Daniel Maximo Corrêa de Alcantara

Filogenia da subfamília Streblinae (Diptera: Streblidae) e associação histórica parasito-hospedeiro

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Co-orientador: Prof. Dr. Daniel José Galafasse Lahr

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Aos pequenos e mal-amados;

Aos desleixados e maltrapilhos;

A todos os filhos bastardos da mesma nação, que

são crianças, porém não são filhos!

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"*De dia à procura de comida / A noite um lugar pra dormir / Carrega no corpo feridas e ainda consegue sorrir / Dizem que o nosso país não vai mal / Porque o povo ainda faz carnaval / Mas os pequenos e mal amados não compartilham da mesma visão / Há tristeza no seu coração / Vivem a margem do nosso país / Assaltando e ferindo quem passa / Tentam gritar do seu jeito infeliz / Que o país os deixou na desgraça / São alvos de uma justiça que só sabe falar / Será que a solução é exterminar / Herodes não morreu e hoje os dias estão piores / Esterilização em massa e chacina de menores / Mas eu queria somente lembrar / Que milhões de crianças sem lar / São frutos do mal que floriu / Num país que jamais repartiu / Pátria amada, Brasil / FUTURO DO PAÍS / Esse é o futuro do país / FUTURO DO PAÍS*"

(Futuro Do País, Planet Hemp)

"*Ninguém pode estar no mundo, com o mundo e com os outros de forma neutra. Não posso estar no mundo de luvas nas mãos constatando apenas. A acomodação em mim é apenas caminho para a inserção, que implica decisão, escolha, intervenção na realidade. Há perguntas a serem feitas insistentemente por todos nós e que nos fazem ver a impossibilidade de estudar por estudar. De estudar descomprometidamente como se misteriosamente, de repente, nada tivéssemos que ver com o mundo, um lá fora e distante mundo, alheado de nós e nós dele.*"

(Paulo Freire, Pedagogia da Autonomia)

Resumo

O paradigma no qual grande parte do arcabouço teórico e empírico tem se baseado para o estudo de associações históricas não tem sido capaz de acomodar as evidências disponíveis. Essas inconsistências permitiram o surgimento do "Paradigma de Estocolmo". Existem diferentes grupos com potencial para ser um sistema modelo para o estudo de associações históricas, como Streblidae. Esta família é caracterizada como um grupo altamente especializado de moscas ectoparasitas de morcegos, que se alimentam de sangue. Embora a associação entre Streblidae e seus hospedeiros possa ser um bom sistema modelo, reconstruir uma hipótese de relacionamento é crucial antes de iniciar um estudo de associação histórica. Para estudar as relações dentro de Streblidae e as associações parasita-hospedeiro, a tese foi dividida em duas partes principais. No capítulo 2, apresentamos a filogenia molecular mais abrangente para Streblidae do Novo Mundo até o momento, incluindo estimativas de tempos de divergência. Nossos resultados suportam a monofilia dos Streblidae do Novo Mundo, Nycterophiliinae e Streblinae, porém não suportam a monofilia de Trichobiinae. As estimativas sugerem que Streblidae do Novo Mundo surgiu no Mioceno Inferior. Novas colonizações foram recuperadas como o principal evento nas associações entre Streblidae e seus hospedeiros. Sugerimos a ecologia de abrigos como um mecanismo importante nas associações parasito-hospedeiro estudadas. No capítulo 3, é apresentada a filogenia da subfamília Streblinae com base em caracteres morfológicos. Foram amostradas todas as espécies válidas de Streblinae. Os resultados suportam a monofilia de Streblinae, com *Anastrebla*, *Metelasmus* e *Paraeuctenodes* também como monofiléticos. Por sua vez, *Strebla* foi recuperado como parafilético em relação a *Metelasmus*. Apresentamos uma visão histórica sobre a delimitação de Streblinae e discutimos a importância de uma nova interpretação dos caracteres para a classificação da subfamília.

Abstract

The paradigm upon which much of the theoretical and empirical framework was based for the study of historical associations has not been able to accommodate the available evidence. These inconsistencies enabled the emergence of the "Stockholm Paradigm". There are different groups that have potential to be a model system for studying historical associations, such as Streblidae. The family is characterized as a highly specialized group of flies that are ectoparasitic on bats, feeding on the blood of their hosts. The association between species of Streblidae and their hosts may be a good model system to understand the host-parasite relationship under the Stockholm Paradigm. However, reconstructing a relationship hypothesis is crucial before starting a study of the historical association. To study the relationship within Streblidae and the host-parasite associations, the thesis was divided into two main parts. In chapter 2, we present the most comprehensive molecular phylogeny of New World Streblidae to date, including a fossil-calibrated estimates of divergence dates. Our analysis supports the monophyly of New World Streblidae, Nycterophiliinae and Streblinae, but a non-monophyletic Trichobiinae. Our estimates suggest that New World Streblidae arose at Lower Miocene. Host-switch is recovered as the main event in the associations between species of Streblidae and their hosts. We suggest roost ecology as an important mechanism in the association between species of Streblidae and their hosts. In chapter 3, the phylogeny of the subfamily Streblinae is presented based on morphological characters. We sampled all valid species of Streblinae. Results strongly support the monophyly of Streblinae, with *Anastrebla*, *Metelasmus* and *Paraeuctenodes* also as monophyletic. In turn, *Strebla* is recovered as paraphyletic in relation to *Metelasmus*. We present a historical overview on the delimitation of Streblinae, and discuss the importance of a new interpretation of the characters.

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Chapter 1

General Introduction

Coevolution is considered one of the main evolutionary forces capable of generating and structuring biological diversity (Brockhurst and Koskella, 2013). Possible coevolutionary interactions are present in many ways within biological communities and are generally placed into three categories: antagonism, mutualism, and commensalism. Within these, the most commonly studied examples of interactions are: host-parasite, predator-prey, and plant-pollinator (Fountain et al., 2017). The role of coevolution in interactions and species diversification is considered important in understanding how microevolutionary processes can result in broad patterns of lineage diversification (Althoff et al., 2014; Gilman et al., 2012; Poisot, 2015). Understanding the factors capable of generating, maintaining and limiting interactions between species may have ecological applications. Examples of applications include emerging disease dynamics, biological control, biological invasion, and biotic responses to climate change (Agosta, 2006; Brooks and Ferrao, 2005; Brooks and Hoberg, 2007; Hoberg and Brooks, 2015). Thus, many authors argue the need for data that integrates microevolutionary processes with macroevolutionary patterns to understand how coevolution affects speciation and extinction. Phylogenetic tools have been a widely used approach to understanding such a link, mainly through comparison of phylogenies, known as cophylogenies (Segraves, 2010; Thompson et al., 2017).

Most of the theoretical and methodological development for macroevolutionary approaches in coevolutionary studies was built on a "maximum cospeciation" paradigm. Within this paradigm, specificity is the cause of coevolution and it is predicted that the potential for new interactions will be minimal (Brooks et al., 2015; Hoberg and Brooks, 2008). However, this paradigm led to the misconception that examination of congruence between phylogenies would be a sufficient evidence of coevolution, even leading to the use

of cospeciation as a synonym for coevolution (Anderson, 2015; Page, 2003; Poisot, 2015; Thompson, 2005; Thompson et al., 2017; Vienne et al., 2013). While coevolution may lead to cospeciation, it is neither necessary nor sufficient to originate and maintain a pattern of parallel speciation (Poisot, 2015; Thompson et al., 2017). In this sense, theoretical and empirical studies are important in understanding how ecological and coevolutionary dynamics can affect interactions between organisms and, in turn, species diversification. Overall, these studies demonstrate that the effect of coevolution on diversification is extremely dependent on the type of ecological interaction present, as well as the phenotypic mechanisms behind these interactions. They suggest that many interactions considered as generators of diversification may not do so. In cases where selection acts on the convergence of phenotypes, coevolution may even reduce diversification. In turn, antagonistic interactions that impose a cost on phenotype matching can increase diversity, such as in host-parasite associations. Given sufficient time, the selection regime in this type of interaction is altered and tends to increase genetic and phenotypic variability. Increased variability promotes the emergence of generalists and the possibility of new ecological opportunities (Best et al., 2010; Gilman et al., 2012; Hall et al., 2011; Paterson et al., 2010; Scanlan et al., 2011; Simmons et al., 2011; Thompson, 2012; Yoder and Nuismer, 2010). However, this opens the opportunity for further colonization (host-switch) and a cophylogenetic structure would emerge through phylogenetic tracking rather than cospeciation (Brockhurst et al., 2005; Poisot, 2015; Vienne et al., 2013).

Currently, the theoretical and empirical framework on which maximum cospeciation was built is unable to accommodate the available evidence on macroevolutionary processes and patterns. Colonization events (host-switch) are the most obtained as an explanation for speciation. (Huyse et al., 2005; Jackson and Charleston, 2004; Vienne et al., 2007, 2013). The inconsistencies between what has been inferred and observed, with what is expected in the current paradigm, makes room for the emergence of new paradigms. One option widely discussed as a substitute is the “Stockholm Paradigm”. This proposal seeks to integrate ecological and evolutionary processes, providing a new theoretical and empirical framework for understanding the mechanisms that lead to diversification on a macroevolutionary scale (Agosta, 2006; Agosta and Klemens, 2008; Agosta et al., 2010; Brooks et al., 2014, 2015; Hoberg and Brooks, 2008). Although it has been elaborated within a context of host-parasite interaction, with a focus primarily on emerging infectious diseases, it allows us to

understand the evolution of interspecific ecological associations as a whole (Araujo et al., 2015; Brooks and Hoberg, 2007; Hoberg et al., 2015; Hoberg and Brooks, 2015).

One of the most commonly used system models for studying historical associations has been organisms of Phthiraptera, the order of insects popularly known as lice (*i.e.*, Johnson et al. 2011, 2009; Sweet et al. 2016; Sweet and Johnson 2016, 2018). Although Phthiraptera is a good model system, there are some other groups that have the same potential to be a model system, such as Streblidae (Graciolli and Carvalho, 2012). Streblidae is a family of obligate ectoparasite bat flies, hematophagous and considered highly specialized in relation to their hosts (Dick et al., 2016). The long and close association between Streblidae species and their hosts provides an interesting scenario from which is possible to study the ecological and coevolutionary dynamics between parasites and hosts, and enhance our knowledge of the coevolutionay process by testing hypothesis in a broad context (Dick and Patterson, 2006; Graciolli and Carvalho, 2012; Tello et al., 2008).

Assuming the Stockholm Paradigm as a theoretical framework for understanding the relationship between species of Streblidae and their hosts on a macroevolutionary scale, it would be expected to find a cophylogenetic pattern that pointed to a greater number of host-switch than cospeciation events, regardless of the specificity considered in the association. In the only cophylogenetic study done so far with Streblidae, Graciolli and Carvalho (2012) analyzed the historical association between *Trichobius phyllostomae* group and their hosts. They found host-switch as the main event in the association between lineages. Streblidae contains five subfamilies, 33 genera and 239 species, and it has a geographic distribution where species, genera and subfamilies that occur in the New World do not occur in the Old World (Dick and Graciolli, 2013). Given this characteristic geographical distribution, even cases of tree congruence need to be carefully analyzed before assuming any hypothesis of coevolution. In such cases, it would be important to look at the host biogeography to understand the patterns. Studies on the main host family of Streblidae in the New World (Phyllostomidae) suggest the importance of biogeographic events, as well as changing in the feeding behaviours, for the diversification of these bats (Pavan and Marroig, 2017; Rojas et al., 2011, 2016; Shi and Rabosky, 2015). However many questions are still open regarding relationships within Streblidae. Most phylogenetic studies with Streblidae have focused on the relationships between the families of Hippoboscoidea, and have been performed based only on some molecular markers (*i.e.*, Nirmala et al. 2001, with

16S and 18S; Dittmar et al. 2006, with 16S, 28S, COI and CAD; Petersen et al. 2007, with 16S, 18S, COII and CytB; Kutty et al. 2010, with 12S, 16S, 18S, 28S, COI, CytB, EF1α and CAD). The relationship between Streblidae subfamilies is still uncertain and poorly defined, as are the internal relationships. Therefore, reconstructing a relationship hypothesis for the interest group in Streblidae is crucial before starting a study of the historical association between the species of Streblidae and their hosts.

Although the initial project proposal was focused on the subfamily Streblinae (Diptera: Streblidae), we decided to expand the scope of the study in the chapter 2 to allow us to make better use of the data at our disposal. Still, in chapter 3 we kept the study focused only on the subfamily Streblinae. Thus, in chapter 2 we present a molecular phylogeny of the New World Streblidae inferred from mitochondrial genes, which includes calibrated divergence time estimate. We sequenced 32 mitochondrial genomes to complement the existing data, representing 34 species of New World Streblidae. The phylogeny is composed by 67 species and 20 genera of the three subfamilies of the New World Streblidae. Based on a much broader taxon sample for the subfamily Streblinae, we combined cophylogenetic analysis with ancestral host reconstruction to address three objectives: (*i*) propose a phylogenetic hypothesis among the three subfamilies of Streblidae of the New World, including an estimate of divergence times, (*ii*) assess the monophyly and relationships among genera of Streblinae, and (*iii*) understand the evolutionary history of associations between Streblidae species and their hosts using the species of Streblinae as a model system. In chapter 3, we perform the phylogenetic analysis of the subfamily Streblinae based on morphological evidence to verify its monophyly, propose a relationship hypothesis between the genera of the subfamily, as well to evaluate the characters used so far to delimit and characterize the subfamily and the genera included in it. Taxonomic sampling for subfamily includes all currently valid genera and species, except the species *Strebla mexicana* Rondani whose the only known type specimen is lost.

Chapter 2

Jump to evolve fast: how host-switch shaped the evolutionary history of New World Streblidae

2.1 Abstract

The paradigm upon which much of the theoretical and empirical framework was based for the study of historical associations has not been able to accommodate the available evidence. Host-switch has been the main event found to explain the association in parasite-host systems. These inconsistencies enabled the emergence of the "Stockholm Paradigm". Streblidae is a family of obligate, blood-feeding ectoparasite flies of bats. The association between species of Streblidae and their hosts may be a good model system to understand the host-parasite relationship under the Stockholm Paradigm. We present the most comprehensive molecular phylogeny of New World Streblidae to date, including all three subfamilies of the New World, a broad sampling for the subfamily Streblinae, and a fossil-calibrated estimates of divergence dates. Our analysis supports the monophyly of New World Streblidae, Nycterophiliinae and Streblinae, but a non-monophyletic Trichobiinae. New World Streblidae arose at Lower Miocene, near the diversification period of the main host clades. Host-switch is recovered as the main event acting in the associations between the species of Streblidae and their hosts,which is congruent with the expectations of the Stockholm Paradigm. Finally, we suggest that roost ecology may be an important mechanism for the events involved in the association between species of Streblidae and their hosts.

2.2 Introduction

Most of the theoretical and methodological development for macroevolutionary approaches in coevolutionary studies has been elaborated on a "maximum cospeciation paradigm". Within this paradigm, specificity is the cause of coevolution and it is hypothesized that the potential for new interactions will be minimal (Hoberg and Brooks, 2008; Brooks et al., 2015). In this sense, it is expected that there would be a congruence between the compared phylogenies, where: (*i*) the phylogeny of one group would be a "mirror" of the phylogeny of the other group, and (*ii*) the interaction occurs between species occupying similar positions in their respective phylogenies. However, this expectation led to the misconception that the examination of congruence between phylogenies would be sufficient evidence of coevolution, in which cospeciation and coevolution are used as synonyms (Bronstein, 2015; Poisot, 2015; Vienne et al., 2013; Thompson, 2005). The problem with this approach is that macroevolutionary patterns are the product of such a large timescale that the events would be difficult to distinguish. Thus, there is no way to assign coevolutionary processes to the recovered patterns of speciation without additional evidence (Bronstein, 2015; Poisot, 2015; Thompson et al., 2017).

Specificity is one of the characteristics commonly observed in parasite-host associations, but colonization of new hosts by parasites is pointed as the main force behind the diversification of interactions as well as species. From these assumptions, emerges the problem: if most parasites appear to be specialized to a particular host, how can they colonize new hosts? This is known as the "Parasite Paradox", which the "Stockholm Paradigm" proposes to solve (Agosta et al., 2010; Brooks et al., 2015). This proposal seeks to integrate ecological and evolutionary processes, providing a new theoretical and empirical framework for understanding the mechanisms that lead to diversification on a macroevolutionary scale (Agosta, 2006; Agosta and Klemens, 2008; Agosta et al., 2010; Araujo et al., 2015). From the perspective of the "Stockholm Paradigm", it is possible to predict that cospeciation will not be the norm, but the exception. Since that, 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 and Brooks, 2008; Forbes et al., 2017; Nieberding et al., 2010; Poisot, 2015; Ricklefs et al., 2014; Vienne et al., 2009).

Species of the family Streblidae are a suitable model to assess these questions. Along-

side with Nycteribiidae, Streblidae is a dipteran family of obligate ectoparasites of bats, hematophagous and considered highly specialized in relation to their hosts (Dick et al., 2016). It has 239 valid species, 33 genera and five subfamilies, three of which are unique to the New World (Nycterophiliinae, Streblinae and Trichobiinae) and two from the Old World (Nycteriboscinae and Ascodipterinae) (Dick and Graciolli, 2013). The relationship between Streblidae subfamilies is uncertain and poorly defined, as are the internal relationships. Of the few phylogenetic studies with Streblidae, almost all focused on interfamilial relationships in Hippoboscoidea (Nirmala et al., 2001; Dittmar et al., 2006; Petersen et al., 2007; Kutty et al., 2010). A study based on morphological characters proposed different hypotheses for the relationship between genera of Streblidae, but with several incongruities between the hypotheses (Guerrero, 2019). Only a single work focused on interspecific relationships in Streblidae, which reconstructed the *Trichobius phylostomae* group phylogeny, based on morphological characters (Graciolli and Carvalho, 2012). Although the New World presents exclusively about 70% of the known diversity of Streblidae (Dick and Patterson, 2006), most phylogenetic studies conducted so far does not take into account such distribution. Due to the reduced sampling, a huge gap remains about the phylogenetic knowledge between and within the families of Hippoboscoidea.

Streblidae species have as hosts bats belonging to the New World families of the suborder Vespertilioniformes, but with the majority of associations on bats of the family Phyllostomidae (Wenzel et al., 1966; Graciolli and Carvalho, 2001; Dick and Patterson, 2006). Most species of Streblidae are considered host specific, but some species have records of occurrence in various host species (Dick and Patterson, 2006, 2007; Dick, 2007). Assuming the "Stockholm Paradigm" as a theoretical framework for understanding the relationship between Streblidae species and their hosts on a macroevolutionary scale, it would be expected to find a cophylogenetic pattern pointing to a greater number of new colonizations than cospeciation events, regardless of the specificity considered in the group. In the only cophylogenetic work performed so far, Graciolli and Carvalho (2012) analyzed the historical association between *Trichobius phyllostomae* group flies and their hosts of the subfamily Stenodermatinae and found new colonization as the main event in the association between lineages. Thus, little is known about the history that connects to its hosts.

In the present study, we present a molecular phylogeny of the New World Streblidae

inferred from mitochondrial genes, which includes calibrated divergence time estimate. We sequenced 32 mitochondrial genomes to complement the existing data, representing 34 species of New World Streblidae. The phylogeny is composed by 67 species and 20 genera of the three subfamilies of the New World Streblidae. Based on a much broader taxon sample for the subfamily Streblinae, we combined cophylogenetic analysis with ancestral host reconstruction to address three objectives: (*i*) propose a phylogenetic hypothesis among the three subfamilies of Streblidae of the New World, including an estimate of divergence times, (*ii*) assess the monophyly and relationships among genera of Streblinae, and (*iii*) understand the evolutionary history of associations between Streblidae species and their hosts using the species of Streblinae as a model system.

2.3 Material and Methods

2.3.1 Sampling for mitogenome sequencing

We sampled specimens of all four genera of Streblinae, including two species of *Anastrebla*, one of *Metelasmus*, one of *Paraeuctenodes* and 20 of *Strebla*. We also sampled eight species of different genera of Trichobiinae. At all, we sequenced 33 specimens, totalizing 32 species (Tables A.1 and A.2). Two of these specimens were from *Strebla mirabilis* (Waterhouse, 1879), but from different locations and hosts.

We used freshly collected specimens, tissue collections specimens and museum specimens of Streblidae. We collected the species *Anastrebla caudiferae* Wenzel, 1976 and *Megistopoda aranea* (Coquillett, 1899) in the Núcleo Pedra Grande, Parque Estadual da Cantareira, São Paulo (Tables A.1 and A.2; Supplemental Material 1)[1]. We fixed the samples in 96% ethanol and stored them at −20 °C, as described in Trevisan et al. (2019). For all other species we obtained samples from the following insect collections: Laboratório de Sistemática e Biogeografia de Insecta, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil (LASBI-USP); Laboratório de Taxonomia e Filogenia de Tripanossomatídeos, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil (LTFT-USP); Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul, Mato Grosso do Sul, Campo Grande, Brazil (ZUFMS).

The samples from LASBI-USP and LTFT-USP were fixed in 96% ethanol and stored

[1] Supplemental Material can be obtained in the link `https://tinyurl.com/yy7dln22`

at −20 °C, however the samples from ZUFMS were fixed in 70% ethanol and stored at room temperature. These museum samples were collected between 2004 and 2017, but for the species *Strebla diaemi* Wenzel, 1966 the collection data are not known (Tables A.1 and A.2).

2.3.2 Mitogenome sequencing

We extracted total genomic DNA using Agencourt DNAdvance – Nucleic Acid Isolation Kit (Beckman Coulter). However, due to the small amount of tissue, we performed changes to the manufacturer's protocol as described in Pinto-da Rocha et al. (2014). The head, thorax and legs were used to avoid cross contamination by the host's blood. We also employed all standard precautions to minimize contamination, and we measured the purity and amount of DNA extractions as described in Trevisan et al. (2019). We deposited the specimen vouchers obtained from LASBI-USP and LTFT-USP at MZUSP (Museu de Zoologia da Universidade de São Paulo, Universidade de São Paulo,São Paulo, SP, Brazil).

We performed the whole-genome skimming approach to sequence the mitogenomes, which sequences the whole genome at low nuclear genome coverage, including both mitochondria and nuclear content, through random shearing and inexpensive multiplexing (Trevisan et al., 2019). To achieve this goal, we used Nextera XT DNA Library Preparation Kit (Illumina) to prepare indexed paired-end libraries and we followed all the steps described in Trevisan et al. (2019), to before starting libraries preparation, to determine the quality, size and concentration of the sequencing libraries, and to library normalization. We pooled the genomic DNA libraries of all 33 samples of Streblidae, with more two libraries of Rhinebothriidae (Trevisan et al., 2019).

We sequenced these libraries using the Illumina NextSeq 550 System, with a High-Output Kit to generate paired-end reads of 150bp. We performed all DNA sequencing in the Core Facility for Scientific Research – University of São Paulo (USP) (CEFAP-USP). Since we manually normalized library concentrations to avoid overdilution of samples, we performed a Spearman correlation for non-parametric data (Zar, 1999) after the sequencing, to evaluate whether the amount of the generated data varied according to the library concentration before dilution for pooling.

2.3.3 Mitogenome assembly, annotation and composition

We perfomed the assembly of mitogenomes through baiting and iterative mapping, using the softwares MIRA v4.0 (Chevreux et al., 1999) and MITOBim v1.9 (Hahn et al., 2013). We used the reference mitogenome sequence of the house fly as bait (*Musca domestica* L., GenBank Accession Number KM200723). After assembly, we made a preliminary annotation using the web server for the annotation of metazoan mitochondrial genomes MITOS2 (Bernt et al., 2013, available at `http://mitos2.bioinf.uni-leipzig.de`), with the genetic code "5 Invertebrate". We used the BLAST online tool (Altschul et al., 1990) to check for possible contamination in the sequences, since some samples had more than ten years, and the origin of some specimens were not known. We performed additional search and validation of tRNA sequences using ARWEN (Laslett and Canback, 2008) and tRNAscan-SE (Lowe and Eddy, 1997; Schattner et al., 2005). For a few tRNA genes that could not be identified by these softwares, we found them by manual inspection. Finally, we confirmed and edited manually the automated annotation by comparison to 15 published reference mitogenomes of flies, from ten different families of Diptera (Table A.3). The reference mitogenomes were obtained through the NCBI Organelle Genome Resourses. For comparison of mitogenomes, we splitted each one by gene and aligned the sequences using MAFFT v7.4 (Katoh, 2002; Katoh and Standley, 2013). After alignment, we examined and edited them using AliView v1.25 (Larsson, 2014), and analyzed the nucleotide base composition with MEGA X (Kumar et al., 2018). We aligned equivalent portions of the control region of all specimens, but due to high levels of variation and the difficulties experienced, we did not include the control region in further analyses.

2.3.4 Phylogenetic analysis and divergence time estimation

2.3.4.1 Taxon sampling and sequence alignment

In addition to the sequenced and the reference mitogenomes, we searched in the GenBank database (Benson et al., 2018) for sequences of mitochondrial genes of Streblinae species of which we could not sequence the mitogenomes. We also searched for other Streblidae species that would be important for understanding the delimitation of Streblinae. We obtained additional sequences for 34 species, comprising three species of Streblinae, 29 species of Trichobiinae and two species of Nycterophiliinae (Supplemental Material 2). Of

the 29 Trichobiinae species, we sampled 24 of the *Trichobius*, the genus with the largest number of species within Streblidae (Dick and Graciolli, 2013). The genus *Trichobius* is traditionally divided into nine morphological groups (Dick and Graciolli, 2013), of which we sampled seven groups. At the end, a total of 80 terminals were used. The ingroup molecular dataset included 67 species and 20 genera of the three subfamilies of the New World Streblidae (26 species and four genera of Streblinae, 39 species and 15 genera of Trichobiinae and two species and one genus of Nycterophiliinae). Additionally, 13 species of nine other families of Diptera were used as outgroup. We aligned the nucleotide sequences using MAFFT v7.4 (Katoh, 2002; Katoh and Standley, 2013) and edited them in AliView v1.25 (Larsson, 2014). We concatenated the nucleotide sequences using the SequenceMatrix v1.8 (Vaidya et al., 2011).

2.3.4.2 Maximum likelihood analyses

For maximum likelihood analyses, we used IQ-TREE v1.6 (Nguyen et al., 2015) implemented on Cipres Science Gateway (Miller et al., 2010). We 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 1,000 replicates. We performed two different analyzes using nucleotides sequences. In one analysis was used only the sequenced mitogenomes, and in the other the mitogenomes and the data obtained from GenBank. Since model selection may be unnecessary when topologies are the desired output (Abadi et al., 2019), we performed two analyses for each dataset. One analysis with the model selection for each gene carried out by ModelFinder (Kalyaanamoorthy et al., 2017) in IQ-TREE, and another under the most parameter-rich model for each gene, GTR+R4+F. The selected models were the same in each analysis and are presented in Table A.4.

2.3.4.3 Bayesian analysis and divergence time estimation

We simultaneously inferred phylogeny and divergence times using BEAST2 v2.5.2 (Bouckaert et al., 2019) implemented on Cipres Science Gateway (Miller et al., 2010). We used the only known fossil of Streblidae to calibrate the phylogeny. *Enischnomyia stegosoma* Poinar and Brown, 2012 belongs to the subfamily Nycterophiliinae and was described from Dominican amber. Although controversial, the latest proposed age range

of Dominican amber was estimated at 20.43–13.65 Ma, dated as Miocene (Iturralde-Vinent and MacPhee, 1996; Poinar and Brown, 2012). We used the Fossilized Birth-Death (FBD) process as the tree prior (Stadler, 2009; Heath et al., 2014), under the lognormal relaxed clock (Drummond et al., 2006). Since *E. stegosoma* belongs to Nycterophiliinae, we incorporate this prior information by creating a monophyletic clade constraint with *E. stegosoma*, *Nycterophilia coxata* and *N. parnelli*. To accommodate uncertainty in the date, we specified the origin as a lognormal distribution, with the mean to 8.5, the standard deviation to 1.0., and the offset to 12.5 (in real space), which translates to a 95% probability range of 13.5–39.2 Ma for the included fossil. To investigate the sensitivity of the estimates, we performed two additional analyses using the Yule process (Yule, 1925; Harding, 1971) as the tree prior, under the lognormal relaxed clock (Drummond et al., 2006). We ran one analysis without any calibration nor clade constraint to compare the obtained topology. The other analysis, we specified a prior distribution on the origen node of the New World Streblidae, using the same parameters as in the FBD estimation. For all analyses, we ran a Markov chain Monte Carlo (MCMC) (Drummond et al., 2002) chain to 200 million generations, logging parameters every 20,000. We used bModelTest (Bouckaert and Drummond, 2017), with the option "namedExtended", to select the most appropriate substitution model for each gene. The selected model for each gene are presented in Table A.4. We also ran an analysis sampled only from priors to verify if the data were generating the posterior probabilities. Sampling from priors allows to check whether the priors were proper or the various priors do not produce an unexpected joint prior in combination (Sanders and Lee, 2007; Drummond and Bouckaert, 2015). We used Tracer v1.7 (Rambaut et al., 2018) to check whether the effective sample sizes (ESS) of all the parameters reached greater than 200. Finally, we calculated the maximum-clade-credibility tree using TreeAnnotator v1.8.4 implemented on Cipres Science Gateway (Miller et al., 2010) and employed FigTree v1.4.4 for visualization of the tree.

2.3.5 Historical association

2.3.5.1 Host phylogeny

We reconstructed a phylogeny containing species from both Emballonuridae and Phyllostomidae, as well all other 12 families of the suborder Vespertilioniformes (Furipteridae, Miniopteridae,

Molossidae, Mormoopidae, Mystacinidae, Myzopodidae, Natalidae, Noctilionidae, Nycteridae, Thyropteridae, Cistugidae and Vespertilionidae). We used as base the sampling of taxa and the genes used by Rojas et al. (2016). We completed the matrix with sequences obtained from GenBank (Benson et al., 2018) for species of the families Miniopteridae, Molossidae, Natalidae, Nycteridae, Cistugidae and Vespertilionidae. At all, we sampled 364 species of all 14 families of Vespertilioniformes (Supplemental Material 3). Futhermore, we used three species as outgroup *Pteropus vampyrus* (Pteropodidae), *Megaderma lyra* (Megadermatidae), *Rhinolophus ferrumequinum* (Rhinolophidae). We aligned sequences using MAFFT v7.4 (Katoh, 2002; Katoh and Standley, 2013) and inspected and edited them in AliView v1.25 (Larsson, 2014). We concatenated the aligned and edited sequences using SequenceMatrix v1.8 (Vaidya et al., 2011). We performed a maximum likelihood analysis, using IQ-TREE v1.6 (Nguyen et al., 2015) implemented on Cipres Science Gateway (Miller et al., 2010). We 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 1,000 replicates. We performed the analysis using the most parameter-rich model for each gene, GTR+R4+F.

2.3.5.2 Associations between Streblinae and the hosts

Wenzel et al. (1966) defined the primary host as the host with the highest number of records for a species of parasite. Although survey studies suggest very high host specificity among bat flies (Dick, 2007), there is no consensus to which would be the primary host for each Streblidae species. In general, the primary host is defined for each area where survey studies are performed, using the values of prevalence and mean intensity (*i.e.*, de Vasconcelos et al. 2016; Durán et al. 2017; Bezerra and Bocchiglieri 2018). However, we are interested in establishing historical associations and not just local or occasional and accidental interactions. Thus, assuming the existence of historical associations and specificity in these associations, we expect to find the same associations occurring in different areas and periods. To achieve this goal, we compiled all studies published from 1966 until June 2019 with records of Streblinae species and their hosts. We used 1966 as the start year because of the work of Wenzel et al. (1966), which defined Streblinae as currently known, as well as described many of the subfamily species. We also compiled records from specimens deposited at two scientific collections: ZUFMS and Field Museum of Natural

History, Chicago, Illinois, USA (FMNH, 2019, Vertebrate ectoparasite collection available at `https://collections-zoology.fieldmuseum.org`). To avoid duplicated data inflating the numbers of associations, we applied the following criteria: (*i*) we used only one host-parasite association record per area of each study; (*ii*) we did not take into account studies that used data from other works; (*iii*) we did not add the records of the scientific collections that were already published; (*iv*) and we did not add records from scientific collections of the same collection area.

To calculate the prevalent association, we used:

$$P = \frac{N}{Nt} \times 100$$

Where P is the prevalent association, N the number of association records between the Streblinae species and the host species, and Nt the total number of association records for the Streblinae species. We used the highest value of P for a single association among all species to establish a threshold and to determine the primary host of each species of Streblinae. We used this threshold to avoid that poorly sampled species may have accidental associations considered as primary associations. Furthermore, we expect the values of P will be above the threshold for Streblinae species with many records, even with a high value of threshold.

2.3.5.3 Cophylogenetic analysis

We conducted the cophylogenetic analysis using distance-based and event-based methods. We used the tree of New World Streblidae generated by both methods, the maximum likelihood and bayesian. Before the cophylogenetic analysis, we pruned the parasite tree and the host tree to remove outgroups and duplicates using the R v3.6.1 (R Core Team, 2019) package "ape" v5.2 (Paradis and Schliep, 2019), so it remained only the Streblinae and the Vespertilioniformes species. We use the R package "phytools" v0.6-60 (Revell, 2012) to produce a tanglegram between Streblinae and Vespertilioniformes trees. For distance-based analyses, we used ParaFit (Legendre et al., 2002) and PACo (Balbuena et al., 2013) to evaluate the congruence between host and symbiont phylogenies. The null hypothesis of ParaFit assumes the relationship pattern of the two groups as independent, assessing how much each individual link contributes to the overall congruence (Legendre et al., 2002). PACo evaluates the congruence of the parasite and the host phylogeny

through a residual sum of square goodness-of-fit test. We used the R implementation of ParaFit in the package "ape", running 100,000 permutations with Cailliez correction for negative eigenvalues. ParaFit runs multiple tests to calculate *p-values* for each individual link, and because of that it is necessary to correct the raw output in order to control the false positive. We corrected individual link *p-values* using the Benjamini-Hochberg correction for false discovery rate (Benjamini and Hochberg, 1995). We ran PACo based on patristic distances for 100,000 permutations, using the packages "ape" and "vegan" v2.5-5 (Oksanen et al., 2019). For the event-based analysis, we used Jane v4 (Conow et al., 2010). We ran the analysis by changing the parameters "Population Size" and "Number of Generations" to see how they affect the quality of the solutions. We used the following values for "Population Size" and "Number of Generations" respectively: 100/1500, 100/350, 100/100, 50/500, 50/100 and 25/100. We run all analyses 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 100 random tip mapping and the same parameters implemented in reconciliation analysis.

2.3.5.4 Ancestral host reconstruction

We used the R package BioGeoBEARS (Matzke, 2013, 2014) to infer possible ancestral hosts. BioGeoBEARS is designed to estimate the fit of models of geographic range evolution to the phylogenies and the distribution of organisms. However, we used to estimate the host association history on the Streblinae phylogeny. It performs model selection to compare the likelihood of different models: Dispersal-Extinction-Cladogenesis (DEC) (Ree, 2005; Ree and Smith, 2008), DEC+J, Dispersal-Vicariance-Analysis (DIVALIKE, with Maximum Likelihood as optimization criteria) (Ronquist and Cannatella, 1997), DIVALIKE+J, BAYAREALIKE (Landis et al., 2013), and BAYAREALIKE+J. The +J model variant implements the inclusion of founder-event speciation (Matzke, 2014). The founder-event speciation allows the daughter lineage to jumps to a new range outside the range of the ancestor at cladogenesis Matzke (2013). We tested the model fit of these six models using the Akaike Information Criterion (AIC) and Akaike weights. To implement BioGeoBEARS, we delimited host groups based on clades recovered in our maximum likelihood phylogeny of hosts and on the classification for the Phyllostomidae family proposed by Baker et al. (2003). At all, we established eight groups: Micronycterinae

(A), Desmodontinae (B), Phyllostominae (C), Glossophaginae (D), Lonchophyllinae (E), Carolliinae + Glyphonycterinae (F), Stenodermatinae (G) and Emballonurinae (H).

BioGeoBEARS was been originally designed to perform inference of biogeographic history on phylogenies (Matzke, 2013, 2014). Thus for comparison, we need to establish a parallel with the cophylogenetic analysis. There is no clear consensus on all events that should be considered in a cophylogenetic analysis. Not even the available softwares have the same events available for analysis. However, they can be divided into four main events: (*i*) cospeciation, when host and parasite species co-diverge; (*ii*) host-switching, when the parasite species successfully colonizes a new host species; (*iii*) duplication, when a parasitic lineage diverges without the stimulation of host speciation, resulting in the co-occurrence of related parasitic species on the same host species; and (*iv*) sorting events, which include events such as extinction and missing the boat (Paterson and Banks, 2001; Page, 2003; Conow et al., 2010; Drinkwater and Charleston, 2014; Baudet et al., 2015). Hereafter, we will consider vicariance as similar to cospeciation, sympatry as duplication, and extinction and range-switching as sorting events. When the +J model is implemented, we believe that the most appropriate would be to interpret the founder-event as host-switch. In its turn, dispersal has been considered by some authors to be analogous to host-switch (Page and Charleston, 1998). Since BioGeoBEARS considers dispersal as range expansion without the need for speciation, we believe the interpretation of dispersal events will need to be analyzed case by case. In this sense, it can be interpreted as host-switch (with speciation) or incomplete host-switch (without speciation, *sensu* Clayton et al. 2003).

2.4 Results

2.4.1 Mitogenome sequencing, organization and structure

We obtained approximately 699 million raw pair-end reads for the 33 Streblidae samples, with a total of 175.1 Gb raw data (Table A.5). Even normalizing library concentrations, the amount of data obtained for each sample was significantly correlated with the concentration of libraries before starting manual normalization ($r = 0.8168$, *p-value* < 0.0001; Figure B.1; Table A.5). However, the number of reads was not crucial for genome assembly. For samples with a value of average consensus quality below 70, we could not recover all the genes. This was the case for the species *Parastrebla handleyi*,

Strebla asternalis, *S. galindoi*, *S. matsoni* and *S. obtusa*, that had a greater number of reads than other species for which we recovered all genes of the mitogenome (Figure B.2; Table A.5; Supplemental Material 4). All five samples were fixed in 70% ethanol and stored at room temperature, although other 13 samples preserved at the same conditions have been successfully sequenced. Unfortunately after assembly and annotation, we found the material sequenced for *Strebla diaemi* and *S. kohlsi* were contaminations. The verification by BLAST and sequence comparison demonstrated the possibility of *S. diaemi* and *S. kohlsi* sequences being from vertebrate genomes.

We recovered the 37 genes found in the typical Metazoan mitogenome for 25 sequenced samples. It includes 13 protein coding genes (PCGs), 22 RNA transporters (tRNAs) and two ribosomal DNAs (rDNAs). However, we recovered a few genes only partially for these samples. They were almost exclusively of ND, with ND4, ND5 and ND6 as the most frequent, and the genes ND2, ND4L and rrnS. For *Strebla galindoi*, with an assembly average quality of 69, we recovered partially seven genes, but we did not recover the tRNA proline. Further, for *Parastrebla handleyi*, with an assembly average quality of 57, we recovered partially 14 genes, and we did not recover three genes; *Strebla asternalis*, with an assembly average quality of 57, we recovered partially 13 genes, and we did not recover 12 genes; *S. matsoni*, with an assembly average quality of 54, we recovered 21 genes, and we did not recover five genes; *S. obtusa*, with an assembly average quality of 63, we recovered partially 15 genes, and we did not recover three genes (Figure B.2; Table A.5; Supplemental Material 4).

The nucleotide composition of all sequenced samples was biased toward A and T for both sequence assembled and individual genes (Supplemental Material 5). We found the canonical ATN start codons for 12 PCGs of the sequenced mitogenomes, while the CO1 had the TCG start codon. We found the stop codon TAA as unique to the PCGs ATP6, ATP8, CO1, CO3, ND2, ND4L and ND6. For the PCGs CYTB, ND3 and ND5 we found the stop codons TAA and TAG. We found incomplete stop codons (T and TA, poly-adenylated to TAA post-transcriptionally) for the PCGs CO2, ND1 and ND4, along with the stop codon TAA (Supplemental Material 4).

2.4.2 Phylogenetic analysis and divergence time estimation

We recovered a well supported monophyly of New World Streblidae in all analyses of maximum likelihood. We also recovered the monophyly of the subfamilies Nycterophiliinae and Streblinae with a high branch support. On the other hand, we recovered Trichobiinae as paraphyletic in all analyses, and with only one morphological group of *Trichobius* as monophyletic (*parasiticus* complex) (Figure B.3). We did not find any differences between the relationships obtained, nor support discrepancies in each maximum likelihood analysis (Supplementals Material 7 and 8). However, the analyses with the most parameter-rich model had always a better score than the analyses performed with model selection (New World Streblidae phylogenies shown in Supplemental Material 7: $\text{log-likelihood}_{\text{ModelFinder}} = -208131.699$, $\text{log-likelihood}_{\text{GTR+R4+F}} = -207971.901$; phylogenies with only the sequenced mitogenomes shown in Supplemental Material 8: $\text{log-likelihood}_{\text{ModelFinder}} = -201160.394$, $\text{log-likelihood}_{\text{GTR+R4+F}} = -201002.344$). Based on the recovered relationships in our analyses, Nycterophiliinae is the sister taxon of all other New World Streblidae, whereas Trichobiinae is a grade divided in at least six clades (Figure B.3). Regarding the relationships within Streblinae, we recovered *Anastrebla* as monophyletic in all analyses and as sister taxon of the other Streblinae genera; *Paraeuctenodes* as sister taxon of the clade containing *Strebla* + *Metelasmus*; and *Strebla* as paraphyletic. Although the clade *Strebla* + *Metelasmus* was strongly supported in all analyses, the positioning of the species was poorly supported as sister taxon of the clade containing *S. consocia*, *S. christinae*, *S. diaemi*, *S. hertigi*, *S. tonatiae*. (Figure B.3).

Despite a few differences in the positioning of some taxa, we recovered a hypothesis from the Bayesian analysis very similar to that obtained with the maximum likelihood analysis (Figure B.4). A comparative figure between phylogenies inferred from maximum likelihood analysis and bayesian analysis is shown in Supplemental Material 6. We also recovered the monophyly of New World Streblidae, Nycterophiliinae and Streblinae. However, we obtained a low support for New World Streblidae and Streblinae. Again, we recovered Trichobiinae as paraphyletic relative to Streblinae, but with two morphological groups of *Trichobius* as monophyletic (*caecus* group and *parasiticus* complex). The relationships within Streblinae were very similar to those obtained in maximum likelihood analysis, with the difference that *S. consocia* were recovered as sister taxon of *S. tonatiae* and *S. curvata*

and *S. galindoi* were not revovered as sister taxa (Figure B.4). The only difference between the topologies inferred from the two processes (FBD and Yule) was the relationship within the clade composed by the *caecus* group (*Trichobius caecus*, *T. galei*, *T. johnsonae* and *T. yunkeri*).

Concerning the divergence time estimation, the results were almost identical. However, we present here only the estimates obtained by Yule process, since it had a better score (mean likelihood$_{\text{FBD}} = -207575.2973$, mean likelihood$_{\text{Yule}} = -207503.9896$) and the estimates obtained by the FBD process were fully included within the Yule process. We estimated the origin of New World Streblidae to the Lower Miocene, about 15.3 Ma (22.7–12.7 Ma 95% highest probability density interval, HPD). For Streblinae, we estimated the origin to the Upper Miocene, at approximately 8.2 Ma (12.6–5.7 Ma 95% HPD) (Figures B.5 and B.6). By our estimation, Streblinae genera may have diverged rapidly within a very short timeframe, between an interval from 12 to 3 Ma. The 95% HPD values for each node are given in Supplemental Materials 9 and 10.

2.4.3 Historical association

2.4.3.1 Host phylogeny

We recovered the monophyly of Vespertilioniformes bats, as well as of its 14 families, with a strong support (Supplemental Material 11). The family Mizopodidae was recovered as sister taxon of all other Vespertilioniformes, which consequently demonstrates that Noctilionoidea is not monophyletic. Emballonuridae and Nycteridae formed a clade (defined here as Clade 1), which is sister taxon of the clade containing the other 11 families (defined here as Clade 2). Clade 2 is divided into two other, defined as Clades 2.a and 2.b. Clade 2.a encompasses Cistugidae, Miniopteridae, Molossidae, Natalidae and Vespertilionidae. Clade 2.b encompasses Mystacinidae and a monophiletic New World Noctilionoidea (Furipteridae, Mormoopidae, Noctilionidae, Phyllostomidae and Thyropteridae). Regarding the two families of interest for the cophylogenetic analyses, we recovered all the Phyllostomidae subfamilies established by Baker et al. (2003) and the two known subfamilies of Emballonuridaethe (Simmons, 2005) as monophyletic (Figure B.7).

2.4.3.2 Associations between Streblinae and their hosts

At all, we compiled records from 90 studies, in addition to labelling data from the two scientific collections, ZUFMS and FMNH (Supplemental Material 12). The threshold was 20%, value of P for a single association obtained for *Strebla asternalis* with *Rhynchonycteris naso*. Thus, associations with values of P above 20% were considered primary and below as accidental. We obtained a total of 40 primary associations, showed in Supplementary Material 8, varying from 100% (*e.g. M. wenzeli* with *Sturnira lilium* and *S. cormurae* with *Cormura brevirostris*) to 22.1% (*e.g.*, *S. mirabilis* with *P. hastatus*).

Considering only the primary associations, Streblinae is almost exclusive to the bat family Phyllostomidae, with only three species of *Strebla* parasitizing bats of the family Emballonuridae. The genus *Anastrebla* presented as primary hosts only bats of the subfamilies Glossophaginae and Lonchophyllinae, while *Metelasmus* was exclusive to the subfamily Stenodermatinae. One of the species of *Paraeuctenodes* presented as a primary host a bat of the subfamily Carolliinae and the other species a bat of the subfamily Glossophaginae. On the other hand, *Strebla* presented primary hosts of nine different subfamilies of Phyllostomidae and one subfamily of Emballonuridae. The subfamily Phyllostominae had the largest number of primary associations, with 14 species of *Strebla* (Table A.6).

2.4.3.3 Cophylogenetic analysis and ancestral host reconstruction

The tanglegrams (Figure B.8) show no obvious congruence between Streblinae species and their hosts, despite the obvious concentration of associations into the Phyllostomidae bat family. Curiously the distance-based tests ParaFit and PACo presented discordant results. We found no significant fit between host and parasite phylogenies with the observed ParaFitGlobal statistic (*p-value* = 0.86 for maximum likelihood phylogeny; and *p-value* = 0.87 for bayesian phylogeny). We found no differences in any of the results of individual link tests, ParaFitLink1 and ParaFitLink2. No tests recovered links as significantly contributing to the global score after the Benjamini-Hochberg correction (Table A.7). In its turn, PACo yielded a $m^2_{XY} = 0.6921604$ with an associated permutational *p-value* = 0.00042 for the Maximum Likelihood phylogeny, and a $m^2_{XY} = 0.7156617$ with an associated permutational *p-value* = 0.00085 for the Bayesian phylogeny. Unlike ParaFit,

PACo rejected the hypothesis that the similarity between the phylogenies of Streblinae and their hosts arised by chance. Procrustes superimposition plot suggests two groups of host-parasite associations, one formed by the association with Phyllostomidae species, and the other by Emballunoridae species (Figures B.9A and B.10A). The bar plots of squared residuals indicate that a few links contributed significantly to m^2_{XY}. The links related to Emballonuridae were clearly incongruent with a history based on cospeciation, possibly being the result of host-switches. On the other hand, a part of the link between Streblinae and Phyllostomidae contributed relatively little to m^2_{XY}, and they are interpreted in PACo as likely coevolutionary links (Figures B.9B and B.10B).

In the event-based inference for both analyses, Jane recovered equally parsimonious solutions with a total of 53 cost events. Further, all analyses performed with different values for the parameters "Population Size" and "Number of Generations" obtained the same cost. The event-cost solutions recovered five cospeciation events, 19 host-switches, ten losses and five cases where Streblinae failed to diverge with the hosts (Supplemental Materials 13 and 14). The observed cost was significantly lower than by chance (*p-value* < 0.001). We obtained DEC+J as the best model to fit our data. The models with the +J founder-speciation were favored over the implementation of these models without +J. Likewise, in all +J models the value of jump dispersals (j) was higher than range-expansions (d) (Table A.8). Based on that, we can state that +J founder-speciation was the main event responsible for the associations between Streblinae species and their hosts. In addition, we recovered Glossophaginae as the most likely ancestral host subfamily for Streblinae, as well as for the clade containing the *Paraeuctenodes* and *Strebla* + *Metelasmus*. However, we recovered Phyllostominae as the most likely ancestral host of the clade *Strebla* + *Metelasmus* (Figure B.11).

2.5 Discussion

2.5.1 Mitogenome sequencing, organization and structure

Multiplexing is usually used to reduce the costs of high-throughput sequencing by poolling libraries in equimolar concentrations. This is particularly important in the case of genome skimming as it allows to start from heterogeneous extracts without prior enrichment, and to sequence a large number of samples at the same time (Timmermans et al.,

2010; Richter et al., 2015; Tilak et al., 2015). However, the libraries are often in a broad range of concentrations, which is overcome by normalizing libraries at a similar concentration. Thus, normalization process is a critical step in multiplex pool construction (Harris et al., 2010), and our results clearly demonstrate how problematic this step can be. In our case, 15 samples out of 33 had 74% of the total data generated for Streblidae (Table A.5; Figure B.1). However, more data did not guarantee that it would be possible to recover the complete mitochondrial genome. In fact, an amount of approximately 1.5–3 Gb (approximately 5–11 Mi reads) for well-preserved material was more than enough to recover all mitochondrial genes (Table A.5). Finally, manual normalization is not an appropriate approach to construction of multiplex pools for genome skimming. It is clear that with a well normalized pool, it would have been possible to sequence a much larger number of samples than those sequenced in the present work. In a comparison of normalization methods, Harris et al. (2010) demonstrated that quantitative DNA binding method yielded the best result for large multiplex amplicon pools. However, there is no comparison of methods focused on genome skimming approach. Once it sequences the whole-genome and not just amplicons, genome skimming can immensely increase the amount of sequencing necessary to fully assess, thus the improvement of the library normalization step is a point that needs further study.

Our results for gene organization and structure are consistent with those found for Streblidae and other dipterans (Li et al., 2015; Junqueira et al., 2016; Liu et al., 2017; Trevisan et al., 2019). The exception is the controlling region, as we do not use it in our analysis. However, an intriguing result was found in subunits 2, 4, 5 and 6 of the NADH dehydrogenase gene. Several samples had indels that could contain up to 268 bp (Figure B.2; Table A.5; Supplemental Material 4). In addition, many of these indels occurred in the form of repeated tandem regions. In general, the beginning and the end of the gene were conserved, with indels occurring more in the middle region. Even the two samples of *Strebla mirabilis* showed differences in the presence of these indels. The mitogenomes obtained by Trevisan et al. (2019) also showed inconsistencies in these genes. Due to the size of some indels, the possibility of introns was raised. The presence of introns in the NADH dehydrogenase gene is known for Cnidaria, for example (Beagley et al., 1996). However, this is a remote possibility in insects, which have a large number of sequenced mitogenomes, but without any intron record. A second possibility is problems with the

mitogenome assembly. Since the median region has a large amount of A and T bases, a mounting error may have occurred, with the insertion of reads containing tandem repeats from other regions. Nonetheless, this possibility still needs to be further evaluated.

2.5.2 On the relationships between subfamilies of the New World Streblidae

This is the first molecular phylogenetic analysis to asses the relationships between the three subfamilies of the New World Streblidae, as well as on a large sampling of species for the subfamily Streblinae. This is also the first estimate of divergence time provided to the family. Some of our results are congruent with previous phylogenetic studies. Although the monophyly of Streblidae has long been refuted (Griffiths, 1972; Dittmar et al., 2006; Kutty et al., 2010, 2019; Šochová et al., 2017), our results along with that of Dittmar et al. (2006) strongly support the monophyly of the New World Streblidae (Figures B.3 and B.4; ; Supplemental Materials 7 and 8). Wenzel et al. (1966) also discussed about the relationships within Streblidae based on morphological similarities. Interpreting the authors' statements within a phylogenetic context, they considered Trichobiinae and Streblinae related to each other than with Nycterophillinae. Our results support this hypothesis, since all analyses we recovered Nycterophillinae as sister taxon of the clade containing Trichobiinae and Streblinae (Figures B.3 and B.4).

The relationships among Trichobiinae and Streblinae have previously been studied by Dittmar et al. (2006). They found Trichobiinae paraphyletic relative to a monophyletic Streblinae. However, the sampling of both Trichobiinae and Streblinae did not allow a broader picture of the relationships between and inside the two subfamilies. Dittmar et al. (2006) pointed out a division of Trichobiinae into two distinct clades, one containing nearctic and neotropical species and another entirely composed of neotropical species. Nonetheless, Dittmar et al. (2006) samples ten species of Trichobiinae, all of them from *Trichobius*. Instead, our results support a division of Trichobiinae in at least four distinct clades, but without a clear division based on neartic and neotropical species. Despite a few differences in the positioning of some terminals in our analyses, the relationships obtained with different reconstruction methods were very consistent for Trichobiinae species (Figures B.3 and B.4).

2.5.3 The relationships within Trichobiinae

2.5.3.1 *Trichobius*

It is the genus of Streblidae with the largest number of species (68 species). Due to the number of species and the morphological differences within the genus, Wenzel et al. (1966) grouped it into eight morphological groups: *caecus*, *dugesii*, *dunni*, *longipes*, *major*, *pallidus*, *phyllostomae* and *uniformis*. However, even though the species may have morphological similarities between and within the groups, different authors report the difficulty to identify the species, which is reflected in the identification keys for the species of the genus (Wenzel et al., 1966; Wenzel, 1976; Guerrero, 1994, 1995). Thus, *Trichobius* as non-monophyletic is not a surprise, and not even a new result. Dittmar et al. (2006) and Graciolli and Carvalho (2012) had already demonstrated it. Even Wenzel et al. (1966), Wenzel (1976) and Guerrero (1994, 1995) were aware of this. All of them stated that the genus would need to be divided, and our results are in agreement with such a position. The problem here is that apparently even the morphological groups are not natural groups. Moreover, many morphological characteristics used to define morphological groups were used by Graciolli and Carvalho (2012) as phylogenetic characters and were recovered as homoplasies. Based on their morphological phylogeny, Graciolli and Carvalho (2012) suggested that *Trichobius* would be composed of the species of the groups *dugesii*, *dunni*, *longipes* and *uniformis*. However, our results do not support this hypothesis. Here, the genus *Trichobius* would be restricted only to the *parasiticus* complex, since it was recovered as monophyletic and presents the type species of the genus (*T. parasiticus*), while other genera would need to be established or revalidated to incorporate the other species (Figures B.3 and B.4). Below we discuss the results recovered for the species groups used in our phylogeny.

Groups caecus and dugesii: The species of the *caecus* group and *T. intermedius*, a species belonging to the *dugesii* complex, were recovered at the base of the clade containing Trichobiinae and Streblinae. Wenzel et al. (1966) stated that morphological group *caecus* probably should be regarded as a separate genus. However, our results do not fully support this hypothesis, given the presence of *T. intermedius* (*dugesii* complex), the breakdown of the group in the maximum likelihood analysis and the low supported monophyly of

the *caecus* group in the Bayesian analysis. The *dugesii* group is the largest in number of species (21 species and two subspecies) and is divided into two subgroups, the *dugesii* complex and the *parasiticus* complex (Dick and Graciolli, 2013). According to Wenzel (1976), the species of the *dugesii* group are the most difficult to identify and similar in most characters, especially the species of the *dugesii* complex. The *parasiticus* complex was the only well supported group recovered in our analysis. In contrast, neither the *dugesii* morphological group nor the *dugesii* complex were recovered as monophyletic. Besides *Trichobius intermedius* recovered along with the species of the *caecus* group, *Aspidoptera phyllostomatis* was recovered nested within the *dugesii* complex. In the morphological phylogeny of Graciolli and Carvalho (2012), the only character supporting the *dugesii* group was a homoplasy, as well as all the autapomorphies for the species *Trichobius tiptoni* (*dugesii* complex) and *T. parasiticus* (*parasiticus* complex). Thus, if the composition of the *dugesii* group is based on homoplastic characteristics and difficult to identify, it is possible that this is not in fact a natural group. If so, it would be necessary to split the *dugesii* group into two or more genera.

Groups longipes, major and uniformis: The *major* group is the second largest in number of species, comprising 18 species (Dick and Graciolli, 2013). Wenzel et al. (1966) supposed that the *major* group could be divided into distinct groups, which is supported here. The species of the *major* group were spread over three distinct and well supported clades, rejecting the hypothesis of Graciolli and Carvalho (2012) in which the morphological groups *caecus*, *major* and *pallidus* would constitute a monophyletic group. As well as obtained by Dittmar et al. (2006), *Trichobius corynorhini* and *T. major* were recovered here as a distinct clade, while *T. hirsutulus* were recovered along with species of the *longipes* group. In turn, *Trichobius parasparsus*, *T. sparsus* and *T. sphaeronotus* were recovered along with the *uniformis* group species *T. lonchophyllae* and *T. uniformis*. *Trichobius hirsutulus* is a species considered poorly known (Guerrero, 1994) and its grouping should be reevaluated in light of the results obtained here and those obtained by Dittmar et al. (2006). Regarding *T. corynorhini* and *T. major*, (Wenzel et al., 1966) hypothesized they could be a subgroup of the *major* group, and closely related to species of the genera *Anatrichobius* and *Joblingia*. However, we had no data available to investigate the relationship between these genera. Wenzel et al. (1966) considered the *longipes* group closely related to the

dugesii group, which is not fully corroborated by our results. They also considered the possibility of the *uniformis* group being related to species of the genus *Speiseria*. Although our results demonstrated that the *uniformis* group is not monophyletic, the clade containing the *uniformis* species is the sister taxon of the clade containing *Speiseria*.

2.5.3.2 Synthesiostrebla

Wenzel et al. (1966) supposed that *Synthesiostrebla* would be closely related to *Trichobius caecus* and *pallidus* groups (Figures B.3 and B.4). Based on the general morphology and female genital structure, they suggested that the genus may be more related to the *pallidus* group. Our results consistently recovered a well supported positioning of *Synthesiostrebla*, but rejected the hypothesis of be related to the *caecus* group. However, a new study using *pallidus* species would be important to further investigate its relationships.

2.5.3.3 Mastoptera and Trichobioides

The clade containing *Mastoptera* and *Trichobioides* recovered in our reconstruction corroborate Wenzel et al. (1966) (Figures B.3 and B.4). Even with extremely different morphologies, the authors cited several characteristics shared between species of both genera.

2.5.3.4 Megistopoda, Neotrichobius and Paratrichobius

These genera form a well known monophyletic group. They have been recovered either in molecular (Petersen et al., 2007) or morphological (Graciolli and Carvalho, 2012) phylogenetic analyses. Wenzel et al. (1966) also believed that these genera were closely related, as well as the *Trichobius phyllostomae* group. Graciolli and Carvalho (2012) also retrieved the genus *Megistapophysis* along with the three genera. Dick and Wenzel (2006) also cited morphological similarities between the four genera and *Trichobius phyllostomae* group. However, the hypothesis of grouping between *Megistopoda*, *Neotrichobius*, *Paratrichobius*, *Megistapophysis* and *Trichobius phyllostomae* group needs to be further investigated.

2.5.3.5 Noctiliostrebla and Paradyschiria

These two genera are exclusive parasites of the bulldog bats genus *Noctilio*, over which they co-occur. With some reservations due to morphological differences, different au-

thors considered them as closely related, forming a monophyletic group with the genus *Xenotrichobius* (not sampled here), also a parasite of *Noctilio* (Wenzel et al., 1966; Wenzel, 1976; Guerrero, 1998). Our results are not consistent about where their position would be within Streblidae. We found them closely related to *Parastrebla*, *Pseudostrebla*, *Speiseria*, *Stizostrebla* and at least some of the *Trichobius* species of the *major* and *uniformis* groups (Figures B.3 and B.4). Although we have recovered *Noctiliostrebla* and *Paradyschiria* as sister taxon, it would be interesting to further investigate the relationship between these genera and *Xenotrichobius*, as well as their placement within Streblidae.

2.5.3.6 Parastrebla, Pseudostrebla, Speiseria and Stizostrebla

These genera along with *Eldunnia* are extremely important to delimit Streblinae. *Pseudostrebla* and *Stizostrebla* have already been included within Streblinae (Jobling, 1936, 1939), but were later removed to Trichobiinae by Wenzel et al. (1966). Although the authors believed they were closely related, they were unsure on the decision to which subfamily these genera should be included. Based on our results (Figures B.3 and B.4), it is clear that Wenzel et al. (1966) were right about the decision of removing them from Streblinae. Concerning *Speiseria*, Wenzel et al. (1966) suggested it may be related to *Trichobius phyllostomae* group, but Wenzel (1976) rejected this suggestion and also presented characteristics shared between the four genera, relationship corroborated by the present analysis.

2.5.3.7 Eldunnia

Monotypic genus that was also part of Streblinae, but it was correctly removed later by Wenzel et al. (1966). For Wenzel et al. (1966) some of the characteristics of the head resembled those found in *Pseudostrebla* and *Stizostrebla*. However, the authors made no assumption about the relationships of the genus, only stating that the relationships of *Eldunnia* were puzzling. From our results it is also not possible to say much about the positioning of *Eldunnia*, since there was no congruence between the results and all of them showed low support, but clearly *Eldunnia* does not belong to Streblinae (Figures B.3 and B.4).

2.5.3.8 Would it be possible to solve Trichobiinae?

Basically, all New World species of Streblidae that do not have a complete ctenidium and a dorsoventrally compressed body, or a flea-like laterally compressed body are considered Trichobiinae. In other words, all that does not fit in Streblinae and Nycterophillinae is Trichobiinae. The characteristics used to define the subfamily are very generalized, as a body sub-cylindrical or dorsoventrally compressed, or the head more or less rounded (Jobling, 1936, 1939; Wenzel et al., 1966; Wenzel, 1976; Guerrero, 1994). The definition goes through the generalized characteristics that resembles *Trichobius*, but composed of genera and species that exhibit a wide morphological diversity (Wenzel, 1976; Guerrero, 1994)(Wenzel 1976; Guerrero 1994). In our opinion, a new classification proposal must go first splitting the polyphyletic *Trichobius* into smaller and phylogenetically defined generic units and also defining their species composition.

2.5.4 The phylogeny of Streblinae and implications for the classification

This is the first phylogenetic analysis to include a large sampling of species for the subfamily. Our results corroborate the decision of Wenzel et al. (1966) to restrict Streblinae only to genera containing a complete ctenidium in the head, extending from the ventral to the dorsal region (Figures B.3 and B.4). On the other hand, the classification proposed by Jobling (1936, 1939) is refuted, since the genera considered by him to be Streblinae were not recovered inside the subfamily clade, such as *Eldunnia*, *Pseudostrebla* and *Stizostrebla*. Thus, Streblinae comprises only: *Anastrebla*, *Metelasmus*, *Paraeuctenodes* and *Strebla*. As pointed out by Wenzel et al. (1966), there are several characteristics that distinguish the genera included by them in Streblinae from the other genera of Streblidae, particularly in the head and anterior portion of the thorax. Wenzel et al. (1966) believed that the definition of Streblinae by Jobling (1936, 1939) was possibly based on convergent characters, as the width of the posterior margin of the head, and the shape and relative size of the mesonotum. Our results corroborate this assumption.

The relationships obtained in our analyses supported some of the statements made by Wenzel et al. (1966). They asserted that *Anastrebla* superficially resemble *Paraeuctenodes*, while the latter contained similarities with *Strebla*. Meanwhile, the results obtained provide strong evidence to reject the current classification of Streblinae. In all analyses, *Strebla* was

recovered as non-monophyletic. Based on that, a new classification proposal would imply either splitting *Strebla* into two genera, or inserting *Metelasmus* inside *Strebla*. Since the two genera are morphologically very distinct, at first the most appropriate option seems to be to split *Strebla*. However, the molecular phylogeny does not allow precise positioning on the decision to be made. In this sense, a morphological study would be extremely important to clarify the relationship between the two genera, and establish new diagnoses regardless of the decision to be taken.

2.5.5 Cospeciation in Streblinae? No, give me a lot of host-switch!

Assuming the maximum cospeciation paradigm to understand the associations between Streblinae and its hosts, we should expect to find congruent phylogenies and a high number of cospeciation events explaining the history between the two groups. However, we found the exact opposite in our analyses. The diversification of Streblinae is more likely influenced by host switching. Although PACo indicated overall host-parasite congruence (Figures B.9 and B.10), we have no reason to take it into account at the moment, since Jane, BioGeoBEARS (Table A.8; Figure B.11) and even ParaFit (Table A.7) indicated the opposite situation. Graciolli and Carvalho (2012) also found a similar result to ours in the cophylogenetic analysis of *Trichobius phyllostomae* group and the host bats of the subfamily Stenodermatinae (Phyllostomidae). Phylogenetic signal studies of associations in bat ectoparasites are also congruent with our results. The hypothesis in such studies assumes that the host-parasite relationships and the composition of the parasite community should reveal phylogenetic signals, i.e. host-switch would be rare. In this sense, Presley et al. (2015) found that most bat ectoparasite species (59%) analyzed showed no phylogenetic signal, including bat flies (Nycteribiidae, Streblidae), bat bugs (Polyctenidae), fleas (Ischnopsyllidae), ticks (Argasidae, Ixodidae), and mites (Chirodiscidae, Macronyssidae, Myobiidae, Spinturnicidae, Trombiculidae). Similarly, Bezerra and Bocchiglieri (2019) found that neither the composition of the Streblidae community, nor the species of Streblidae that parasitize more than one host were associated with phylogenetically closer hosts. Thus, all these results provide strong evidence that host-switch is perhaps the main event acting on the association between Streblidae and their hosts. They are totally consistent with a growing view that cospeciation is neither the dominant, nor the determining factor in host-parasite systems (Hoberg and Brooks, 2008; Vienne et al., 2013; Brooks et al.,

2015).

Host specificity is usually hypothesized as a possible indication of congruent phylogenetic histories, since the parasites would be less prone to exploit other hosts (Krasnov et al. 2007, 2014). Streblidae species are considered highly host-specific, and this is well recorded for countless areas (*i.e.*, Dick 2007; Cuxim-Koyoc et al. 2015; Barbier and Bernard 2017; Durán et al. 2017). Despite of obligate parasites with a remarkable host specificity, they have a notable dispersal ability (Dick and Patterson, 2007). Streblidae species are pupiparous (viviparous), which means eggs are incubated internally and all larval stages develop inside the female. The female deposit the larva in a late stage (prepupa) on a substrate in bat roosting sites, which immediately turns into a puparium. Consequently, females actively leave their hosts for larviposition, even at considerable distances from the host. Males in turn leave their hosts in few situations, yet only in the vicinity of the host (Dittmar et al. 2009, 2011). In addition, most Streblidae species (≈79%) have the ability to fly and tend to leave their hosts when disturbed (Dick and Patterson, 2006). To explain this apparent incongruity between specificity and dispersibility, Dick and Patterson (2007) used the Filter Concept (Combes, 1991; Poulin, 2007) along with the Reproductive Filter as mechanisms enabling the host specificity. Briefly, the parasite needs to be able to find a new host (Encounter Filter), colonize (Compatibility Filter), and have mates available to reproduce and stay on the new host (Reproductive Filter). Thus, these filters together would act as a strong constrain limiting the chance of new associations, regardless of the dispersal capacity (see details in Dick and Patterson 2007).

Still, how could host-switch be such an event in the historical association between Streblidae and their hosts? Graciolli and Carvalho (2012) provided a likely explanation: roost ecology. Although the influence of environmental variables such as rainfall and vegetation over the interactions are still under discussion (Pilosof et al., 2012; Rivera-García et al., 2017; Barbier et al., 2019; Saldaña-Vázquez et al., 2019), it seems clear the role of bat roosts as a key mechanism. Roosting habits were positively and significantly related to prevalence, mean intensity and number of associated fly species. Bat species roosting in more permanent, enclosed structures were found to be more likely to have the highest values for these three measures of parasitism (Patterson et al., 2007), while reducing the specialization of interactions (Saldaña-Vázquez et al., 2019). In contrast, these longer-lived roost, such as caves and mines, usually shelter simultaneously several bat species

(Avila-Flores and Medellín, 2004). Since bat ectoparasitic species richness are positively correlated with bat host species richness (Barbier and Bernard, 2017), high-density roosters of large colonies may act weakening the filters argued by Dick and Patterson (2007). Some studies present evidence that may demonstrate and support how this filter weakening could happen. Based on experiments and observations, Dick et al. (2009) demonstrated that when removing dispersal barriers, bat ectoparasites can accepted a secondary host species, and even remain if a primary host is immediately available. In the same way, Wenzel et al. (1966) cited observations for different species of Streblidae that parasitized secondary hosts, but only when these hosts shared the same roost as the primary hosts. Our results for primary hosts also demonstrated how much interactions can vary (Supplemental Material 12). Although we did not consider all associations for analytical purposes, most species had records with different hosts. Besides, Lourenço and Palmeirim (2008) studied the sensory cues of nycteribiids involved in locating hosts from a distance. The authors found that carbon dioxide and body heat were the most efficient cues used to locate the hosts. Thus, if general cues are used rather than specific host cues, there is a considerable chance that the ectoparasites do not directly find their primary bat hosts Lourenço and Palmeirim (2008), causing new host-parasite interactions. Finally, if dense populations of bat flies on high-density bat roosts are favored over small-density or solitary roosts (Dick and Patterson, 2006), then it is possible that host-switch is also favored over cospeciation.

2.5.6 Jumping and evolving: Is the timeframe of Streblidae associated with host-switch?

This is the first divergence time estimation provided to any group of Streblidae (Figures B.5 and B.6). Earlier estimates for Diptera and internal groups took into account only the origin for Hippoboscoidea, sampling solely specimens of Glossinidae and Hippoboscidae. Wiegmann et al. (2011) estimated the origin of Hippoboscoidea at approximately 40 Ma, while Cerretti et al. (2017) at 46 Ma. The difference between the studies is very small, with both estimating an origin in the Middle Eocene. Dittmar et al. (2015) argued that the origin of bat flies (Streblidae and Nycteribiidae) would probably be associated with the most dramatic diversification in bats at 50–30 Ma, from the Lower Eocene to the Lower Oligocene. Although our analyses focused only on New World Streblidae, they are not inconsistent with the estimates for Hippoboscoidea. We are aware that having only one fossil available for calibration may increase uncertainty in the rate estimates

(Drummond and Bouckaert, 2015). Despite that, we can say that they are in line with previous estimates, especially those for hosts.

New World Streblidae species are mainly associated with bats of New World Noctilionoidea, which includes Furipteridae, Mormoopidae, Noctilionidae, Phyllostomidae and Thyropteridae. The greatest diversity of associations are upon Phyllostomidae, though a few species are associated with bats from Emballonuridae, Molossidae and Vespertilionidae (Wenzel et al., 1966; Wenzel, 1976; Dick and Patterson, 2006; Dick et al., 2016). New World Noctilionoidea is clearly monophyletic and its origin is estimated from the Middle Eocene (≈42 Ma) (Rojas et al., 2016). However, the Miocene may have been an important period for Noctilionoidea diversification, particularly for Phyllostomidae in which diet diversification may have played a crucial role. Different analyses revealed that all subfamilies started diversification and about half the genera of phyllostomid bats had arisen during the Miocene, with a prominent role of dispersal and founder events (Rojas et al., 2011, 2016; Shi and Rabosky, 2015). These results provide strongly support to our estimates, in particular for Streblinae origin which is consistent with the diversification period for the estimated ancestral host.

Glossophaginae bats (21.1 Ma, 23.7–17 Ma 95% HPD; Rojas et al. 2011, 2016) are older than Streblinae flies (8.2 Ma, 12.6–5.7 Ma 95% HPD). Based on the gap between the intervals of each estimate, it can be assumed that the association between them was not the result of cospeciation. After Glossophaginae, Streblinae was associated with nine other subfamilies by host switching. Thus, host diversification may have provided an abundant and underexploited source of possible associations, accessed through host-switch and which enabled rapid diversification within Streblinae, as well as in New World Streblidae. In this view, we suggest that our findings are consistent with the expectations of the "Stockholm Paradigm" (Brooks et al., 2015). Ecological isolation may have occurred through mechanisms such as diet diversification of the hosts. In contrast, episodes of expansion may have been promoted by dispersal and new occurrences. The breakdown in isolation increases host sympatry and density (Hoberg and Brooks, 2008, 2010), allowing events such as roost sharing. These dynamics of change between episodes of isolation and expansion by the host, along with their diversification and events host-switch, may have strongly determined patterns of diversification and associations in New World Streblidae.

2.6 Conclusions

This study comprises the first molecular phylogeny with the largest sample of species and genera of New World Streblidae to date, especially for the subfamily Streblinae. We consistently recovered the monophyly of New World Streblidae, as well as of the subfamilies Nycterophiliinae and Streblinae. We corroborate the well-known non-monophyly of the subfamily Trichobiinae and the genus *Trichobius*. We also indicated that many of the *Trichobius* morphological groups proposed by Wenzel et al. (1966) are not monophyletic. The proposed classification of Wenzel et al. (1966) for Streblinae is corroborated, but for the first time the monophyly of *Strebla* is contested, indicating it is a paraphyletic taxon in relation to *Metelasmus*. We also provide the first estimates of divergence times of the New World Streblidae, with the estimated age of origin to the Lower Miocene (15.3, 22.7–12.7 Ma 95% HPD). Our results further provide an insight into the historical association between the species of Streblidae and their bat hosts. The origin of the New World Streblidae, in particular of the subfamily Streblinae, is consistent with the period of diversification of the main host clades. Our study supports host-switch as the main process operating in the associations, even though the group is considered highly specialized and host-specific. This is in congruence with the results previously found by Graciolli and Carvalho (2012), and with the expectations of the "Stockholm Paradigm" (Brooks et al., 2015). Regarding associations, we hypothesize that host roosts may act as a key mechanism, weakening the filters argued by Dick and Patterson (2007) to keep host-parasite specificity, and favoring host-switch over cospeciation. This demands future studies to better understand the role of roost ecology in shaping the associations between hosts and parasites.

Chapter 3

Morphological phylogeny of Streblinae (Diptera: Streblidae)

3.1 Abstract

With a complicated delimitation and a complex morphology, the subfamily Streblinae underwent several changes in its composition. Currently with four genera and 35 species, there are still doubts regarding the delimitation of the subfamily. The phylogeny of the subfamily is presented based on morphological characters. We sampled all valid species of Streblinae. Results strongly support the monophyly of Streblinae, with *Anastrebla*, *Metelasmus* and *Paraeuctenodes* also as monophyletic. In turn, *Strebla* is recovered as paraphyletic in relation to *Metelasmus*. We present a historical overview on the delimitation and characterization of Streblinae, and discuss the importance of a new interpretation of the characters for subfamily classification.

3.2 Introduction

Streblidae is a family of obligate, blood-feeding ectoparasite bat flies that have a broad morphological diversification. Despite being cosmopolitan and distributed across all biogeographic regions, Streblidae has a distribution pattern in which no species, genus or even subfamily occurs in either the Old World or the New World (Dick and Patterson, 2006; Dittmar et al., 2015). The family has 239 described species, 33 genera and five subfamilies, three of which are endemic to the New World: Nycterophiliinae, Streblinae and Trichobiinae; and two from the Old World: Nycteriboscinae and Ascodipterinae (Dick and Graciolli, 2013; Dick et al., 2016).

With 156 species in 26 genera, the New World uniquely accounts for approximately 70% of the known diversity of Streblidae, most of which is restricted to the Neotropics (Dick and Graciolli, 2013; Dick and Patterson, 2006). However, much of the phylogenetic work performed so far does not take into account such geographical representation, including a very small sample of neotropical specimens and consequently of the family itself. Most phylogenetic studies with Streblidae have focused on interfamilial relationships in Hippoboscoidea. Futhermore, the low sampling so far has a clear impact on discrepancies between current hypotheses, with conflicting relationships even for large clades of the Hippoboscoidea superfamily, to which Streblidae belongs (*i.e.* Dittmar et al. 2006; Petersen et al. 2007; Kutty et al. 2010).

The long and intimate association between the species of Streblidae and their host bats has produced a variety of adaptations in Streblidae, possibly the result from ecological interactions and evolutionary relationships (Dick and Patterson, 2007; Tello et al., 2008). Some authors argued that morphological and behavioral characteristics may be related to the host body part they occupy. However, without detailed phylogeny, it is not possible to ascertain whether the morphological characteristics are solely a reflection of the evolutionary history of flies or they may reflect convergent adaptations for the host habitat (Dick and Patterson, 2006; Hiller et al., 2018). In this sense, one group that has been neglected is the subfamily Streblinae. Only two phylogenetic studies sampled species of Streblinae, both using species of *Strebla*. One study used two species (Dittmar et al., 2006), while the other a single species as the root of the phylogeny (Graciolli and Carvalho, 2012). A third study focused on the relationship between the genera of New World Streblidae, but without using species as terminals (Guerrero, 2019).

Streblinae are restricted to the Neotropics and comprise four genera and 35 species: *Anastrebla* Wenzel 1966 with five species, *Metelasmus* Coquillett 1907 and *Paraeuctenodes* Pessôa & Guimarães, 1937 with two species each and *Strebla* Wiedemann 1824 with 26 species. The family is mainly characterized by the body strongly dorsoventrally flattened, the palps with a shield-like shape, and the presence of a complete ctenidium (extending from the ventral surface to the dorsal surface of the head). However, the delimitation of the subfamily is quite complicated and controversial among authors. It has been delimited from different morphological characteristics and comprised different genera until it reaches its present conformation (Speiser, 1900; Kessel, 1924, 1925; Jobling, 1936, 1939; Wenzel et al.,

1966; Wenzel, 1970, 1976; Guerrero, 1996). It is currently restricted only to genera that have a complete ctenidium in the head (Guerrero 1996). Regarding the hosts, the species of Streblinae have as their primary hosts (*sensu* Wenzel et al. 1966, host with the highest number of records for one parasite species) mainly bats of the family Phyllostomidae, except for three species of *Strebla* that parasitize bats of the family Emballonuridae.

The objective of the present work was to perform the phylogenetic analysis of the subfamily Streblinae based on morphological evidence to verify its monophyly, propose a relationship hypothesis between the genera of the subfamily, as well to evaluate the characters used so far to delimit and characterize the subfamily and the genera included in it. Taxonomic sampling for subfamily includes all currently valid genera and species, except the species *Strebla mexicana* Rondani whose the only known type specimen is lost.

3.3 Material and Methods

3.3.1 Taxon sampling, terminology and character coding

As in chapter 2, we sampled all four genera of Streblinae. We included all species of Streblinae, except *Strebla mexicana* Rondani whose holotype is lost and there are no known specimens of the species. We selected three species of Trichobiinae as outgroup based on the phylogeny of chapter 2, *Speiseria ambigua* Kessel, *Pseudostrebla ribeiroi* Costa Lima and *Eldunnia breviceps* Curran. We also included a species of an undescribed genus genus as outgroup for having a ventral ctenidium. We used a total of 38 terminals, 34 for the ingroup and 4 as outgroup (Supplemental Material 15). We used *S. ambigua* as root, based on the result obtained in chapter 2. The terminology used for the head followed Jobling (1929), and for external morphology of thorax and abdomen followed Wenzel (1976) and Wenzel and Peterson (1987). The terminology adopted for the gonopod setae followed Graciolli and Dick (2004).

We performed the morphological study by examining specimens preserved in ethanol, as well specimens mounted on slides. We examined the material sequenced in chapter 2, as well as specimens obtained from Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul, Mato Grosso do Sul, Campo Grande, Brazil (ZUFMS) and Coleção Entomológica Padre Jesus de Santiago Moure, Paraná, Curitiba, Brazil (DZUP). We could not examine specimens of *Anastrebla mattadeni* Wenzel, *A. nycteridis* Wenzel, *E. breviceps*

Curran, *Strebla cormurae* Wenzel and *S. hoogstraali* Wenzel. For these species, we obtained their characters from descriptions, original illustrations and images available on the Vertebrate ectoparasite collection (Bat Flies catalog) website of the Fiel Museum of Natural History (FMNH, 2019, available at `https://collections-zoology.fieldmuseum.org`) and on the website "The Bat Flies of La Selva (Diptera: Nycteribiidae, Streblidae)" (Miller and Tschapka, 2009, available at `http://www.biologie.uni-ulm.de/bio3/Batfly/index.html`).

Most of the phylogenetic characters constructed were based on observations of the authors. We treated all characters as unordered (Fitch, 1971). We constructed the data matrix using Mesquite v3.6 (Maddison and Maddison, 2018). Missing data were coded as "?" and nonapplicable data as "–". We took pictures on the Leica MZ16 microscope, and later stacked them with the Helicon Focus software (Helicon Soft Ltda.). Next, we edited the images and organized the plates in the Inkscape software.

3.3.2 Phylogenetic analysis

We performed parsimony analysis using the software TNT 1.5 (Goloboff and Catalano, 2016), with equal weighting of the characters. The heuristic search was performed with New Technology Search with the following algorithms and configurations: sectorial search (Goloboff, 1999) with default configuration, ratchet (Nixon, 1999) with 200 interactions, tree drifting (Goloboff, 1999) with 50 cycles and tree fusing (Goloboff, 1999) with default configuration. These algorithms were used to solve the problems of "local optimal" and "composite optimal", which the old algorithms suffer, breaking the islands where the trees are limited and thus reaching the best result quickly (Nixon, 1999; Goloboff, 1999). Branch support estimated through non-parametric bootstrap calculation (Felsenstein, 1985) was performed in TNT 1.5 (Goloboff and Catalano, 2016) with the following configurations: 1000 pseudoreplicates; standard resampling (sample with replacement); output as frequency differences; Traditional Search tree search (TBR / 1000 replicates / 10 trees per replication). Bremer support (Bremer, 1994) was also performed in the software on TNT 1.5 (Goloboff and Catalano, 2016), using trees with 20 additional steps. Character visualization and optimization were performed with the software Winclada (Nixon, 2002). The editing of the cladograms was done in the software Inkscape.

3.4 Results

3.4.1 Characters

We obtained a total of 57 morphological characters: 42 characters from the head, 13 from the thorax, one from the wings, and one from the male genitalia. The list of characters is shown in Appendix C, along with length, CI and RI for each character. Head, thorax and abdomen images are presented in Supplemental Materials 16 and 17.

3.4.2 Parsimony Analysis

We obtained only one most parsimonious tree, with 78 steps, CI = 93 and RI = 97 (Figure B.12). Bootstrap and Bremer supports are shown in Figure B.13. The analysis recovered Streblinae as monophyletic and supported by 18 unambiguous synapomorphies, of which 13 are of head characters (1:1, 2:1, 3:2, 5:1, 6:1, 9:1, 28:1, 30:1, 32:1, 35:1, 36:1, 37:1, 42:1) and five of thorax characters (43:1, 48:1, 50:1, 53:1, 55:1). Including the ambiguous characters, Streblinae was supported by nine additional synapomorphies, three of head characters (4:1, 38:1, 39:1) and six of thorax characters (45:1, 46:1, 47:3, 49:3, 51:1, 52:1). Mapped unambiguous character states are shown in Figure B.14, while mapped ambiguous character states are shown in Figure B.15. Regarding internal relationships, we recovered *Anastrebla* as monophyletic, sister taxon of the clade containing the other genera, and supported by one unambiguous homoplastic character (27:1), as well as by three ambiguous synapomorphies (4:1, 38:1, 39:1) and one ambiguous homoplastic character (26:1). The clade *Paraeuctenodes* + *Metelasmus* + *Strebla* was supported by three unambiguous synapomorphies (8:1, 21:1, 25:2), six ambiguous synapomorphies (4:2, 10:1, 29:1, 31:1, 38:2, 39:2) and one ambiguous homoplastic character (26:0). *Paraeuctenodes* was recovered as sister taxon of the clade containing *Strebla* + *Metelasmus*, and supported by only one unambiguous synapomorphy (40:1). *Strebla* + *Metelasmus* was supported by one unambiguous synapomorphy (7:1) and one additional ambiguous synapomorphy (11:1).

Unlike the other genera of Streblinae, we recovered *Strebla* as non-monophyletic in relation to *Metelasmus*. A clade containing the species of *Metelasmus* and the species of *Strebla* with a complete frontoclypeus (*S. christinae*, *S. consocia*, *S. diaemi*, *S. hertigi*, *S. hoogstraali*, *S. tonatiae*) was supported by one unambiguous synapomorphy (34:1),

one unambiguous homoplastic character (44:0) and one ambiguous synapomorphy (22:1). *Metelasmus* was supported by four unambiguous synapomorphies (23:3, 39:3, 47:2, 56:1), one unambiguous homoplastic character (27:1), one ambiguous synapomorphy (22:1) and one ambiguous homoplastic character (11:1). The clade containing the species of *Strebla* with a complete frontoclypeus was supported by one unambiguous synapomorphy (33:1), one ambiguous synapomorphy (22:2) and one ambiguous homoplastic character (11:0). On the other hand, the clade containing the other species of *Strebla* was supported by one unambiguous homoplastic character (51:2) and one additional ambiguous homoplastic character (11:1). The species of this clade, characterized mainly by the frontoclypeus with anterior detached plates, were divided into two clades. One clade (*S. asternalis*, *S. chrotopteri*, *S. diphyllae*, *S. kohlsi*, *S. machadoi*, *S. mirabilis*, *S. obtusa*, *S. paramirabilis* and *S. wiedemanni*) was supported by one unambiguous synapomorphy (54:1) and one ambiguous synapomorphy (52:1), while the other (*S. altmani*, *S. alvarezi*, *S. carvalhoi*, *S. cormurae*, *S. curvata*, *S. galindoi*, *S. guajiro*, *S. harderi*, *S. matsoni*, *S. proxima*) by one unambiguous synapomorphy (12:1) and one ambiguous synapomorphy (52:2).

3.5 Discussion

3.5.1 What defines Streblinae? The classification of the subfamily over time

The delimitation and characterization of Streblinae always had as one of the main bases the characteristics of the head. Speiser (1900) proposed Streblinae to include two genera, *Euctenodes* Waterhouse, 1879 (= *Strebla* Wiedemann, 1824) and *Strebla sensu* Speiser, 1900 (= *Anastrebla* Wenzel, 1966). Among the characters used by Speiser in his classification, he used the flattened head and the presence of ctenidium to define Streblinae:

> "Kopf flach gewölbt, mit je einem „Kragen"oben und unten, deren unterer am Hinterrande eine Reihe starker Chitindornen trägt" (Speiser, 1900, p. 65)

Here translated as:

> "Head flat, with a 'collar' above and below, the lower part of which bears a series of strong chitin spines on the posterior margin"

After the description of *Metelasmus* Coquillett, 1907, both Coquillett (1907) and Speiser (1908) considered it as a genus of Streblinae. Thus, Streblinae came to be composed of three

genera: *Euctenodes* (= *Strebla*), *Metelasmus* and *Strebla sensu* Speiser (= *Anastrebla*). Although, Kessel (1924) did not mention *Metelasmus* in his "Notes on the Streblinae", Kessel (1924, 1925) maintained the definition of Streblinae, mainly using head characteristics and highlighting the ctenidium.

Dissatisfied with the classification at the time, Jobling (1936) redefined the subfamily Streblinae to include *Pseudostrebla* Costa Lima, 1921 and *Eldunnia* Curran, 1934, reducing the importance of ctenidium and assigning these two genera as an exception for head characters:

> "Head triangular or trapezoidal, its posterior part as broad as the anterior part of the thorax, usually with ctenidium; palps triangular, contiguous and horizontal, except in *Pseudostrebla* and *Eldunnia*. Thorax rectangular, the mesonotum slightly convex or flat, broader than the sternopleura" (Jobling, 1936, p. 364)

Later, Jobling (1939) redefined Streblinae once more to accommodate the new described genus, *Stizostrebla* Jobling, 1939:

> "Head subtrapezoidal or trapezoidal, as broad in its posterior part as the anterior margin of the thorax; with or without ctenidium. Palps subtriangular,contiguous and horizontal, except in *Eldunnia* and *Stizostrebla*. Thorax rectangular, with a broad, shallow longitudinal groove in each antero-lateral part, where lie the femora of the fore-legs when at rest. The mesonotum slightly convex or flat, broader than the sterno-pleurae" (Jobling, 1939, p. 269)

Moreover, *Paraeuctenodes* Pessôa & Guimarães, 1937 was present for the first time as part of the subfamily. Thereby, Streblinae came to encompass seven genera: *Eldunnia*, *Euctenodes* (= *Strebla*), *Strebla sensu* Speiser (= *Anastrebla*), *Metelasmus*, *Paraeuctenodes*, *Pseudostrebla* and *Stizostrebla*. This classification of Streblinae remained until 1966.

According to Wenzel et al. (1966), based on the established definition of Jobling (1936, 1939), the subfamily would have to include additional genera, such as *Parastrebla* Wenzel, 1966. After a comprehensive taxonomic review and despite some skepticism, Wenzel et al. (1966) redefined Streblinae. The authors restricted the subfamily to species with a complete ctenidium (extending from the ventral surface to the dorsal surface of the head), and reassigned *Eldunnia*, *Pseudostrebla* and *Stizostrebla* to the subfamily Trichobiinae. From

this study, the ctenidium once again is highlighted as an important characteristic to define the subfamily. Futhermore, *Euctenodes* was synonymized under *Strebla* and *Anastrebla* was proposed for *Strebla sensu* Speiser and subsequent authors, not Wiedemann (1824). Thus, the subfamily was attributed to its current composition, with four genera: *Anastrebla*, *Metelasmus*, *Paraeuctenodes* and *Strebla*. All subsequent studies followed the delimitation proposed by Wenzel et al. (1966), such as in Dick and Graciolli (2013), Dick et al. (2016), Graciolli and Carvalho (2001), Guerrero (1996, 2019) and Wenzel (1970, 1976).

Guerrero (2019)[2] performed a phylogenetic analysis based on morphological characters in order to understand the relationships between the genera of the New World Streblidae. The author sampled 25 genera of Streblidae, which include the four genera of the subfamily Streblinae, as well as *Eldunnia*, *Parastrebla*, *Pseudostrebla* and *Stizostrebla*. Although not presenting an optimization of characters[2], the study presents analyses performed with the sets of characters separated (head, thorax and abdomen), which allows us to interpret each character set. The consensus tree shows Streblinae as monophyletic with the four subfamily genera. In contrast, the analysis with the head characters recovered Streblinae along with *Eldunnia* and *Stizostrebla*, while the analysis with thorax characters also recovered *Synthesiostrebla* Townsend, 1913 along with the mentioned taxa. Analysis with abdomen characters was the most incongruous, with Streblinae forming a clade with *Pseudostrebla*, *Speiseria*, *Stizostrebla*, *Trichobioides* Wenzel, 1966 and three morphological groups of *Trichobius* Gervais, 1844. Guerrero (2019) believed that because *Eldunnia* has a ventral ctenidium and it was usually recovered close to Streblinae, the genus should be included within the subfamily. However, aware of the inconsistencies between the hypotheses, Guerrero (2019) opted to maintain the classification proposed by Wenzel et al. (1966).

Finally, both morphological (this study) and molecular (chapter 2) data corroborate Wenzel et al. (1966) decision, as well as reinforce the importance of head characters for the delimitation of Streblinae. The monophyly of Streblinae in chapter 2 is highly supported and refutes the hypothesis that *Eldunnia*, *Parastrebla*, *Pseudostrebla* and *Stizostrebla* belong to the subfamily. Moreover, the results of chapter 2 show that the simple presence of a

[2] Although Guerrero's publication is from 2019, the study was originally done in 1990 with the computational resources and the phylogenetic inference softwares available at the time, such as the softwares PHYLIP v3.0 and PAUP v2.4, and the personal computer IBM PS/2 (Model 50).

ctenidium, regardless of whether it is complete or not, is a homoplasic character. We even believe that the results of Guerrero (2019) is in accordance with the classification proposed by Wenzel et al. (1966), since the consensus tree clearly demonstrates it. The inconsistency between hypotheses generated with sets of characters analyzed separately may well be the result of homoplastic characters. Here, we recovered many synapormorphies supporting the subfamily that assisted to corroborate the delimitation of Streblinae (Figures B.14 and B.15). One of these synapomorphies is the presence of a complete ctenidium (Figure B.14, 42:1), a character attributed by Wenzel et al. (1966) as an important characteristic that defines the subfamily. Other characteristics of the head discussed by previous authors have also been recovered here as synapomorphies of Streblinae, such as the flattened head and the shape of the palpi (Figure B.14, 1:1 and 2:1). Thus, the characters of the head were found to be important in the delimitation of Streblinae, and no reason was found to change the classification of the subfamily as suggested by Guerrero (2019).

3.5.2 The relationships within Streblinae

3.5.2.1 The position of Anastrebla is unanimous

Our results are totally congruent with those presented in chapter 2, and corroborate some assumptions and results from previous studies. The idea that *Anastrebla* would be a sister taxon to the other genera of Streblinae is not new. Speiser (1908) presented assumptions about the relationships within Streblidae, including the genera of Streblinae. In his representation, Speiser (1908) placed *Anastrebla* (= *Strebla sensu* Speiser) at the base of Streblinae, from which *Strebla* (= *Euctenodes*) and *Metelasmus* would derive. Similarly, Jobling (1939) had an interpretation of the sequence of morphological changes in Streblinae which resembles that assumed by Speiser (1908). For Jobling (1939), *Anastrebla* (= *Strebla sensu* Speiser) would present a more general morphology in relation to *Strebla* (= *Euctenodes*), *Paraeuctenodes* and *Metelasmus*, as can be seen below:

> "According to the structural modifications, the genera of this subfamily can be arranged in the following order: *Pseudostrebla*, *Eldunnia*, *Stizostrebla*, *Strebla*, *Eudenodes*, *Paraeuctenodes* and *Metelasmus*" (Jobling, 1939, p. 269)

. Within a context based on phylogenetic analysis, the consensus tree obtained by Guerrero (2019) also presented *Anastrebla* as a sister taxon of the clade formed by the other genera.

Based on our analysis, it seems clear why different authors assumed that *Anastrebla* would be either basal or with a more general morphology in relation to the other subfamily genera. Some characteristics of *Anastrebla* appear to be an intermediate stage between a general morphology of Trichobiinae and the pattern found in *Metelasmus*, *Paraeuctenodes* and *Strebla*, as the palpus that does not strongly bend over itself, appearing to be longer than wide (Figure B.14, 4:1), and the shape of the frontoclypeus, which is not flattened as in the other genera of the subfamily (Figure B.14, 8:1).

3.5.2.2 Metelasmus, Paraeuctenodes and Strebla: no more contradictions in their relationships?

Despite the relationships of *Anastrebla* with the other genera seem to be well recognized, the same cannot be said about the relationship among *Metelasmus*, *Paraeuctenodes* and *Strebla*. The hypotheses proposed by previous studies are not congruent. In the first study to take into account the four genera of Streblinae, Jobling (1939) assumed that *Metelasmus* and *Paraeuctenodes* would be more closely related than with *Strebla*. In turn, the consensus tree of Guerrero (2019) obtained *Metelasmus* as sister taxon of the clade containing *Paraeuctenodes* and *Strebla*, whereas in chapter 2 *Paraeuctenodes* was recovered as the sister taxon of the clade *Metelasmus* + *Strebla*. Here, we recovered a result congruent with that obtained chapter 2, in which *Metelasmus* and *Strebla* formed a clade supported by one unambiguous synapormophy (Figure B.14, 7:1), and *Strebla* as non monophyletic in relation to *Metelasmus* (Figure B.12). Although Wenzel et al. (1966) do not explicitly state a relationship hypothesis between the genera of Streblinae, the comments presented for each genus show some of the insight into the subject. When comparing the genera in the comments, Wenzel et al. (1966) always presented *Anastrebla* and *Strebla* at opposite ends, while *Metelasmus* and *Paraeuctenodes* would be between the two extremes. Likewise, Guerrero (2019) states that although not proposing a relationship hypothesis, all studies conducted by Wenzel (Wenzel et al., 1966; Wenzel, 1970, 1976, in) follow the same order of presentation of taxa, which could be interpreted as a supposed relationship scheme. Indeed, the order of taxons presented by Wenzel is always the same, and Wenzel et al. (1966) states that the order of subfamilies indicates their assumption of relationships. However, it cannot be stated with certainty that the same applies to the order of genera and species. Nevertheless, if we consider this to be true, we could interpret

that Wenzel considered the following relationship: (*Strebla*, (*Paraeuctenodes*, (*Anastrebla*, *Metelasmus*))), which contrasts with all other hypotheses presented.

We are aware that the morphological characters of our analysis still need to be better explored, given the low support of branches (Figure B.13) and the low number of characters from thorax, abdomen and genitalia. However, we believe the results obtained so far provide a great insight into the problems of understanding the relationship between *Metelasmus*, *Paraeuctenodes* and *Strebla*. In our opinion, this confusion goes through a major issue: the interpretation of characters. An example of how character interpretation can be problematic in understanding relationships in Streblinae is our character 27 (Appendix C): presence or absence of a broad scale on the dorsal surface of the gena. *Strebla* and *Paraeuctenodes* do not have this scale, whereas *Anastrebla* and *Metelasmus* do. If the presence or absence or absence of this scale is considered as an important character to group the genera, two options are possible: 1) *Strebla* and *Paraeuctenodes* can be grouped by absence, similarly to that obtained by Guerrero (2019), or 2) *Anastrebla* and *Metelasmus* would be grouped together, as Wenzel et al. (1966) supposedly would have thought. However, both the molecular phylogeny in chapter 2 and the morphological phylogeny presented here show that this is a homoplastic character (Figure B.14, 27:1). Similarly, the use of homoplastic characters was possibly one of the reasons that led Jobling (1936, 1939) to include more genera within Streblinae, as discussed earlier in chapter 2, as well as by Wenzel et al. (1966).

Another point that demonstrates how character interpretation may have led to contradictions between hypotheses, it is the fact that no previous study has raised the possibility that *Strebla* could not be monophyletic. The phylogenies carried out so far have not had a sampling capable of detecting such a condition (*i.e.*, Dittmar et al. 2006; Petersen et al. 2007; Kutty et al. 2010). However, all identification keys for the species of *Strebla* (*i.e.*, Wenzel 1976; Guerrero 1996) have a very striking feature, they are divided into two parts, one for species with a complete frontoclypeus and the anterior pigmented prescutal suture absent, and other for species with frontoclypeus with anterior detached plates and the anterior pigmented prescutal suture present. Interestingly, the division shown in the identification key is nearly the same as that recovered in the morphological phylogeny and the molecular phylogeny of chapter 2. The division is not exactly the same because of a single species, *Strebla christinae*. This species has always been considered to have a frontoclypeus

with anterior detached plates, so it is found on one side of the identification key, while in phylogeny it has been recovered with species of the other side of the key. A closer look at the identification key shows that there is a note stating that *Strebla christinae* is an exception for not having the anterior pigmented prescutal suture present, a characteristic of the species with a complete frontoclypeus. This is also a homoplastic character, along with the shape of the anterior margin of the gena (Figure B.14, 34:1, 44:0), support the clade containing *Metelasmus* and the species of *Strebla* species with a complete frontoclypeus. Likewise, upon careful examination of the frontoclypeus it is possible to realize that there are no detached plate, but is complete with unpigmented areas that give the impression of being divided. Therefore, it can be argued that a more careful examination of characters, with a reinterpretation of them when necessary, can and should do much to resolve the contradictions between existing relationship hypotheses.

3.5.3 What to do with Strebla?

Due to the results obtained in chapter 2, it was argued that a new classification proposal would imply either splitting *Strebla* into two genera, or inserting *Metelasmus* inside *Strebla*. Morphological knowledge at the time did not allow us to take the best decision, although we had the notion that *Metelasmus* was morphologically very distinct. If the result remains after the morphological analysis has been improved, with a greater number of characters, we are sure that the best decision will be to propose a new genus for the species of *Strebla* with the complete frontoclypeus (Figure B.12), since the genus type-species (*S. wiedemanni*) has been recovered within the clade of species with detached plates.

3.6 Conclusions

This is the first phylogeny to rely on all valid species of the subfamily Streblinae. The monophyly of Streblinae is well supported by numerous synapomorphies. Despite the low branch support, we have recovered *Anastrebla*, *Metelasmus* and *Paraeuctenodes* as monophyletic. We corroborate the result of chapter 2, demonstrating that *Strebla* is not monophyletic. The species of *Strebla* have been splitted into species with a complete frontoclypeus and the anterior pigmented prescutal suture absent, and species with frontoclypeus with anterior detached plates and the anterior pigmented prescutal suture present.

In addition, species with a complete frontoclypeus were recovered more closely related to *Metelasmus*. Based on our results, we propose a new interpretation of the head characters for the species of *Strebla* with the complete frontoclypeus. However, an improvement in the morphological analysis is important to increase the number of characters from thorax, abdomen and genitalia.

Chapter 4

General conclusion

The present study contributes with unprecedented results to understand the evolutionary history of Streblidae, as well as the association with their hosts. In chapter 2, we presented the first molecular phylogeny with the largest sample of species and genera of New World Streblidae to date, especially for the subfamily Streblinae. We recovered the monophyly of the New World Streblidae, and of the subfamilies Nycterophiliinae and Streblinae. Futhermore, we corroborate the well-known non-monophyly of the subfamily Trichobiinae and the genus *Trichobius*. The proposed classification of Wenzel et al. (1966) for Streblinae is corroborated, but for the first time the monophyly of *Strebla* is contested, indicating it is a paraphyletic taxon in relation to *Metelasmus*. We also provide the first estimates of divergence times of the New World Streblidae, with the estimated age of origin to the Lower Miocene (15.3, 22.7–12.7 Ma 95% HPD). Our results further provide an insight into the historical association between the species of Streblidae and their bat hosts. The origin of the New World Streblidae, in particular of the subfamily Streblinae, is consistent with the period of diversification of the main host clades. Our study supports host-switch as the main process operating in the associations, even though the group is considered highly specialized and host-specific. This is in congruence with the results previously found by Graciolli and Carvalho (2012), and with the expectations of the Stockholm Paradigm (Brooks et al., 2015). Regarding associations, we hypothesized that host roosts may act as a key mechanism, weakening the filters argued by Dick and Patterson (2007) to keep host-parasite specificity, and favoring host-switch over cospeciation.

The morphological phylogeny of chapter 3 is the first to rely on all valid species of the subfamily Streblinae. The monophyly of Streblinae is well supported by numerous synapomorphies. Despite the low branch support, we have recovered *Anastrebla*, *Metelasmus* and

Paraeuctenodes as monophyletic. We corroborate the result of chapter 2, demonstrating that *Strebla* is not monophyletic. The species of *Strebla* have been splitted into species with a complete frontoclypeus and the anterior pigmented prescutal suture absent, and species with frontoclypeus with anterior detached plates and the anterior pigmented prescutal suture present. In addition, species with a complete frontoclypeus were recovered more closely related to *Metelasmus*. Based on our results, we propose a new interpretation of the head characters for the species of *Strebla* with the complete frontoclypeus. However, an improvement in the morphological analysis is important to increase the number of characters from thorax, abdomen and genitalia.

Finally, we believe that the data presented in this thesis provide a valuable contribution to the growing knowledge of both the group in question (Streblidae) and the understanding of the dynamics of host-parasite associations. Our study along with Graciolli and Carvalho (2012) establishes Streblidae as a good model to answer questions in a broad context, such as: the role of ecological timescale interactions over evolutionary timescale associations; how host-switches impact the eco-evolutionary dynamics of highly specialized parasites; what is the role of host ecology on parasite diversification, and so on. In addition, our study demonstrates that numerous aspects of Streblidae systematics remain to be explored, including the classification of Trichobiinae, *Trichobius* and *Strebla*, besides the need of morphological studies that improve the interpretation of the characters.

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Appendix

Appendix A

Tables

Table A.1 - Species of Streblinae, including information about samples with the sequenced mitogenome (mtDNA), their respective date of collection and the acromym of the collection where the material is deposited. See further specimen information in Supplemental Material 1.

Genera	Species	mtDNA	Date	Ownership
Anastrebla (5 species)	*A. caudiferae* Wenzel, 1976	Sequenced	12.iii.2017	MZSP
	A. mattadeni Wenzel, 1966	-	-	-
	A. modestini Wenzel, 1966[1]	Sequenced	22.xii.2008	ZUFMS
	A. nycteridis Wenzel, 1966	-	-	-
	A. spurrelli Wenzel, 1976	-	-	-
Metelasmus (2 species)	*M. pseudopterus* Coquillett, 1907[1]	Sequenced	?	LTFT-USP
	M. wenzeli Graciolli & Dick, 2004	-	-	-
Paraeuctenodes (2 species)	*P. longipes* Pessôa & Guimarães, 1937[1]	Sequenced	?	ZUFMS
	P. similis Wenzel, 1976	-	-	-
Strebla (26 species)	*S. altmani* Wenzel, 1966	-	-	-
	S. alvarezi Wenzel, 1966	-	-	-
	S. asternalis Wenzel, 1976	Sequenced	25.ix.2004	ZUFMS
	S. carvalhoi Graciolli, 2003	Sequenced	08.ii.2016	ZUFMS
	S. christinae Wenzel, 1966	Sequenced	18.viii.2013	MZSP
	S. chrotopteri Wenzel, 1976	Sequenced	08.ii.2016	ZUFMS
	S. consocia Wenzel, 1966	Sequenced	17.viii.2013	MZSP
	S. cormurae Wenzel, 1976	-	-	-
	S. curvata Wenzel, 1976	Sequenced	26.i.2017	ZUFMS
	S. diaemi Wenzel, 1966	Not worked	Unknown	ZUFMS
	S. diphyllae Wenzel, 1966	Sequenced	?	ZUFMS
	S. galindoi (García & Casal, 1965)	Sequenced	11.v.2005	ZUFMS
	S. guajiro Wenzel, 1966	Sequenced	24.viii.2013	LASBI-USP
	S. harderi Wenzel, 1976	Sequenced	04.ix.2011	ZUFMS
	S. hertigi Wenzel, 1966	Sequenced	20.viii.2013	LASBI-USP
	S. hoogstraali Wenzel, 1966	-	-	-
	S. kohlsi Wenzel, 1966	Not worked	2004	ZUFMS
	S. machadoi Wenzel, 1966	Sequenced	25.vii.2012	LASBI-USP
	S. matsoni Wenzel, 1976	Sequenced	14.xi.2004	ZUFMS
	S. mexicana Rondani, 1878	-	-	-
	S. mirabilis (Waterhouse, 1879)[2]	Sequenced	18.viii.2013	LASBI-USP
		Sequenced	18.viii.2013	LASBI-USP
	S. obtusa Wenzel, 1976	Sequenced	04.ix.2010	ZUFMS
	S. paramirabilis Wenzel, 1976	-	-	-
	S. proxima Wenzel, 1976	Sequenced	?	ZUFMS
	S. tonatiae (Kessel, 1924)	Sequenced	21/08/2013	LASBI-USP
	S. wiedemanni Kolenati, 1856[1]	Sequenced	20.i.2017	ZUFMS

Table A.2 - Species of Trichobiinae with sequenced mitogenome (mtDNA), showing sample information about their respective date of collection and the acromym of the collection where the material is deposited. See further specimen information in Supplemental Material 1.

Species	Date	Ownership
Megistopoda aranea (Coquillett, 1899)	22.ii.2017	MZSP
Neotrichobius delicatus Machado-Allison, 1966	21.viii.2013	LASBI-USP
Noctiliostrebla morena Alcantara et al., 2019	03.iii.2013	LASBI-USP
Parastrebla handleyi Wenzel, 1966	04.ix.2010	ZUFMS
Pseudostrebla ribeiroi Costa Lima, 1921	29.i.2013	LASBI-USP
Speiseria ambigua Kessel, 1925	22.viii.2013	LASBI-USP
Stizostrebla longirostris Jobling, 1939	?	ZUFMS
Synthesiostrebla cisandina Graciolli & Azevedo, 2011	?	ZUFMS

Table A.3 - Mitogenomes obtained through NCBI Organelle Genome Resources and used to compare with the generated annotation by MITOS2.

Superfamily	Family	Species	Accession Number
Culicoidea	Culicidae	*Aedes aegypti* (Linnaeus, 1762)	EU352212
Culicoidea	Culicidae	*Anopheles arabiensis* Giles, 1902	KT382816
Tephritidae	Tephritoidea	*Bactrocera dorsalis* (Hendel, 1912)	DQ845759
Oestroidea	Calliphoridae	*Calliphora vicina* Robineau-Desvoidy, 1830	JX913760
Oestroidea	Calliphoridae	*Chrysomya albiceps* (Wiedemann, 1819)	JX913736.1
Ephydroidea	Drosophilidae	*Drosophila melanogaster* Meigen, 1830	KJ947872
Oestroidea	Tachinidae	*Elodia flavipalpis* Aldrich, 1933	JQ348961
Oestroidea	Tachinidae	*Exorista sorbillans* (Wiedemann, 1830)	HQ322500
Oestroidea	Calliphoridae	*Lucilia cuprina* (Wiedemann, 1830)	JX913744
Platypezoidea	Phoridae	*Megaselia scalaris* Loew, 1866	KF974742
Hippoboscoidea	Hippoboscidae	*Melophagus ovinus* (Linnaeus, 1758)	NC_037368
Muscoidea	Muscidae	*Musca domestica* Linnaeus, 1758	KM200723
Hippoboscoidea	Streblidae	*Paradyschiria parvula* Falcoz, 1931	NC_044702
Hippoboscoidea	Streblidae	*Paratrichobius longicrus* (Miranda Ribeiro, 1907)	NC_044652
Oestroidea	Sarcophagidae	*Sarcophaga albiceps* Meigen, 1826	KT444443

[1] Type species

[2] Species with more than one sequenced specimen

Table A.4 - Substitution model selected for each gene by ModelFinder in IQTREE, and by bModelTest in BEAST2 for the for the analyzes under FBD and Yule process.

Gene	ModelFinder	$\text{bModelTest}_{\text{FBD}}$	$\text{bModelTest}_{\text{Yule}}$
rrnS	GTR+F+I+G4	TVM+I+G	TVM+I+G
rrnL	GTR+F+R3	K81+I+G	K81+I+G
ATP6	GTR+F+I+G4	GTR+I+G	GTR+I+G
ATP8	TIM3+F+I+G4	TN93+I+G	TN93+I+G
CO1	GTR+F+I+G4	TIM+I+G	TIM+I+G
CO2	GTR+F+I+G4	GTR+I+G	GTR+I+G
CO3	GTR+F+I+G4	TIM+I+G	TIM+I+G
CYTB	GTR+F+I+G4	TIM+I+G	TIM+I+G
ND1	TIM+F+I+G4	GTR+I+G	GTR+I+G
ND2	TIM+F+R4	GTR+I+G	GTR+I+G
ND3	TIM+F+I+G4	GTR+I+G	GTR+I+G
ND4	GTR+F+R4	GTR+I+G	GTR+I+G
ND4L	K3Pu+F+I+G4	TVM+I+G	TVM+I+G
ND5	GTR+F+R4	GTR+I+G	GTR+I+G
ND6	TIM+F+I+G4	GTR+I+G	GTR+I+G
trnA	TPM2u+F+G4	TVM+I+G	TVM+I+G
trnC	TPM2+F+G4	TVM+I+G	TVM+I+G
trnD	TPM2u+F+I+G4	K80/HKY+I+G	K80/HKY+I+G
trnE	F81+F+G4	K80/HKY+I+G	K80/HKY+I+G
trnF	K3Pu+F+I+G4	K81+I+G	K81+I+G
trnG	K3Pu+F+G4	TVM+I+G	TVM+I+G
trnH	K3Pu+F+G4	K81+I+G	K81+I+G
trnI	K3Pu+F+G4	K81+I+G	K81+I+G
trnK	TPM2+F+R2	TVM+I+G	TVM+I+G
trnL1	K3Pu+F+G4	TN93+I+G	K80/HKY+I+G
trnL2	TIM2+F+G4	GTR+I+G	GTR+I+G
trnM	TIM+F+I+G4	GTR+I+G	GTR+I+G
trnN	TVM+F+G4	TVM+I+G	TVM+I+G
trnP	K3Pu+F+I+G4	K80/HKY+I+G	K80/HKY+I+G
trnQ	TIM+F+I+G4	TIM+I+G	TIM+I+G
trnR	TVM+F+G4	TVM+G	TVM+G
trnS1	TVM+F+G4	TVM+I+G	TVM+I+G
trnS2	TIM2+F+R2	TVM+G	TVM+G
trnT	K3Pu+F+G4	TVM+G	TVM+G
trnV	TIM+F+G4	K81+I+G	K81+I+G
trnW	GTR+F+R2	GTR+I+G	GTR+I+G
trnY	K3Pu+F+G4	K81+I+G	K81+I+G

Table A.5 - Concentration of libraries before manual normalization, size of data generated in sequencing, and MIRA (Chevreux et al., 1999) / MITOBim (Hahn et al., 2013) assembly statistics result.

Species	Conc (ng/μl)	Size (Gb)	Total Reads	Length (bp)	Av. Quality	Max. Coverage	Av. Coverage	GC%
Anastrebla caudiferae	5.86	3.9	13,681,494	18,052	77	7,270	331.23	21.54
Anastrebla modestini	13.7	18.0	88,553,862	16,568	76	171,024	2,790.83	20.82
Megistopoda aranea	13	6.1	22,713,068	16,972	76	53,425	1,109.85	20.50
Metelasmus pseudopterus	4.18	1.8	6,402,864	16,393	76	1,370	201.95	17.04
Neotrichobius delicatus	10.6	6.3	22,319,876	16,661	75	151,503	2,055.51	20.22
Noctiliostrebla morena	24.2	13.0	45,114,316	16,376	78	3,567	428.78	19.12
Paraeuctenodes longipes	4.38	3.1	11,840,126	16,464	77	1,633	293.24	20.00
Parastrebla handleyi	6.6	4.4	18,605,100	16,092	57	284	39.28	21.72
Pseudostrebla ribeiroi	8.86	5.1	18,594,658	16,360	77	9,374	340.01	19.63
Speiseria ambigua	3.66	2.7	10,477,142	17,006	70	14,884	2,509.45	19.75
Stizostrebla longirostris	16.5	14.0	57,085,898	16,611	74	4,569	161.85	21.40
Strebla asternalis	6.22	7.0	37,782,168	17,801	57	54,433	3,441.71	31.18
Strebla carvalhoi	8.32	5.1	17,787,860	18,508	74	11,956	448.24	16.85
Strebla christinae	5.08	2.6	9,166,832	16,305	76	28,352	500.50	18.76
Strebla chrotopteri	4.12	2.1	7,457,868	16,737	73	19,005	1,444.12	19.34
Strebla consocia	9.62	2.3	7,191,790	16,563	77	918	213.33	19.31
Strebla curvata	12.1	5.5	18,972,226	17,115	75	3,726	644.13	20.98
Strebla diaemi	22.8	19.0	70,147,354	16,548	54	9,790	503.56	41.42
Strebla diphyllae	4.26	2.0	6,968,274	16,479	76	15,487	769.33	18.89
Strebla galindoi	8.48	9.0	41,165,192	17,326	69	106,377	1,312.55	19.94
Strebla guajiro	2.69	1.6	5,920,026	17,841	75	7,204	471.52	19.81
Strebla harderi	1.99	1.5	5,680,512	16,434	77	3,948	182.23	19.75
Strebla hertigi	5.92	1.9	6,056,586	16,169	78	759	156.34	19.16
Strebla kohlsi	1.76	2.8	17,679,010	19,049	44	40,777	2,657.64	36.94
Strebla machadoi	5.18	2.5	9,066,520	16,245	75	14,379	453.91	19.50
Strebla matsoni	5.58	5.4	27,386,728	16,200	54	70,827	1,856.98	23.80
Strebla mirabilis (*Phyllostomus*)	6.18	4.3	16,988,350	16,530	76	31,754	921.96	19.49
Strebla mirabilis (*Trachops*)	14.2	7.8	27,306,996	16,868	75	30,542	1,356.73	19.43
Strebla obtusa	4.24	2.3	8,090,552	16,710	63	8,176	140.30	19.24
Strebla proxima	3.92	2.9	11,021,742	16,197	79	2,251	236.19	20.15
Strebla tonatiae	5.46	2.8	9,550,484	20,645	76	1,591	332.28	23.13
Strebla wiedemanii	4.86	2.4	8,297,868	16,280	73	6,864	926.30	19.17
Synthesiostrebla cisandina	5.54	3.9	13,671,058	17,012	79	2,777	194.04	19.86

Table A.6 - Primary associations between genera of Streblinae and their respective host families and subfamilies. Ns: number of species in the genus; Nf: number of Streblinae species that have primary hosts belonging to the bat family; Nsf: number of Streblinae species that have primary hosts belonging to the bat subfamily. See species information in Supplemental Material 12.

Streblinae genera (Ns)	Host family	Nf	Host subfamily	Nsf
Anastrebla (5)	Phyllostomidae	5	Glossophaginae	3
			Lonchophyllinae	2
Metelasmus (2)	Phyllostomidae	2	Stenodermatinae	2
Paraeuctenodes (2)	Phyllostomidae	2	Carolliinae	1
			Glossophaginae	1
Strebla (25)	Phyllostomidae	22	Carolliinae	1
			Desmodontinae	3
			Glossophaginae	3
			Glyphonycterinae	1
			Lonchorhininae	1
			Micronycterinae	2
			Phyllostominae	10
			Stenodermatinae	1
	Emballonuridae	3	Emballonurinae	3

Table A.7 - Results from the ParaFit analysis between the Vespertilioniformes phylogeny and the Streblinae phylogeny of both analyzes, Maximum Likelihood (ML) and Bayesian. The global test (ParaFitGlobal) results and each individual link tests (ParaFitLink1 and ParaFitLink2) results are indicated for both, raw *P-value* and the Benjamini-Hochberg correction (BH). Since the individual link tests obtained the same values, they are presented only once for each analysis. Bold values indicate significance ($\alpha = 0.05$).

Host	Parasite	ML		Bayesian	
		Raw	BH	Raw	BH
Anoura caudifer	*Anastrebla caudiferae*	0.621	0.937	0.649	0.965
Anoura geoffroyi	*Anastrebla modestini*	0.643	0.937	0.664	0.965
Lonchophylla robusta	*Anastrebla nycteridis*	0.753	0.937	0.715	0.965
Artibeus jamaicensis	*Metelasmus pseudopterus*	0.905	0.937	0.830	0.965
Glossophaga soricina	*Paraeuctenodes longipes*	0.913	0.937	0.978	0.978
Micronycteris megalotis	*Strebla alvarezi*	0.838	0.937	0.803	0.965
Saccopteryx bilineata	*Strebla asternalis*	0.286	0.937	0.416	0.965
Anoura caudifer	*Strebla carvalhoi*	0.883	0.937	0.913	0.965
Phylloderma stenops	*Strebla christinae*	0.544	0.937	0.469	0.965
Chrotopterus auritus	*Strebla chrotopteri*	0.650	0.937	0.610	0.965
Phyllostomus elongatus	*trebla consocia*	0.185	0.937	0.146	0.965
Phyllostomus hastatus	*Strebla consocia*	0.211	0.937	0.168	0.965
Glossophaga soricina	*Strebla curvata*	0.862	0.937	0.891	0.965
Diaemus youngi	*Strebla diaemi*	0.689	0.937	0.560	0.965
Diphylla ecaudata	*Strebla diphyllae*	0.576	0.937	0.491	0.965
Tonatia bidens	*Strebla galindoi*	0.727	0.937	0.766	0.965
Tonatia saurophila	*Strebla galindoi*	0.725	0.937	0.764	0.965
Carollia perspicillata	*Strebla guajiro*	0.840	0.937	0.854	0.965
Anoura geoffroyi	*Strebla harderi*	0.880	0.937	0.907	0.965
Phyllostomus discolor	*Strebla hertigi*	0.207	0.937	0.215	0.965
Micronycteris minuta	*Strebla machadoi*	0.909	0.937	0.878	0.965
Macrophyllum macrophyllum	*Strebla matsoni*	0.867	0.937	0.876	0.965
Phyllostomus hastatus	*Strebla mirabilis*	0.783	0.937	0.732	0.965
Trachops cirrhosus	*Strebla mirabilis*	0.612	0.937	0.588	0.965
Trinycteris nicefori	*Strebla obtusa*	0.937	0.937	0.933	0.965
Peropteryx macrotis	*Strebla proxima*	**0.021**	0.273	**0.020**	0.199
Peropteryx kappleri	*Strebla proxima*	**0.015**	0.273	**0.015**	0.199
Peropteryx trinitatis	*Strebla proxima*	**0.019**	0.273	**0.019**	0.199
Lophostoma brasiliense	*Strebla tonatiae*	0.350	0.937	0.268	0.965
Desmodus rotundus	*Strebla wiedemanii*	0.770	0.937	0.676	0.965
Global Test		0.860		0.869	

Table A.8 - Results of the multimodel event-based analysis with BioGeoBEARS. LnL, logarithmic Likelihood; *d*, dispersion; *e*, extinction; *j*, founder event/long dispersal; AIC, Akaike information criterion; wAIC, weighted Akaike information criterion; AICc, corrected Akaike information criterion; wAICc, weighted corrected Akaike information criterion. Bold highlight indicates the best model fit.

Model	LnL	*d*	*e*	*j*	AIC	wAIC	AICc	wAICc
DEC	-72.05	0.02	0.06	0	148.1	4.00E-12	148.6	5.00E-12
DEC+J	**-45.53**	**1.00E-12**	**1.00E-12**	**0.12**	**97.05**	**0.47**	**98.19**	**0.47**
DIVALIKE	-72.58	0.01	0.01	0	149.2	2.00E-12	149.7	3.00E-12
DIVALIKE+J	-45.67	1.00E-12	1.00E-12	0.13	97.34	0.41	98.48	0.41
BAYAREALIKE	-75.13	0.02	0.31	0	154.3	2.00E-13	154.8	2.00E-13
BAYAREALIKE+J	-46.95	1.00E-07	1.00E-07	0.11	99.9	0.11	101	0.11

Appendix B

Figures

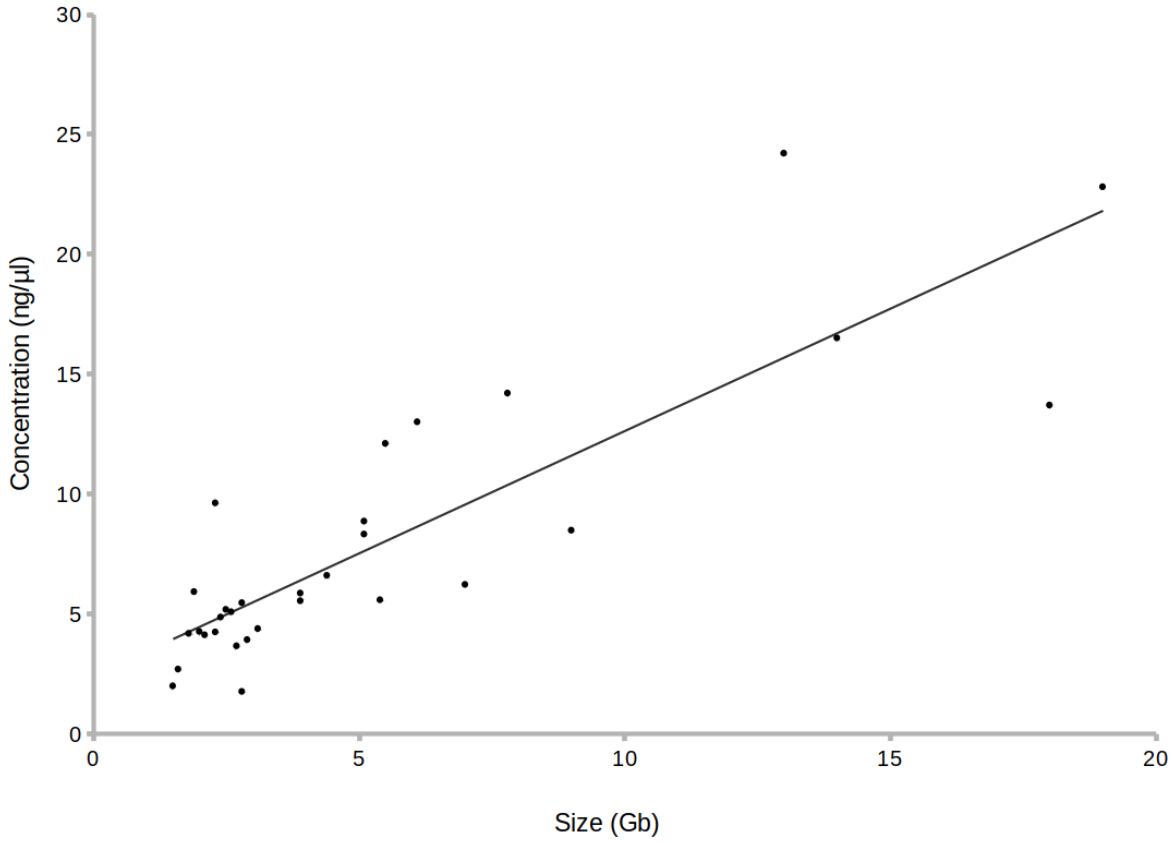

Figure B.1: Correlation plot based on 33 samples between the library concentration before pooling dilution (ng/μl) and the size of the generated data (Gb) ($r = 0.8168$, *P-value* < 0.0001). See further information in Table A.5.

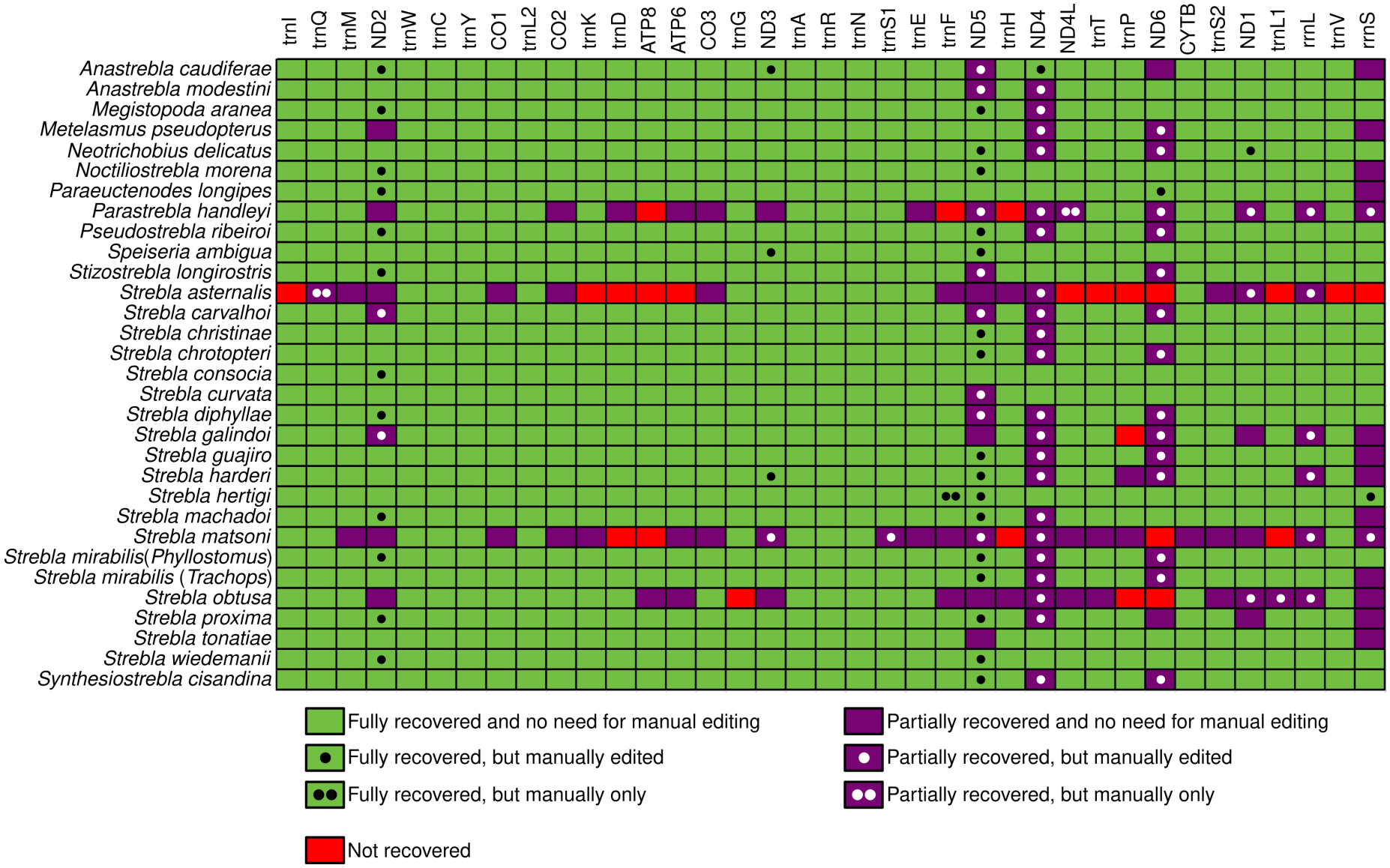

Figure B.2: Illustrative summary of the final result of the mitogenome sequencing, assembly and annotation. It shows the genes obtained for each species, as well as whether they were completely or partially recovered and needed manual editing. The species *Strebla diaemi* and *S. kohlsi* are not shown as they were excluded from analysis due to contamination. See detailed information in Supplemental Material 4.

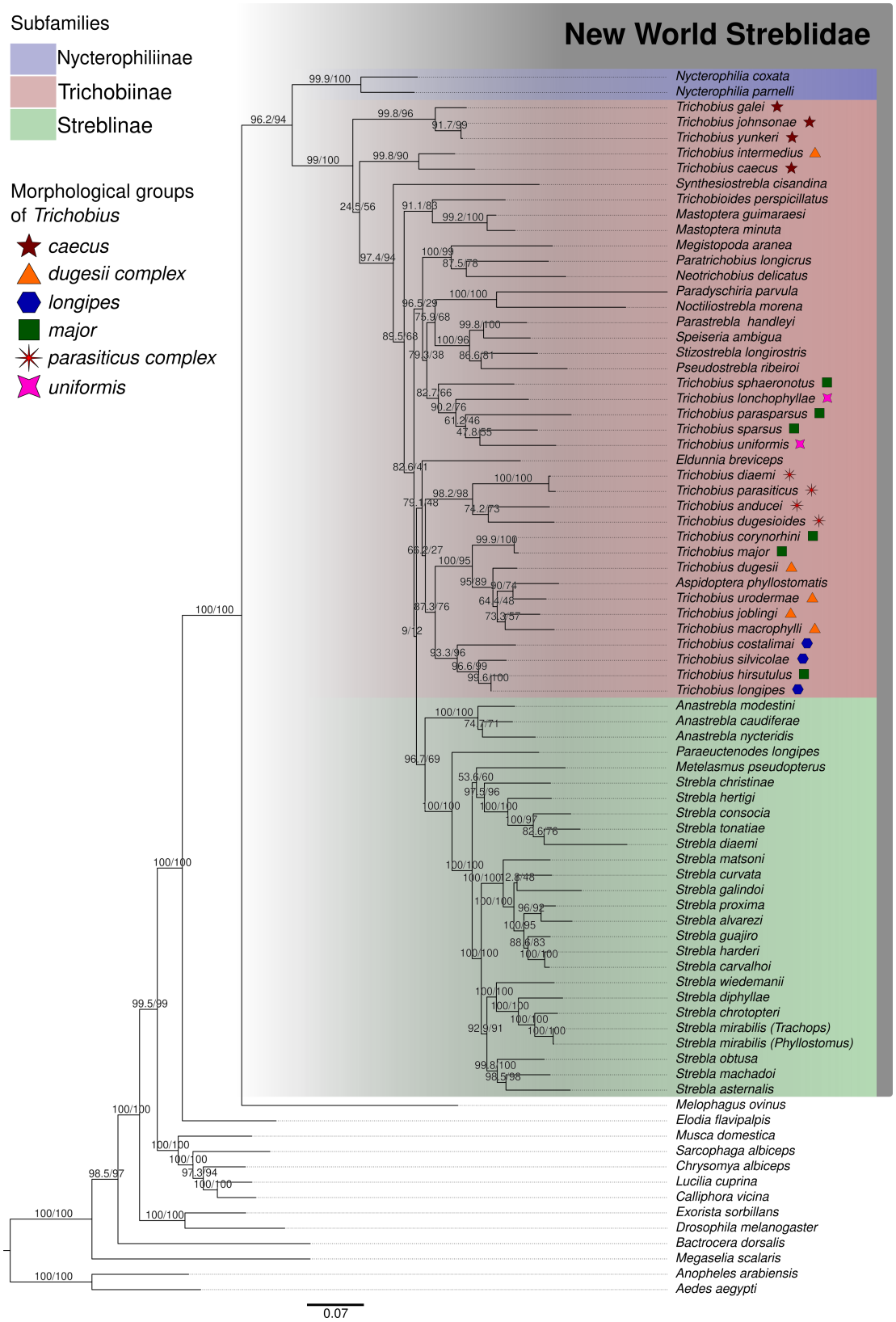

Figure B.3: Phylogeny of New World Streblidae inferred with maximum likelihood using IQTREE (Nguyen et al., 2015) under the most parameter-rich model for each gene, GTR+R4+F. Support values indicate respectively "SH-aLRT branch test" (Guindon et al., 2010) and "UFBoot" (Minh et al., 2013; Hoang et al., 2018).

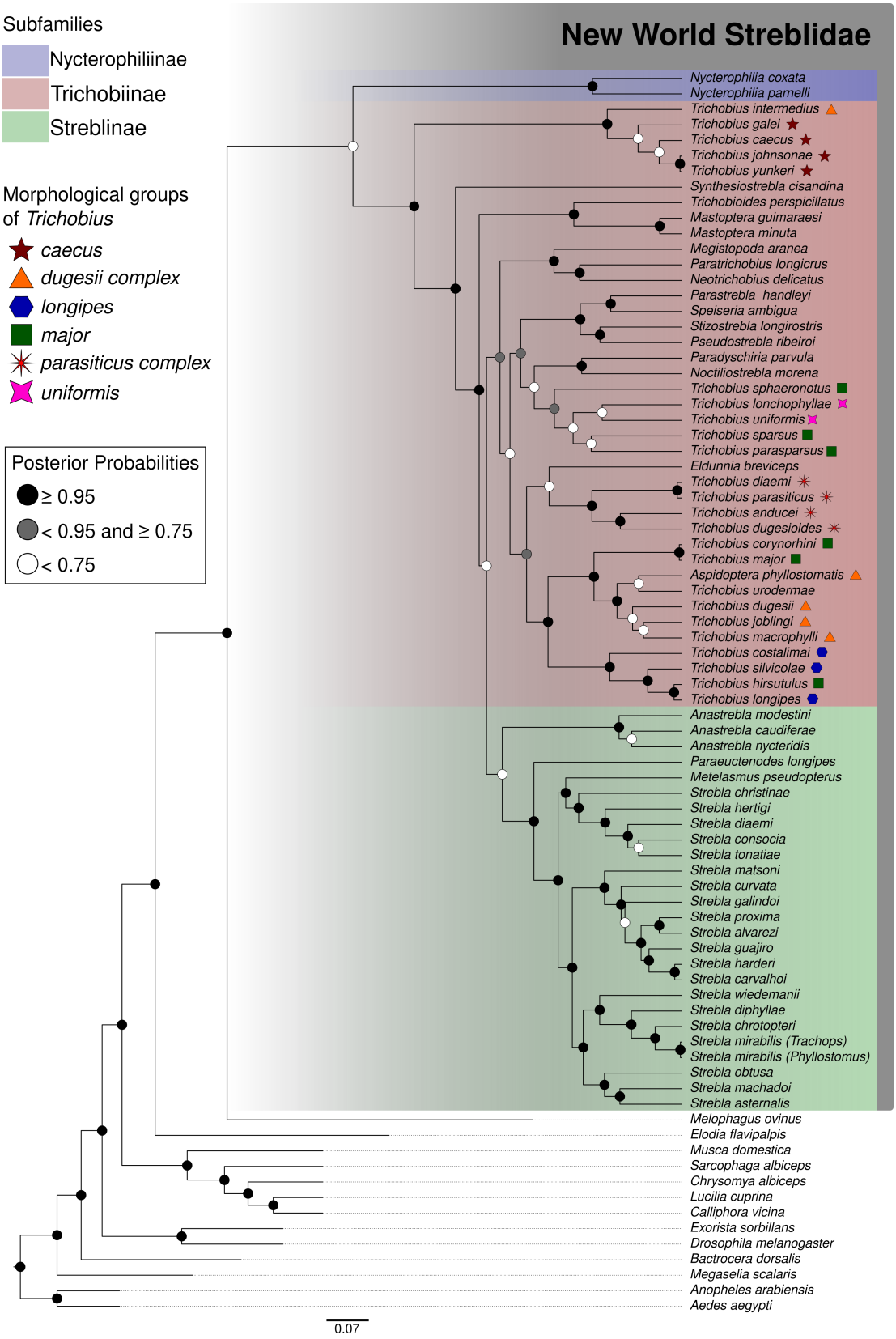

Figure B.4: Phylogeny of New World Streblidae inferred with bayesian inference using BEAST2.5 (Bouckaert et al., 2019) under the Yule process (Yule, 1925; Harding, 1971), the lognormal relaxed clock (Drummond et al., 2006), and without constrains and divergence time estimation (B). Circles indicate posterior probabilies.

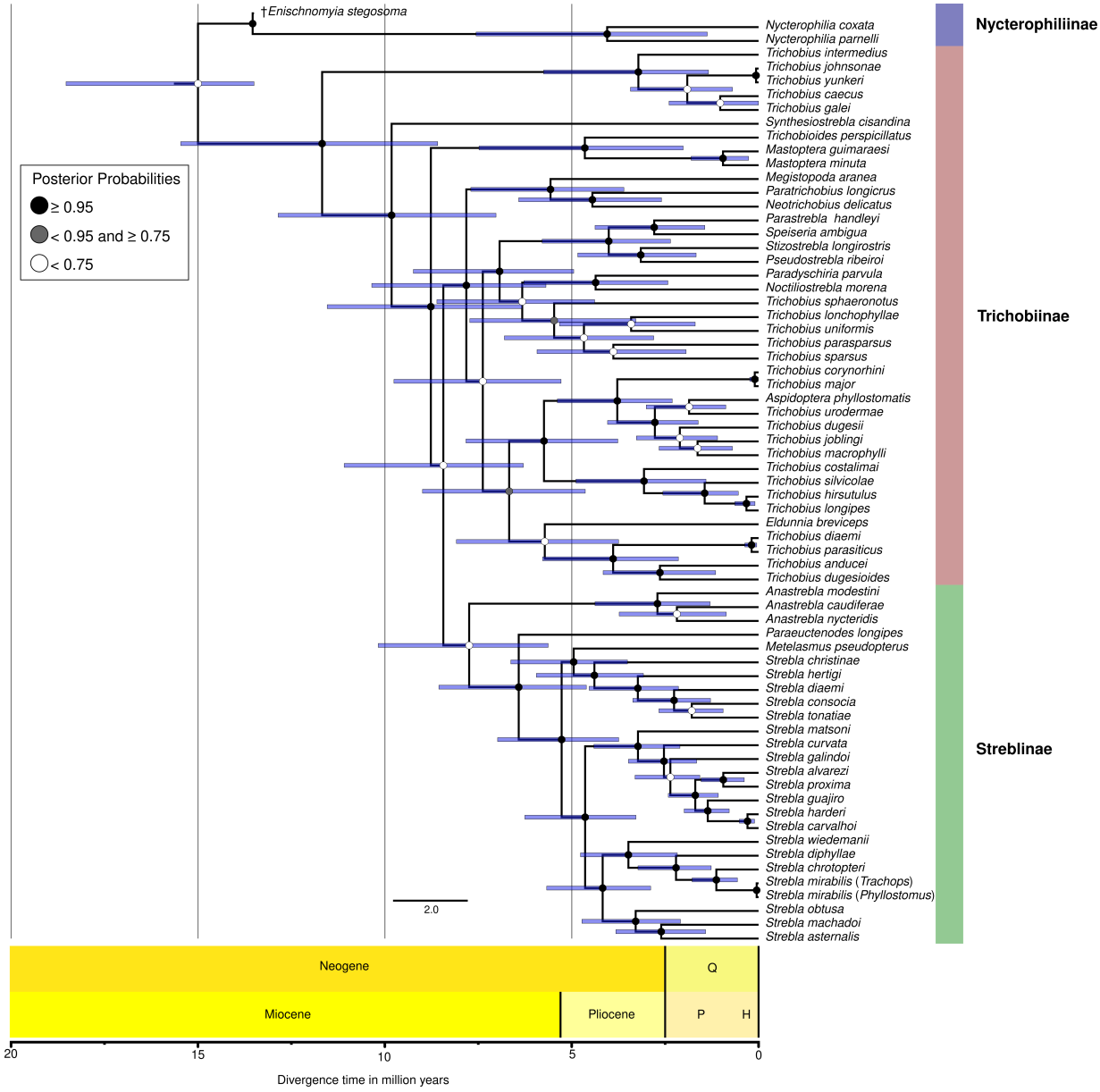

Figure B.5: Divergence times among lineages of New World Streblidae estimated in BEAST 2 (Bouckaert et al., 2019), under the FBD process (Stadler, 2009; Heath et al., 2014) and the lognormal relaxed clock (Drummond et al., 2006). Bars depict the 95% highest posterior density (HPD) intervals of each estimate. Mean ages and ranges are provided in Supplemental Material 9.

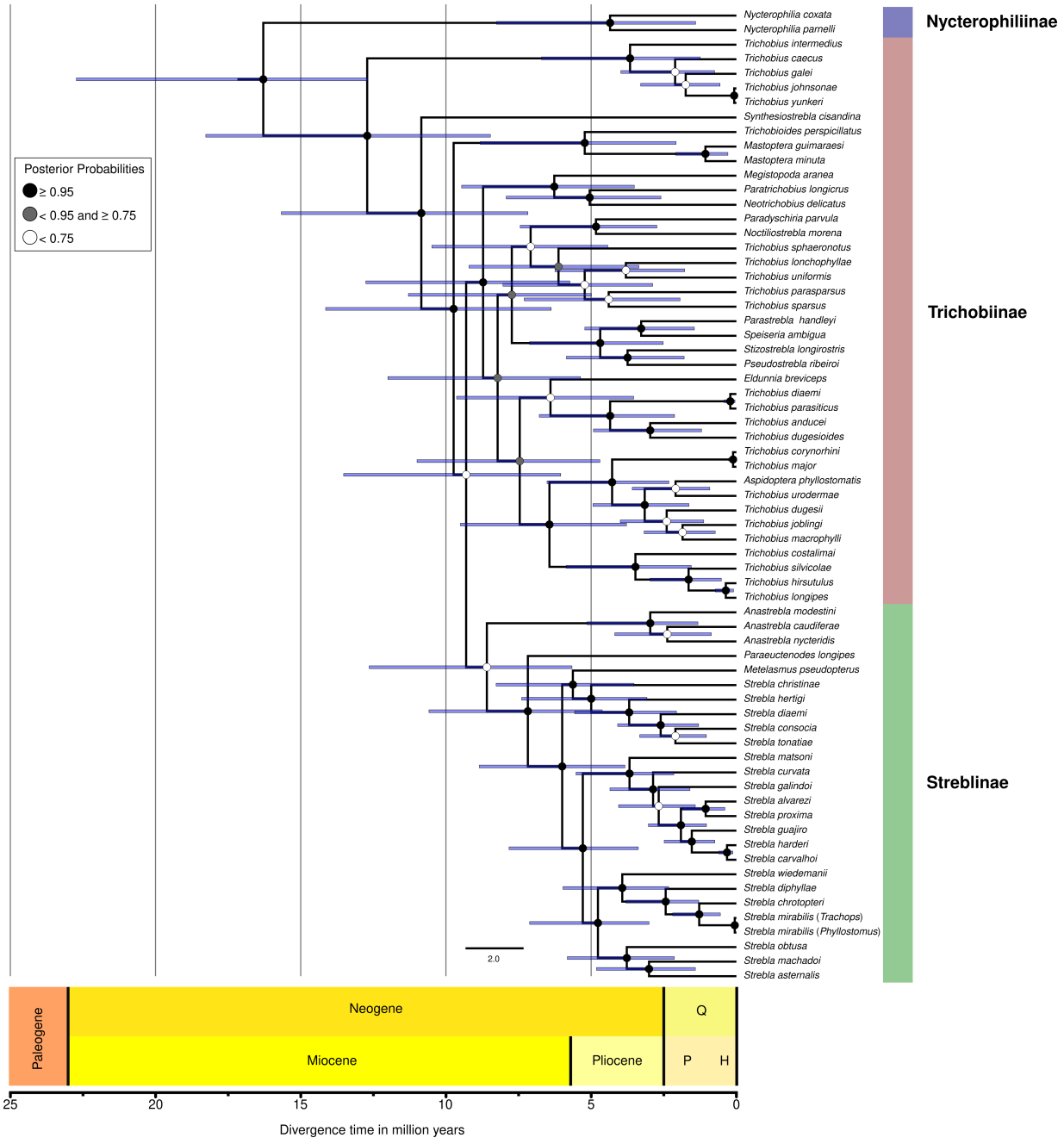

Figure B.6: Divergence times among lineages of New World Streblidae estimated in BEAST 2 (Bouckaert et al., 2019), under the Yule process (Yule, 1925; Harding, 1971) and the lognormal relaxed clock (Drummond et al., 2006). Bars depict the 95% highest posterior probability density of each estimate. Mean ages and ranges are provided in Supplemental Material 10.

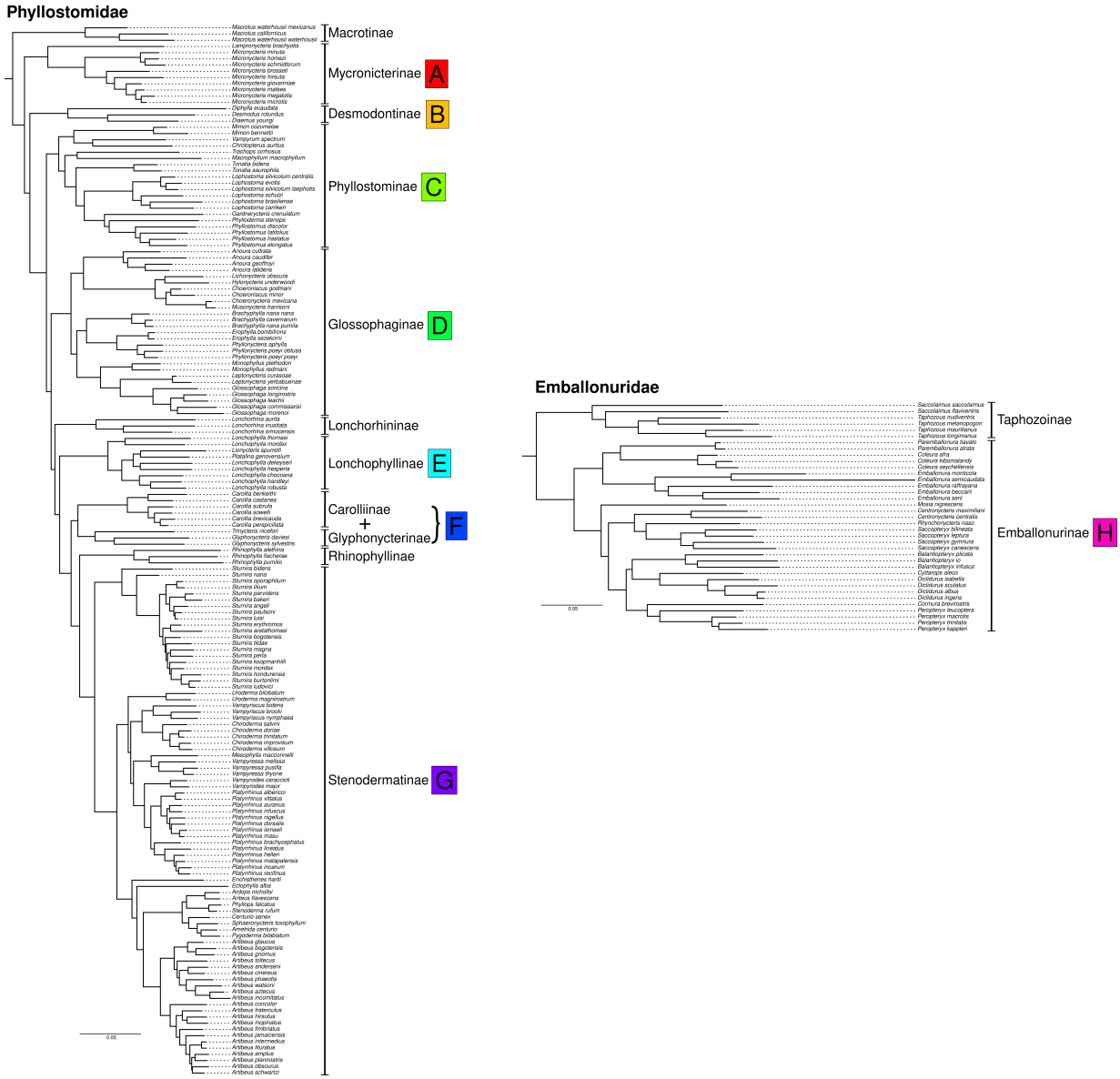

Figure B.7: Relationships of species and subfamilies within the two families parasitized by Streblinae, Phyllostomidae and Emballonuridae. The letters inside the colored frames indicate the groups defined to infer ancestral hosts by BioGeoBEARS (Matzke, 2013, 2014)

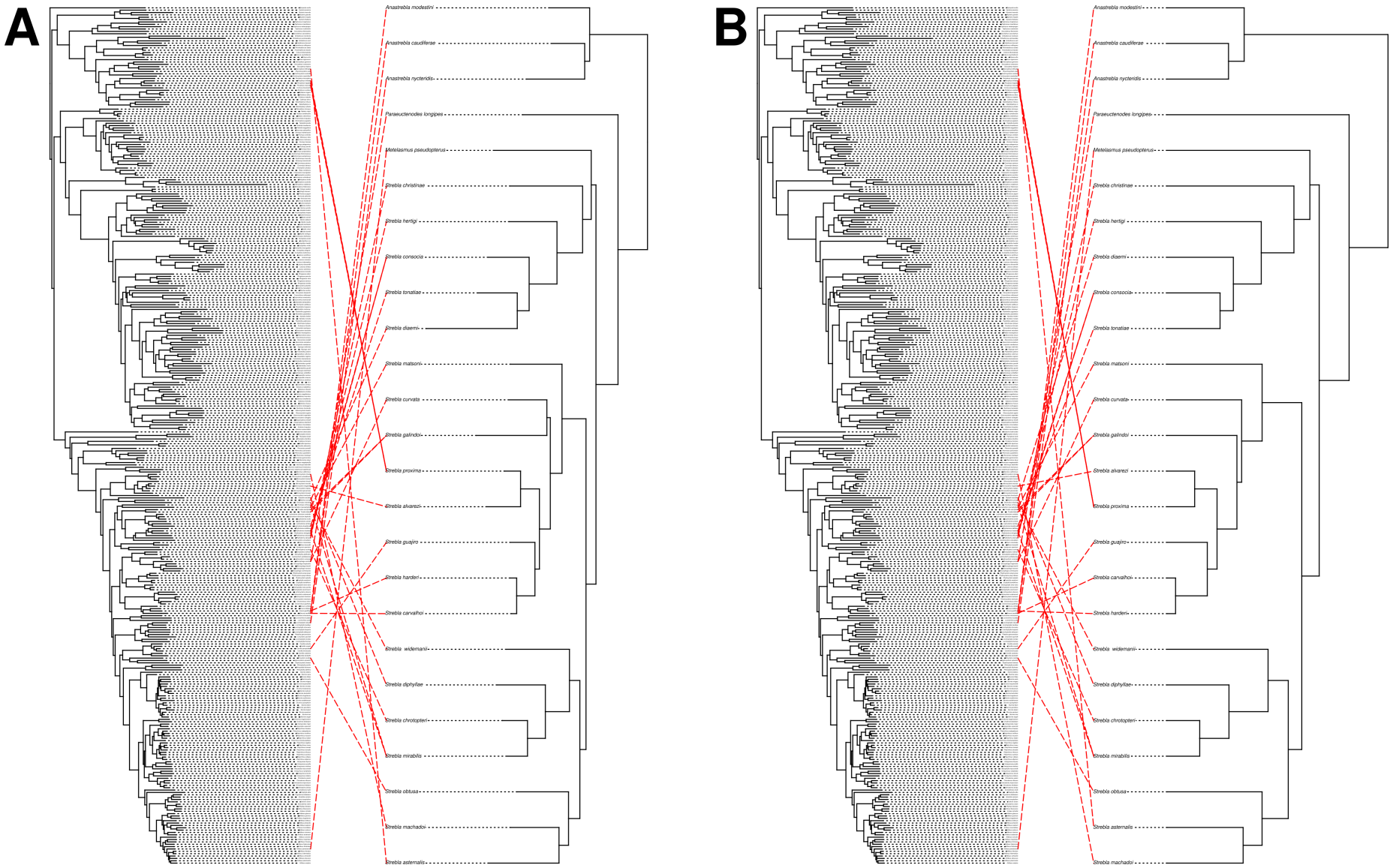

Figure B.8: Tanglegrams showing the associations between Streblinae (right) species and their bat hosts (left). (A) Maximum likelihood phylogeny of Vespertilioniformes (left) compared to a maximum likelihood phylogeny of Streblinae. (B) Maximum likelihood phylogeny of Vespertilioniformes (left) compared to a bayesian phylogeny of Streblinae.

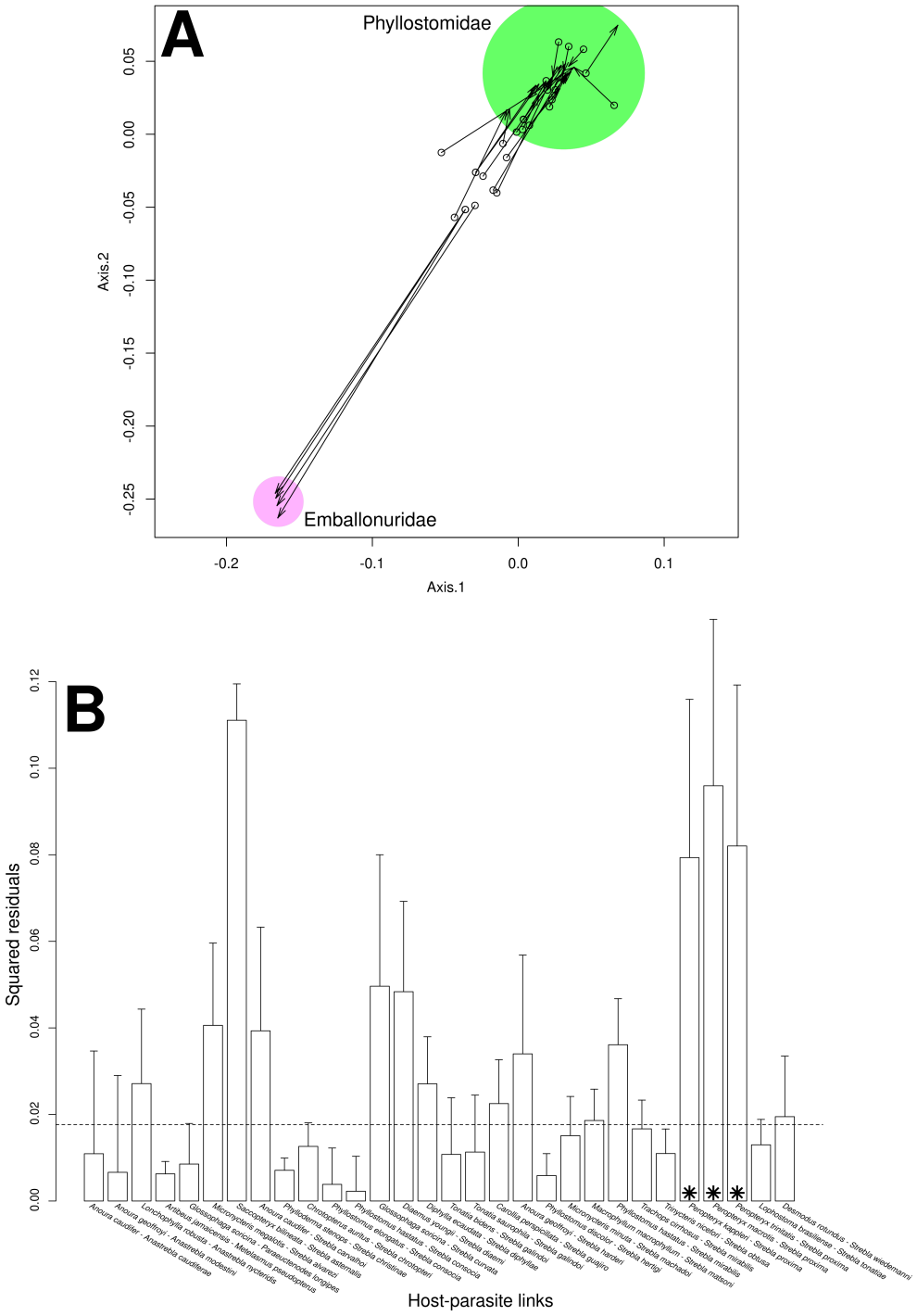

Figure B.9: Procrustean superimpostion plot and contributions of individual host-parasite links to the Procrustean fit of Streblinae species and their hosts. Analysis performed using PACo (Balbuena et al., 2013), between the maximum likelihood phylogeny of Vespertilioniformes and the maximum likelihood phylogeny of Streblinae. (A) The ordinations of bats and Streblinae species are Principal Correspondence Coordinates of patristic distances; Streblinae species configuration (dots) has been rotated and scaled to fit the host ordination (arrow tips); length of arrows represents the projection of residuals onto the first two axes. (B) Jacknifed squared residuals (bars) and upper 95% confidence intervals (error bars) resulting from applying PACo to patristic distances; asterisks identify links significantly supported ($\alpha < 0.05$) by individual link tests without Benjamini-Hochberg correction, and the dashed line indicates the median squared residual value.

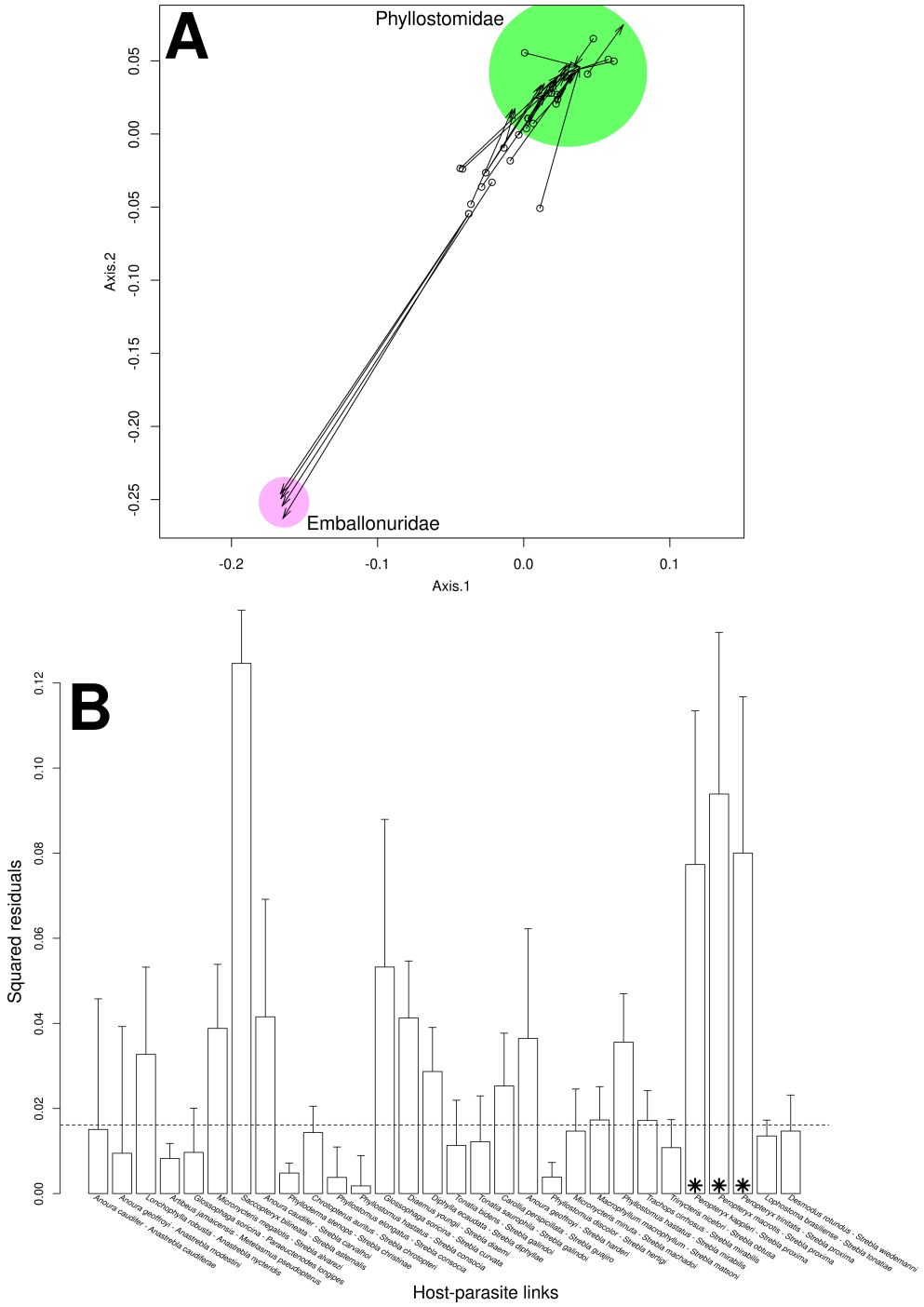

Figure B.10: Procrustean superimpostion plot and contributions of individual host-parasite links to the Procrustean fit of Streblinae species and their hosts. Analysis performed using PACo (Balbuena et al., 2013), between the maximum likelihood phylogeny of Vespertilioniformes and the bayesian phylogeny of Streblinae. (A) The ordinations of bats and Streblinae species are Principal Correspondence Coordinates of patristic distances; Streblinae species configuration (dots) has been rotated and scaled to fit the host ordination (arrow tips); length of arrows represents the projection of residuals onto the first two axes. (B) Jacknifed squared residuals (bars) and upper 95% confidence intervals (error bars) resulting from applying PACo to patristic distances; asterisks identify links significantly supported ($\alpha < 0.05$) by individual link tests without Benjamini-Hochberg correction, and the dashed line indicates the median squared residual value.

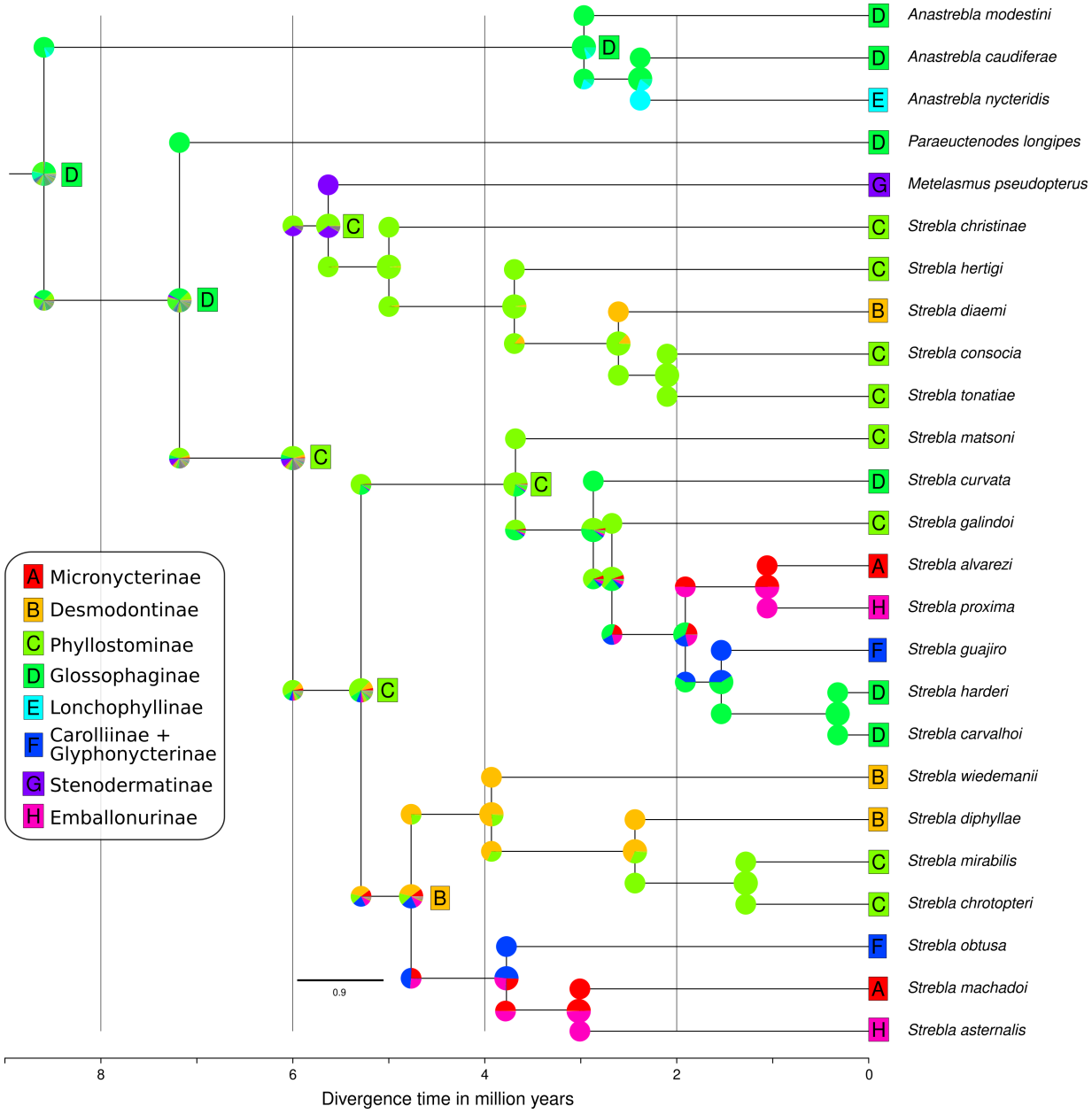

Figure B.11: Results of Dispersal-Extinction-Cladogenesis (DEC+J) chronogram of the time-calibrated analysis obtained by BioGeoBEARS (Matzke, 2013, 2014). It shows the most likely ancestral host for lineages of the subfamily Streblinae.

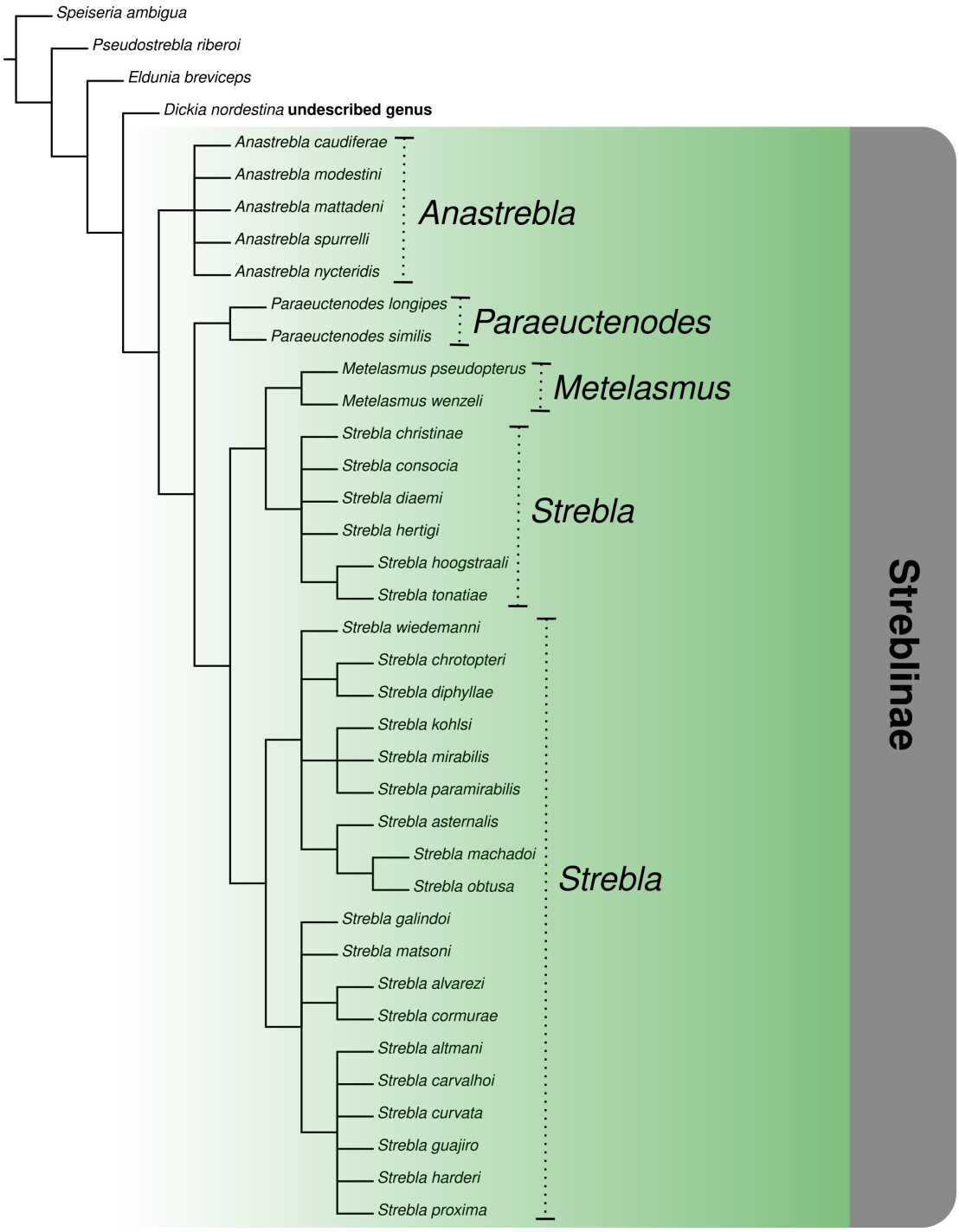

Figure B.12: Single most parsimonious tree (L = 76, CI = 93 and RI = 97) of Streblinae inferred by parsimony analysis of morphological characters and equal character weight, using TNT1.5 (Goloboff and Catalano, 2016).

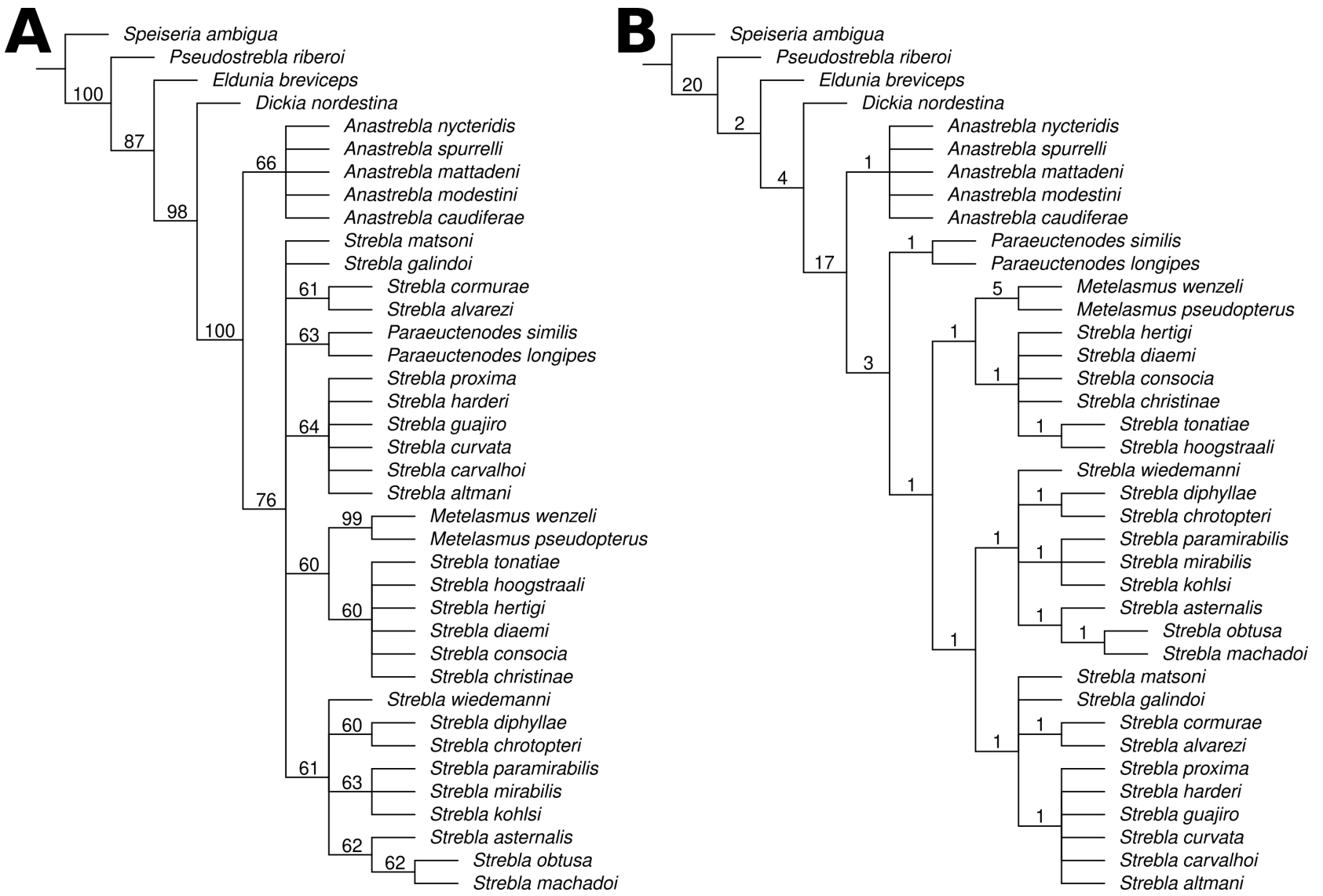

Figure B.13: Branch support of the most parsimonious tree. (A) Bootstrap values over 50%, with the values shown on the branches representing percentages. (A) Bremer support values, with nodes collapsed below 1.

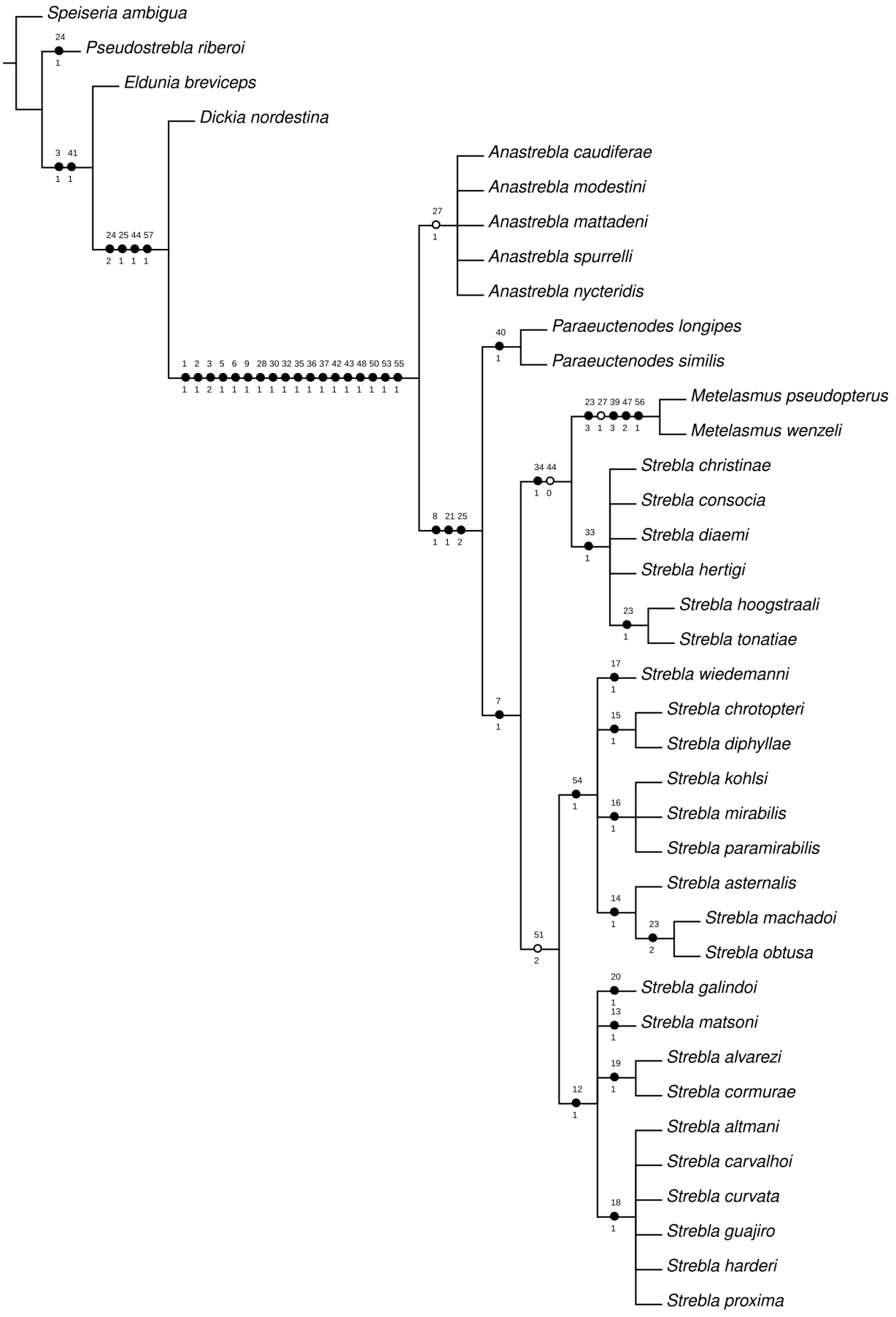

Figure B.14: Most parsimonious tree with unambiguous character state changes mapped. Solid circles = synapomorphies, blank circles = homoplasies.

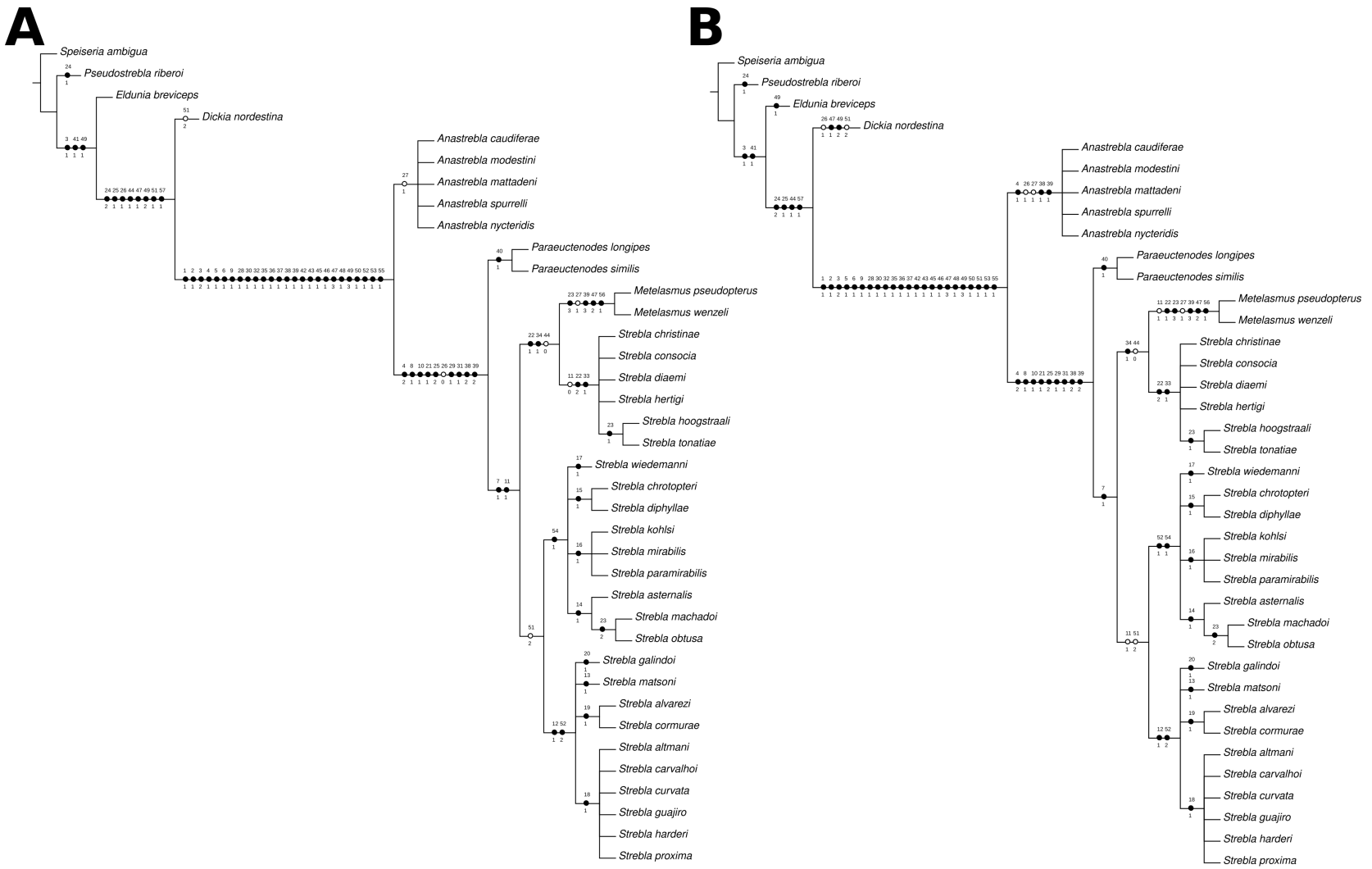

Figure B.15: Most parsimonious tree with ambiguous character state changes mapped. (A) Mapping under fast optimization. (B) Mapping under slow optimization. Solid circles = synapomorphies, blank circles = homoplasies.

Appendix C

List of characters

Head

1. Head capsule shape: (0) oval and slightly flattened dorsoventrally; (1) triangular and strongly flattened dorsoventrally. Ci=100, Ri=100.

2. Palpus shape (0) foliaceous; (1) shieldlike. Ci=100, Ri=100.

3. Direction of palpus: (0) up; (1) back; (2) forward. Ci=100, Ri=100.

4. Anterior portion of the palpus: (0) straight or slightly arched; (1) folded over itself, and longer than wide; (2) folded over itself, and wider than long. Ci=100, Ri=100.

5. Clypeus: (0) Projected along with the anterior portion of the face; (1) Projected solely. Ci=100, Ri=100.

6. Chitinised cone: (0) indistinguishable; (1) distinguishable. Ci=100, Ri=100.

7. Sclerotinized anterior margin position of the face relative to the chitinised cone: (0) not reaching the anterior margin of the chitinised cone; (1) reaching the anterior margin of the chitinised cone. Ci=100, Ri=100.

8. Frontoclypeus shape: (0) concave; (1) flattened. Ci=100, Ri=100.

9. Width of the anterior margin of face in relation to clypeus: (0) longer than clypeus; (1) as wide as clypeus. Ci=100, Ri=100.

10. Anterior portion of the face close to clypeus: (0) wide and short; (1) funneled and elongated. Ci=100, Ri=100.

11. Anterior portion of frontoclypeus plate: (0) entire; (1) detached plates. Ci=50, Ri=93.

12. Detached plates shape: (0) conspicuous and strong; (1) inconspicuous and thin. Ci=100, Ri=100.

13. Detached plates shape as indistinct: (0) no; (1) yes. Ci=0, Ri=0.

14. Detached plates shape as L (0) no; (1) yes. Ci=100, Ri=100.

15. Detached plates shape trapezoid wider than long: (0) no; (1) yes. Ci=100, Ri=100.

16. Detached plates shape parallelogram with inclinated anterior margin: (0) no; (1) yes. Ci=100, Ri=100.

17. Detached plates shape parallelogram: (0) no; (1) yes. Ci=0, Ri=0.

18. Detached plates shape comma: (0) no; (1) yes. Ci=100, Ri=100.

19. Detached plates shape acute trapezoid: (0) no; (1) yes. Ci=100, Ri=100.

20. Detached plates shape fused: (0) no; (1) yes. Ci=0, Ri=0.

21. Post palpal plates: (0) flat; (1) swollen. Ci=100, Ri=100.

22. Post palpal plates shape: (0) beans; (1) drop; (2) bulbous. Ci=100, Ri=100.

23. Eyes: (0) multi-faceted; (1) single wide hyaline lens, ending before the anterior laterovertex; (2) single wide hyaline lens, ending after the anterior laterovertex; (3) single narrow hyaline lens, with a tip on the anterior margin. Ci=100, Ri=100.

24. Number of laterovertex plates: (0) 1; (1) 2; (2) 3. Ci=100, Ri=100.

25. Size of the laterovertex posterior plate in relation to post-ocular: (0) without post-ocular plate; (1) similar; (2) at least three times wider and longer. Ci=100, Ri=100.

26. Laterovertex anterior plate shape: (0) without a recognizable anterior plate; (1) ellipsoid; (2) rectangular. Ci=50, Ri=80.

27. Scale on dorsal head: (0) absent; (1) present. Ci=50, Ri=83.

28. Delimitation among gena and posgena: (0) indistinguishable; (1) distinguishable. Ci=100, Ri=100.

29. Separation between gena and posgena: (0) membranous; (1) closed. Ci=100, Ri=100.

30. Gena position: (0) laterally; (1) dorso-ventrally. Ci=100, Ri=100.

31. Gena size dorsally: (0) narrow, with no portion wider than the width of the laterovertex; (1) at least a portion wider than laterovertex width. Ci=100, Ri=100.

32. Gena structure: (0) single plate; (1) divided into two plates. Ci=100, Ri=100.

33. Separation of the two gena plates: (0) membranous and well defined; (1) closed. Ci=100, Ri=100.

34. Lateral margin shape of the anterior gena: (0) subacuminate; (1) acuminate. Ci=100, Ri=100.

35. Occiput: (0) not flat and not fully dorsal; (1) totally dorsal and flat. Ci=100, Ri=100.

36. Median occipital sclerite: (0) reduced; (1) well developed and elongated. Ci=100, Ri=100.

37. Occiput setae arrangement: (0) throughout; (1) posterior margin. Ci=100, Ri=100.

38. Number of marginal setae in the occipital lobe: (0) 0; (1) 4; (2) 7-8. Ci=100, Ri=100.

39. Number of occipital lobe setae wider than the setae in the median occipital sclerite: (0) indistinguishable; (1) all the same width; (2) no more than one setae with the same width (3) 3-4 Ci=100, Ri=100.

40. Three marginal setae before the longest seta in the occipital lobe: (0) absent; (1) present. Ci=100, Ri=100.

41. Posgenal ctenidium: (0) Absent; (1) Present. Ci=100, Ri=100.

42. Posgenal ctenidium delimitation: (0) Only ventral; (1) Ventral and dorsal. Ci=100, Ri=100.

Thorax

43. Epaulets: (0) Absent; (1) Present. Ci=100, Ri=100.

44. The anterior (second) pigmented prescutal suture: (0) absent; (1) present. Ci=50, Ri=90.

45. Anterior suture extension: (0) Not reaching the lateral margin; (1) Reaching the lateral margin. Ci=0, Ri=0.

46. Anterior suture direction: (0) downward facing; (1) upward facing. Ci=0, Ri=0.

47. Longitudinal suture: (0) reaching the transverse suture; (1) absent; (2) reaching the scutellum; (3) vestigial. Ci=100, Ri=100.

48. Scutelum setae: (0) Not in line; (1) In line. Ci=100, Ri=100.

49. Number of episternal setae: (0) 4; (1) 3; (2) 5; (3) 2. Ci=100, Ri=100.

50. Endometasternite shape: (0) fork; (1) T. Ci=100, Ri=100.

51. Transversal suture: (0) Entire; (1) Obliterated but no pigmented; (2) Obliterated and pigmented. Ci=66, Ri=93.

52. Middle of the transversal suture: (0) arched down pigmentation; (1) arched up

pigmentation; (2) straight pigmentation. Ci=100, Ri=100.

53. Prescutal arc of setae: (0) absent; (1) present. Ci=100, Ri=100.

54. Pair of metasternal setae: (0) absent; (1) present. Ci=100, Ri=100.

55. Length of the scutellar arrows: (0) outer pair smaller than inner pair; (1) outer pair of similar length to the inner pair. Ci=100, Ri=100.

56. Wing development: (0) macropterous; (1) brachypterous. Ci=100, Ri=100.

Abdomen

57. Gonopophyseal setae: (0) distal seta longer than proximal; (1) proximal seta longer than distal. Ci=100, Ri=100.