Biogeographical events, not cospeciation, might be the main drivers in the historical association between *Noctiliostrebla* species (Streblidae) and their bulldog bat hosts

DANIEL MAXIMO CORREA ALCANTARA[1,2,*,], GUSTAVO GRACIOLLI[3,], MANUEL ANTUNES JUNIOR[1,], RONALDO TOMA[2,] and SILVIO SHIGUEO NIHEI[1,]

[1]*Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil*
[2]*Fundação Oswaldo Cruz de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil*
[3]*Setor de Zoologia, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil*

The genus *Noctiliostrebla* Wenzel (Diptera: Streblidae) stands out for its high degree of specificity exhibited in relation to bat species of the genus *Noctilio* Linnaeus and provides an exciting system for understanding the history behind host–parasite associations. Here, we present a phylogeny of *Noctiliostrebla* based on an analysis of DNA sequences and morphological characters, along with cophylogenetic and biogeographical analyses. Our results strongly support the monophyly of *Noctiliostrebla*, but with uncertainties within the genus. With a low frequency of cospeciation events explaining the associations between hosts and parasites, cophylogenetic analyses did not show an overall congruence between the host and parasite phylogenies. Indeed, two parallel histories were recovered in the host–parasite associations, which might indicate that niche segregation is determined evolutionarily, facilitating the coexistence of parasites and promoting diversification. Biogeographical analysis showed a strong spatial congruence between disjunct distributions of *Noctiliostrebla* and major river basins in South America and with areas of higher elevation, which might be associated with the glacial periods throughout the Pliocene and Pleistocene. Overall, our findings suggest an agreement with the expectations of the 'Stockholm paradigm' framework, in which biogeographical events and ecological factors act as important components to explain the associations, instead of cospeciation events.

ADDITIONAL KEYWORDS: Chiroptera – cophylogeny – Hippoboscoidea – host–parasite associations – host switching – parasitism – systematics – Trichobiinae – vicariance.

INTRODUCTION

Most of the theoretical and methodological development for macroevolutionary approaches in co-evolutionary studies has been elaborated on a 'maximum cospeciation paradigm' (Hoberg & Brooks, 2008; Brooks *et al.*, 2015). The influence of the maximum cospeciation way of thinking in parasitology research has been around for more than a century. At the core of this traditional paradigm in parasitology is the assumption that parasites typically are highly specialized on a single host species (Nylin *et al.*, 2018). Within this paradigm, specificity is the cause of

co-evolution, and it is hypothesized that the potential for new interactions will be minimal. The associations between parasites and hosts are so intimate and persistent over time that the interacting lineages should cospeciate, leading to congruent phylogenies. However, this expectation led to the misconception that the examination of congruence between phylogenies would be sufficient evidence of co-evolution, in which cospeciation and co-evolution are used as synonyms (Poisot, 2015).

Specificity is a traditional assumption in parasitology, but colonization is indicated as the main event for acquisition of new hosts. From these assumptions emerges the problem: if most parasites appear to be specialized to a particular host, how can

*Corresponding author. E-mail: danielmxm@gmail.com

583

they colonize new hosts? This is known as the 'parasite paradox', which the 'Stockholm paradigm' proposes to solve. This paradigm seeks to integrate ecological and evolutionary processes, providing a theoretical and empirical framework for understanding the mechanisms that lead to diversification on a macroevolutionary scale (Agosta *et al.*, 2010; Brooks *et al.*, 2015). In the perspective of this paradigm, the structure and diversification of complex faunas has been substantially driven by recurrent geographical events and host colonizations, emerging from ecological perturbations manifested across spatial and temporal scales (Brooks *et al.*, 2015). Therefore, it is possible to predict that cospeciation will not be the norm, because cases of new colonization are now explained through a progressive increase in associations and subsequent isolation rather than spontaneous acquisition of a new association (Hoberg & Brooks, 2008; Poisot, 2015).

Bat flies are a suitable model to study host–parasite associations under the 'Stockholm paradigm' framework. They are divided into two cosmopolitan dipteran families of obligate ectoparasites of bats, Streblidae and Nycteribiidae (Diptera: Hippoboscoidea), although their taxonomic status is under debate (Dittmar *et al.*, 2015; Haelewaters *et al.*, 2021). Bat flies are often presented as highly specialized, particularly those on Neotropical bats (Haelewaters *et al.*, 2021). There are even hypotheses regarding the possibility of cospeciation between bats and bat flies in the Western Hemisphere (Patterson *et al.*, 1998). Within Streblidae, the genus *Noctiliostrebla* Wenzel, 1966 stands out for the high degree of specificity exhibited in relationship to bat species of the genus *Noctilio* Linnaeus, 1758 (Moura *et al.*, 2003; Dick & Gettinger, 2005). The unique genus within Noctilionidae, *Noctilio*, has only two species, *Noctilio albiventris* Desmarest, 1818 and *Noctilio leporinus* (Linnaeus, 1758), which occur in the Neotropics (Hood & Pitocchelli, 1983; Hood & Jones Jr, 1984). However, there is debate over whether each of the two species should be a species complex. Recent studies have provided evidence to support the identification of independent evolutionary lineages (Pavan *et al.*, 2013; Khan *et al.*, 2014). Many of these lineages are strongly associated with major river basins in South America (Pavan *et al.*, 2013). There is a growing body of studies demonstrating the role of watersheds as barriers, mainly attributable to the difference in elevation and the organization of drainage. As barriers, watersheds would prevent dispersal and gene flow, fragmenting populations and causing diversification in many organisms. However, most studies for vertebrates have focused on fish and birds (Frable *et al.*, 2022; Musher *et al.*, 2022). Thus, bats and bat flies provides an exciting system for understanding host–parasite associations and the role of biogeography.

We present phylogenetic hypotheses of *Noctiliostrebla* inferred from a morphological dataset, in addition to a molecular dataset composed of four genes, the nuclear coding region of the domain carbamoyl synthetase phosphate (CPS) of the *CAD* gene, and three mitochondrial genes, the ribosomal 12S rDNA, a coding region of cytochrome *c* oxidase subunit I (*COI*) and a coding region of cytochrome B (*CytB*). Our taxonomic sampling for *Noctiliostrebla* encompasses all 11 described species of the genus. We also used available host sequences to infer a robust phylogeny of *Noctilio* and to identify potential populations. Based on our defined host populations and on available information on their geographical distributions, we established host–parasite associations. Thus, we have integrated phylogenetic, cophylogenetic and biogeographical approaches to address the following goals: (1) to propose a phylogenetic hypothesis of *Noctiliostrebla* species; (2) to assess the monophyly of the hosts, defining possible populations; (3) to understand the evolutionary history of associations between *Noctiliostrebla* species and their hosts; and (4) to assess the role of river basins and differences in elevation as potential barriers inferred from disjunct distributions of sister taxa.

MATERIAL AND METHODS

ABBREVIATION OF TAXON NAMES

To avoid confusion owing to the similarity of the names *Noctilio* and *Noctiliostrebla*, herein, we use the abbreviated generic form (*N.*) only for *Noctiliostrebla* species. For *Noctilio* host species, we always use the full genus name.

TAXON SAMPLING

We sampled all 11 described species of *Noctiliostrebla* for morphological data, using the specimens present in the most recent taxonomic revision of the genus (Alcantara *et al.*, 2019). This includes the lectotype of *Noctiliostrebla dubia* (Rudow, 1871), in addition to the holotypes of all other species. For molecular data, we obtained DNA samples from nine species. We have sequenced samples of seven species, *N. aitkeni* Wenzel, 1966, *N. dubia*, *N. falsispina* Alcantara et al., 2019, *N. guerreroi* Alcantara et al., 2019, *N. lamasi*, Alcantara et al., 2019 *N. morena* Alcantara et al., 2019 and *N. pantaneira*, Alcantara et al., 2019 and for *N. maai* Wenzel, 1966 and *N. traubi* Wenzel, 1966, we obtained sequences available on GenBank (Sayers *et al.*, 2021). We did not sample all species for molecular data owing to availability and preservation of specimens. When possible, we sampled more than one specimen per species, to evaluate the monophyly of representatives from different populations. Thus, we obtained a total of 28 specimens of *Noctiliostrebla*, of which two were

of *N. aitkeni*, one of *N. dubia*, four of *N. falsispina*, one of *N. guerreroi*, six of *N. lamasi*, three of *N. maai*, three of *N. morena*, five of *N. pantaneira*, and three of *N. traubi* (Supporting Information, Appendix S1). We conducted the study by following the permit guidelines issued by 'Sistema de Autorização e Informação em Biodiversidade – SISBIO', Brazil (35177-1, 4568-1 and 4596-1, issued in 2012; 35177-3, 5076-1 and 5184-1, issued in 2013). More details about collection, identification and deposition of *Noctiliostrebla* specimens are available elsewhere (Alcantara *et al.*, 2016; 2019).

We included five species as the outgroup: *Aspidoptera phyllostomatis* (Perty, 1833), *Paradyschiria parvula* Falcoz, 1931, *Paratrichobius longicrus* (Miranda Ribeiro, 1907), *Speiseria ambigua* Kessel, 1925 and *Xenotrichobius noctilionis* Wenzel, 1976 (Supporting Information, Appendix S1). We chose the outgroup and the root in *S. ambigua* based on the morphological grouping proposed by Wenzel *et al.* (1966) and existing relationship hypotheses (Dittmar *et al.*, 2006; Petersen *et al.*, 2007; Graciolli & Carvalho, 2012). Additionally, we selected the genera *Paradyschiria* and *Xenotrichobius* because they are considered to have a single origin with *Noctiliostrebla*, and *Paradyschiria* is the hypothetical sister-group of *Noctiliostrebla* (Guerrero, 1998).

From the sampling obtained for molecular and morphological data, we generated four different datasets to perform the analyses: dataset 1, combined molecular + morphology of all 11 described species; dataset 2, morphological data of all 11 described species; dataset 3, molecular data of 28 specimens from nine *Noctiliostrebla* species; and dataset 4, molecular data of nine species, but only one specimen per species.

MORPHOLOGICAL TERMINOLOGY AND CHARACTER CODING

We followed Wenzel (1976) and Wenzel & Peterson (1987) for the terminology of general morphology, with modifications as in the paper by Alcantara *et al.* (2019). For the terminology of the gonopodal setae, we followed Graciolli & Dick (2004). We coded the characters from the examination of the external morphology and male genitalia of adults. For the female of *X. noctilionis*, we obtained information from the literature, because there were no specimens available for examination. We constructed and edited the data matrix using WINCLADA v.1.00.08 (Nixon, 2002) and MESQUITE v3.61 (Maddison & Maddison, 2018). When relevant, we used contingent coding in relationship to the others. We coded missing data with a question mark ('?') and inapplicable data with a hyphen ('-'). Inapplicable data have been assumed when character states were absent or reduced in some of the taxa (Hawkins *et al.*, 1997;

Strong & Lipscomb, 1999; Forey & Kitching, 2000). All characters were given equal weights and treated as unordered (Fitch, 1971). We defined a total of 50 morphological characters for males and females: seven characters for the head, eight for the thorax, one for the wings, six for the legs, 22 for the abdomen, and six for the male genitalia. The list of characters and the matrix of taxa/characters are appended in the Supporting Information (Appendix S2), which provides consistency index (CI) and retention index (RI) for characters, under equal weighting.

DNA EXTRACTION, SEQUENCING AND ALIGNMENT

We used freshly collected or ethanol-preserved specimens. We extracted total genomic DNA using the Agencourt DNAdvance System kit (Beckman Coulter, CA, USA). Owing to the small size of the specimens, we performed a longitudinal incision in the thoracic sternum and inserted the entire specimen into the lysis solution, without any further damage to the specimen. From this approach, we managed to allow a larger amount of tissue to come in contact with the lysis solution, removing the specimen from the solution at the end of the lysis step, for later storage as a voucher.

We targeted four genes for amplification and sequencing, one nuclear, coding region of the domain carbamoyl synthetase phosphate (CPS) of the *CAD* gene; and three mitochondrial, namely the ribosomal 12S rDNA gene, a coding region of cytochrome *c* oxidase subunit I (*COI*) and a coding region of cytochrome B (*CytB*).

The PCR protocols followed Pinto-da-Rocha *et al.* (2014) and are described in the Supporting Information (Appendix S1). We obtained the consensus sequences using the CONSED/PHREDPHRAP package (Ewing & Green, 1998; Ewing *et al.*, 1998; Gordon *et al.*, 1998, 2001). We aligned the sequences in MAFFT v.7.4 (Katoh *et al.*, 2002; Katoh & Standley, 2013) and edited the sequences using ALIVIEW v.1.25 (Larsson, 2014). We inspected the sequences of the coding genes (*CAD*, *COI* and *CytB*) in search of stop codons by means of the DNA to Protein Translation online resource (Bikandi *et al.*, 2004), in which all sequences were cut so that the first base matched the first codon position. The fully concatenated data comprised 2399 bp of aligned gene sequence (12S, 420 bp; *CAD*, 567 bp; *COI*, 776 bp; *CytB*, 636 bp). Gene sequences were deposited in GenBank, and the respective accession numbers are presented in the Supporting Information (Appendix S1).

BAT FLIES PHYLOGENY

We conducted the phylogenetic analysis of the four datasets under two optimality criteria: maximum parsimony and maximum likelihood. We performed

parsimony analysis using TNT v.1.5 software (Goloboff *et al.*, 2008, 2016), with equal weighting of characters. Owing to the number of terminals, we performed a heuristic search using the implicit enumeration algorithm, 'ie'. We estimated the support of the branches by non-parametric bootstrapping (Felsenstein, 1985) in TNT with the following settings: 1000 bootstrap replicates; standard resampling; output as frequency differences; search trees with Traditional Search (TBR, 1000 replicates and ten trees saved by replication). We performed Bremer support (Bremer, 1994) analysis in TNT, using trees with 20 additional steps. When analysing datasets 1, 3 and 4, we also conducted a partitioned Bremer support analysis for each partition (Gatesy *et al.*, 1999; Peña *et al.*, 2006). We also provided the length (L), CI and RI of the trees resulting from the parsimony analysis. We performed the visualization and optimization of morphological characters on parsimony analyses using the software WINCLADA (Nixon, 2002). For maximum likelihood analyses, we used IQ-TREE v.2.0.4 (Minh *et al.*, 2020; Nguyen *et al.*, 2015). For the analysis of molecular data in datasets 1, 3 and 4, we considered each gene as a different partition. Given that we were mainly interested in cladistic relationships, we used the most parameter-rich model for each gene (GTR+R4+F), following Abadi *et al.* (2019). For the morphological data in datasets 1 and 2, we used the Jukes–Cantor-type model for morphological data (MK), applying the ascertainment bias correction (+ASC) model (Lewis, 2001). We used ultrafast bootstrap approximation (UFBoot) (Minh *et al.*, 2013; Hoang *et al.*, 2018) and Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) (Guindon *et al.*, 2010) to assess the branch supports, both with 1000 replicates. We edited the phylogenies in R software (R Core Team, 2021), using the packages 'tidytree' (Yu, 2021), 'ggtree' (Yu *et al.*, 2017, 2018; Yu, 2020) and 'treeio' (Wang *et al.*, 2020) and in the software INKSCAPE.

HOST PHYLOGENY AND POPULATION STRUCTURE

Although there are two recognized species of *Noctilio*, studies have indicated a complex of species yet poorly defined (Pavan *et al.*, 2013; Khan *et al.*, 2014). Thus, we used the sequences sampled by Khan *et al.* (2014), owing to the wide distribution of sampling. Based on studies by Davis (1973, 1976), Khan *et al.* (2014) have selected the specimens in order to sample the geographical range of the four subspecies of *Noctilio albiventris* (*Noctilio a. albiventris* Desmarest, 1818, *Noctilio a. minor* Osgood, 1910, *Noctilio a. affinis* D'Orbigny, 1836 and *Noctilio a. cabrerai* Davis, 1976) and the three subspecies of *Noctilio leporinus* [*Noctilio l. leporinus* (Linnaeus, 1758), *Noctilio*

l. mastivus (Vahl, 1797) and *Noctilio l. rufescens* Olfers, 1818].

To reconstruct a bat host phylogeny, we used the same four genes as Khan *et al.* (2014): cytochrome B (*CytB*), cytochrome *c* oxidase subunit I (*COI*), zinc finger X (*ZFX*) and zinc finger Y (*ZFY*). However, we removed the specimens with data for only one gene to avoid noise by missing data. All in all, we sampled 86 specimens of *Noctilio* (44 of *Noctilio albiventris* and 42 of *Noctilio leporinus*) and eight specimens for the outgroup [two of *Carollia perspicillata* (Linnaeus, 1758), two of *Mormoops megalophylla* (Peters, 1864), and one specimen of each of the following species: *Pteronotus gymnonotus* Natterer, 1843, *Pteronotus parnelli* (Gray, 1843), *Pteronotus personatus* (Wagner, 1843) and *Pteronotus quadridens* (Gündlach, 1840)]. We obtained sequences from GenBank (Sayers *et al.*, 2021), presented in the Supporting Information (Appendix S1).

We aligned sequences using MAFFT v.7.4 (Katoh *et al.*, 2002; Katoh & Standley, 2013) and inspected and edited them in ALIVIEW v.1.25 (Larsson, 2014). We concatenated the aligned and edited sequences using SEQUENCEMATRIX v.1.8 (Vaidya *et al.*, 2011). We performed a maximum likelihood analysis using IQ-TREE v.2.0.4 (Nguyen *et al.*, 2015; Minh *et al.*, 2020), and used ultrafast bootstrap approximation (UFBoot) (Minh *et al.*, 2013; Hoang *et al.*, 2018) and SH-aLRT branch tests (Guindon *et al.*, 2010) to assess the branch supports, both with 1000 replicates. We performed the analysis using the most parameter-rich model for each gene, GTR+R4+F, following Abadi *et al.* (2019). We edited the resulting phylogenies in R, using the packages 'tidytree' (Yu, 2021), 'ggtree' (Yu *et al.*, 2017, 2018; Yu, 2020) and 'treeio' (Wang *et al.*, 2020), and the software INKSCAPE.

In addition to phylogenetic analysis, we used *k*-means clustering to infer the population structure and identify groups of more closely related individuals resulting from reduced gene flow. However, owing to the large difference in the number of sequences available per individual, we performed the analysis individually for each gene. We assessed the number of clusters using the 'find.clusters' function, based on the lowest associated Bayesian information criterion (BIC), and available in the package 'poppr' (Kamvar *et al.*, 2014). We also analysed the population structure by discriminant analysis of principal components (DAPC) (Jombart *et al.*, 2010) using the 'adegenet' package (Jombart & Ahmed, 2011). We chose the number of retained principal components (PCs) based on the a-score optimization, in order to manage the trade-off between the power of discrimination and over-fitting. We conducted all analysis of *k*-means clustering and DAPC in R.

Based on the results obtained by both phylogenetic analysis of individuals and population structure analysis, we construct a population structure phylogeny to use in cophylogenetic and biogeographical analyses. For that, we collapsed the individuals of the same population into a single tip, providing the population was monophyletic.

COPHYLOGENETIC ANALYSES

We conducted the cophylogenetic analysis using distance-based and event-based methods. For a full view of the cophylogenetic history, we considered only the phylogenetic trees from the analysis of datasets 1 and 2, which include all *Noctiliostrebla* species. To accommodate uncertainties regarding relationships between species, we considered the phylogenies recovered from both maximum parsimony and maximum likelihood. In the case of parsimony, when more than one most parsimonious tree had been recovered, we considered all phylogenies, including the consensus tree. Before the cophylogenetic analyses, we pruned the bat flies phylogeny to remove outgroup taxa, using the package 'ape' (Paradis & Schliep, 2019) in R. For each cophylogenetic method, we analysed each considered *Noctiliostrebla* phylogenetic hypothesis with the population structure phylogeny of hosts. For the associations between *Noctiliostrebla* and their hosts, we used the information available in the paper by Alcantara *et al.* (2019). However, we added a new record for *N. lamasi*, not shown anywhere else (Supporting Information, Appendix S1). We defined the associations from the overlapping distribution between *Noctiliostrebla* species and *Noctilio* populations, providing the *Noctilio* species recognized as host was respected. However, given that there were no samples of individuals of *Noctilio leporinus* that overlapped the distribution of *Noctiliostrebla caissara* Alcantara et al., 2019, we attributed it to the host with the closest distribution. We used the R package 'phytools' (Revell, 2012) to produce a tanglegram between *Noctiliostrebla* and *Noctilio* trees.

For the distance-based analysis, we used PARAFIT (Legendre *et al.*, 2002), which assesses overall congruence between the host and parasite phylogenies, in addition to the relative contribution of individual host–parasite links (associations) to the overall congruence. The null hypothesis of PARAFIT assumes the relationship pattern of the two groups to be independent, assessing how much each individual link contributes to the overall congruence (Legendre *et al.*, 2002). We used the R implementation of PARAFIT in the package 'ape', running 100 000 permutations, with Cailliez correction for negative eigenvalues. We used the 'cophenetic' command to convert the host and parasite phylogenies to patristic distance matrices, and

we sorted each distance matrix according to the host–parasite association matrix. We tested the contribution of each individual link using the PARAFITLINK1 and PARAFITLINK2 tests. A significant link suggests that a particular host–parasite association contributes to the global congruence between the host and parasite trees.

We used JANE v.4 (Conow *et al.*, 2010) for the event-based analysis. The software JANE uses a genetic algorithm to reconstruct the optimal set of evolutionary events and is based on a priori event costs. We ran the analysis using the following genetic algorithm parameters: 1000 'Number of Generations' and 10 000 'Population Size', under default event costs (0 cospeciation, 1 duplication, 2 duplication and host switch, 1 loss, and 1 failure to diverge). We assessed statistical significance using 999 random tip mapping, with 100 'Number of Generations' and 10 000 'Population Size'. A significant result from the statistical test indicates congruence between the phylogenies (Sweet & Johnson, 2016).

BIOGEOGRAPHICAL ANALYSIS

We studied *Noctiliostrebla* and bats independently to hypothesize disjunct distributions (allopatric or vicariant), using the vicariance inference program (VIP) (Arias, 2010). The VIP assumes that the only evidence left from a speciation process in a geographical context is an allopatric distribution (Hovenkamp, 1997, 2001; Arias, 2010; Arias *et al.*, 2011). High elevations were hypothesized as potential barriers between *Noctiliostrebla* species (Alcantara *et al.*, 2019), whereas *Noctilio* lineages were hypothesized to be correlated with the major river basins of South America and their nearby lowlands (Pavan *et al.*, 2013). Thereby, we intended to assess whether hypothetical vicariance and dispersal events, inferred from disjunct distributions, could provide an explanation for the relationship pattern in the two groups, and whether these barriers could be related to high elevations and watersheds. For this purpose, we used elevation information and watershed boundaries to determine whether the hypothetical barriers could be related to this geospatial information. We used a georeferenced tagged image file format (GeoTIFF), with embedded elevation information from Shuttle Radar Topography Mission (SRTM), and hydrographical data layers of watershed boundaries (Lehner & Grill, 2013).

The analysis required phylogenies without branch lengths and the coordinates for each terminal. For bat flies, we used the same phylogenetic trees from the cophylogenetic analysis, and for hosts we used the population structure phylogeny. We obtained the georeferencing data for bat flies from the study by Alcantara *et al.* (2019), including the new record for *N. lamasi*. For the hosts, we obtained

the data from the locality information recorded in museums (Supporting Information, Appendix S1). To run VIP, some parameters needed to be defined a priori. Different sets of parameters can significantly alter the results of the analyses regarding the reconstruction of the disjoint nodes. Thus, to evaluate the effect of the parameters, we used eight sets of different parameters on the same set of hypothetical data, phylogeny plus distribution data. We performed the searches allowing 10 000 interactions, and keeping 200 reconstructions per interaction. Barriers were represented by Voronoi lines on the map. To avoid a large number of hypotheses to be discussed owing to the number of analyses, we used only disjunction recovered in half or more of the consensus reconstruction.

RESULTS

BAT FLIES PHYLOGENY

We recovered a monophyletic and well-supported *Noctiliostrebla* clade in all analyses (bootstrap, UFBoot and SH-aLRT ≥ 90%; Bremer ≥ 10; Figs 1, 2). From parsimony analysis of dataset 2 (morphological data), we obtained only one most parsimonious tree (L = 85, CI = 0.800 and RI = 0.852; Fig. 1), with *Noctiliostrebla* supported by nine unambiguous synapomorphies and one homoplastic character (h) (9:1, 10:1, 11:1, 14:2, 25:1, 26:1, 35:1, 40h:1, 45:1 and 46:1; see Supporting Information, Appendix S2). We obtained two most parsimonious trees from the parsimony analysis of dataset 1 (combined data), with 1404 steps, CI = 0.701 and RI = 0.654 (trees 1 and 2, in Fig. 1). The maximum likelihood analysis of the dataset 1 recovered a similar topology to tree 1 from the parsimony analysis using the same dataset. Based on partitioned Bremer support, the morphological characters were important in supporting the clade containing the genus; *COI* was important in the resolution of the internal relationships, whereas the other genes were more important in the relationship of *Noctiliostrebla* with the outgroup (Figs 1, 2). In the molecular analysis with multiple conspecific specimens, we recovered all sampled species as monophyletic, with many well-supported clades (bootstrap, UFBoot and SH-aLRT ≥ 70%; Bremer ≥ 1; Fig. 2). Moreover, we recovered the species *N. lamasi* structured into two clades composed of specimens from two different areas. Although we recovered *N. maai* and *N. traubi* as monophyletic species, their positioning within the genus differed considerably between the analyses, and always with low or no support. In this regard, the parsimony analysis of the combined data demonstrates well the uncertainties over the positioning of these species (Fig. 1).

Within *Noctiliostrebla*, we consistently recovered three clades in the phylogenetic analyses containing all species: (*N. pantaneira*, (*N. dubia* and *N. falsispina*)), (*N. aitkeni*, (*N. lamasi* and *N. caissara*)) composed only by parasites of *Noctilio leporinus*, and (*Noctiliostrebla ecuadorensis* Alcantara et al., 2019, *N. guerreroi* and *N. morena*) composed only by parasites of *Noctilio albiventris*. The clade (*N. pantaneira*, (*N. dubia* and *N. falsispina*)) was supported mainly by molecular data. In the parsimony analysis of morphological data, this clade was supported by one unambiguous synapomorphy (30:1). The clade (*N. dubia* and *N. falsispina*) was well supported by both morphological and molecular data, although the combined analysis demonstrated a greater importance of the morphological data, which in the parsimony analysis was recovered with three unambiguous synapomorphies and one homoplastic character (h) (32:1, 34:1, 45h:0 and 46:3). We obtained the clades (*N. aitkeni*, (*N. lamasi* and *N. caissara*)) and (*N. ecuadorensis*, *N. guerreroi* and *N. morena*) as sister groups by the analyses of datasets 1 and 2, but usually with low support. In the parsimony analysis of morphological data, we recovered the clade (*N. aitkeni*, (*N. lamasi* and *N. caissara*)), supported by one unambiguous synapormophy (46:2), and the clade (*N. lamasi* and *N. caissara*), also supported by one unambiguous synapormophy (36:1). We recovered (*N. ecuadorensis*, *N. guerreroi* and *N. morena*) as a polytomy supported by two unambiguous synapomorphies (28:1 and 29:1) (Supporting Information, Appendix S2). In the molecular analysis, we recovered *N. aitkeni* and *N. lamasi* as sister species with a high support, in addition to *N. guerreroi* and *N. morena* (Figs 1, 2).

HOST PHYLOGENY AND POPULATION STRUCTURE

For the hosts, we recovered a monophyletic and well-supported clade for *Noctilio* and for both species, *Noctilio albiventris* and *Noctilio leporinus* (UFBoot ≥ 87% and SH-aLRT ≥ 97%; Fig. 3). The 'find. clusters' function indicated eight clusters in *COI* and *CytB* and three clusters in *ZFX* and *ZFY*. Basically, phylogenetic and population structure analyses demonstrated that we can recognize both *Noctilio* species as valid. The results of the DAPC analysis and the retained PCs based on the a-score optimization are shown in the Supporting Information (Appendix S3).

Concerning *Noctilio albiventris*, both the phylogeny and the population structure analysis were congruent and presented four well-structured populations. Population 1 of *Noctilio albiventris* comprised the eastern portion of the Andes (Ecuador and Peru), northern Brazil, reaching the Guyana region. Population 2 comprised central west of South America, which included among the
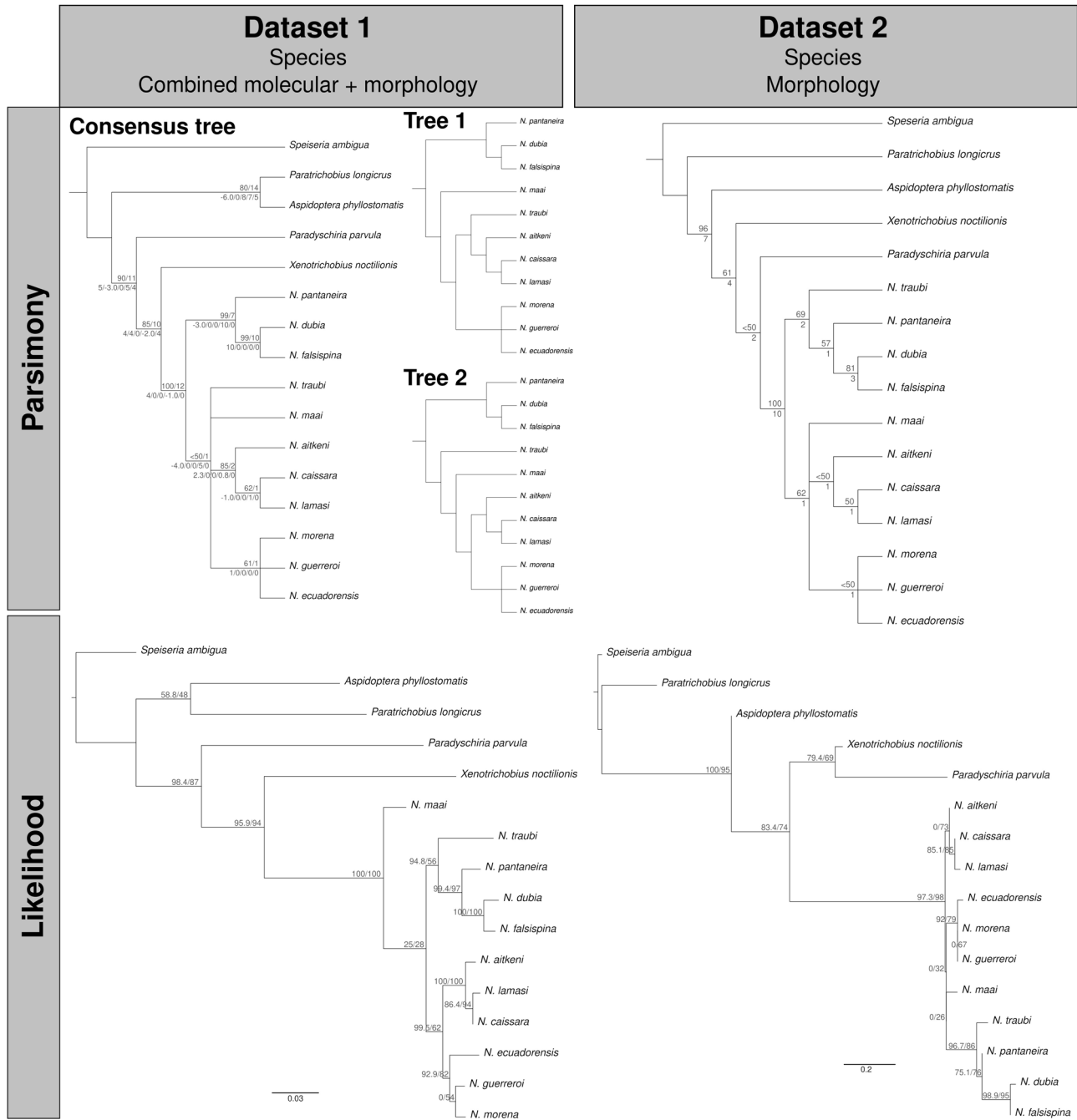
Figure 1. Phylogenetic hypotheses for datasets 1 and 2 using maximum parsimony and maximum likelihood. Dataset 1, top, consensus tree and the two most parsimonious trees (L = 1404, CI = 0.701 and RI = 0.654) inferred by parsimony analysis under equal weighting of characters; bottom, phylogeny inferred with maximum likelihood. Dataset 2, top, single most parsimonious tree (L = 85, CI = 0.800 and RI = 0.852) inferred by parsimony analysis under equal weighting of characters; bottom, phylogeny inferred with maximum likelihood. For maximum parsimony trees, the numbers above branches indicate bootstrap values and Bremer values, respectively, and the numbers below branches indicate partitioned Bremer support values for each partition. For maximum likelihood trees, numbers above branches indicate the SH-aLRT branch test and UFBoot, respectively.
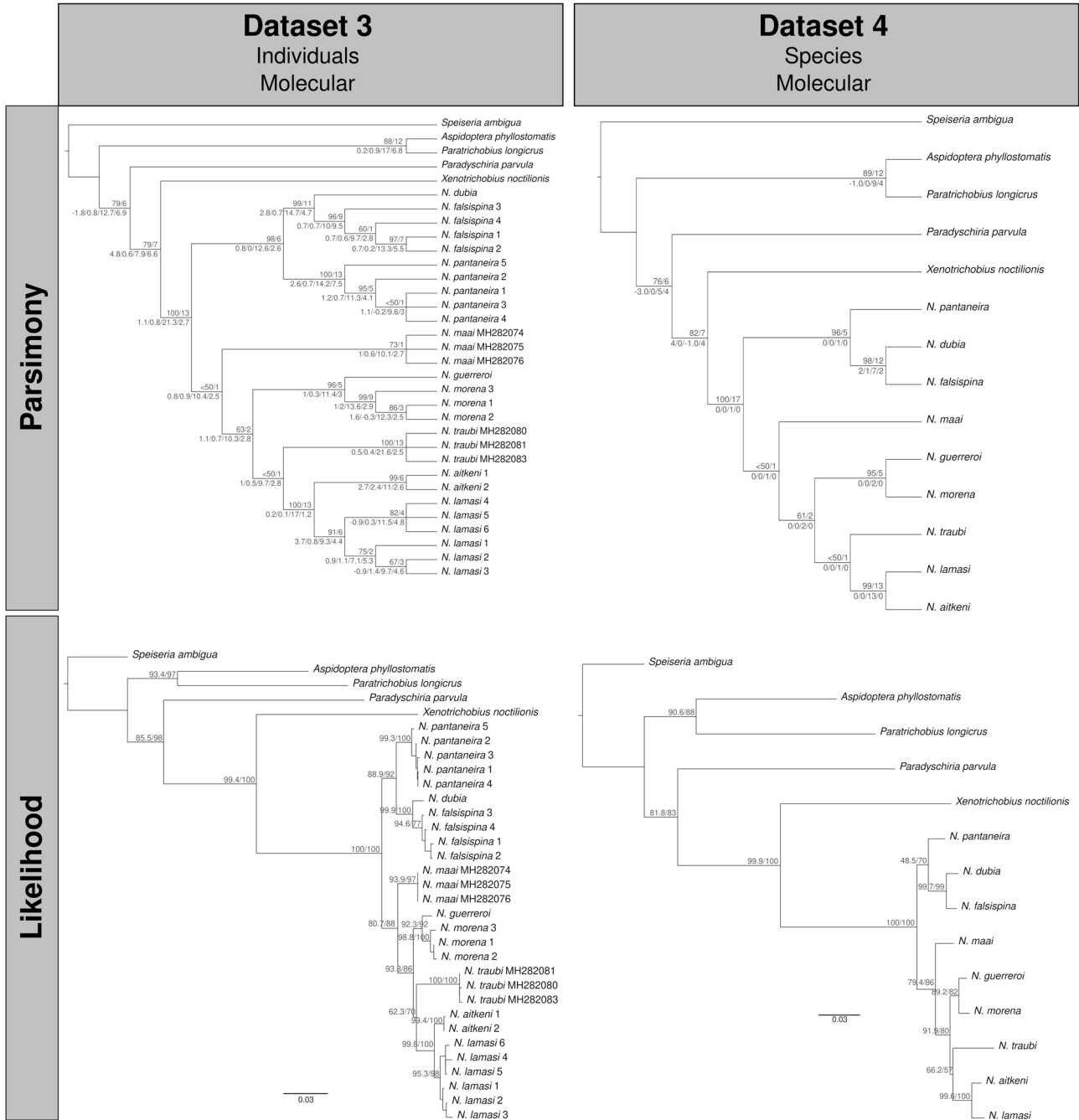
Figure 2. Phylogenetic hypotheses for datasets 3 and 4 using maximum parsimony and maximum likelihood. Dataset 3, top, single most parsimonious tree (L = 1397, CI = 0.677 and RI = 0.781) inferred by parsimony analysis under equal weighting of characters; bottom, phylogeny inferred with maximum likelihood. Dataset 4, top, single most parsimonious tree (L = 1305, CI = 0.701 and RI = 0.643) inferred by parsimony analysis under equal weighting of characters; bottom, phylogeny inferred with maximum likelihood. For maximum parsimony trees, the numbers above branches indicate bootstrap values and Bremer values, respectively, and the numbers below branches indicate partitioned Bremer support values for each partition. For maximum likelihood trees, numbers above branches indicate SH-aLRT branch test and UFBoot, respectively.
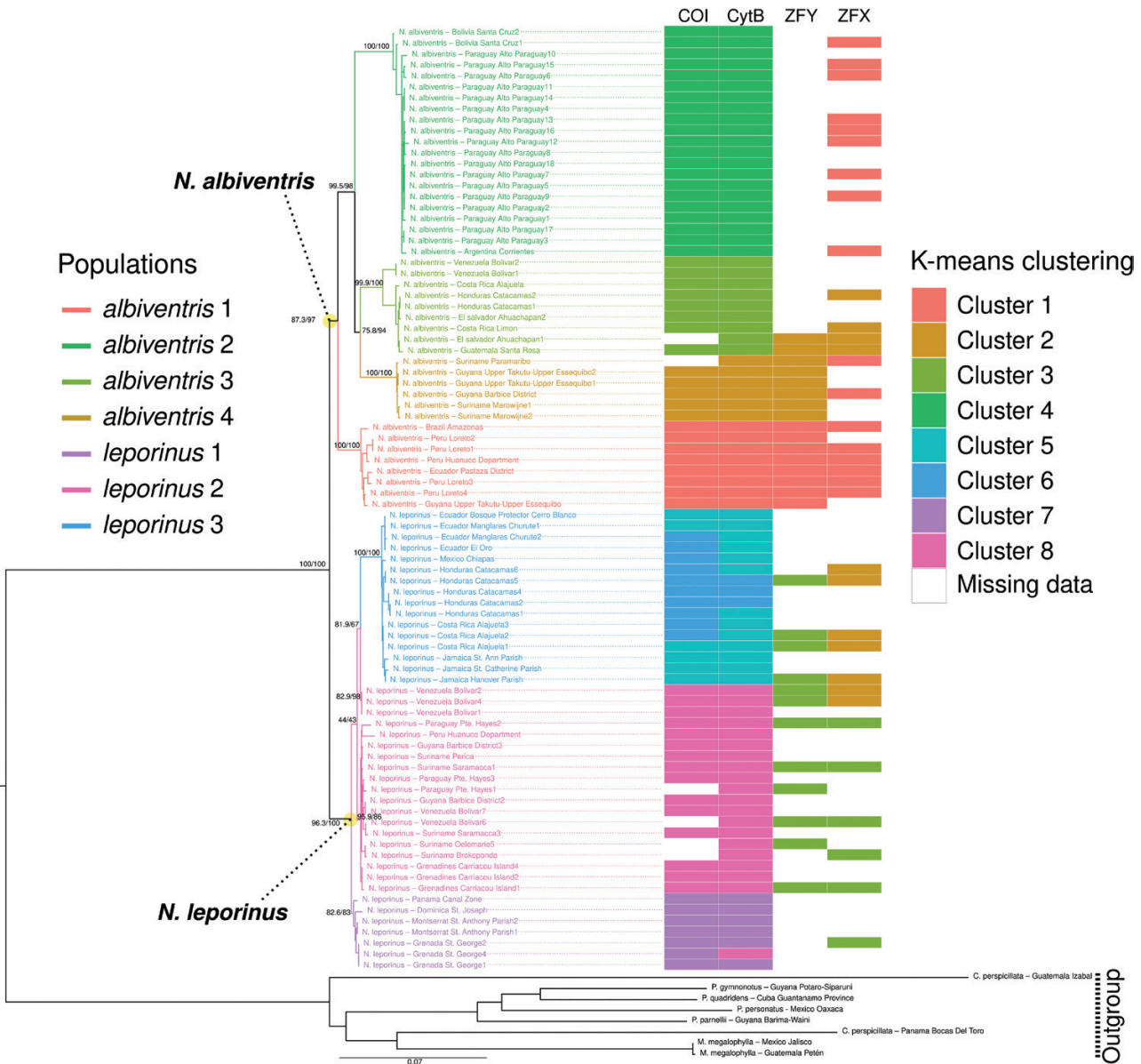
Figure 3. Phylogenetic hypothesis for hosts using maximum likelihood, showing the populations considered for each species, which were defined based on phylogenetic analysis and *k*-means clustering analysis. Support values indicate SH-aLRT branch test and UFBoot, respectively.

sampled countries Argentina, Bolivia and Paraguay. Population 3 encompassed the northernmost portion of South America (Venezuela) and Central America. Lastly, population 4 comprised the north-eastern coast of South America, which included the countries of Guyana and Suriname.

For *Noctilio leporinus*, three populations were defined. Population 1 of *Noctilio leporinus* included the Lesser Antilles and Panama. Population 2 of *Noctilio leporinus* was the only one within the genus that was not recovered as monophyletic. It was divided into two clades, one composed of individuals from Venezuela and the other with individuals from midwestern to northern South America, including Venezuela and Lesser Antilles. Although population 3 was divided into two clusters, both the phylogenetic analysis and the DAPC analysis showed that it could be considered as a single population. Thus, this population included Central America (excluding Panama), Greater Antilles and the western portion of the Andes, including Ecuador (Fig. 3).

COPHYLOGENETIC ANALYSES

For the cophylogenetic analyses, we used the *Noctiliostrebla* trees obtained from the parsimony analysis of dataset 1 (combined data consensus tree, tree 1 and tree 2) and dataset 2 (morphological data). Therefore, a total of four analyses (four ectoparasite trees × one host tree) were performed for each method, the distance-based method PARAFIT and the event-based method JANE. Distance-based tests were not significant across our entire dataset (PARAFIT global $P ≥ 0.1$), thus accepting the independence of the host and parasite phylogenies. Both PARAFITLINK1 and PARAFITLINK2 tests recovered only one host–parasite link as significant in three of four cophylogenetic analysis ($α = 0.05$) (Fig. 4; Supporting Information, Appendix S3). Likewise, the event-based method recovered no congruence in all analysed combinations between *Noctiliostrebla* and host trees, whereupon the observed cost was not significantly lower than by chance. Considering all reconstructions, cospeciation was recovered with low frequency and, in general, at the base of reconstructions. Losses were the most frequent events (30%), followed by duplication and failure to diverge (23% each), duplications and host switches (14%) and cospeciation (9%) (Table 1; Supporting Information, Appendix S4).

BIOGEOGRAPHICAL ANALYSIS

Using the same *Noctiliostrebla* trees as in the cophylogenetic analysis, the search for disjunct distributions performed with VIP resulted in 294 reconstructions over all *Noctiliostrebla* trees and 24 in the *Noctilio* population structure tree. Among the four *Noctiliostrebla* trees, we obtained six disjunctions, independently confirmed by more than one phylogeny, and between different sets of parameters. These disjunctions were spatially congruent with areas of elevation > 700 m a.s.l., which in turn were congruent with the boundaries of the tropical Andes and the watersheds of South America (Fig. 5; Supporting Information, Appendix S5). The nodes that composed the clade (*N. pantaneira*, (*N. falsispina* and *N. dubia*)) supported a disjunction between the two largest basins in South America, the Amazon and La Plata. The node between *N. lamasi* and *N. caissara* supported a disjunction of the Uruguay–Brazil, South Atlantic Coast basin from the rest. In this case, there was remarkable delimitation of *N. caissara* to the eastern area of the Serra do Mar, a system of mountain ranges and escarpments in south-eastern Brazil. The node between *N. aitkeni* and (*N. lamasi* and *N. caissara*) supported a disjunction between the extreme north and north-east of South America with the south area of this disjunction. The analyses that relied on

phylogenies with bifurcations for *N. maai* and *N. traubi* demonstrated the importance of the Andes as a barrier, regardless of the phylogenetic position of these species. The species *N. traubi* was totally restricted to the North and Northwest of the Andes, also occurring in Central America. Despite having an overlapping distribution with *N. traubi*, *N. maai* was not limited by the Andes in its eastern portion and occurred over the entire area of the following basins: Caribbean Coast, Orinoco and north-east South America–South Atlantic Coast, but without records beyond these areas (Fig. 5; Supporting Information, Appendix S5).

In the *Noctilio* population structure tree, we recovered four disjunctions, confirmed between different sets of parameters, which showed similarities to those found for bat flies (Fig. 6; Supporting Information, Appendix S5). The node between populations 2 (Venezuela) and 3 of *Noctilio leporinus* supported a disjunction between the two edges of the Andes. Population 3 overlapped the occurrence area of the bat fly *N. traubi*, whereas population 2 was within the occurrence range of *N. aitkeni*. The node between populations 3 and 4 of *Noctilio albiventris* supported a disjunction corresponding to the basins of the Orinoco and north-east South America–South Atlantic Coast. However, the occurrence area of the bat fly *N. maai* overlapped with these two populations. Thus, if the two populations harbour *N. maai*, there must be contact possible between the two populations. The node separating population 2 from populations 3 and 4 supported the disjunction of La Plata basin from the basins north of it, which was similar to the pattern found in the nodes of the bat fly clade (*N. pantaneira*, (*N. falsispina* and *N. dubia*)). Lastly, the node separating population 1 from the other populations of *Noctilio albiventris* supported the disjunction between the Amazon and La Plata basins, and between the Amazon basin in relationship to the Orinoco and north-east South America–South Atlantic Coast basins (Fig. 6; Supporting Information, Appendix S5).

DISCUSSION

FROM TAXONOMY TO PHYLOGENY OF *NOCTILIOSTREBLA*: CONGRUENCES AND UNCERTAINTIES

Using morphological and molecular data and performing analyses with separate and combined data, we were able to identify strongly supported clades, in addition to characters that can help us to understand character evolution and improve species identification. *Noctiliostrebla* was recovered as monophyletic and well supported by all datasets (Figs 1, 2). Many species were recovered as monophyletic (Fig. 2), and the different datasets
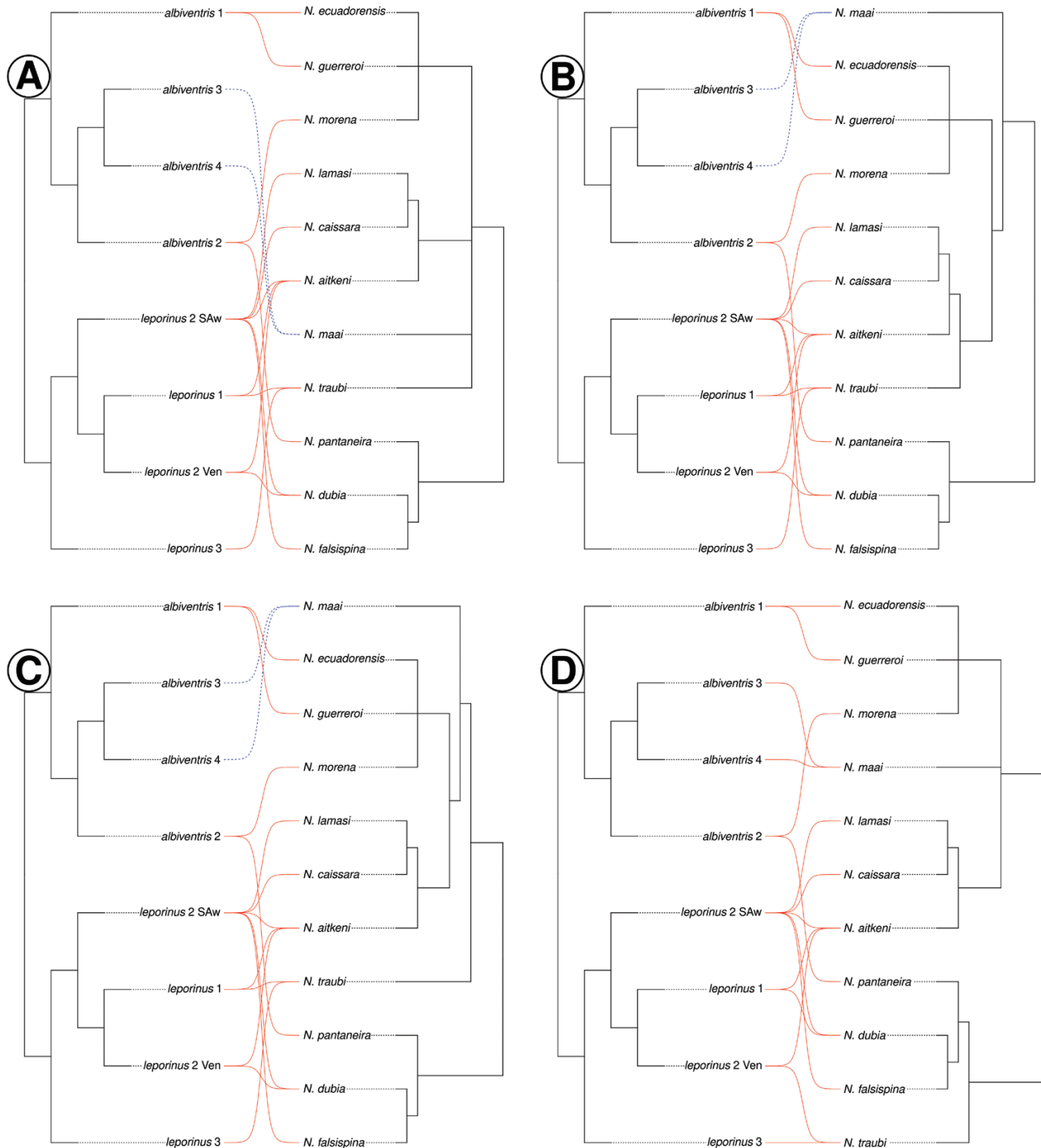
Figure 4. Tanglegrams between the relationship hypothesis of *Noctilio* populations and different phylogenetic hypotheses of *Noctiliostrebla*. A, *Noctiliostrebla* tree from dataset 1, consensus tree (ParaFitGlobal = 2700.069, *P*-value = 0.1). B, *Noctiliostrebla* tree from dataset 1, tree 1 (ParaFitGlobal = 4815.769, *P*-value = 0.1). C, *Noctiliostrebla* tree from dataset 1, tree 2 (ParaFitGlobal = 3068.416, *P*-value = 0.3). D, *Noctiliostrebla* tree from dataset 2, morphological tree (ParaFitGlobal = 3297.879, *P*-value = 0.2). Blue and dashed lines indicate significant host–parasite links estimated by both ParaFitLink1 and ParaFitLink2. Red continuous lines indicate no significant host–parasite links estimated by both ParaFitLink1 and ParaFitLink2. Abbreviations: '*leporinus* 2 SAw', '*leporinus* 2 South America widespread'; '*leporinus* 2 Ven', 'leporinus 2 Venezuela'.

Table 1. Summary of the Jane v.4 results for *Noctiliostrebla* species and their hosts, between the population structure host phylogeny and different phylogenetic hypotheses of *Noctiliostrebla*

Bat flies tree	Events					Cost	Isomorphic solutions
	Cospeciation	Duplications	Duplications and host switches	Losses	Failure to diverge		
Consensus tree (dataset 1)	4	6	0	11	5	22	1
Tree 1 (dataset 1)	1–2	5	3–4	5–7	5	23	5
Tree 2 (dataset 1)	3	5	2	9	5	23	3
Morphological tree (dataset 2)	1–2	4–6	3–4	4–6	5	22	10
Events sum	40 (9%)	100 (23%)	60 (14%)	128 (30%)	–	–	–

Ranges indicate the range of the number of events found for different solutions. For detailed information for each isomorphic solution, see the Supporting Information (Appendix S4).

presented congruences on the relationship of part of the species, such as (*N. morena* and *N. guerreroi*), (*N. aitkeni* and *N. lamasi*) and (*N. pantaneira*, (*N. dubia* and *N. falsispina*)). Additionally, diagnostic characters described by Wenzel (Wenzel *et al.*, 1966; Wenzel, 1976), and later by Alcantara *et al.* (2019), were recovered as synapomorphies of the genus and species within it (see Supporting Information, Appendix S2).

However, we also identified incongruence in the positioning of *N. maai* and *N. traubi*. Part of the incongruence might be explained by the fact that we had only one gene available for these species. Another part might be explained by the morphology. Both species have the simplest abdominal morphology when compared with the other species in the genus, with few recognizable diagnostic characters to identify the species reliably (Alcantara *et al.*, 2019). Although extremely useful, it is necessary to examine the morphology carefully, because the low morphological variation among many species of the genus makes the recognition of morphological characters difficult (Alcantara *et al.*, 2019). For example, polytomies were recovered owing to the lack of synapomorphic character states under the morphological analysis. The low morphological variation is very evident when examining males, which are very similar interspecifically. This homogeneity is possibly caused by the high number of morphological reductions, associated with the parasitic habit, which can also lead to the evolution of convergent characters (Wenzel *et al.*, 1966; Wenzel, 1976; Guerrero, 1995; Alcantara *et al.*, 2019). Therefore, we consider the relationship of *N. maai* and *N. traubi* within *Noctiliostrebla* to be uncertain, until more data can be used to support a relationship hypothesis for these species.

NOCTILIO SPECIES, HOST–PARASITE ASSOCIATION AND THE ASSUMPTION OF INTERGRADATION ZONES

The two most recent phylogenetic studies recovered hypotheses with evidence pointing to the paraphyly of *Noctilio albiventris* (Pavan *et al.*, 2013; Khan *et al.*, 2014). Although we have used sequences obtained by Khan *et al.* (2014), our analysis recovered two well-supported clades for *Noctilio* species. We performed the analysis with all genes concatenated, whereas Khan *et al.* (2014) performed individual analyses for *ZFX*, *ZFY* and the mitochondrial genes. Thus, we believe that our result provides a robust hypothesis for the relationships within *Noctilio*, in view of the full data analysis and the support obtained for the clades. Besides, we found correspondence of some populations with subspecies delimited by Davis (1973, 1976). For *Noctilio albiventris*, population 1 has a distribution equivalent to *Noctilio a. affinis*, population 2 to *Noctilio a. cabrerai*, population 3 to *Noctilio a. minor* and population 4 to *Noctilio a. albiventris*. However, despite the recognition of different populations, it is important to highlight that Davis (1976) and Khan *et al.* (2014) presented evidence of hybridization between the equivalents of *Noctilio a. albiventris*, *Noctilio a. affinis* and *Noctilio a. cabrerai*.

For *Noctilio leporinus*, we found three populations, but they do not match the subspecies defined by Davis (1973). Population 1 was exclusively within the area corresponding to *Noctilio l. mastivus*, but restricted to the Lesser Antilles and Panama. Population 3 was also exclusively within the area corresponding to *Noctilio l. mastivus*, but restricted to Central America (excluding Panama), Greater Antilles and the western portion of the Andes. Lastly, population 2 covered the areas of both *Noctilio l. leporinus* and *Noctilio l. rufescens*, widely distributed through South America. Khan *et al.* (2014) also found no evidence that distinguished
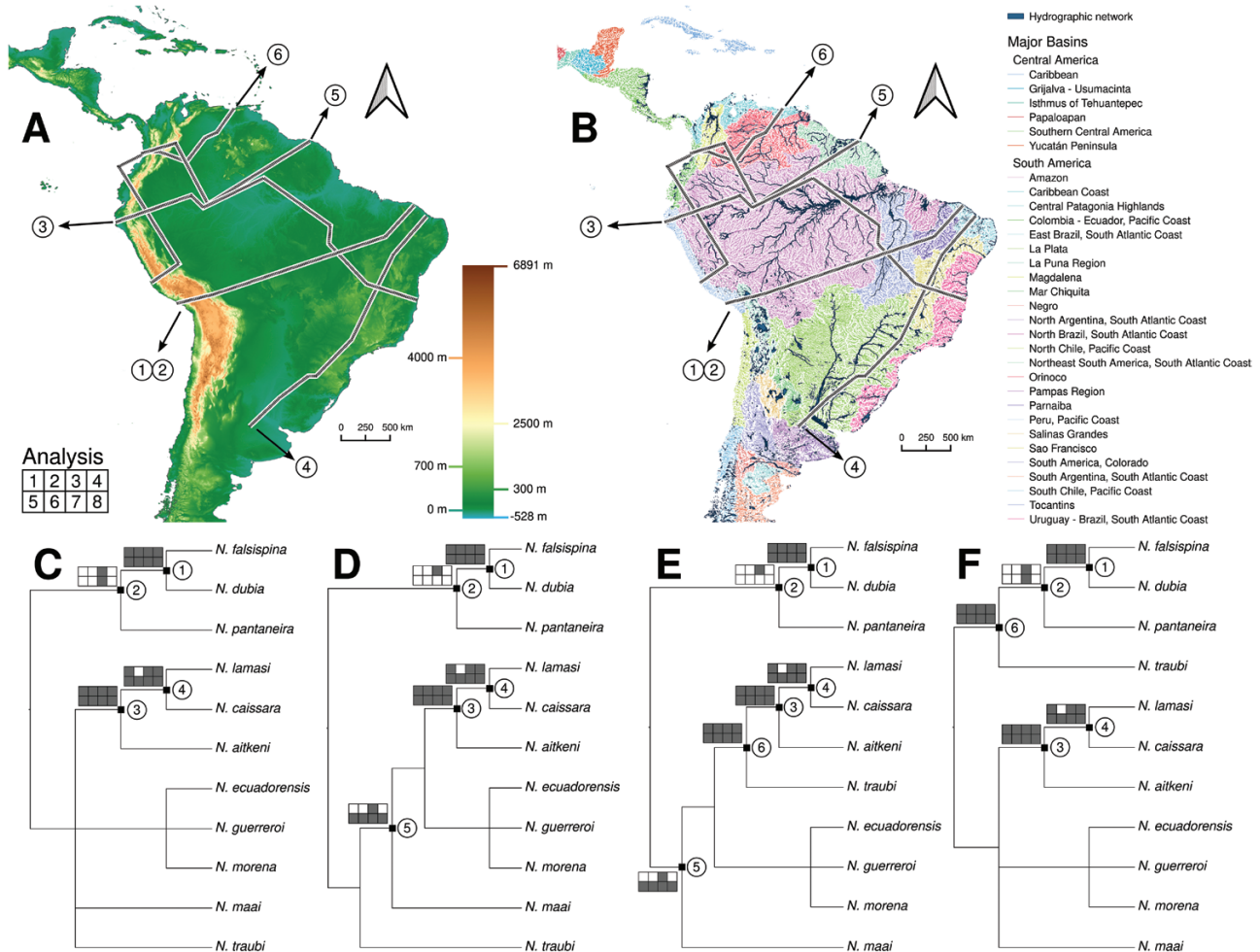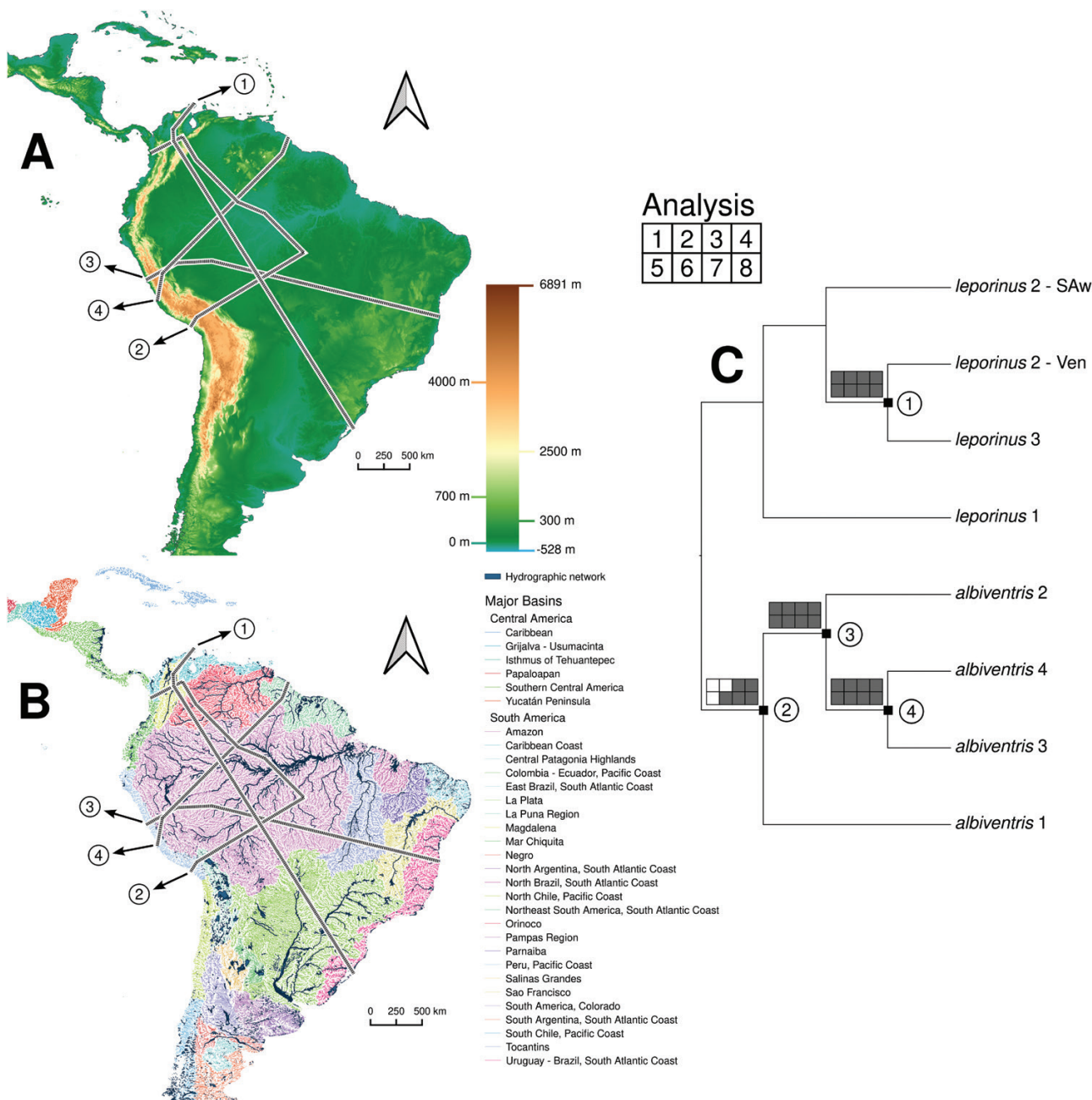
Figure 5. Hypothetical barriers recovered for *Noctiliostrebla* by VIP, with the indication of the corresponding nodes. A, elevation data map, showing the recovered barriers. B, watershed boundaries map, showing the recovered barriers. C, *Noctiliostrebla* tree from dataset 1, consensus tree. D, *Noctiliostrebla* tree from dataset 1, tree 1. E, *Noctiliostrebla* tree from dataset 1, tree 2. F, *Noctiliostrebla* tree from dataset 2, morphological tree. Numbers in the circles indicate corresponding nodes. Lines indicate disjunctions. Navajo rugs represent the results of each set of parameters run in VIP, in which a grey square indicates that the barrier represented on the map has been recovered for that specific analysis. The eight parameter sets implemented in VIP and a detailed basin boundary map are available in the Supporting Information (Appendix S5).

between the correspondents of *Noctilio l. leporinus* and *Noctilio l. rufescens*. Furthermore, Khan *et al.* (2014) presented evidence of hybridization between a lineage corresponding to *Noctilio l. mastivus* and another from South America. This could explain why we found two clusters for *Noctilio leporinus* population 3 in the *k*-means clustering analysis, and the clade of *Noctilio leporinus* population 2 from Venezuela closer to the clade containing population 3 than to the rest of the population 2.

The hypothesis of hybridization between *Noctilio* populations has consequences for the understanding of associations with ectoparasites. Although the works of Davis (1973, 1976) were subsequent to the description of *Noctiliostrebla*, many subspecies of *Noctilio* were

already recognized. Aware of discussions involving hosts, Wenzel (in Wenzel *et al.*, 1966) suggested the possibility of hybridization in *Noctiliostrebla* species, if the same were true for the hosts. His assumption was based on the high degree of specificity exhibited by *Noctiliostrebla* in relationship to hosts, in addition to the observation of the correspondence between the distributions of *Noctiliostrebla* species with host subspecies. The observation of correspondence between parasites and *Noctilio* subspecies led Wenzel (in Wenzel *et al.*, 1966) to designate *Noctilio* subspecies as hosts in descriptions of *N. traubi*, *N. aitkeni* and *N. maai*. Based on the study by Alcantara *et al.* (2019) and the morphological characters presented here, it was possible to identify some similarities regarding the

Figure 6. Hypothetical barriers recovered for *Noctilio* by VIP, with an indication of the corresponding nodes. A, elevation data map, showing the recovered barriers. B, watershed boundaries map, showing the recovered barriers. C, *Noctilio* population structure tree. Numbers in the circles indicate corresponding nodes. Lines indicate disjunctions. Navajo rugs represent the results of each set of parameters used to run VIP, in which a grey square indicates that the barrier represented on the map has been recovered for that specific analysis. The eight parameter sets implemented in VIP and a detailed basin boundary map are available in the Supporting Information (Appendix S5).

morphological issues involving the hosts (Davis, 1973, 1976) and speculated by Wenzel (in Wenzel *et al.*, 1966).

Despite the uncertainties about the relationships, the species of the parasite clade of *Noctilio albiventris* (*N. ecuadorensis*, *N. guerreroi* and *N. morena*) presented

a morphological relationship resembling that shown by Davis (1976) for the subspecies of *Noctilio albiventris*. The species *N. morena* (distribution overlaps that of *Noctilio a. cabrerai*) is similar to *N. guerreroi* (distribution overlaps that of *Noctilio a. albiventris*),

with the sternite VI and the genitalia practically similar in males, while the females present differences in the setae around spiracle III. The species *N. guerreroi* is very similar to *N. ecuadorensis* (distribution overlaps that of *Noctilio a. affinis*), with practically identical females. Sternite VI of the *N. ecuadorensis* male appears to be an intermediate between *N. guerreroi* and *N. maai* (distribution overlaps that of *Noctilio a. minor* and *Noctilio a. affinis*), and the genitalia are identical to those of *N. maai*. Based on the evidence presented by Khan *et al.* (2014) about hybridization between three strains that would correspond to *Noctilio a. albiventris*, *Noctilio a. affinis* and *Noctilio a. cabrerai*, the intermediate characteristics found in the clade of *Noctilio albiventris* parasites (*N. ecuadorensis*, *N. guerreroi* and *N. morena*) could be the result of hybridization in the parasites. However, even so, the morphology allowed the distinction between these species, and the analyses here performed did not allow that hybridization cases were measured.

In the *Noctilio leporinus* parasite clade (*N. aitkeni*, (*N. caissara* and *N. lamasi*)), the morphological examination of *Noctiliostrebla* species showed issues resembling that presented by Davis (1973) for the subspecies of *Noctilio leporinus*. The specimens of *N. aitkeni* (distribution overlaps that of *Noctilio l. leporinus* and *Noctilio l. mastivus*) and *N. lamasi* (distribution overlaps that of *Noctilio l. rufescens* and *Noctilio l. leporinus*) were morphologically distinct. However, specimens from locations close to the overlap zone between *Noctilio l. leporinus* and *Noctilio l. rufescens* created problems in determining their identities. In this work, specimens from these hypothetical zones were examined (four males and one female), in addition to a pair of *N. aitkeni* paratypes from Peru, which Alcantara *et al.* (2019) identified as belonging to *N. lamasi*. Males are difficult to identify, while females appeared to be on a threshold between *N. aitkeni* and *N. lamasi*. This situation for females is reminiscent of the intergradation speculated by Davis (1973) for the Bolivian specimens of *Noctilio leporinus*. One explanation for these morphological issues might lie in the hypothesis that *Noctilio leporinus* colonized South America rapidly (Pavan *et al.*, 2013). The founder effect caused by the rapid demographic expansion of *Noctilio leporinus* might have reduced the genetic variability of populations, consequently decreasing the evolutionary potential of these species (Frankham *et al.*, 1999) and thus maintaining the observed similarities. This expectation is reinforced by the fact that it was not possible to distinguish populations that would be equivalent to *Noctilio l. leporinus* and *Noctilio l. rufescens*. Even so, it is possible to distinguish *N. aitkeni* and *N. lamasi* morphologically, and the phylogenetic analyses reinforce this distinction.

THE HISTORICAL ASSOCIATION OF *NOCTILIOSTREBLA* AND EVOLUTION OF ECOLOGICAL NICHES ON HOSTS

When looking at the tree reconciliation analysis, it is evident that two parallel histories took place within hosts, in addition to at least one host-switch event (Table 1; Supporting Information, Appendix S4). Among the two recovered parallel histories, one involves the clade (*N. pantaneira*, (*N. dubia* and *N. falsispina*)) and the other involves two clades, ((*N. morena*, *N. guerreroi* and *N. ecuadorensis*) and (*N. aitkeni*, (*N. caissara* and *N. lamasi*))) (Supporting Information, Appendix S4). As shown in the Results, we recovered three clades consistently in the phylogenetic analyses containing all species (datasets 1 and 2; Fig. 1). When two species coexist on the same host, the coexistence is always of species present in the clade (*N. pantaneira*, (*N. dubia* and *N. falsispina*)) with species present in the clade (*N. aitkeni*, (*N. lamasi* and *N. caissara*)) or (*N. ecuadorensis*, *N. guerreroi* and *N. morena*). Species of the same clade never co-occur, suggesting a potential effect of niche restriction and segregation between clades. *Noctiliostrebla maai* and *N. traubi* have no record of co-occurrence with other species and are the only known species that have a distribution restricted to Central America and the extreme north of South America (Alcantara *et al.*, 2019).

Habitat segregation within hosts is widely recognized for bat flies and is strongly correlated with ecomorphological characteristics. Given that the space on a host is limited, its partitioning into niches has been considered a major factor for the coexistence of different species of parasites on the same host species, with specialization to a particular niche acting as a barrier to competition among parasites (Presley, 2007; Tello *et al.*, 2008; Hiller *et al.*, 2018; Alcantara *et al.*, 2022). Morphological differences between coexisting *Noctiliostrebla* species have been speculated as possible adaptations to inhabit different microhabitats on the body of the host, although observational and experimental studies are needed for confirmation (Dick & Gettinger, 2005). On that account, the assumption of niche segregation in *Noctiliostrebla* species parasitizing the same host (Dick & Gettinger, 2005) is strongly reinforced by the tree reconciliation analysis. Based on our results, niche segregation appears to be determined evolutionarily, facilitating species coexistence and promoting species diversification.

Lastly, the role of losses and failure to diverge is puzzling. The problem with establishing distribution-based associations is that areas with sympatric host populations pose challenges for interpretation. Based on the distributions of hosts and bat flies, *N. maai* overlaps the distributions of *Noctilio albiventris* populations 1 (= *Noctilio a. affinis*) and 3 (= *Noctilio a. minor*). In the same way, *N. aitkeni* overlaps the

distributions of *Noctilio leporinus* populations 1 (= *Noctilio l. mastivus*) and 2 (= *Noctilio l. leporinus* and *Noctilio l. rufescens*), and *N. traubi* overlaps the distributions of *Noctilio leporinus* populations 1 (= *Noctilio l. mastivus*) and 3 (= *Noctilio l. mastivus*). Losses and failure to diverge events are present exclusively in these associations (Table 1; Supporting Information, Appendix S4). Given that it is not possible to define whether *Noctiliostrebla* species are restricted or not to specific populations in areas of sympatry between hosts, it is doubtful whether these events are being overestimated.

ELEVATION, WATERSHEDS AND THE LOW RELEVANCE OF COSPECIATION EVENTS: THE HISTORICAL ASSOCIATION EXPLAINED UNDER THE STOCKHOLM PARADIGM

The disjunctions show a strong spatial congruence with major river basins in South America and with areas of higher elevations. Most of the localities recorded by Davis (1976) for *Noctilio albiventris* were at elevations < 500 m, with the highest elevation ≤ 1100 m a.s.l. Also, the species has a wide, but discontinuous distribution range from Guatemala and Honduras to northern Argentina and western Uruguay. However, it is absent from large areas of South America characterized by seasonally dry vegetation (savannah type, xerophytic vegetation, seasonal deciduous or semideciduous forests), including the Brazilian Cerrado (Smith, 2008; Pavan *et al.*, 2013). Although *Noctilio leporinus* does not have such detailed information, its diet is much more restrictive and therefore influenced by higher elevations and dry vegetation (Hood & Jones Jr, 1984). Thus, geographical features end up influencing the foraging area of the hosts, and consequently of distribution, acting as strong evidence for vicariance processes. Their roles are reinforced by taking into account that the hypothesis of origin of *Noctilio* is between the Pliocene and the Pleistocene (Pavan *et al.*, 2013; Khan *et al.*, 2014). Four glacial periods are reported for this period. Accordingly, from this period onwards a series of isolation and expansion processes might have occurred, possibly related to temperature oscillation, elevation discontinuities in the Andes, and expansion of seasonally dry vegetation that ends up limiting the foraging area of hosts (Gregory-Wodzicki, 2000; Mayle *et al.*, 2004; Hoorn *et al.*, 2010). Although there is no consensus on the biogeographical history of *Noctilio*, the hypothesis of colonization of South America and the Caribbean Islands from Central America, followed by a rapid process of demographic expansion (Pavan *et al.*, 2013; Khan *et al.*, 2014), finds support when considering the processes of Andean uplift and dispersal facilitated by temperature oscillation of the Pleistocene. During the glacial periods, processes of Andean uplift and retraction of rain forests could favour isolation. Already over interglacial periods, the appearance of warm valleys and sharp elevation discontinuities could be associated with dispersal events (Bedoya *et al.*, 2021; Muñoz-Valencia *et al.*, 2022).

We did not find any indication of an overall congruence between the host and parasite trees, nor any evidence that could determine cospeciation events as relevant. Based on the potential importance of biogeographical events in the isolation and colonization of both bat flies and hosts, associated with ecological factors, we suggest that our findings are consistent with the expectations of the 'Stockholm paradigm' (Brooks *et al.*, 2015). In this sense, ecological factors are an important component to explain how an expansion of associations between bat flies and different host species/populations would be possible. *Noctilio* species are known to form stable, long-lasting shelters containing dozens of individuals (Hood & Jones Jr, 1984; Aguirre *et al.*, 2003; Bernard & Fenton, 2003), usually forming harems or groups of females (Brooke, 1997). However, some individuals show a lower fidelity and carry out periodic exchanges of shelter, which tends to be more frequent among males (Brooke, 1997; Bernard & Fenton, 2003). Moreover, *Noctiliostrebla* generally has a high abundance on hosts (Moura *et al.*, 2003; Presley, 2007) and a dispersal capacity limited by reduced wings. Thus, the sharing of shelters by different *Noctilio* populations and periodic changing of shelters by some individuals would allow the exchange of parasites between hosts of different roosts and different populations. This hypothesis gains support with the evidence of hybridization between *Noctilio* populations. If there is hybridization, there is contact between populations, probably at shelters, and a chance for the ectoparasites to come into contact with new hosts. In this sense, geographical events might act in the isolation of populations, allowing specialization and diversification of ectoparasites. In turn, perturbations allowing the contact of previously isolated populations would promote the chance of duplication and host switching, owing to the ecological characteristics of the hosts, such as shelter sharing.

Lastly, taking into account the role of high elevation as a barrier and the similar disjunct distributions between *Noctiliostrebla* species and hosts, we speculate that the host of *N. caissara* might belong to a population not yet described and discussed in relationship to the other populations/subspecies. According to Alcantara *et al.* (2019) and with our results, *N. caissara* does not co-occur with any other species of *Noctiliostrebla* and is totally restricted to the south-eastern coast of South America, at the eastern region of Serra do Mar in Brazil. The Serra do Mar is a system of escarpments and mountains that stretch > 1500 km between the states of Santa Catarina

and Rio de Janeiro ([Vieira & Gramani, 2015](#)) and can act perfectly as a barrier. Considering that *Noctilio leporinus* specimens from the eastern region of Serra do Mar have never been compared morphologically and molecularly with specimens from other regions, we emphasize their potential to be a new population.

CONCLUSION

We have presented an integrated approach to understanding the evolutionary history of *Noctiliostrebla* and the historical association with the hosts. The genus is monophyletic, with some well-defined internal relationships, but also with some inconsistencies, especially in relationship to the position of *N. traubi* and *N. maai*. We expect that additional phylogenetic studies might assist in the understanding of the relationships inside the genus.

The difficulty in attributing well-delimited host associations raises the value of seeking to assess more often the role of populations in processes involving historical associations. Population-based tree reconciliation analysis can reveal intricate processes, such as host switching, that are not so evident when addressed by a species-based approach. Based on our results, it is evident that at least two parallel histories have developed in the association between *Noctiliostrebla* species and their bat hosts. These two parallel histories involve clades whose species coexist on the same host, suggesting a potential effect of niche restriction and segregation between clades. However, it is important to establish more carefully how restrictive the association is between populations, especially when there are sympatric host populations. This is evidenced by [Khan *et al.* (2014)](#), who raised the possibility that potential hybridization cases might be the result of incorrect assignment of individuals with relatively unique genotypes.

Our findings are in agreement with the expectations of the 'Stockholm paradigm'. We have demonstrated the potential importance of biogeographical events in the isolation and colonization for both groups, bat flies and hosts, whereas cospeciation events had no relevance in practice. We assume that geographical features, such as high elevations and watersheds, play an important role as barriers, owing to the similar disjunct distributions between *Noctiliostrebla* species and hosts. However, it would be important to sample the areas of disjunctions pointed out in the present work, seeking to evaluate the roles of elevation and vegetation in these areas. Historical association and biogeographical studies of Streblidae are still in their infancy, and there is a long way to go. Additional studies are important and are needed urgently for the understanding of the host–parasite relationship of streblid flies and their bat hosts.

DATA AVAILABILITY

The data underlying this article are available in the article and in its online Supporting Information.

The gene sequences are available on the GenBank Nucleotide Database at: https://www.ncbi.nlm.nih.gov/genbank/ and can be accessed with the access numbers shown in the Supporting Information.

REFERENCES

Abadi S, **Azouri D**, **Pupko T**, **Mayrose I. 2019.** Model selection may not be a mandatory step for phylogeny reconstruction. *Nature Communications* **10:** 934.

Agosta SJ, **Janz N**, **Brooks DR. 2010.** How specialists can be generalists: resolving the parasite paradox and implications for emerging infectious disease. *Zoologia (Curitiba)* **27:** 151–162.

Aguirre LF, **Lens L**, **Matthysen E. 2003.** Patterns of roost use by bats in a neotropical savanna: implications for conservation. *Biological Conservation* **111:** 435–443.

Alcantara DMC, **Graciolli G**, **Nihei SS. 2019.** Revision of *Noctiliostrebla* (Diptera: Streblidae), parasites of bulldog bats (Chiroptera: Noctilionidae: *Noctilio*). *Zootaxa* **4560:** 483–521.

Alcantara DMC, **Graciolli G**, **Toma R**, **Souza CS. 2022.** Sex-biased parasitism, host mass and mutualistic bat flies: an antagonistic individual-based network of bat-bat fly interactions. *International Journal for Parasitology* **52:** 217–224.

Alcantara DMC, **Souza CS**, **Graciolli G**, **Nihei SS. 2016.** New records of tree roosts of *Noctilio albiventris* from the Pantanal, Brazil. *Studies on Neotropical Fauna and Environment* **51:** 235–238.

Arias JS. 2010. *VIP: Vicariance Inference Program, software and documentation published by the author*. Available at: http://www.lillo.org.ar/phylogeny/VIP/

Arias JS, **Szumik CA**, **Goloboff PA. 2011.** Spatial analysis of vicariance: a method for using direct geographical information in historical biogeography. *Cladistics* **27:** 617–628.

Bedoya AM, **Leaché AD**, **Olmstead RG. 2021.** Andean uplift, drainage basin formation, and the evolution of plants living in fast-flowing aquatic ecosystems in northern South America. *New Phytologist* **232:** 2175–2190.

Bernard E, **Fenton MB. 2003.** Bat mobility and roosts in a fragmented landscape in Central Amazonia, Brazil. *Biotropica* **35:** 262–277.

Bikandi J, **San Millán R**, **Rementeria A**, **Garaizar J. 2004.** *In silico* analysis of complete bacterial genomes: PCR, AFLP–PCR and endonuclease restriction. *Bioinformatics* **20:** 798–799.

Bremer K. 1994. Branch support and tree stability. *Cladistics* **10:** 295–304.

Brooke AP. 1997. Social organization and foraging behaviour of the fishing bat, *Noctilio leporinus* (Chiroptera: Noctilionidae). *Ethology* **103:** 421–436.

Brooks DR, **Hoberg EP**, **Boeger WA. 2015.** In the eye of the cyclops: the classic case of cospeciation and why paradigms are important. *Comparative Parasitology* **82:** 1–8.

Conow C, **Fielder D**, **Ovadia Y**, **Libeskind-Hadas R. 2010.** Jane: a new tool for the cophylogeny reconstruction problem. *Algorithms for Molecular Biology* **5:** 16.

Davis WB. 1973. Geographic variation in the fishing bat, *Noctilio leporinus*. *Journal of Mammalogy* **54:** 862–874.

Davis WB. 1976. Geographic variation in the lesser *Noctilio*, *Noctilio albiventris* (Chiroptera). *Journal of Mammalogy* **57:** 687–707.

Dick CW, **Gettinger D. 2005.** A faunal survey of streblid flies (Diptera: Streblidae) associated with bats in Paraguay. *Journal of Parasitology* **91:** 1015–1024.

Dittmar K, **Morse SF**, **Dick CW**, **Patterson BD. 2015.** Bat fly evolution from the Eocene to the present (Hippoboscoidea, Streblidae and Nycteribiidae). In: Morand S, Krasnov BR, Littlewood DTJ, eds. *Parasite diversity and diversification: evolutionary ecology meets phylogenetics*. Cambridge: Cambridge University Press, 246–264.

Dittmar K, **Porter ML**, **Murray S**, **Whiting MF. 2006.** Molecular phylogenetic analysis of nycteribiid and streblid flies (Diptera: Brachycera, Calyptratae): implications for host associations and phylogeographic origins. *Molecular Phylogenetics and Evolution* **38:** 155–170.

Ewing B, **Green P. 1998.** Base-calling of automated sequencer traces using *Phred*. II. Error probabilities. *Genome Research* **8:** 186–194.

Ewing B, **Hillier L**, **Wendl MC**, **Green P. 1998.** Base-calling of automated sequencer traces using *Phred*. I. Accuracy assessment. *Genome Research* **8:** 175–185.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

Fitch WM. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Biology* **20:** 406–416.

Forey PL, **Kitching IJ. 2000.** Experiments in coding multistate characters. In: Scotland R, Pennington T, eds. *Homology and systemactics: coding characters for phylogenetic analysis*. London: CRC Press, 58–80.

Frable BW, **Melo BF**, **Fontenelle JP**, **Oliveira C**, **Sidlauskas BL. 2022.** Biogeographic reconstruction of the migratory Neotropical fish family Prochilodontidae (Teleostei: Characiformes). *Zoologica Scripta* **51:** 348–364.

Frankham R, **Lees K**, **Montgomery ME**, **England PR**, **Lowe EH**, **Briscoe DA. 1999.** Do population size bottlenecks reduce evolutionary potential? *Animal Conservation* **2:** 255–260.

Gatesy J, **O'Grady P**, **Baker RH. 1999.** Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. *Cladistics* **15:** 271–313.

Goloboff PA, **Catalano SA. 2016.** TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics* **32:** 221–238.

Goloboff PA, **Farris JS**, **Nixon KC. 2008.** TNT, a free program for phylogenetic analysis. *Cladistics* **24:** 774–786.

Gordon D, **Abajian C**, **Green P. 1998.** *Consed*: a graphical tool for sequence finishing. *Genome Research* **8:** 195–202.

Gordon D, **Desmarais C**, **Green P. 2001.** Automated finishing with Autofinish. *Genome Research* **11:** 614–625.

Graciolli G, **Carvalho CJB. 2012.** Do fly parasites of bats and their hosts coevolve? Speciation in *Trichobius phyllostomae* group (Diptera, Streblidae) and their hosts (Chiroptera, Phyllostomidae) suggests that they do not. *Revista Brasileira de Entomologia* **56:** 436–450.

Graciolli G, **Dick CW. 2004.** A new species of *Metelasmus* (Diptera: Streblidae: Streblinae) from southern South America. *Zootaxa* **509:** 1–8.

Gregory-Wodzicki KM. 2000. Uplift history of the Central and Northern Andes: a review. *Geological Society of America Bulletin* **112:** 1091–1105.

Guerrero R. 1995. Catalogo de los Streblidae (Diptera: Pupipara) parásitos de murciélagos (Mammalia: Chiroptera) del Nuevo Mundo. V. Trichobiinae con alas reducidas o ausentes y miscelaneos. *Boletín de Entomología Venezolana. Nueva Serie* **10:** 135–160.

Guerrero R. 1998. Notes on Neotropical batflies (Diptera, Streblidae). II. Review of the genus *Xenotrichobius*. *Acta Parasitologica* **43:** 142–147.

Guindon S, **Dufayard JF**, **Lefort V**, **Anisimova M**, **Hordijk W**, **Gascuel O. 2010.** New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59:** 307–321.

Haelewaters D, **Dick CW, Cocherán Pittí KP**, **Dittmar K**, **Patterson BD. 2021.** Bats, bat flies, and fungi: exploring uncharted waters. In: Lim BK, Fenton MB, Brigham RM, Mistry S, Kurta A, Gillam EH, Russell A, Ortega J, eds.

50 years of bat research. Fascinating life sciences. Cham: Springer, 349–371.

Hawkins JA, **Hughes CE**, **Scotland RW. 1997.** Primary homology assessment, characters and character states. *Cladistics* **13:** 275–283.

Hiller T, **Honner B**, **Page RA**, **Tschapka M. 2018.** Leg structure explains host site preference in bat flies (Diptera: Streblidae) parasitizing neotropical bats (Chiroptera: Phyllostomidae). *Parasitology* **145:** 1475–1482.

Hoang DT, **Chernomor O**, **von Haeseler A**, **Minh BQ**, **Vinh LS. 2018.** UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* **35:** 518–522.

Hoberg EP, **Brooks DR. 2008.** A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography* **35:** 1533–1550.

Hood CS, **Jones JK Jr**. **1984.** *Noctilio leporinus*. *Mammalian Species* **21:** 1–7.

Hood CS, **Pitocchelli J. 1983.** *Noctilio albiventris*. *Mammalian Species* **197:** 1–5.

Hoorn C, **Wesselingh FP**, **ter Steege H**, **Bermudez MA**, **Mora A**, **Sevink J**, **Sanmartín I**, **Sanchez-Meseguer A**, **Anderson CL**, **Figueiredo JP**, **Jaramillo C. 2010.** Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330:** 927–931.

Hovenkamp P. 1997. Vicariance events, not areas, should be used in biogeographical analysis. *Cladistics* **13:** 67–79.

Hovenkamp P. 2001. A direct method for the analysis of vicariance patterns. *Cladistics* **17:** 260–265.

Jombart T, **Ahmed I. 2011.** *adegenet 1.3-1*: new tools for the analysis of genome-wide SNP data. *Bioinformatics* **27:** 3070–3071.

Jombart T, **Devillard S**, **Balloux F. 2010.** Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11:** 94.

Kamvar ZN, **Tabima JF**, **Grünwald NJ. 2014.** *Poppr*: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* **2:** e281.

Katoh K, **Misawa K**, **Kuma KI**, **Miyata T. 2002.** MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30:** 3059–3066.

Katoh K, **Standley DM. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30:** 772–780.

Khan FAA, **Phillips CD**, **Baker RJ. 2014.** Timeframes of speciation, reticulation, and hybridization in the bulldog bat explained through phylogenetic analyses of all genetic transmission elements. *Systematic Biology* **63:** 96–110.

Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* **30:** 3276–3278.

Legendre P, **Desdevises Y**, **Bazin E. 2002.** A statistical test for host–parasite coevolution. *Systematic Biology* **51:** 217–234.

Lehner B, **Grill G. 2013.** Global river hydrography and network routing: baseline data and new approaches to study the world's large river systems. *Hydrological Processes* **27:** 2171–2186.

Lewis PO. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* **50:** 913–925.

Maddison WP, **Maddison DR. 2018.** *Mesquite: a modular system for evolutionary analysis. Version 3.61*. Available at: http://www.mesquiteproject.org

Mayle FE, **Beerling DJ**, **Gosling WD**, **Bush MB. 2004.** Responses of Amazonian ecosystems to climatic and atmospheric carbon dioxide changes since the last glacial maximum. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359:** 499–514.

Minh BQ, **Nguyen MAT**, **von Haeseler A. 2013.** Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* **30:** 1188–1195.

Minh BQ, **Schmidt HA**, **Chernomor O**, **Schrempf D**, **Woodhams MD**, **von Haeseler A**, **Lanfear R. 2020.** IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* **37:** 1530–1534.

Moura MO, **Bordignon MO**, **Graciolli G. 2003.** Host characteristics do not affect community structure of ectoparasites on the fishing bat *Noctilio leporinus* (L., 1758) (Mammalia: Chiroptera). *Memórias do Instituto Oswaldo Cruz* **98:** 811–815.

Muñoz-Valencia V, **Vélez-Martínez GA**, **Montoya-Lerma J**, **Díaz F. 2022.** Role of the Andean uplift as an asymmetrical barrier to gene flow in the neotropical leaf-cutting ant *Atta cephalotes*. *Biotropica* **54:** 191–204.

Musher LJ, **Giakoumis M**, **Albert J**, **Del-Rio G**, **Rego M**, **Thom G**, **Aleixo A**, **Ribas CC**, **Brumfield RT**, **Smith BT**, **Cracraft J. 2022.** River network rearrangements promote speciation in lowland Amazonian birds. *Science Advances* **8:** eabn1099.

Nguyen LT, **Schmidt HA**, **von Haeseler A**, **Minh BQ. 2015.** IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32:** 268–274.

Nixon K. 2002. *WinClada, version 1.00.08*. Ithaca: Published by the author, 734–745.

Nylin S, **Agosta S**, **Bensch S**, **Boeger WA**, **Braga MP**, **Brooks DR**, **Forister ML**, **Hambäck PA**, **Hoberg EP**, **Nyman T**, **Schäpers A**, **Stigall AL**, **Wheat CW**, **Österling M**, **Janz N. 2018.** Embracing colonizations: a new paradigm for species association dynamics. *Trends in Ecology & Evolution* **33:** 4–14.

Paradis E, **Schliep K. 2019.** ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35:** 526–528.

Patterson BD, **Ballard JWO**, **Wenzel RL. 1998.** Distributional evidence for cospeciation between Neotropical bats and their bat fly ectoparasites. *Studies on Neotropical Fauna and Environment* **33:** 76–84.

Pavan AC, **Martins FM**, **Morgante JS. 2013.** Evolutionary history of bulldog bats (genus *Noctilio*): recent diversification and the role of the Caribbean in Neotropical biogeography. *Biological Journal of the Linnean Society* **108:** 210–224.

Peña C, **Wahlberg N**, **Weingartner E**, **Kodandaramaiah U**, **Nylin S**, **Freitas AVL**, **Brower AVZ. 2006.** Higher level phylogeny of Satyrinae butterflies (Lepidoptera: Nymphalidae) based on DNA sequence data. *Molecular Phylogenetics and Evolution* **40:** 29–49.

Petersen FT, **Meier R**, **Kutty SN**, **Wiegmann BM. 2007.** The phylogeny and evolution of host choice in the Hippoboscoidea (Diptera) as reconstructed using four molecular markers. *Molecular Phylogenetics and Evolution* **45:** 111–122.

Pinto-da-Rocha R, **Bragagnolo C**, **Marques FPL**, **Antunes Junior M. 2014.** Phylogeny of harvestmen family Gonyleptidae inferred from a multilocus approach (Arachnida: Opiliones). *Cladistics* **30:** 519–539.

Poisot T. 2015. When is co-phylogeny evidence of coevolution? In: Morand S, Krasnov BR, Littlewood DTJ, eds. *Parasite diversity and diversification. Evolutionary ecology meets phylogenetics*. Cambridge: Cambridge University Press, 420–433.

Presley S. 2007. Streblid bat fly assemblage structure on Paraguayan *Noctilio leporinus* (Chiroptera: Noctilionidae): nestedness and species co-occurrence. *Journal of Tropical Ecology* **23:** 409–417.

R Core Team. 2021. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org/

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3:** 217–223.

Sayers EW, **Cavanaugh M**, **Clark K**, **Pruitt KD**, **Schoch CL**, **Sherry ST**, **Karsch-Mizrachi I. 2021.** GenBank. *Nucleic Acids Research* **49:** D92–D96.

Smith P. 2008. Lesser bulldog bat *Noctilio albiventris* Desmarest, 1818. *Fauna Paraguay: handbook of the mammals of Paraguay* No. **22:** 1–15. Available at: http://www.faunaparaguay.com/noctilioalbiventris.html

Strong EE, **Lipscomb D. 1999.** Character coding and inapplicable data. *Cladistics* **15:** 363–371.

Sweet AD, **Johnson KP. 2016.** Cophylogenetic analysis of New World ground-doves (Aves: Columbidae) and their parasitic wing lice (Insecta: Phthiraptera: *Columbicola*). *Molecular Phylogenetics and Evolution* **103:** 122–132.

Tello JS, **Stevens RD**, **Dick CW. 2008.** Patterns of species co-occurrence and density compensation: a test for interspecific competition in bat ectoparasite infracommunities. *Oikos* **117:** 693–702.

Vaidya G, **Lohman DJ**, **Meier R. 2011.** SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **27:** 171–180.

Vieira BC, **Gramani MF. 2015.** Serra do Mar: the most 'Tormented' relief in Brazil. In: Vieira BC, Salgado AAR, Santos LJC, eds. *Landscapes and landforms of Brazil*. Dordrecht: Springer Netherlands, 285–297.

Wang LG, **Lam TTY**, **Xu S**, **Dai Z**, **Zhou L**, **Feng T**, **Guo P**, **Dunn CW**, **Jones BR**, **Bradley T**, **Zhu H**, **Guan Y**, **Jiang Y**, **Yu G. 2020.** *Treeio*: an R package for phylogenetic tree input and output with richly annotated and associated data. *Molecular Biology and Evolution* **37:** 599–603.

Wenzel RL. 1976. The streblid bat flies of Venezuela. *Brigham Young University Science Bulletin, Biological Series. Provo* **20:** 1–177.

Wenzel RL, **Peterson B. 1987.** Streblidae. In: MacAlpine J, Peterson B, Shewell G, Teskey H, Vockeroth J, Wood D, eds. *Manual of Nearctic Diptera, Vol. 2*. Ottawa: Canadian Government Publishing Centre, 1293–1301.

Wenzel RL, **Tipton VJ**, **Kiewlicz A. 1966.** The streblid bat flies of Panama (Diptera: Calyptrate: Streblidae). In: Wenzel RL, Tipton VJ, eds. *Ectoparasites of Panama*. Chicago: Field Museum of Natural History, 405–675.

Yu G. 2020. Using ggtree to visualize data on tree-like structures. *Current Protocols in Bioinformatics* **69:** e96.

Yu G. 2021. *tidytree: a tidy tool for phylogenetic tree data manipulation. R package version 0.3.4*. Available at: https://CRAN.R-project.org/package=tidytree

Yu G, **Lam TTY**, **Zhu H**, **Guan Y. 2018.** Two methods for mapping and visualizing associated data on phylogeny using *ggtree*. *Molecular Biology and Evolution* **35:** 3041–3043.

Yu G, **Smith DK**, **Zhu H**, **Guan Y**, **Lam TTY. 2017.** ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution* **8:** 28–36.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supporting information for the Material and Methods section on molecular and distributional data.
Appendix S2. Supporting information for morphological data and phylogenetic analysis of morphological characters.
Appendix S3. Supporting information for host population structure analyses and ParaFit analyses.
Appendix S4. Supporting information on the analyses performed with the Jane v.4 software.
Appendix S5. Supporting information on the analyses performed with the VIP software.