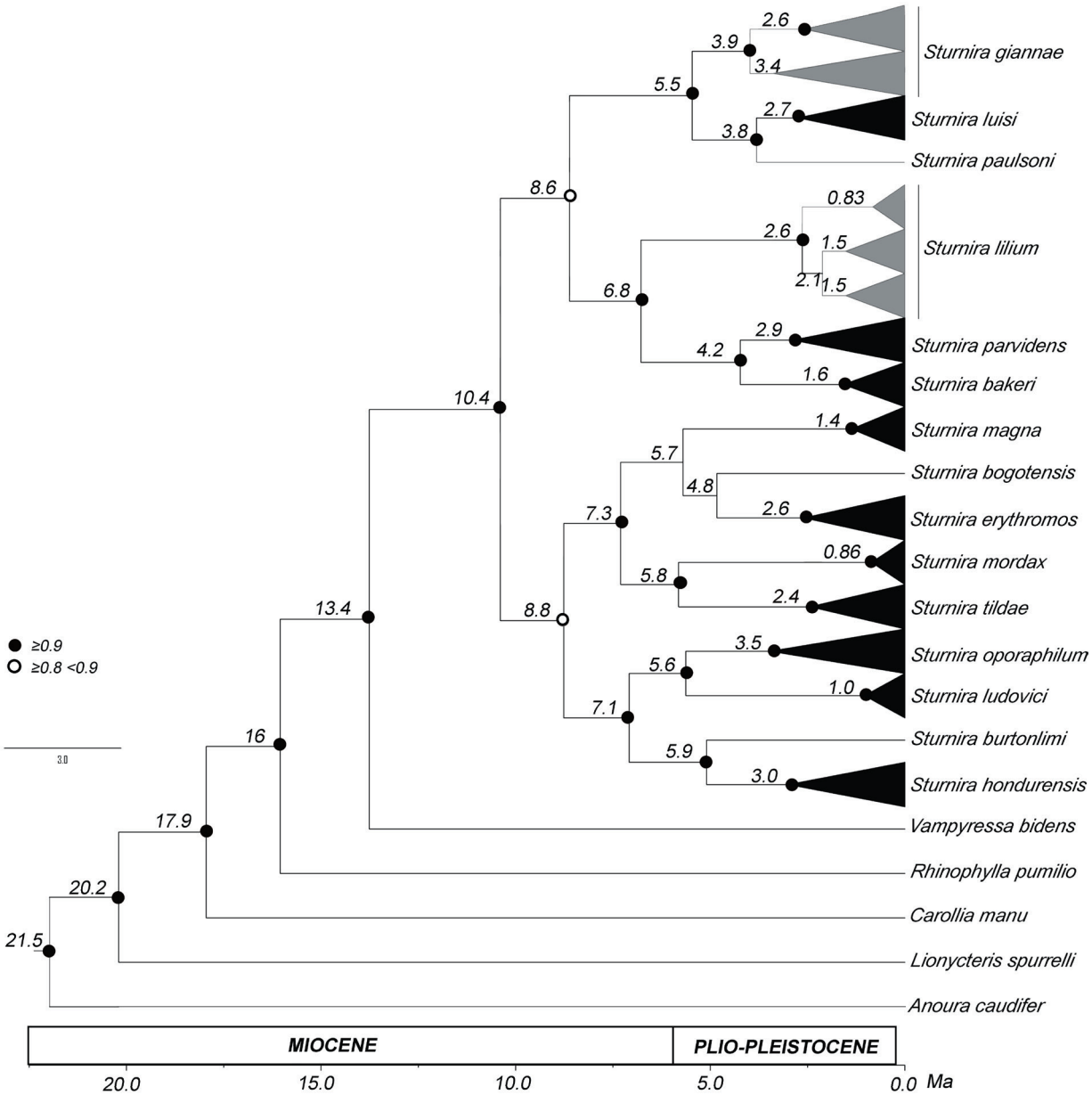

Figure 3. Bayesian dating tree inference performed with cytochrome b gene for *Sturnira*. Numbers are the nodes ages and values of posterior probability are represented by circles in black (pp ≥ 0.9) and white (pp ≥ 0.8 < 0.9). See the list of haplotypes in Table S1 and Fig. S1.

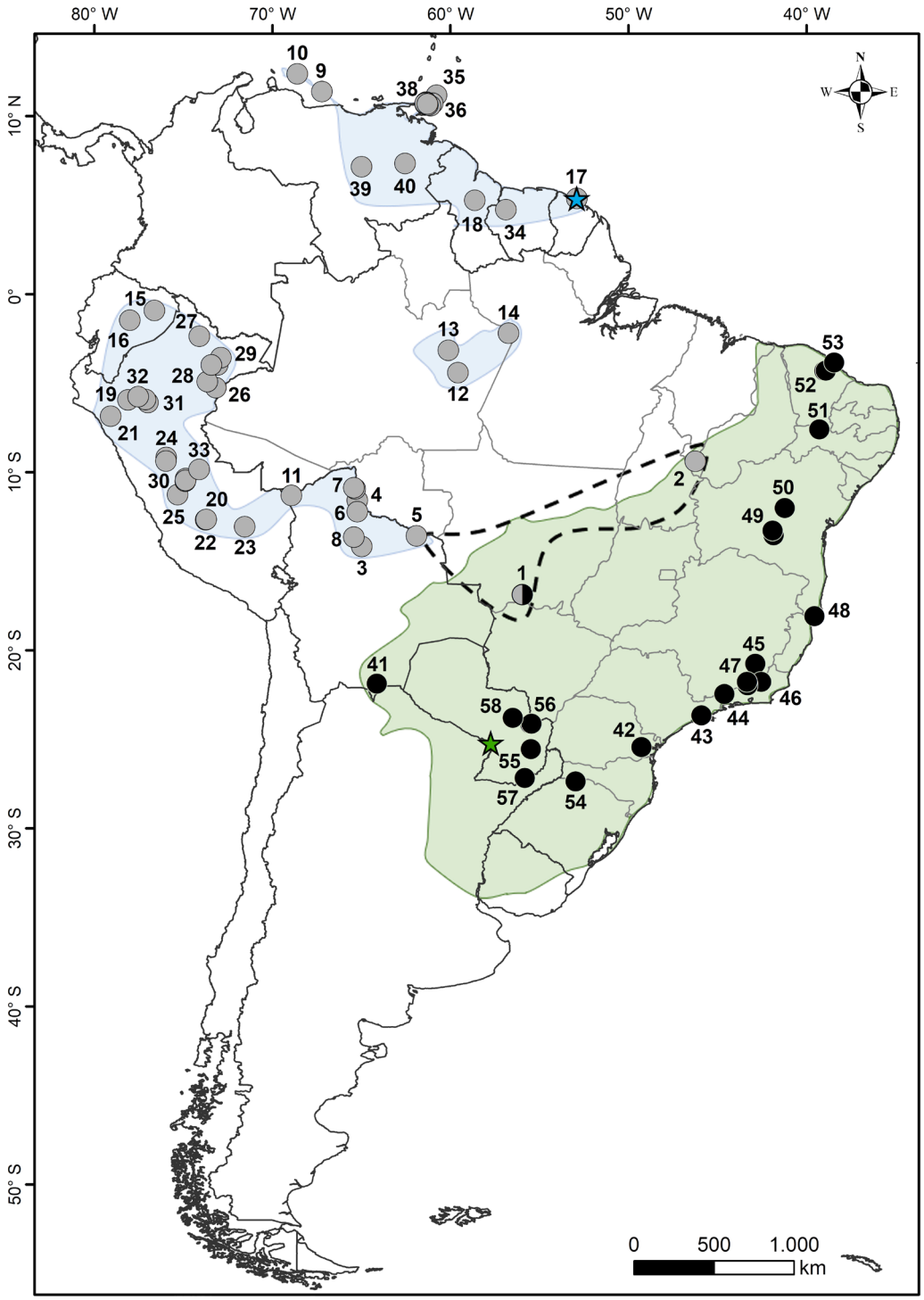

Figure 4. Map showing the distribution and location of analyzed samples of *S. giannae* in gray circles and blue boundaries, and of *S. lilium* in black circles and green boundaries. The blue star is the type locality of *S. giannae*, and the green star is the type locality of *S. lilium*. The dotted black line marks the extent of *S. giannae*'s territory in Brazil: Mato Grosso State (1) Barão de Melgaço, and Maranhão State (2) Alto Parnaíba. Other localities are in the Appendix 1.

sidering the high and very high suitability scale based on georeferenced occurrence data for *S. giannae*. The potential distribution covers the central Amazon and extends towards the western portion of northern South America. The model also indicates a moderate potential distribution, reaching the central part of the open area corridor, where we recorded new records of *S. giannae*, including a potential distribution to some areas in the south of the state of Bahia, Brazil. For *S. lilium*, the models illustrating moderate, high, and very high potential distribution align well with the documented range of the species. This potential range spans from southern, central, and northeastern Bolivia, encompassing eastern Paraguay, northwestern and northeastern Argentina, and extending into northern Uruguay. In Brazil, the distribution spans the southern and central regions, covering the entire east coast, traversing through the southeast, and reaching the central part of the northeastern region (Fig. 5).

The occurrence of *S. giannae* is associated with isothermality (BIO3), a characteristic that is prevalent in tropical regions. Additionally, it is linked to a relatively high precipitation during the driest month of the year (BIO14), implying a potential occurrence in less seasonal and wet areas (BIO18, Fig. 6). Consequently, the species displays a tropical pattern, indicating a potential distribution in higher latitudes. This distribution is particularly associated with lower thermal variability and relatively dry periods, especially in regions where precipitation does not coincide with lower temperatures, such as in south-central and eastern Brazil.

The association of *S. lilium* with isothermality (BIO3) is less pronounced, indicating a potential altitudinal distribution in the highlands of eastern and southern Brazil, and in the wet regions of eastern Paraguay and the coastal areas

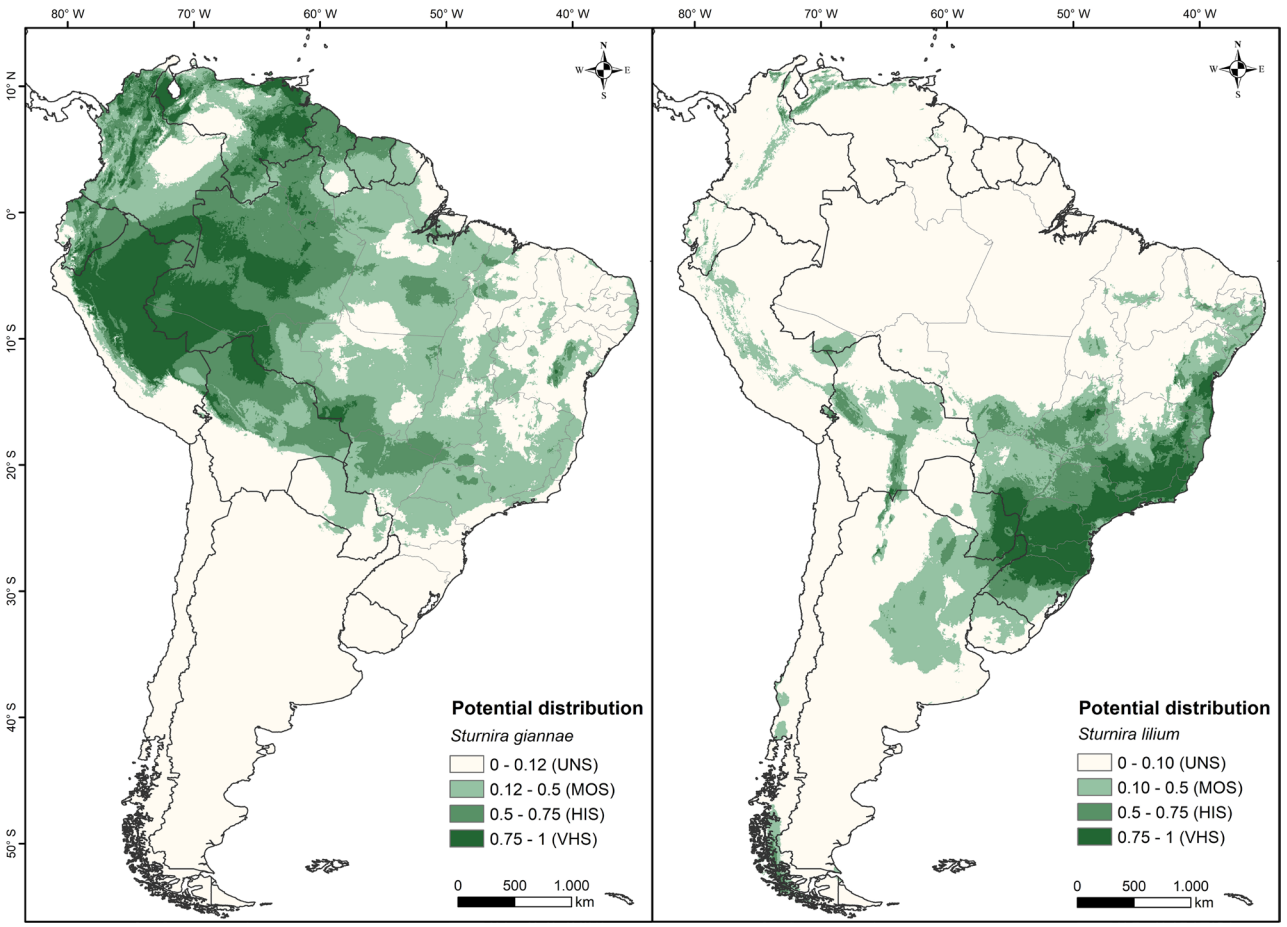

Figure 5. Maps of South America with the potential distribution (between years 1970–2000) of *S. giannae* and *S. lilium*. Unsuitable (0 – MTP value), moderate suitability (MTP value – 0.50), high suitability (0.50–0.75), and very high suitability (0.75–1.00).

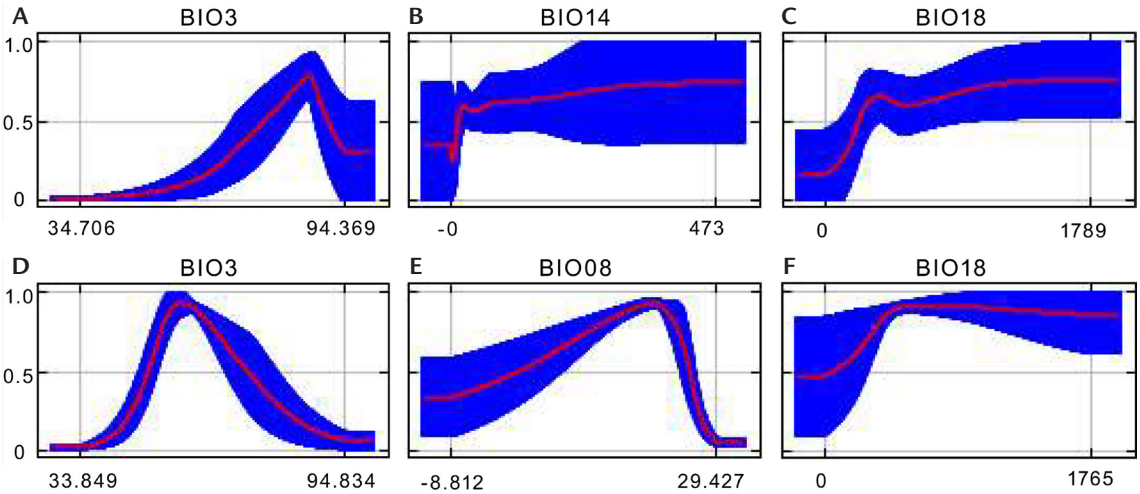

Figure 6. Response curve showing how each environmental variable affected the Maxent prediction. Variables A, B, and C were the ones that most affected the potential distribution of *S. giannae*, and D, E, and F most affected the potential distribution of *S. lilium*. The curves show the average response of the 10 replicate Maxent runs (red) and the standard deviation (blue).

of eastern Brazil. The latter region includes a subtropical climate and coastal regions in the southeast, which are among the wettest areas in South America. These areas experience high precipitation in the austral summer (BIO08, BIO18), averaging more than 3,000 mm of annual rainfall (Fig. 6). This is primarily influenced by the orographic effect caused by the Serra do Mar mountains (Conti and Furlan 2011). The species' potential distribution extends from northeastern Argentina, including the Yungas, a southernmost extension of the Andean tropical forests, to central Bolivia along the slopes of the Andes. This suggests that *S. lilium* is able to withstand temperature fluctuations throughout the year, with notable differences observed during the southern winter and the rainiest months.

DISCUSSION

The integrative approach of this study, encompassing phylogenetics, genetic divergence, morphology, and distribution modeling, confirmed the syntopy between *S. giannae* and *S. lilium* in the northern Pantanal of Mato Grosso state, Brazil. Furthermore, it extended the distribution of *S. giannae* by 2,075 km to the northeast. The average forearm length variation in *S. giannae* males reported in the literature, 43.8 mm (41–45 mm, Velazco and Patterson 2019), is smaller than the 41.80 mm (38.89–44.83 mm) found in the present study. Our results expand the known range of variation in the length of the forearm of *S. giannae* males, leading to an increased overlap with the measurements of *S. lilium* and *S. tildae* (Velazco and Patterson 2019, Carneiro et al. 2022). The external and skull-dental measurements reported here for *S. giannae* are, on average, similar to those found in *S. lilium* (Reis et al. 2017, Velazco and Patterson 2019), and smaller than the those reported for *S. tildae* (Carneiro et al. 2022). These findings highlight that it may be difficult to distinguish between *S. lilium* and *S. giannae* due to morphological similarities and sympatry.

The results of Bayesian inference were consistent with previous phylogenetic arrangements (Velazco and Patterson 2013, Hernández-Canchola and León-Paniagua 2017). In all analyses, the clade containing *S. giannae*, *S. luisi*, and *S. paulsoni* emerged as the sister group of the clade containing *S. lilium*, *S. parvidens*, and *S. bakeri*, topologically well-separated from the clade that includes *S. tildae* and the remaining species (Fig. 3, Hernández-Canchola and León-Paniagua 2017). *Sturnira* diversified during the Middle Miocene; however, it was during the Pliocene that most extant species emerged. Our divergence time results suggest a recent diversification between *S. lilium* and *S. giannae*, according to previous publications (Velazco and Patterson 2013, Hernández-Canchola and León-Paniagua 2017). Although the diagnostic morphological characters of *S. lilium* and *S. giannae* individuals overlap in areas where they are sympatric, the interspecific genetic distance estimates between the two taxa are high (ranging from a minimum of 4.9% and a maximum of 7.0%), surpassing those observed between other *Sturnira* species.

This finding adds support to their distinct species status (Velazco and Patterson 2013, 2019, Hernández-Canchola and León-Paniagua 2017).

The neutrality tests suggest that *S. giannae* and *S. lilium* show a pattern that fits a model of exponential population growth, suggesting an excess of rare mutations in populations. This observation may provide evidence of recent population expansion (Aris-Brosou and Excoffier 1996). *Sturnira giannae* overlaps with *S. tildae* in the Central Andes, Amazon, and Caribbean lowlands (Velazco and Patterson 2019, Carneiro et al. 2022). With the extension of its range, *S. giannae* is now also found in central Brazil. Thus, *S. giannae*, previously restricted to the Amazon Forest and the islands of Trinidad and Tobago (Velazco and Patterson 2019), also occurs in the Pantanal and Cerrado. While *S. lilium* has a potential distribution in regions with an average annual temperature between 12 and 24 °C, *S. giannae* occupies regions with an average annual temperature between 24 and 26 °C. This set of conditions, based on climate zones (Alvares et al. 2013), along with varying resource availability, may allow for niche differentiation in these species. Another potential limiting factor could be the higher altitude in the regions where *S. lilium* occurs. Regions of sympatry and potential syntopy have intermediate average annual temperatures and altitudes between the two extremes shown by both species.

The potential distribution of *S. giannae* in the southern part of the Brazilian state of Bahia is associated with the Hileia Baiana, an Atlantic Forest formation relatively similar to the Amazon. The forests in southern Bahia contain a unique and highly diverse biota, marked by significant levels of endemism. The vegetation in this region has been influenced by past connections with the Amazon biome (Faria et al. 2021, Silva et al. 2023). In this region, temperatures range from 20 to 26 °C (Alvares et al. 2013), falling within the optimal range for *S. giannae*, when compared to its current and potential distribution, as described here. Bat species with a distribution pattern akin to *S. giannae* have previously been recorded in Hileia Baiana (Gregorin and Rossi 2005, Dávalos et al. 2018). The pattern of moderate suitability for the species is associated with extensive savannas in the central part of the continent and areas with seasonally dry forests. These regions tend to be drier during the middle of the year (austral winter), and include central Amazonia and the coastal regions of eastern Brazil. Conversely, the central and eastern Amazon, a particular region, although considered humid over large areas, has short periods of drought, such as the monsoon-type climate (Alvares et al. 2013). These suggest an association with low-latitude seasonal regions.

Sturnira lilium occurs in open vegetation formations in the Pantanal, Cerrado and caatinga, as well forest formation in the areas of Atlantic Forest. This species appears to support sudden differences in temperature throughout the coldest months, with average annual temperatures varying between 22 and 26 °C (Alvares et al. 2013), and sudden differences in the rainiest months, with annual rainfall varying from around 700 to 2,500 mm (Alvares et al. 2013). The potential distribution model also highlights areas along the foothills of the Andes in Bolivia where the species is documented (Sanborn 1932, Poma-Urey et al. 2023), extending into the Argentine Yungas (Sánchez and Giannini 2014) as a north-south corridor. These results show greater suitability for habitats where precipitation and temperature exhibit considerable variation throughout the year.

External characters, even if subtle, are important for species identification in the field across their geographic distribution and in known or potential sympatric areas (Gregorin et al. 2008, Mendes et al. 2019, Paes et al. 2022). Morphologically, *S. lilium* and *S. giannae* can be considered cryptic (Bickford et al. 2007). The characters considered diagnostic for each species are shared by both species in areas of sympatry; hence, morphometric and molecular data are necessary for their precise differentiation. Recognizing cryptic species is essential for accurately estimating the real biodiversity of the world, understanding evolutionary processes, geographic variation in species distribution, and species coexistence, and assisting in biodiversity conservation planning.

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Competing Interests

The authors have declared that no competing interests exist.

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Supplementary material 1

Table S1. List of *Sturnira* samples used in morphological and cytochrome b gene analyses, specifying the taxon, haplotype (H), GenBank accession number, museum or field or tissue number (ID, for acronyms see material and methods), locality, and reference (Ref). (*) sample used only in morphology, (**) sample used in morphology and cytochrome b gene analyzes.

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Data type: sample information.

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Supplementary material 2

Figure S1. Bayesian dating tree inference with cytochrome b gene for *Sturnira*. In bold specimens sequenced in the present study from the states of Mato Grosso and Maranhão. Posterior probability values are represented by black (pp ≥ 0.9) and white (pp ≥ 0.8 < 0.9) circles. See the list of haplotypes in Table S1.

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Data type: molecular analysis topology.

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Appendix 1. *Sturnira* records used in the analysis of potential distribution.

Sturnira giannae BOLIVIA: Beni department, (3) Cercado, ca. 4 km from Tijamuchi River mouth (AMNH 210732), (4) General José Ballivian, ca. 5 km SW of Buena Hora (AMNH 210723), ca. 8 km N of Santa Cruz (AMNH 210730), (5) Iténez, ca. 1 km below Paragua River mouth, Remansos (AMNH 209424), (6) Mamoré, Mamoré River, opposite Cascajal (AMNH 210724), (7) Vaca Diez, ca. 5 km S of Guayaramerin, Mamoré River (AMNH 209418), (8) Yacuma, ca. 2 km from Yacuma River mouth (AMNH 210733); Pando department, (9) Abuna, Bella Vista (AMNH 262473), (10) Manuripi, Madre de Díos River, Gargantua Island (AMNH 262483), (11) Nicolas Suárez, Nareuda River (AMNH 248862, 248873). BRAZIL: Amazonas state, (12) Borba, Madeira River (AMNH 92200, AMNH 92227), (13) Manaus, Igarapé Cacao Pereira, Negro River (AMNH 91467–91469); Maranhão state, (2) Alto Parnaíba (MN 84766); Mato Grosso state, (1) Barão de Melgaço (MN 82217–82218, MN 82232, MN 82308); Pará state, (14) Faro, Amazon River, N bank, Serra do Espelho (AMNH 93896–93897). ECUADOR: Orellana province, (15) Parque Nacional Yasuní, Estación Científica Onkone Gare, 38 km S Pompeya Sur (ROM 105875); Pastaza province, (16) 5 km E of Puyo, Safari Hosteria Park (TTU 84983, TTU 85109–85110, TTU 85121). FRENCH GUIANA: Sinnamary, (17) Paracou (AMNH 266207, AMNH 266210, AMNH 266236, AMNH 268545). GUYANA: Upper Demerara-Berbice Region, (18) Mabura Hill (ROM 103552). PERU: Amazonas department, (19) Luya, Río Utcubamba, 11 km NW (by road) of Pedro Ruíz (FMNH 128825); Ayacucho department, (20) La Mar, Hacienda Luisiana on Apurimac River (AMNH 208063–208064); Cajamarca department, (21) Santa Cruz, Río Zaña, 2 km N of Monte Seco (FMNH 128845); Cuzco department, (22) La Convención, Cordillera Vilcabamba, Mapitunari River (AMNH 233541), (23) Paucartambo, San Pedro (FMNH 172153); Huánuco department, (24) Leoncio Prado, 11 km N and 6 km E of Tingo Maria (TTU 46263–46264, TTU 46267–46269), 9 km S and 2 km E of Tingo Maria (TK 22784, TTU 46270, TTU 46265–46266, TTU 46271–46272); Junín department, (25) Chanchamayo, 2 mi NE of San Ramon (AMNH 230526, AMNH 230529, AMNH 230545–230546); Loreto department, (26) Alto Amazonas, Gálvez River, Nuevo San Juan (MUSM 13260), (27) Maynas, Curaray River (AMNH 71691–71695), Maynas, Estación Biológica Isla Muyuy (MUSM 21266), Río Maniti, Santa Cecilia (FMNH 87058), (28) Requena, Jenaro Herrera, Centro de Investigaciones Jenaro Herrera (MUSM 5922, MUSM 5924–5925), (29) Reserva Nacional Allpahuayo-Mishana, Estación Biológica "José Álvarez" (ROMF 63353); Pasco department, Oxapampa, (30) Nevati Mission (AMNH 230558), San Juan (AMNH 230567–230568), San Pablo (AMNH 230582); San Martín department, Moyobamba, (31) Área de Conservación Municipal Mishquiyacu-Rumiyacu y Almendra, Orquidiario Waqanki (FMNH 203408, FMNH 203410, FMNH 203412, FMNH 203416, FMNH 203582, FMNH 203584, FMNH 203586, FMNH 203590, MUSM 39223–39227), Tingana (FMNH 203415, FMNH 203420, MUSM 39229), Rioja, (32) Pardo Miguel, Naranjos, Caserio El Diamante (FMNH 203414, FMNH 203587–203588; MUSM 39228); Ucayali department, (33) Atalaya, Tahuania, Shahuaya (AMNH 230535–230537). SURINAME: Sipaliwini, (34) Blanche Marie Vallen (ROM 117574, ROM 117642). TRINIAD & TOBAGO: Tobago, (35) Saint Patrick, Grange (TK 25163, TTU 44085); Trinidad, Saint Andrew, (36) Balandra (AMNH 204710), Sangre Grande, Rio Grande Forest (AMNH 204717), Saint George, (38) 5 mi N in Arima (TK 25100, TTU 44090), road Churchill Roosevelt (AMNH 179953), La Fillette (AMNH 204712), Las Cuevas (AMNH 204723), Maracas (AMNH 204726), Maracas, Waterfalls Road (AMNH 178652), Simla Research Center, 4 mi N of Arima (TK 25035, TTU 44092). VENEZUELA: Bolívar state, (39) 3 km E of Puerto Caballo del Caura (ROM 107936), (40) 8 km S and 5 km E of El Manteco (TK 19138, CM 78567).

Sturnira lilium. BOLIVIA: Tarija department, (41) Chiquiacá (FMNH 162524, FMNH 162542). BRAZIL: Bahia state, (48) Mucuri (MN 46844), (49) Rio de Contas, Chapada Diamantina (MN 67601, MN 67602, MN 67603, MN 67604), Abaíra, Chapada Diamantina (MN 67828, MN 67833), (50) Bonito (MN 67840); Ceará state, (51) Jardim (MN 79942), (52) Pacoti (MHNCE-MAM 00313), Guaramiranga (MHNCE-MAM 00323), Mulungu (MHNCE-MAM 00524), Baturité (MHNCE-MAM 00550), (53) Eusébio (MCHN-MAM 00176); Mato Grosso state, (1) Barão de Melgaço (MN 82212, MN 82215–82216, MN 82228, MN 82230, MN 82235, MN 82245–82246, MN 82248, MN 82953, MN 82255); Minas Gerais state, (45) Viçosa (MN 3393, MN 3396), (46) Volta Grande (MN 71629), (47) Matias Barbosa (MN 81305), Simão Pereira (MN 81352, MN 83354), Juiz de Fora (MN 81563, MN 83312); Paraná state, (42) Curitiba (LBCE 21164–21165, LBCE 21192–21193, LBCE 21218–21220); Rio de Janeiro state, (44) Parque Nacional do Itatiaia (LBCE 22841); Rio Grande do Sul state, (54) Parque Estadual de Nonoai (MN 58792, MN 58798–58800, MN 58809–58811, MN 58813–58814, MN 58822, MN 58829, MN 58831–58833, MN 58836–58842); São Paulo state, (43) Estação Ecológica de Boracéia (BDP 3174). PARAGUAY: Caazapá department, (55) Estancia Golondrina (TTU 99168); Canindeyú department, (56) Reserva natural del Bosque Mbaracayu (TTU 99277, TTU 94024, TTU 94259); Itapúa department, (57) El Tirol, 19.5 km N-NE of Encarnación (MVZ 154711); San Pedro department, (58) Yaguarete forests, Puente rio Verde (TTU 96816).