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Molecular and Morphological Systematics of Phyllostomid Bats : Insights from complete mitochondrial genomes

María Alejandra Camacho Monge

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Doctorat de l'Université de Toulouse

préparé à l'Université Toulouse III - Paul Sabatier

Systematique moléculaire et morphologique des chauves-souris Phyllostomidae : aperçus à partir des génomes mitochondriaux complets

Thèse présentée et soutenue, le 3 juillet 2024 par

María Alejandra CAMACHO MONGE

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Thèse dirigée par

Jérôme MURIENNE et Merino-Viteri ANDRÉS

Composition du jury

M. Frédéric DELSUC, Président, CNRS Occitanie Est

M. Alexandre HASSANIN, Rapporteur, Muséum national d'Histoire naturelle

M. Sergio SOLARI, Rapporteur, Universidad de Antioquia

Mme Patricia BALARESQUE, Examinatrice, CNRS Occitanie Ouest

M. Jérôme MURIENNE, Directeur de thèse, CNRS Occitanie Ouest

M. ANDRÉS MERINO-VITERI, Co-directeur de thèse, Pontificia Universidad Católica del Ecuador

Résumé de la thèse

Cette thèse explore la systématique moléculaire et morphologique des chauves-souris Phyllostomidées, utilisant des génomes mitochondriaux complets pour éclairer les relations évolutives, le statut taxonomique et la spéciation cryptique au sein de cette famille diverse. Après une Introduction Générale, au Chapitre 2 je présente une phylogénie révisée des Phyllostomidae basée sur des génomes mitochondriaux complets de 39 espèces, dont 23 ont été nouvellement séquencées pour cette étude. Grâce à une analyse minutieuse, j'ai établi un cadre évolutif robuste qui clarifie les relations auparavant incertaines parmi les sous-familles, notamment celles des Lonchorhininae et des Macrotinae. Ce travail pose les bases pour une classification plus précise au sein de la famille. Le Chapitre 3 se concentre sur le genre *Trachops*, réévaluant son statut taxonomique à travers une approche intégrée combinant des données morphologiques, morphométriques et moléculaires. Les résultats suggèrent l'élévation de *T. cirrhosus ehrhardti* au statut d'espèce, reconnaissant sa particularité génétique et morphologique dans le sud-est du Brésil. En plus, je ne trouve aucun soutien pour maintenir *T. c. coffini* comme une sous-espèce distincte. Le genre *Trachops* comprendrait alors deux espèces monotypiques, *T. cirrhosus* et *T. ehrhardti*. Le quatrième chapitre enquête sur la biodiversité et la spéciation cryptique potentielle au sein des genres *Phylloderma* et *Macrophyllum*, avec un accent particulier sur la révélation d'une nouvelle espèce cryptique d'Amérique centrale. Les analyses complètes indiquent la présence de deux clades fortement soutenus au sein de *Phylloderma stenops* et *Macrophyllum macrophyllum*, menant à la proposition d'élever la population d'Amérique centrale du Nord de *Phylloderma*, *P. s. septentrionalis*, au rang d'espèce, et l'introduction d'une *Macrophyllum* nouvelle espèce endémique de la région. Cette thèse souligne la complexité de la diversité des chauves-souris Phyllostomidées et le rôle crucial des analyses moléculaires et morphologiques intégrées pour découvrir la diversité cachée. Cette

contribution fournit une compréhension plus claire des processus de spéciation chez les chauves-souris tropicales, soulignant l'importance d'une classification taxonomique précise pour des stratégies de conservation efficaces.

Mots clés: Chauves-souris Phyllostomidées, Génomes mitochondriaux, Systématique, Espèces cryptiques, Classification taxonomique, Analyse morphologique, Relations évolutives, Biodiversité néotropicale.

Molecular and Morphological Systematics of Phyllostomid Bats: Insights from Complete Mitochondrial Genomes

Thesis abstract

This thesis explores the molecular and morphological systematics of Phyllostomid bats utilizing complete mitochondrial genomes to illuminate the evolutionary relationships, taxonomic status, and cryptic speciation within this diverse family. After a General Introduction, in Chapter 2 I present a revised phylogeny of Phyllostomidae based on complete mitochondrial genomes from 39 species, 23 of which are newly sequenced for this study. Through meticulous analysis, I achieved a robust evolutionary framework that clarifies previously uncertain relationships among subfamilies, notably those of Lonchorhinae and Macrotinae. This work sets the stage for a more accurate classification within the family. Chapter 3 focuses on the genus *Trachops*, reassessing its taxonomic status through an integrated approach that combines morphological, morphometric, and molecular data. The findings suggest the elevation of *T. cirrhosus ehrhardti* to species status, recognizing its genetic and morphological distinctiveness in southeastern Brazil. Additionally, I find no support for maintaining *T. c. coffini* as a distinct subspecies. The genus *Trachops* would then consist of two monotypic species, *T. cirrhosus*, and *T. ehrhardti*. The fourth chapter investigates the biodiversity and potential cryptic speciation within the genera *Phylloderma* and *Macrophyllum*, with a particular emphasis on revealing a new cryptic species from Central America. Our comprehensive analyses indicate the presence of two highly supported clades within both *Phylloderma stenops* and *Macrophyllum macrophyllum*, leading to the proposal of elevating the North Central American population of *Phylloderma*, *P. s. septentrionalis*, to species status, and the introduction of a *Macrophyllum* new species endemic to the region. This thesis underscores the complexity of Phyllostomid bat diversity and the critical role of integrated molecular and morphological analyses in uncovering hidden diversity.

This contribution provides a clearer understanding of speciation processes in tropical bats, emphasizing the importance of accurate taxonomic classification for effective conservation strategies.

Keywords: Phyllostomid Bats, Mitochondrial genomes, Systematics, Cryptic species, Taxonomic classification, Morphological analysis, Evolutionary relationships, Neotropical Biodiversity.

Do what you can, with what you have, where you are.
Theodore Roosevelt

Para JuanSe, Santi, Marisol, Alejandro, Juan José y José David... somos un equipo.

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When I embarked on this adventure of pursuing a PhD degree later in life compared to my peers, the end seemed distant. Beginning my studies during the pandemic, amidst partial confinement, with limited funding and alongside full-time university teaching duties, the task seemed nearly impossible.

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Chapter 1. General introduction

Diversity and general characteristics of the Order Chiroptera

Bats (order Chiroptera) represent the second-largest mammalian group after rodents (order Rodentia). Encompassing a vast taxonomic diversity, Chiroptera includes over 1,470 species distributed across 21 families and 236 genera worldwide, accounting for approximately one-quarter of the 6,718 known mammalian species (MDD, 2024). Bats are distributed from high latitudes to deserts and isolated islands, with the majority found in the Neotropics—from approximately 23.5°N, near the Tropic of Cancer in Mexico, to 55°S in the southern extremities of South America (Fouquet et al., 2007; Myers et al., 2000) (Figure 1-1). Amazonia, in particular, is a region of chiropteran megadiversity, hosting over 200 recognized species. The Amazon basin and some Central Amazonian localities are home to one in ten known bat species, with some localities recording more than 100 species found in the same area (Lopez-Baucells et al., 2016).

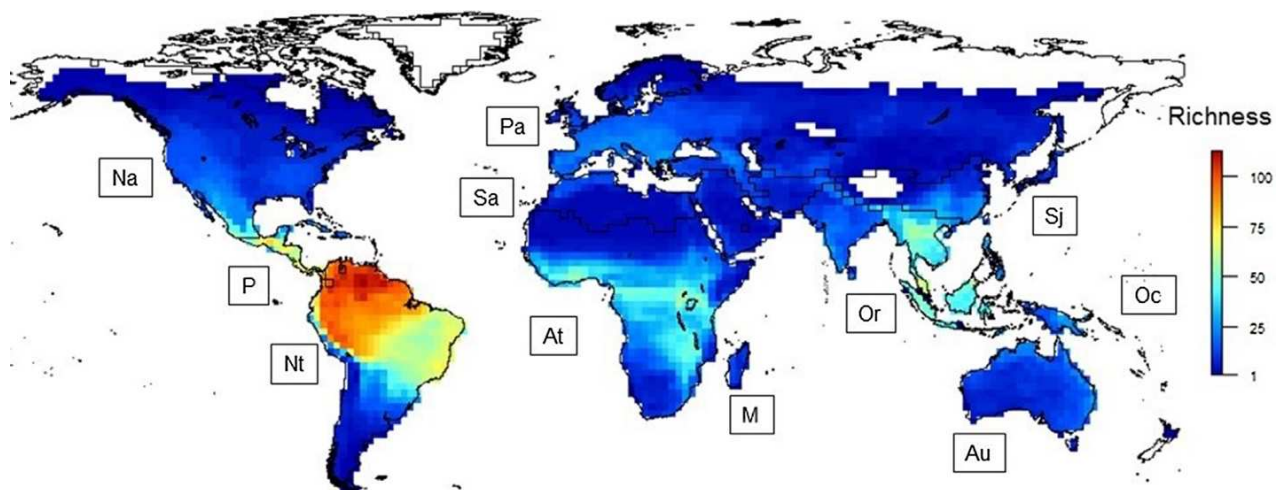


Figure 1-1. Global pattern of bat species richness. Legend corresponds to the number of bat species per 2°× 2°grid cell. Letters represent the zoo-geographic realms: Na = Nearctic, P = Panamanian, Nt = Neotropical, Pa = Palearctic, Sa = Saharo-Arabian, At = Afrotropical, M = Madagascan, Or = Oriental, Au = Australian, Sj = Sino-Japanese, Oc = Oceanian. Figure from Alves et al. (2018).

Throughout their evolutionary history (+50 million years), bats have developed various adaptations in sensory and motor systems, behavior, and ecology, that have enabled them to exploit nocturnal niches uninhabited by other animals during daylight hours (Denzinger & Schnitzler, 2013). Above all, bats have adapted powered flight and echolocation, which have allowed them to colonize nearly all terrestrial habitats.

Powered flight in bats is a mode of existence presumably adopted to exploit aerial insectivorous resources (Smith, 1976). The bat wing is a modified forearm and a skin membrane supported by the arm. The radius bone in the arm is long and curved. Apart from the bones of the hand (carpals) and the thumb, all other bones are thin and very long. The ability to fly is related to the structure of the wing, influencing the type of foraging and niches that bats can associate with (Freeman, 1981).

Besides their ability to fly, bats have echolocation abilities that are also responsible for their success and diversity in the world. Echolocation is a biological sonar system that produces and receives high-frequency pulses, allowing animals to perceive the surrounding environment. In vertebrate history, echolocation has evolved multiple times in bats, whales, rodents, shrews, tenrecs, and birds (Nojiri et al., 2021). The echoes that occur in echolocation allow bats to evaluate the distance, shape, size, and surface structure of objects, especially in the dark (Falk et al., 2011). The majority of bats are capable of a broad range of echolocating strategies, possessing one of the broadest frequency ranges of vocalizations and hearing sensitivities among mammals (Jones & Holderied, 2007).

Extant bats are classified into two groups: Yinpterochiroptera (Pteropodidae + Rhinolophoidea) and Yangochiroptera (Emballonuroidea + Noctilionoidea + Vespertilionoidea). Among these, laryngeal echolocation is found in Rhinolophoidea and Yangochiroptera (see Evolutionary History section). Pteropodidae is the only group among bats that lacks the ability of laryngeal

echolocation, with some members of the genus *Rousettus* possessing echolocating systems based on tongue clicks (Nojiri et al., 2021).

Until a few years ago, no consensus had been reached on the origin of laryngeal echolocation (Teeling et al., 2016; Wang et al., 2017), however, a comprehensive study by Nojiri et al. (2021) conducted embryological comparisons of 34 bat species, encompassing eight families and five species of non-bat outgroup mammals, demonstrated that there is no developmental difference between pteropodids and non-bat mammals and that yangochiropterans and rhinolophoids exhibit contrasting developmental patterns, providing embryological evidence for the convergent evolution of echolocation in bats. The components of the mammalian ear have possibly evolved multiple times through convergent evolution, shedding new light on its complex evolutionary history (Urban et al., 2017). Nojiri et al. (2021) supported the multiple-origin hypothesis of laryngeal echolocation, suggesting that a basic form of this trait may have evolved in a common ancestor. This primitive echolocation then diverged into the distinct forms observed today in yinpterochiropteran and yangochiropteran bats.

Besides these extraordinary capabilities, bats show incomparable dietary diversity, reproductive strategies, habitat preferences, and roles in the ecosystem (Potter et al., 2021; Santana & Cheung, 2016).

Evolutionary history of Chiroptera

Understanding bat evolution has been complicated, not only because of the unresolved phylogenetics, but also because the lack of fossil evidence (Teeling et al., 2018). The fragile nature of bat skeletons, combined with their preference for living in environments such as caves, holes, and secluded spaces in forests, seems to have led to a scarcity of fossil records (Smith, 1976). The lightweight structure of bats, essential for flight, results in bones that are small and

easily decomposed rather than fossilized (Eiting & Gunnell, 2009; Gunnell & Simmons, 2005; Morgan & Czaplewski, 2012).

The chiropteran fossil record goes back to the Eocene, 50 million years ago, but it is characterized by a sparse collection of samples (Gunnell & Simmons, 2005; Sears et al., 2006). The presence of bats fossils from this period is evidenced by some genera as *Icaronycteris* from the early Eocene of United States and France; *Palaeochiropteryx* and *Archaeonycteris* from the early Eocene of Austria; *Cecilonycteris* from the middle Eocene of Germany; *Ageina* from the early Eocene of France; and the extant genus *Hipposideros* from the middle Eocene of Europe. By the Oligocene and Miocene, some chiropteran families including Pteropodidae, Rhinolophidae, Emballonuridae, Phyllostomidae, Vespertilionidae, and Molossidae are represented in the fossil record (Gunnell & Simmons, 2005; Smith, 1976). More recently Morgan et al. (2023) described a new genus and species of fossil that was named *Americanycteris cyrtodon*, from the early Miocene of Panama and was attributed to the subfamily Phyllostominae.

From their split from other mammal lineages *ca.* ~65 million years ago, bats underwent a rapid diversification in the Eocene (O’Leary et al., 2013). Current phylogeny-based classifications recognize Chiroptera in the superorder Laurasiatheria along with Eulipotyphla (e.g., shrews, moles, hedgehogs), Carnivora (e.g., dogs, cats), Pholidota (pangolins), Perissodactyla (e.g. horses, rhinos, tapirs), and Cetartiodactyla (e.g. camels, pigs, whales, dolphins) (Murphy et al., 2001). The order was, for an extended period of time, and widely accepted, subdivided into the suborders Microchiroptera (microbats) and Megachiroptera (megabats) (Koopman, 1993; Simmons, 2005). Microbats were understood generally as smaller than megabats and often (although erroneously) characterized by poor vision, along with the echolocation system. Megabats comprised a single family (Pteropodidae) and were commonly known as ‘Old World

fruit bats', with members that can weigh as much as 1.5 kilograms and have a well-developed vision and a different mechanism of echolocation in some species, which involves tongue clicks rather than laryngeal echolocation as in microbats (Springer, 2013).

Teeling et al. (2002) highlighted the paraphyly of Microchiroptera by phylogenetically analyzing nuclear DNA data from representatives of 11 chiropteran families and detected strong support for the traditionally recognized microchiropteran families Megadermatidae, Rhinolophidae, and Rhinopomatidae being more closely related to the megachiropteran Pteropodidae than to other microchiropterans. This resulted in a new classification for bats comprised the two suborders mentioned before: Yangochiroptera and Yinpterochiroptera (Hutcheon & Kirsch, 2004; Teeling et al., 2002, 2005; Van Den Bussche & Lack, 2013), which is now widely accepted. Moreover, the subdivision had implications, among other things, on the question of the origin of echolocation in bats and whether the absence of echolocation in Pteropodids is secondary or was acquired independently in both suborders (Kruskop & Artyushin, 2021). According to Nojiri et al. (2021), the scenario that independent acquisition of echolocation is more likely.

Ongoing molecular systematic studies have established the classification of the 21 families within the two suborders, including the three newly confirmed families Miniopteridae, Cistugidae, and Rhinonycteridae, in addition to the traditional 18 subfamilies (Hofer & Van Den Bussche 2003). The most current study by Hao et al. (2023) used four mitochondrial genes and five nuclear genes to provide a robust family-level phylogenetic tree of bats based on the integrated datasets from Amador et al. (2018), which resulted in a phylogeny with almost all inter-familial nodes in the phylogenetic tree as strongly supported. This work confirmed the relationships between families such as Rhinonycteridae, Rhinolophidae, and Hipposideridae within the superfamily Rhinolophoidea, but also to locate two other of the three new families

Miniopteridae and Cistugidae (in addition to Rhinonycteridae) and resolve the phylogenetic relationship of the family Mizopodidae whose status has remained ambiguous. Specifically, the works of Rojas et al. (2016), Teeling et al. (2018) and Álvarez-Carretero et al. (2022) supported the position of Myzopodidae as an early lineage of the superfamily Noctilionoidea, whereas Amador et al. (2018) placed Myzopodidae into Emballonuroidea. Hao et al. (2023) found strong support to place the Emballonuroidea superfamily as the basal lineage of Yangochiroptera, and that Myzopodidae as the basal lineage of Emballonuroidea.

Thus, the suborder Yinpterochiroptera is comprised of Pteropodidae and the superfamily Rhinolophoidea (Rhinonycteridae, Rhinolophidae, Hipposidaridae, Megadermatidae, Craseonycteridae, and Rhinopomatidae); while the suborder Yangochiroptera comprised the superfamilies Emaballonuroidea (Myzopodidae, Nycteridae, Emballonuridae), Vespertilionoidea (Cistugidae, Vespertilionidae, Miniopteridae, Molosidae, and Natalidae); and Noctilionoidea (Phyllostomidae, Mormoopidae, Noctilionidae, Furipteridae, Thyropteridae, and Mystacinidae) (Figure 1-2).

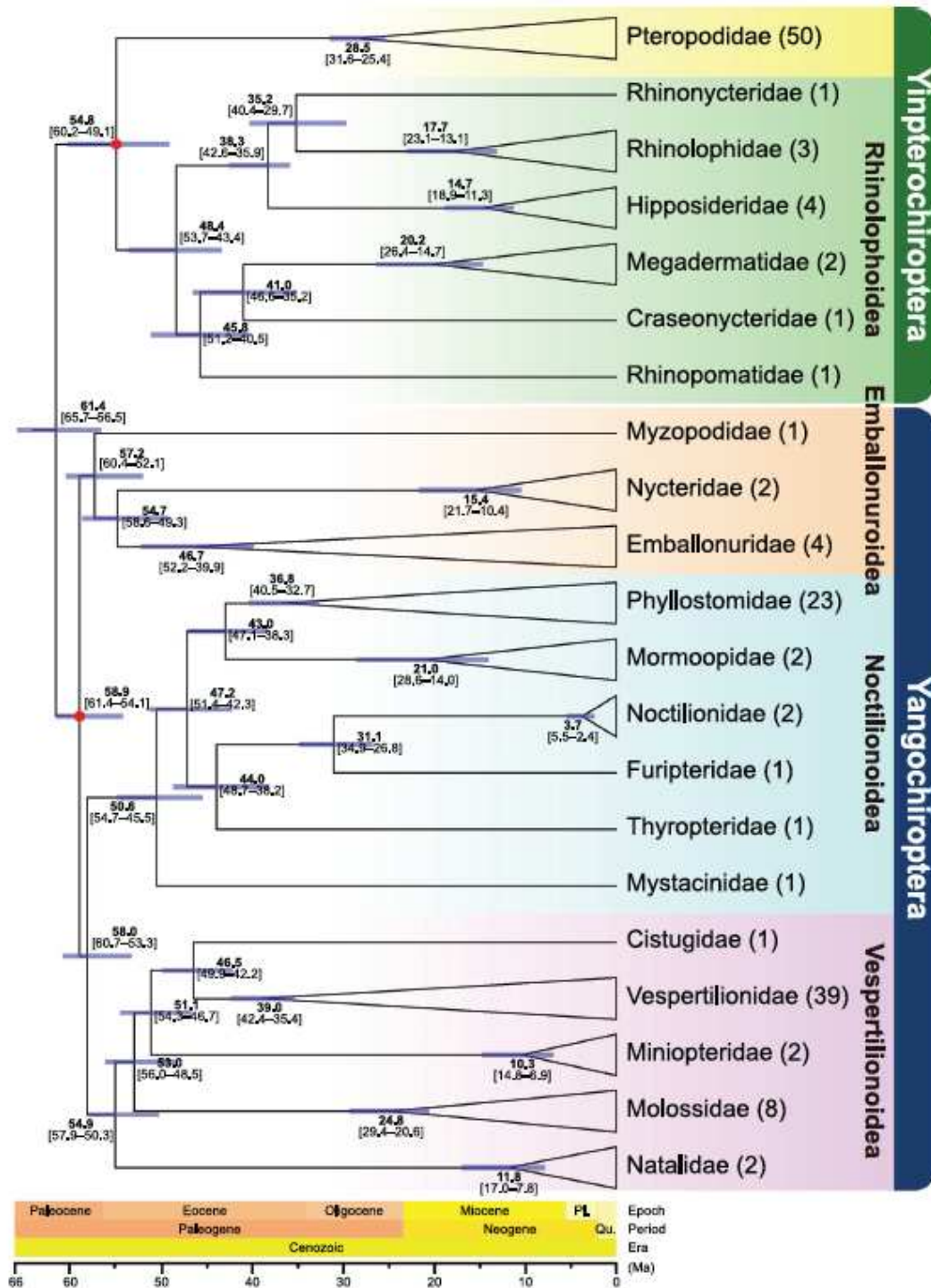


Figure 1-2. Time-calibrated phylogenetic tree of family-level relationships of bats. Multiple branches in each family are collapsed to display the family-level relationships. Bold numbers on the nodes indicate the molecular dates in millions of years. Values in the intervals and the blue bars represent the 95% credibility interval of divergence time estimates. Numbers in parentheses after each family name indicate the number of species selected for each family. The most recent common ancestors of Yinpterochiroptera and Yangochiroptera are marked with a red dot, respectively. Figure from Hao et al. (2023).

The superfamily Noctilionoidea

Within the Neotropics, the Noctilionoidea superfamily, comprising the families Phyllostomidae, Mormoopidae, Noctilionidae, Furipteridae, and Thyropteridae, represents a significant portion of bat diversity. These families collectively account for approximately 17% of the global species richness among bats, with at least 254 species identified. In contrast, the family Mystacinidae, which is endemic to New Zealand, includes only one genus and two species (MDD, 2024).

For some of these families, more than others, there is concrete evidence of their origin and patterns of richness and endemism. For Phyllostomidae, there is an important support of the hypothesis of separate diversification processes in South America and North America in the Miocene, and a subsequent mixing coinciding with the Great American Interchange (GABI), with a predominant south to north movement. For Mormoopidae it has been suggested a North American origin, followed by diversification in the West Indies and Central America (Arita et al., 2014). For the remaining families, data are insufficient, and the origins are difficult to resolve because the GABI model is insufficient to explain present-day patterns, and that at least some of the major families have had dual diversification centers, in some cases associated with the xeric habitats of the Miocene (Arita et al., 2014; Ramos-Pereira & Palmeirim, 2013).

More recently, analyses such as Rojas et al. (2016), have supported that the New World noctilionoids are thought to have reached the Neotropics from Australia via Antarctica during the Eocene, approximately 37 to 47 million years ago, which aligns with findings of mystacinid fossils in Australia from the late Oligocene and early Miocene (Hand et al., 2005), and that the ancestral origin for New World Noctilionoids is South America. Biogeographic studies in a few genera of Phyllostomids have supported this South American origin for most species, followed by dispersals to Central America and the Caribbean (Velazco & Patterson, 2008, 2013).

The family Phyllostomidae: relationships and diversification

The current taxonomic diversity of the Leaf-nosed Bats comprises at least 227 species in 61 genera assembled in 12 tribes and 11 subfamilies (Baker et al., 2016; MDD, 2024). Most species of Phyllostomidae are primarily restricted to the Neotropical region in South America, Central America, and the Caribbean Islands, although *Macrotus californicus*, *Choeronycteris mexicana*, *Leptonycteris nivalis*, and *L. yerbabuena*, extend northward into northern Mexico and the southwestern United States (Morgan et al., 2023).

Phyllostomids exhibit a wide range of morphological and ecological diversity and display most of the broad spectrum of dietary adaptations observed within the Chiroptera order. The range of diversity in morphology and feeding among Phyllostomidae is thought to be originated from long isolation of this family in South America, dating to the early Miocene (Lim, 2009; Rojas et al., 2016). However, the current distribution of Phyllostomidae in Central America, Mexico, and the Southwestern United States, alongside the presence of endemic genera and species in Central America (Solari et al., 2019a), suggests that North America was not only a recipient of these species during the GABI following the formation of the Isthmus of Panama, as mentioned earlier, but rather, the existence of palaeo-endemism in Central America indicates a historical range reduction pointing to an ancient diversification center in Central America (Arita et al., 2014). Such evidence could signify that Phyllostomidae might have already been present in Central and North America by the Pliocene, if not earlier (Morgan et al., 2023).

The systematic and phylogenetic relationships among phyllostomids have long been subjects of debate, posing significant challenges to understanding both the biogeographic history and evolutionary connections within the family. This complexity is intensified by the ancient diversification and subsequent dispersal events that were facilitated by continental connections in the Americas. These factors not only make inferences about systematics and biogeography

difficult but also continue to drive the focus of most chiropteran studies, as evidenced by the research presented in this thesis.

Miller (1907) classified 51 genera of phyllostomid bats into seven subfamilies, an arrangement that was well accepted until the mid-1970s. At that time, new systematic studies utilizing allozymic, karyological, and morphological data began, which subsequently led to significant revisions of this family's taxonomy. The studies by Wetterer et al. (2000) on morphology and Baker et al. (2003) on DNA sequences from both nuclear and mitochondrial genomes have been particularly influential. These works provided a comprehensive framework that has helped establish a consensus on the number of genera and their classification into subfamilies (Van Den Bussche & Lack, 2013).

The modern taxonomy of the family Phyllostomidae was upbuilt by some authors based on molecular and morphological approaches (Baker et al., 2003; Cirranello et al., 2016; Dávalos et al., 2014). In particular, the paraphyletic nature of the subfamily Phyllostominae was treated by Baker et al., (2003) and Dumont et al. (2012). As a result, the genus *Macrotus* was recognized as representing an early lineage and was categorized as a distinct subfamily, while Micronycterinae, Glyphonycterinae, and Lonchorhininae were also designated as separate subfamilies (Cirranello et al., 2016). However, the relationships of these families with respect to the others in the different phylogenies were not clear (Rojas et al., 2011).

Likewise, with the integration of more evidence, it was demonstrated that nectarivory evolved independently in the two subfamilies Glossophaginae and Lonchophyllinae, both of which include non-nectar-feeders (Baker et al., 2016; Kruskop & Artyushin, 2021). Instead, Lonchophyllinae was sister to a clade comprising Carollinae, Glyphonycterinae, Rhinophyllinae, and Stenodermatinae (Datzmann et al., 2010; Rojas et al., 2016). Herein, in Chapter 1 we corroborate these relationships and notably resolve the position of Lonchorrhinae.

Disagreements as to phylogenetic relationships and delimitation of taxonomic units are more pronounced at lower taxonomic levels (genus and species), which is evident in the prevalence of taxonomic revisions and ongoing debates over phylogenetic relationships and taxonomic boundaries within the family (Solari et al., 2019b). What is clear to systematists, and taxonomists is that Phyllostomidae has undergone a diversification unparalleled in other mammalian families in terms of ecological and morphological diversity (Dumont et al., 2012; Freeman, 2000). The nearly 230 recognized species have most probably evolved from an insectivorous ancestor; nevertheless, although insectivory is still the predominant dietary strategy amongst Phyllostomidae, it encompasses a range of dietary strategies larger than that seen in any other mammal family, including omnivorous, insectivorous, carnivorous, nectarivorous, frugivorous, and hematophagous species (Gardner, 1977; Potter et al., 2021). Ecological variation in diets is associated with extensive morphological diversity that involves skeletal, muscle, digestive, sensory systems, and behavior (Baker et al., 2016). Particularly, drastic craniofacial evolution has involved both lengthening and shortening of the skull and significant variation in the size and shape of mandibles and teeth, among other structures (Santana et al., 2012). The study of these characters and the statistical significance of their variation have traditionally been important to understand the relationships between species. In Chapters 2 and 3 of this thesis, I use morphological and morphometric evidence, among others, to resolve the taxonomic status of three genera of the Phyllostominae subfamily.

Phyllostominae

Phyllostominae is characterized by a well-defined noseleaf and a molar configuration in which the cusps show a W-pattern (Miller 1907). The interfemoral membrane is usually well developed, and the tail may differ among taxa, from total absence, to being long and extending to the margin of the uropatagium (Williams & Genoways, 2008). The group is distributed from

the southern United States southward into northern Argentina, Paraguay, and southern Brazil (Williams & Genoways, 2008).

Prior to molecular studies of the past decade or so, phylogenies based primarily on morphology indicated a monophyletic Phyllostominae (as mentioned earlier), with all members of this group possessing broadly similar insectivorous teeth. Currently, classical Phyllostominae is considered non-monophyletic (Baker et al., 2016) and that the genera previously included within the Phyllostominae, belong to five different subfamilies: Glyphonycterinae (*Glyphonycteris*, *Neonycteris*, *Trinycteris*); Lonchorhininae (*Lonchorhina*); Macrotinae (*Macrotus*); Micronycterinae (*Lampronnycteris*, *Micronycteris*); and Phyllostominae that do constitute a monophyletic group (*Chrotopterus*, *Gardnerycteris*, *Lophostoma*, *Macrophyllum*, *Mimon*, *Phylloderma*, *Phyllostomus*, *Tonatia*, *Trachops*, *Vampyrum*). Macrotinae, Micronycterinae, and Lonchorhininae, as well as the monophyletic Phyllostominae, are placed near the base of the phyllostomid tree in most molecular phylogenies (Morgan et al., 2023).

Collectively, the subfamily Phyllostominae, as defined by Baker et al. (2016), comprises 25 species in ten genera. Five of these are monotypic genera: *Chrotopterus*, *Macrophyllum*, *Phylloderma*, *Trachops*, and *Vampyrum*, and share the characteristic of having broad geographic distributions. Among them, *Trachops* has been the focus of more extensive taxonomic scrutiny without a definitive resolution (see Clare et al., 2011; Ditchfield, 1996; Fonseca, 2019), and more recently *Chrotopterus* (Stevens, 2023). However, other genera such as *Phylloderma* and *Macrophyllum* have historically been neglected. In this thesis (Chapters 2 and 3), we propose taxonomic resolutions for these groups previously accepted as monotypic (see hereafter).

The Fringe-lipped Bat, *Trachops cirrhosus* (Spix, 1823)

This bat can be easily distinguished from other bats by its finger-like dermal projections on the chin and lips. This species has a wide distribution in the Neotropics, ranging from southern Mexico to Brazil, and occurring at mid to high elevations at both sides of the Andes Cordillera, across the Amazon, and in the Atlantic Forest (Cramer et al., 2001; Jones & Carter, 1976; Solari et al., 2019a; Williams & Genoways, 2008). The species inhabits humid tropical and subtropical forests, and forages in primary, secondary, disturbed and gallery forests, at woodland edge and near cultivated areas (Ditchfield, 1996; Fenton et al., 1992).

Trachops taxonomy has undergone numerous changes. Initially described by Spix in (1823) within the genus *Vampyrus*, the name was reassigned to what we currently recognize as *Vampyrum* (Williams & Genoways, 2008). Gray (1825) clarified the taxonomy led to the classification of the species into the new genus *Trachops* (Gray, 1847). Some taxonomical changes included *Phyllostoma angusticeps* Gervais (1956), and *Tylostoma mexicana* (de Saussure, 1860), as junior synonyms following a reevaluation of their classification. The genus was further refined when *Trachops fuliginosus* Gray 1947 was recognized as a junior synonym of *T. cirrhosus*. Goldman (1925) provided a description of *Trachops coffini*. Later, Felten (1955a) described *Trachops cirrhosus ehrhardti*, and reevaluated the taxonomic classification of *T. coffini*, designating it as a subspecies of *T. cirrhosus* (Felten, 1955b).

Currently, *Trachops* includes three subspecies: *Trachops cirrhosus cirrhosus* (Spix 1823), occurring from Costa Rica to northeastern Brazil; *Trachops cirrhosus coffini* (Goldman 1925), distributed from Mexico to Nicaragua; and *Trachops cirrhosus ehrhardti* (Felten 1955b), which is found only in southeastern Brazil (Jones & Carter 1976; Solari et al., 2019a). The descriptions of these subspecies are brief, based solely on aspects of size, and were conducted with few samples without comparisons between specimens from Central and South America. Attempting

to clarify the taxonomy, geographic ranges, and diagnostic characters of the subspecies, Ditchfield (1996) analyzed partial sequences of the mitochondrial Cytochrome b gene, showing five unique haplotypes with a range sequence divergence of 5.5% to 11%. This work revealed significant geographic structuring and a low incidence of haplotype sharing among *Trachops* populations. Subsequent research by Clare et al. (2007) and Clare (2011) investigated genetic divergence using the mitochondrial marker COI, which yielded high divergence values, but failed to align these results with the topology produced by the nuclear marker Dby. Their findings indicated intraspecific groups with overlapping distributions, implying potential ongoing or historical speciation processes. Most recently, the genus was revised by Fonseca's PhD thesis (2019), who questioned the subspecific level of *T. c. ehrhardti* with a combination of morphological, ecological, and genetic evidence, suggesting a redefinition of the subspecies within *T. cirrhosus*. Nonetheless, the taxonomic changes proposed by Fonseca (2019) have not yet been formalized in a peer-reviewed publication, and the specific morphological characters necessary to differentiate subspecies of *Trachops cirrhosus* remain unclear.

The Pale-faced Bat, *Phylloderma stenops* (W. Peters, 1865)

Phylloderma stenops has a broad distribution in the Neotropics and occurs from South Mexico through Central America and South America to Southeast Brazil and North Bolivia. The species inhabits primarily lowland and humid forest, but also mesic or riparian, inundated floodplain, and deciduous forests, and also extends into drier regions such as dry, xeric shrublands and agricultural habitats (Bomfim et al., 2017; Carrera et al., 2010; Ramírez-Fráncel et al., 2015; Salas et al., 2014; Timm et al., 1989; Williams & Genoways, 2008). The species is believed to be omnivorous, consuming fruits, insects, and possibly small vertebrates (Esbérard, 2012; Giannini & Kalko, 2004).

Morphologically, *P. stenops* is recognized as a large and robust bat, with a robust skull, a flat face profile, and a V-shaped chin bordered with rounded papillae. The species' fur is typically reddish-brown, with short dorsal hairs and whitish wingtips (Barquez & Ojeda, 1979; Díaz et al., 2011). Although similar in appearance to *Phyllostomus*, *P. stenops* can be identified by its nasal leaf, which is fused to the upper lip and rectilinear at the base, unlike the free and semicircular nasal leaf of *Phyllostomus*; its lighter facial color; and the presence of three lower premolars instead of two, distinguishing it further from *Phyllostomus* (de Souza et al., 2022).

The natural history and the morphological and genetic diversity of this species have been not studied. Its taxonomy has remained unchanged since 1979, with three recognized subspecies whose descriptions were based solely on morphometric data and distributional records: the nominal subspecies *P. s. stenops*, which has a widespread distribution from Panamá throughout most of South America to southeastern Brazil; *P. s. septentrionalis* (Goodwin, 1940) distributed in Central America, from Mexico to Costa Rica; and *P. s. boliviensis* (Barquez & Ojeda, 1979) endemically distributed in central east Bolivia. Posterior to that, only new records filling geographic distribution gaps rather than expanding our understanding of the species' biology or ecology, have been published (Bomfim et al., 2017; de Souza et al., 2022; Esbérard & Faria, 2006; Martínez-Cerón et al., 2019; Salas et al., 2014).

The Long-legged Bat, *Macrophyllum macrophyllum* (Schinz, 1821)

Macrophyllum macrophyllum is widespread from Southern Mexico through Central and South America to northern Argentina. The species was discovered during the explorations of the German explorer Prince Maximilian of Wied-Neuwied in Brazil (1815-1817) who documented its distinct morphological traits such as large feet and elongated legs, as well as a tail extending to the edge of the interfemoral membrane, adorned with saw-like dermal denticles (Harrison & Pendleton, 1974; Wied-Neuwied, 1826). The species was then described as *Phyllostoma*

macrophyllum by Schinz in 1821, who reviewed the collection made by Prince Maximilian zu Wied, with the type locality "In the forests of Brazil" and then reassigned to the genus *Macrophyllum* by Gray in (1838), under the new name *Macrophyllum nieuwiedii* and "Brazil" as the type locality. Subsequent name changes included *Dolichophyllum macrophyllum* by Allen (1900), and finally, the species was named *Macrophyllum macrophyllum* by Nelson in (1912), which is the current valid name.

This bat can be easily recognized by its long and powerful legs and feet and a developed interfemoral membrane that is lined with rows of dermal denticles (Harrison, 1975). These adaptations are thought to be specialized for foraging, specifically for skimming the surfaces of bodies of water, like for the bulldog bats *Noctilio* spp. (Noctilionidae), the proboscis bat *Rhynchonycteris naso* (Emballonuridae), and some evening bats *Myotis* spp. (Vespertilionidae) (Gardner, 1977; Harrison & Pendleton, 1974; Weinbeer et al., 2006).

M. macrophyllum has been observed in diverse habitats ranging from rainforest watercourses to semi-arid scrublands (Bloedel, 1955; Feijó et al., 2015; Harrison, 1975). Its presence in these ecosystems, often near water, suggests that its diet primarily consisted of insects captured in flight near water surfaces (Davis et al., 1964; Gardner, 1977).

Despite its distinctive morphological features which facilitate easy identification, significant gaps remain in the scientific literature concerning the phylogeographic patterns and taxonomic status of *Macrophyllum macrophyllum*. This research aims to address these gaps by conducting a comprehensive phylogeographic and taxonomic analysis of the species, given the increasing availability of both morphological and molecular data.

Contribution of this thesis to the advance in the systematics of nose-leaf bats

The understanding of bat systematics in the Neotropics has significantly advanced thanks to the integration of various approaches and methodologies. Studies based on morphology (Wetterer et al., 2000) and DNA sequence data (Baker et al., 2003; Rojas et al., 2011; Dumont et al., 2012; Dávalos et al., 2014) have been fundamental in this process, as mentioned previously.

The classifications proposed by Wetterer et al. (2000) were challenged by molecular studies that revealed phylogenetic trees in which feeding guilds were not necessarily monophyletic (Baker et al., 2003). These molecular studies proposed new nomenclatures for family groups and redefined the content of already established family-level names, as evidenced in the work of Baker et al. (2003), who sequenced fragments of mtDNA and the nuclear gene RAG2. Analyzes based on a larger number of genes provided additional support for many of the clades detected by Baker et al. (2003), as demonstrated by Datzmann et al. (2010), Rojas et al. (2011) and Dumont et al. (2012). Subsequently, a combined analysis of molecular and morphological data (Dávalos et al., 2014), recovered most of the clades obtained by Baker et al. (2003). The two major areas of disagreement among these analyses concerned the relationships of Lonchorhininae and the shared common ancestor of nectar feeders.

The work of Baker et al. (2016) proposed revised classification of the family with an emphasis on ensuring that all family-group names were available, clearly defined, and comprehensively diagnosed. In their results, and despite the uncertainty regarding its position in the phyllostomid tree, *Lonchorhina* was recovered as a supported branch distinct from all other subfamilies and genera which supported the decision to recognize the taxon as its own subfamily. Botero-Castro et al. (2013, 2018) employed complete mitochondrial genomes to provide additional evidence. Their mitogenomic analysis confirmed the findings of Baker et al. (2003), retrieving the 11 subfamilies. However, they noted that some subfamilies were represented by single taxa,

resulting in isolated branches and unstable nodes. Importantly, their data showed that Lonchophyllinae never clustered with Glossophaginae, thereby confirming the independent evolution of nectarivory in phyllostomid bats, as previously suggested by Datzmann et al. (2010).

In this thesis, we further explore the phylogenetic relationships of phyllostomids with the use of a greater number of complete mitochondrial genomes, also highlighting the importance of continuing to carry out studies with mitochondrial DNA to deepen the understanding of the diversity and phylogenetic relationships of Neotropical bats.

Bats represent a challenging group for mammalian systematics, as many taxa are almost or completely indistinguishable in morphological, acoustic, or biometric form (Clare, 2011; Preatoni et al., 2005). These similarities can result in underestimated diversity, as already pointed out in several molecular studies (Mota et al., 2022). Thus, the study of genome-scale datasets emerges as a potentially powerful tool in this context, providing detailed phylogenetic resolution and helping to clarify complex evolutionary relationships. The increasing accessibility of complete genomic data offers an opportunity to explore deeper into bat systematics, emphasizing the importance of continuing the generation and analysis of mitogenome sequences to address taxonomic and phylogenetic questions.

Why mitogenomes and its sequencing method

The mitochondrial DNA (mtDNA) is a compact, circular molecule of 14-20 kilobases and contains 37 genes. The typical gene set encodes 13 protein subunits for the enzyme complexes involved in oxidative phosphorylation—a critical energy-yielding pathway. This process efficiently oxidizes carbohydrates and fats, resulting in the production of carbon dioxide, water, and adenosine triphosphate (ATP). Additionally, mtDNA encodes for two types of ribosomal

RNA (rRNA) that form part of the mitochondrial ribosome, essential for protein synthesis within the mitochondria; and 22 transfer RNAs (tRNAs), which are crucial for the proper assembly of proteins by translating the mitochondrial genetic code (Boore, 1999). Alongside these functional genes, mtDNA includes a crucial regulatory segment, the D-loop, which plays a role in the replication and expression of mitochondrial genes (Ballard & Whitlock, 2004). Unlike basic features of nuclear DNA, which vary drastically among organisms from size to genomic architecture to genetic content, mtDNA remains very consistent in size throughout most of the animal kingdom (Carter et al., 2023).

Despite the relatively constant gene content across animal mtDNA, the arrangement of these genes shows considerable variation across major taxonomic groups. The mechanisms responsible for these rearrangements are not well understood, but it is apparent that transfer RNA (tRNA) genes change position more frequently than either rRNAs or protein-coding genes (Macey et al., 1997). It has been suggested that such rearrangements can be used as cladistic characters for studying relationships among higher-level taxa (Curole & Kocher, 1999). Furthermore, the unique characteristics of mitochondrial genes, including their effective existence in a single copy and the clonal pattern of maternal inheritance typical in most animals, facilitate the direct reconstruction of phylogenetic trees. The uniparental mode of inheritance also narrows the effective population size for mitochondrial genes, accelerating the fixation of genetic variants during speciation events. Additionally, the mitochondrial DNA sequence divergence rate in vertebrates, which is estimated to be 5–10 times faster than that of single-copy nuclear DNA, enables the identification of closely related species or taxa (Harrison, 1989). This rapid divergence rate is a critical tool for studying recent evolutionary events and relationships (Shipp-Pennock et al., 2005). These characteristics have made mtDNA a popular genetic marker for evolutionary studies and a reliable and easy-to-use phylogenetic marker

(Curole & Kocher, 1999; Mueller, 2006), both for population-level studies and for broadscale comparative analyses (Smith, 2016).

There are also limitations associated with mtDNA. Due to its molecular structure, mtDNA may function as a single locus, which may not always accurately represent the true evolutionary history of the taxa under study. The phenomenon of introgression can complicate the understanding of relationships among taxa, especially at deeper evolutionary divergences (Garg et al., 2023). Furthermore, the maternal inheritance of mtDNA introduces the risk of misidentifying a social group as a distinct taxonomic unit, a situation that arises from female philopatry and male-mediated gene flow (Rosel et al., 2017).

Technologies for sequencing DNA have evolved rapidly over the past two decades (Kumar et al., 2019). This rapid progress has set the stage for significant advancements in the field of DNA sequencing, leading to the emergence of three generations of sequencing technologies: The first generation was represented by Sanger sequencing, providing the foundation for DNA sequencing; the second generation introduced massively parallel sequencing, enabling High-throughput sequencing (HTS); and the third generation, offering long-read and single-molecule sequencing capabilities (Satam et al., 2023).

Throughout this thesis we have employed the Illumina platform, which utilizes a sequencing-by-synthesis method based on reversible dye terminators (Satam et al., 2023). This method is recognized as a Second-generation sequencing technique. These second-generation, or high-throughput sequencing techniques, have profoundly transformed biodiversity research, from phylogenomic and systematic studies to population genetics, barcoding, and ecological investigations (Dodsworth, 2015). Among these technologies, the application of low-coverage and cost-effective “genome skimming” stands out for its efficiency and effectiveness (Trevisan et al., 2019).

The concept of “genome skimming” was originally introduced by Straub et al. (2012) to describe the process of obtaining a shallow-pass genome sequence to recover highly repetitive regions of the genome, such as ribosomal DNA (rDNA) or organelle genomes. Despite the relatively low depth of sequencing, it can still provide highly valuable sequence data at sufficient sequence depth for repetitive regions, and these regions include the usual suite of DNA barcoding markers (Dodsworth, 2015; Zeng et al., 2018). The integration of HTS platforms, such as the Illumina platform, significantly increased the yield and cost-effectiveness of genome skimming and has eliminated the need for systematists to choose between analyzing more loci or more taxa, thus broadening the scope of genomic studies (Garg et al., 2023).

One particular advantage of genome skimming is that has proven particularly effective for the analysis of degraded genomic DNA, which is a common challenge with samples from museum collections and herbaria. Despite the degradation, high-copy regions of the genome are still recoverable, making genome skimming an optimal method for such samples (Dodsworth, 2015). In addition to its efficacy with degraded samples, genome skimming minimizes sample destruction, allowing for the preservation of precious material while still facilitating access to crucial genetic information (Zeng et al., 2018).

In the context of highly debated phylogenies, such as that of the Phyllostomidae, the use of a multitude of genetic markers may still be insufficient due, among other reasons, to the presence of short branches at deep divergences, reticulated evolution, and recent speciation events. In these cases, high-throughput sequencing technologies offer a valuable alternative, providing datasets significantly larger than those achievable through traditional Sanger sequencing methods, and at increasingly affordable costs (Phillips & Shazwani-Zakaria, 2021; Toussaint et al., 2021). This technological advancement enables a more detailed and comprehensive exploration of phylogenetic relationships, even in the face of complex evolutionary scenarios.

In this study, I have taken into account these considerations and analyzed not only whether the inclusion of a substantial amount of newly sequenced mitogenomic data aids in enhancing resolution at the Phyllostomidae family level—given the analysis of an unprecedented number of complete mitogenomes—but also how different analytical conditions affect the results. These conditions include partition schemes, the selection of outgroups, the types of data employed, and the phylogenetic methodologies used.

Conceptual and geographical framework

Species are the fundamental unit of analysis in biodiversity. Consistent delimitation of species is a necessary component of many biological inferences, for it allows appropriate comparisons of data sets (Rosel et al., 2017). Besides, having a well-supported and stable classification for any group reduces confusion among non-experts, enabling productive discussion, debate, and research (Cirranello et al., 2016)

In this thesis, the taxonomic decisions are founded on the general definition of species proposed by Simpson (1951) and reworded by Molinari (2023) in which a species is a phyletic lineage (ancestral-descendent sequence of populations) evolving independently of others, and diagnosably distinct based on heritable morphological characters, genetic markers, or both.

Likewise, we recognize a species as cryptic if it is superficially morphologically indistinguishable from its sister species (and has been classified as a single nominal species), but genetic markers provide sufficient differentiation for species-level recognition (Bickford et al., 2007; Molinari, 2023). Subtle or non phenotypic differences pose a problem for biological classification, not only in the case of cryptic species, but also in cases of allopatry and/or lineages that have diverged recently (Fujita et al., 2012). In this context, genetic data provide robust approaches to delimit species that are difficult to recognize solely with morphological

traits (Baker & Bradley, 2006; de Ferran et al., 2024). The exercise of recognizing new species, either resulting from taxonomic amendments or from taxa never described before, remains important because, despite the greatest local species richness of vertebrates is found in northern South America, especially in the Amazonian region, and in Central America (Rocha-Méndez et al., 2019; Silva et al., 2023), there is still a significant number of species that are underrepresented in scientific literature or not yet formally recognized. This gap in knowledge impedes a comprehensive understanding of biodiversity and ecosystem dynamics in these regions of the Neotropics, which are known for their complex habitats and high rates of endemism (Mittermeier et al., 2011).

Central America possesses a complex geological history, accompanied by cyclic changes in vegetation and climate, and constant orogenic processes have promoted a highly broken topography characterized by highland isolated patches of humid montane forest between 600 and 3000 m, which includes both humid pine-oak forest and cloud forest (Morales et al., 2016). South America, meanwhile, has eighty percent of its land within the tropical zone, yet it extends into the subantarctic. Essentially, all life zones and vegetation formations are represented. The principal vegetation types are tropical evergreen and semi-evergreen moist forest, dry forest to woodland (Cerrado or woody savanna), open grassy savanna, desert and arid steppe, Mediterranean-climate communities, temperate evergreen forest, and several montane formations (e.g. páramo, stone fields or campos rupestres, puna) (Morellato, 2003). Continuous orogenic processes have given rise to a diversified topography with isolated and humid mountain forests from lowlands to altitudes above 5,000 m. Particularly, the presence of mountain chains (including the Andes Mountains) and mountain ranges throughout the region makes relief and altitude factors of special interest (Díaz et al., 2021).

These mosaics landscapes has been associated to centers of diversification along elevational gradients, in which both a high species richness and endemism have evolved for the last 2 Myr, likely as a result of both environmental and geological complexity, as well as Pleistocene climatic fluctuations (Ramírez-Barahona & Eguiarte, 2013), which have been invoked to explain patterns of genetic differentiation in the increasing rates of speciation and diversification in Neotropical biota (Rocha-Méndez et al., 2019).

General objectives of the thesis

Because of their diversity, abundance, and ubiquity across the Neotropics, phyllostomid bats are the focus of several research efforts; therefore, a well-supported and stable classification is desirable.

This thesis offers an exploration of the phylogenetic relationships, taxonomic classification, and evolutionary patterns within Phyllostomidae, particularly focusing on the genera *Trachops*, *Phylloderma*, and *Macrophyllum*. Through an integrative use of molecular, morphological, and geographic evidence, this work aims to investigate the evolutionary history and clarify the taxonomic statuses within these groups. By employing complete mitochondrial genomes and extensive morphological datasets, the study seeks to resolve longstanding uncertainties in the subfamilial classifications, reevaluate species and subspecies designations, and unveil cryptic diversity.

After a General Introduction in Chapter 1, Chapter 2 addresses the revised phylogeny of phyllostomid bats, highlighting the role of complete mitochondrial genomes in resolving subfamilial classifications. The chapter aims to assess the impact of newly sequenced mitogenomic data on the phylogenetic resolution at the subfamily level and evaluating the influence of various analytical conditions, including partition schemes, choice of outgroups, and phylogenetic methods. The study provides a more definitive classification within the Phyllostomidae, clarifying previously uncertain relationships.

Chapter 3 focuses on the Fringe-lipped Bat, *Trachops cirrhosus*, to reevaluate its taxonomic status and geographic distribution through an integrated study that combines morphological and molecular data. The objectives are to reassess the validity of the current subspecies classifications, identify distinct and diagnosable characters, and explore the potential for identifying cryptic species within *Trachops* through comprehensive analyses. By expanding the

taxon sampling to include significant geographic coverage, this study refines our understanding of this species taxonomic framework.

Chapter 4 further explores the biodiversity and cryptic speciation within *Phylloderma* and *Macrophyllum*, proposing the elevation of North Central American populations to species status based on genetic and morphological evidence. By examining specimens from across their entire distributional ranges, this research seeks to evaluate cryptic diversity, proposing the recognition of a new species based on significant genetic and morphological divergence. The findings aim to contribute to our understanding of the complex factors driving bat diversity in the Neotropics, particularly in Central America.

Chapter 2. Revised phylogeny from complete mitochondrial genomes of Phyllostomids bats resolves subfamilial classification.

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M. Alejandra Camacho^{1,2*}, Dániel Cadar³, Balázs Horváth³, Andrés Merino-Viteri^{1,4} and

Jérôme Murienne²



Lonchorhina aurita. Ecuador, Napo, Tena. Foto por Rubén D. Jarrín E.

¹Museo de Zoología (QCAZ), Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Quito, Pichincha, Ecuador

²Laboratoire Evolution et Diversité Biologique (UMR5174), CNRS, IRD, Université Paul Sabatier, Toulouse, France

³WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Bernhard Nocht Institute for Tropical Medicine, 20359 Hamburg, Germany

⁴Laboratorio de Ecofisiología, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Quito, Pichincha, Ecuador.

Abstract

Classically, molecular phylogenetic trees of Phyllostomidae have been inferred using a combination of a few mitochondrial and nuclear markers. However, there is still uncertainty in the relationships, especially among deep clades within the family. In this study, we provide newly sequenced complete mitochondrial genomes from 26 bat species, including genomes of 23 species reported here for the first time. By carefully analyzing these genomes using maximum likelihood and Bayesian methods and different ingroup and outgroup samples, partition schemes and data types, we investigated the robustness and sensitivity of our phylogenetic results. The optimal topologies were those inferred from the complete data matrix of nucleotides, with complex and highly parameterized substitution models and partition schemes. Our results show a statistically robust picture of the evolutionary relationships between phyllostomid subfamilies and clarify hitherto uncertain relationships of Lonchorhinae and Macrochinae.

Keywords: Leaf-nosed bats, Lonchorhinae, Macrochinae, mitogenomics, phylogenetics, Phyllostomidae.

Introduction

The New World leaf-nosed bats of the family Phyllostomidae Gray, 1825, are found ubiquitously in all regions of the Neotropics. The most recent classification recognizes 227 species in 61 genera (Simmons & Cirranello, 2022), making it the second most species-rich chiropteran family after Vespertilionidae Gray, 1821. This group of bats is characterized by its rapid early radiation and recent speciation events (Velazco, 2005; Solari et al., 2009; Larsen et al., 2013; Velazco & Patterson, 2013, 2019) that led to an unparalleled morphological, behavioural and ecological diversity (Freeman, 2000; Dumont et al., 2012; Dávalos et al., 2012,

2014; Baker et al., 2016) and an exceptional diversification of feeding habits, with six different strategies: sanguivory, insectivory, frugivory, nectivory, carnivory and omnivory (Baker et al., 2012). According to Baker et al. (2016), Phyllostomidae are divided into 11 subfamilies, 12 tribes and nine subtribes. Several phylogenetic studies have examined the relationships among phyllostomid bats using nuclear and mitochondrial loci obtained from classical Sanger sequencing (Baker et al., 2003; Datzmann et al., 2010; Rojas et al., 2011; Dumont et al., 2012; Dávalos et al., 2014; Rojas et al., 2016). Among these studies, Rojas et al. (2016) inferred a phylogenetic tree from seven nuclear and five mitochondrial genes, which has been used as a backbone for evolutionary inferences in phyllostomids (Potter et al., 2021). Despite these comprehensive analyses, there is still considerable debate at a deeper level about the position of Lonchorhininae Gray, 1866, Micronycterinae Van Den Bussche, 1992, and Macrotinae Van Den Bussche, 1992. In addition, further studies are required to solve the systematics of certain groups at the genus level, especially those that have been underrepresented traditionally owing to limited taxonomic sampling, limited datasets, and incongruences among and within different types of data, leading to conflicts when drawing taxonomic or systematic conclusions (Solari et al., 2009, 2019b; Baker et al., 2016; Morales-Martínez et al., 2021).

Botero-Castro et al. (2013) used high-throughput sequencing to obtain complete mitogenomes via a genome-skimming approach. Using shallow sequencing, this approach is able to recover repeated regions of the genome fully, which are typically in organelles, such as the mitochondria (Straub et al., 2012; Trevisan et al., 2019). Botero-Castro et al. (2013) sequenced the complete mitochondrial genome of 11 bat species, including representatives of seven phyllostomid subfamilies, and compared the phylogenies from mitochondrial genomes vs. nuclear exons (*rag2* and *vWF*), and single genes vs. concatenation for both types of genes. They found that the phylogenetic trees inferred from concatenated mitogenomic sequences were better resolved and well supported. Following their previous study, Botero-Castro et al. (2018) sequenced eight

new chiropteran mitogenomes and added sequences from GenBank, consolidating a sample of 39 bat species, including 19 phyllostomid bats representing each subfamily as defined by Baker et al. (2003).

Complete mitochondrial genomes in vertebrates might provide a better resolution of phylogenetic relationships on various taxonomic levels than the short nuclear and mitochondrial fragments typically used with Sanger sequencing data (Meganathan et al., 2012; Finstermeier et al., 2013; Fabre et al., 2017; Botero-Castro et al., 2018; Pan et al., 2020; Hassanin et al., 2020). In addition, advances of high-throughput sequencing technologies in the last decade provide efficient access to mitochondrial genome sequences (Springer et al., 2004; Pajmans et al., 2013; Morgan et al., 2014; Phillips & Shazwani-Zakaria, 2021; Toussaint et al., 2021).

The seminal work of Botero-Castro et al. (2013, 2018) provided the foundation for mitochondrial phylogenomics of phyllostomids. However, their results were based on a limited dataset of bats, representing 2.6% of the diversity of the order. Using high-throughput sequencing, we here generated several complete bat mitogenomes to reconstruct a subfamily-level phylogenetic tree of phyllostomid bats. We herein address the following questions:

1. Do the newly sequenced mitogenomic data help to improve the resolution at the subfamily level?
2. What is the influence of various analytical conditions on the results?

In particular, we investigated the influence of partition schemes, choice of outgroups, types of data and phylogenetic methods.

Materials and methods

Taxon sampling and DNA extraction and sequencing

We sequenced 32 mitogenomes from 26 species, of which 23 belong to Phyllostomidae, two to Vespertilionidae and one to Molossidae Gervais, 1856. The tissues used for DNA extraction belonged to specimens collected during various field trips to western provinces of Ecuador (Figure 2-1), as part of research projects of the Mammalogy Section of the Museum of Zoology of the Pontifical Catholic University of Ecuador (QCAZ). Taxonomic and collection data from the specimens analyzed is provided in Supplementary data 1; additional information about these specimens is available at: <https://bioweb.bio/faunaweb/mammaliaweb/>. We also used 39 complete mitogenome sequences generated by Botero-Castro et al. (2013, 2018), comprising 37 sequences from eight chiropteran and two non-chiropteran families. Our dataset was thus formed by 71 complete mitogenomes (Figure 2-1). Only three species have reported mitogenomes in previous studies: *Artibeus lituratus* Leach, 1821 (Phyllostomidae), *Desmodus rotundus* Wied-Neuwied, 1826 (Desmodontinae J.A. Wagner, 1840) and *Micronycteris megalotis* (Micronycterinae Van Den Bussche, 1992).

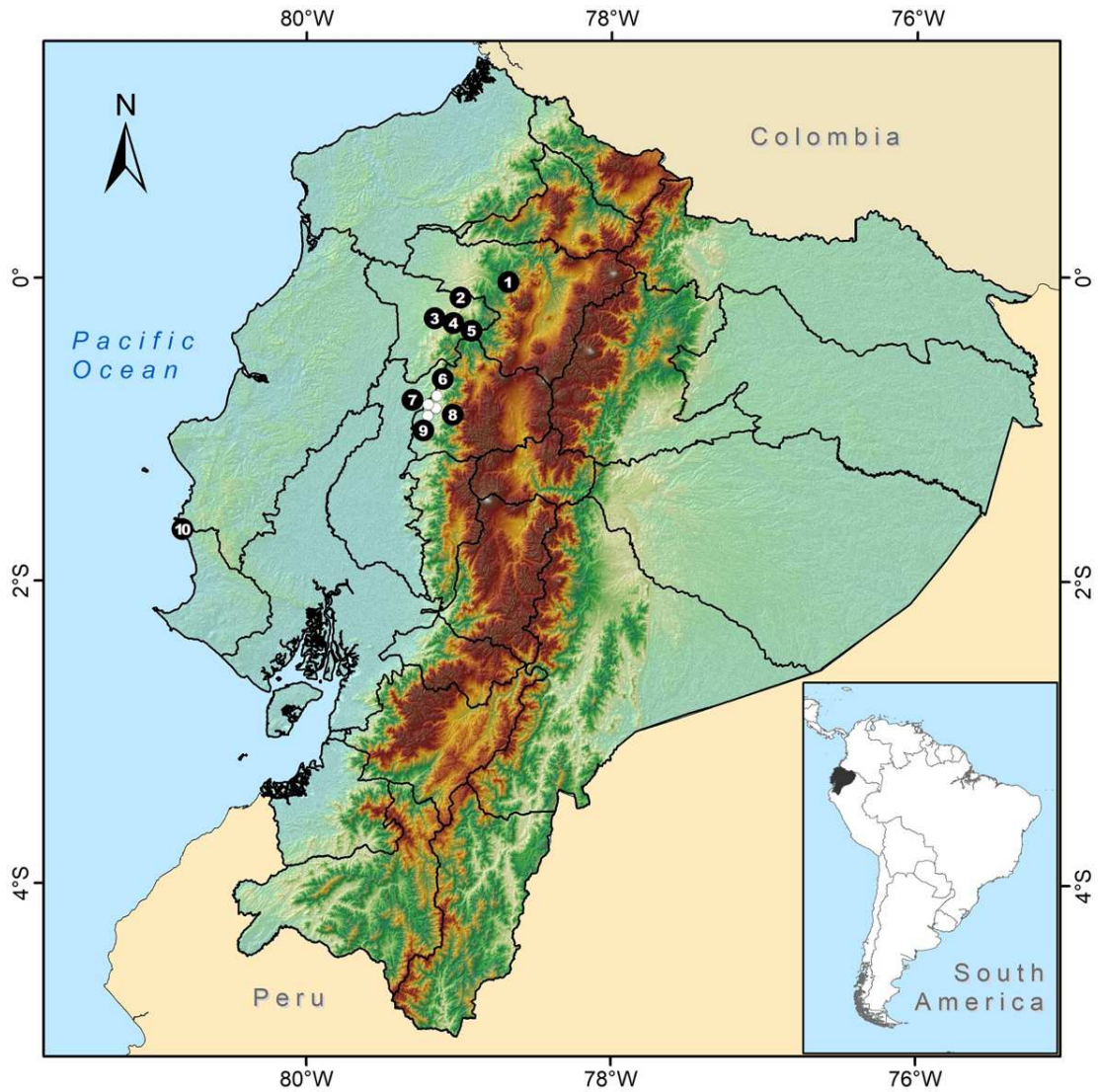


Figure 2-1. Geographical distribution of Phyllostomidae specimens. Sampling localities: Pichincha: 1) Alambi (Lon: -78.680733, Lat: -0.030217); Santo Domingo de Los Tsáchilas: 2) Río Mulaute (Lon: -78.993183, Lat: -0.131917); 3) La Lorena (Lon: -79.139733, Lat: -0.2747998); 4) Hacienda Tinalandia (Lon: -79.054433, Lat: -0.2979668); 5) Reserva Otongachi (Lon: -78.9518828, Lat: -0.3212998); Cotopaxi: 6) Guasaganda (Lon: -79.1468667, Lat: -0.7798167); 7) Jardín de los Sueños (Lon: -79.2045668, Lat: -0.8372); 8) San Cristóbal (Lon: -79.1532668, Lat: -0.8607); 9) Manguilita El Triunfo (Lon: -79.20735, Lat: -0.9117498); Manabí: 10) Las Tunas (Lon: -80.8152861, Lat: -1.6621917). White dots are the precise localities.

Table 2-1. List of specimens used for Phyllostomidae phylogenetic analysis. Records include taxonomic information, voucher identification, GenBank accession number, and bibliographic reference.

Family	Subfamily	Species	Voucher ^a	Accession Number	Reference
Chiroptera					
Hipposideridae	–	<i>Hipposideros armiger</i>	–	NC018540	Xu <i>et al.</i> 2012
Molossidae	Molossinae	<i>Molossus molossus</i>	QCAZ18284	ON357729	This study
Molossidae	Molossinae	<i>Molossus molossus</i>	QCAZ18287	ON653196	This study
Mormoopidae	–	<i>Pteronotus rubiginosus</i>	ISEM-V-2322	NC022425	Botero-Castro <i>et al.</i> 2013
Mystacinidae	–	<i>Mystacina tuberculata</i>	–	NC006925	Unpublished
Noctilionidae	–	<i>Noctilio leporinus</i>	ISEM-V-1890	KU743910	Botero-Castro <i>et al.</i> 2018
Phyllostomidae	Carollinae	<i>Carollia brevicauda</i>	QCAZ18221	ON527784	This study
Phyllostomidae	Carollinae	<i>Carollia castanea</i>	QCAZ18219	ON310505	This study
Phyllostomidae	Carollinae	<i>Carollia perspicillata</i>	MHNG1972-003	HG003309	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Carollinae	<i>Carollia brevicauda</i>	QCAZ18226	ON527785	This study
Phyllostomidae	Desmodontinae	<i>Desmodus rotundus</i>	EBRG-L-1874	HG003310	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Desmodontinae	<i>Desmodus rotundus</i>	QCAZ18371	ON527786	This study
Phyllostomidae	Desmodontinae	<i>Diaemus youngii</i>	MSB-56205	KU743906	Botero-Castro <i>et al.</i> 2018
Phyllostomidae	Desmodontinae	<i>Diphylla ecaudata</i>	MSB-211697	KU743911	Botero-Castro <i>et al.</i> 2018
Phyllostomidae	Glossophaginae	<i>Anoura caudifer</i>	ROM-113962	HG003307	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Glossophaginae	<i>Anoura cultrata</i>	QCAZ18217	ON310503	This study
Phyllostomidae	Glossophaginae	<i>Anoura geoffroyi</i>	QCAZ18218	ON310504	This study
Phyllostomidae	Glossophaginae	<i>Brachyphylla cavernicum</i>	ISEM-V-2350	NC022421	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Glossophaginae	<i>Choeroniscus godmani</i>	QCAZ18233	ON357720	This study
Phyllostomidae	Glossophaginae	<i>Glossophaga soricina</i>	QCAZ18230	ON321893	This study
Phyllostomidae	Glyphonycterinae	<i>Glyphonycteris daviesi</i>	ROM-41125	KU743912	Botero-Castro <i>et al.</i> 2018
Phyllostomidae	Lonchophyllinae	<i>Hsunnycteris thomasi</i>	ISEM-V-1646	KU743907	Botero-Castro <i>et al.</i> 2018
Phyllostomidae	Lonchophyllinae	<i>Lonchophylla concava</i>	QCAZ18274	ON357727	This study
Phyllostomidae	Lonchophyllinae	<i>Lonchophylla concava</i>	QCAZ18273	ON653195	This study
Phyllostomidae	Lonchophyllinae	<i>Lonchophylla robusta</i>	QCAZ18236	ON357721	This study
Phyllostomidae	Lonchorhininae	<i>Lonchorhina aurita</i>	MVZ-185587	KU743908	Botero-Castro <i>et al.</i> 2018
Phyllostomidae	Macrotinae	<i>Macrotus californicus</i>	MSB-140888	KU743909	Botero-Castro <i>et al.</i> 2018
Phyllostomidae	Micronycterinae	<i>Micronycteris hirsuta</i>	QCAZ18237	ON357722	This study
Phyllostomidae	Micronycterinae	<i>Micronycteris megalotis</i>	ISEM-V-2620	HF947304	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Micronycterinae	<i>Micronycteris megalotis</i>	QCAZ18280	ON357728	This study
Phyllostomidae	Phyllostominae	<i>Chrotopterus auritus</i>	AMNH-M-272843	KU743905	Botero-Castro <i>et al.</i> 2018

Family	Subfamily	Species	Voucher ^a	Accession Number	Reference
Phyllostomidae	Phyllostominae	<i>Lophostoma brasiliense</i>	QCAZ18085	ON310506	This study
Phyllostomidae	Phyllostominae	<i>Lophostoma silvicola</i>	MNHN2004-352	NC022424	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Phyllostominae	<i>Phyllostomus discolor</i>	QCAZ18297	ON357733	This study
Phyllostomidae	Phyllostominae	<i>Tonatia maresi</i>	MNHN2004-376	NC022428	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Phyllostominae	<i>Vampyrum spectrum</i>	EBRG-L-1896	NC022429	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Rhinophyllinae	<i>Rhinophylla pumilio</i>	ISEM-V-1992	NC022426	Botero-Castro <i>et al.</i> 2013
Phyllostomidae	Stenodermatinae	<i>Artibeus aequatorialis</i>	QCAZ18246	ON357726	This study
Phyllostomidae	Stenodermatinae	<i>Artibeus jamaincensis</i>	–	NC002009	Pumo <i>et al.</i> 1998
Phyllostomidae	Stenodermatinae	<i>Artibeus literatus</i>	QCAZ18245	ON357725	This study
Phyllostomidae	Stenodermatinae	<i>Artibeus lituratus</i>	–	NC016871	Meganathan <i>et al.</i> 2012
Phyllostomidae	Stenodermatinae	<i>Artibeus ravus</i>	QCAZ18228	ON321891	This study
Phyllostomidae	Stenodermatinae	<i>Chiroderma salvini</i>	QCAZ18227	ON321890	This study
Phyllostomidae	Stenodermatinae	<i>Enchisthenes hartii</i>	QCAZ18229	ON321892	This study
Phyllostomidae	Stenodermatinae	<i>Platyrrhinus matapalensis</i>	QCAZ18238	ON357723	This study
Phyllostomidae	Stenodermatinae	<i>Platyrrhinus nigellus</i>	QCAZ18299	ON357734	This study
Phyllostomidae	Stenodermatinae	<i>Sturnira bakeri</i>	QCAZ18306	ON357735	This study
Phyllostomidae	Stenodermatinae	<i>Sturnira ludovici</i>	QCAZ18312	ON357738	This study
Phyllostomidae	Stenodermatinae	<i>Sturnira luisi</i>	QCAZ18241	ON357724	This study
Phyllostomidae	Stenodermatinae	<i>Sturnira bakeri</i>	QCAZ18307	ON357736	This study
Phyllostomidae	Stenodermatinae	<i>Sturnira bakeri</i>	QCAZ18308	ON357737	This study
Phyllostomidae	Stenodermatinae	<i>Sturnira tildae</i>	ISEM-V-2412	NC022427	Botero-Castro <i>et al.</i> 2013
Pteropodidae	–	<i>Pteropus dasymallus</i>	–	NC002612	Nikaido <i>et al.</i> 2000
Pteropodidae	–	<i>Pteropus scapulatus</i>	–	NC002619	Lin & Penny, 2001
Pteropodidae	–	<i>Rosetus aegyptiacus</i>	–	NC007393	Unpublished
Rhinolophidae	Rhinolophinae	<i>Rhinolophus ferrumequinum</i>	–	NC016191	Yoon <i>et al.</i> 2011
Rhinolophidae	Rhinolophinae	<i>Rhinolophus formosae</i>	–	NC011304	Unpublished
Rhinolophidae	Rhinolophinae	<i>Rhinolophus luctus</i>	–	NC018539	Xu <i>et al.</i> 2012
Rhinolophidae	Rhinolophinae	<i>Rhinolophus monoceros</i>	–	NC005433	Lin <i>et al.</i> 2002
Rhinolophidae	Rhinolophinae	<i>Rhinolophus pumilus</i>	–	NC005434	Nikaido <i>et al.</i> 2001
Vespertilionidae	Vespertilioninae	<i>Lasiurus borealis</i>	LSUMZ CSM 020	NC016873	Meganathan <i>et al.</i> 2012
Vespertilionidae	Vespertilioninae	<i>Myotis albescens</i>	QCAZ18292	ON357731	This study
Vespertilionidae	Vespertilioninae	<i>Myotis formosus</i>	–	NC015828	Kim <i>et al.</i> 2011
Vespertilionidae	Vespertilioninae	<i>Myotis riparius</i>	QCAZ18293	ON357732	This study
Vespertilionidae	Vespertilioninae	<i>Myotis riparius</i>	QCAZ18288	ON357730	This study

Family	Subfamily	Species	Voucher ^a	Accession Number	Reference
Vespertilionidae	Vespertilioninae	<i>Pipistrellus abramus</i>	–	NC005436	Nikaido <i>et al.</i> 2001
Vespertilionidae	Vespertilioninae	<i>Plecotus auritus</i>	–	NC015484	Unpublished
Vespertilionidae	Vespertilioninae	<i>Plecotus rafinesquii</i>	LSUMZ (field number M8121)	NC016872	Meganathan <i>et al.</i> 2012
Vespertilionidae	–	<i>Chalinolobus tuberculatus</i>	–	NC002626	Lin & Penny, 2001
Other mammals					
Bovidae	–	<i>Bos taurus</i>	–	NC006853	Unpublished
Canidae	–	<i>Canis lupus</i>	–	NC002008	Kim <i>et al.</i> 1998

^aKey to institutional abbreviations: **AMNH** – American Museum of Natural History, New York, USA; **EBRG** – Museum “Estación Biológica de Rancho Grande”, Aragua, Venezuela; **ISEM** – University of Montpellier, Montpellier, France; **LSUMZ** – Louisiana State University Museum of Natural Science, Baton Rouge, USA; **MHNG** – Natural History Museum of Geneva, Geneva, Switzerland; **MNHN** – Museum National d’Histoire Naturelle, Paris, France; **MSB** – Museum of Southwestern Biology, Albuquerque, USA; **MVZ** – Museum of Vertebrate Zoology at the University of California, Berkeley, USA; **QCAZ** – Zoology Museum, Pontifical Catholic University of Ecuador, Quito, Ecuador; **ROM** – Royal Ontario Museum, Toronto, Canada.

Tissue samples from the heart, liver and lungs were pooled per specimen and placed into 2 mL safe-lock tubes (Eppendorf, Hamburg, Germany) with tungsten carbide beads (3 mm in diameter; Qiagen, Hilden, Germany) and 0.7 mL chilled high-glucose (4.5 g/L) Dulbecco’s modified Eagle’s medium (Sigma-Aldrich, St. Louis, MO, USA). Bat tissues were homogenized with a TissueLyser II (Qiagen, Hilden, Germany) for 2 min at 30–50 Hz. The suspension was clarified by centrifugation for 1 min at 11269 G at 4 °C. DNA and RNA were extracted using the MagMAX CORE Nucleic Acid Purification Kit (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer’s recommendations. The quality and quantity of extracted nucleic acids were measured using a Qubit DNA/RNA HS Assay Kit (Thermo Fisher Scientific, Austin, TX, USA). The extracted DNA (120 ng starting amount) was subjected to library preparation using a QIAseq FX DNA Library Kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. Normalized samples were pooled and sequenced on an Illumina NextSeq550 sequencing platform using a 300-cycle (2 × 150 bp paired-end) NextSeq550 reagent kit (Illumina, San Diego, CA, USA).

Mitogenome assembly and annotations

The paired-end Illumina reads were quality checked, automatically trimmed and merged using QIAGEN CLC Genomics Workbench v.20.0 (<https://digitalinsights.qiagen.com/>). Additional adapter trimming and deduplication were performed using the BBTools software package (<https://sourceforge.net/projects/bbmap/>). The resulting high-quality reads were assembled de novo with MEGAHIT, which in this case was found to give the best output (Li et al., 2015). We used reference-guided mapping, circularization validation and manual curation, also using the CLC Genomics Workbench v.20.0 software. All the assembled mitochondrial genomes were annotated using the MITOS2 metazoan pipeline (Bernt et al., 2013; Al Arab et al., 2017; Donath et al., 2019), followed by manual adjustment in Geneious v.9.0.5 (<https://www.geneious.com>). We relied on the publicly available mitochondrial sequences as reference (Botero-Castro et al., 2013, 2018). In order to validate the morphological identification of the bat species, we used the Barcoding of Life Database Identification Engine on the 5' region of the COI sequence using the BOLD web server (Ratnasingham & Hebert, 2007).

Alignment and phylogeny

Ribosomal RNA (rRNA) and transfer RNA (tRNA) loci were aligned using MUSCLE (Edgar, 2004). Sequences of protein-coding genes were aligned using TranslatorX (Abascal et al., 2010) to preserve the reading frame. We used trimAl (Capella-Gutiérrez et al., 2009) to remove poorly aligned regions. Following Botero-Castro et al. (2018), the protein-coding gene *nd6* and the control region were excluded from the analyses. The *nd6* gene has frequently been omitted, because it is coded on the light strand, and its properties differ from those of the other 12 protein-coding genes (Springer et al., 2001; Gao et al., 2018); the control region is the most variable, because of a faster rate of evolution compared with the rRNA and protein-coding genes of the mitochondrial genome (Gong et al., 2015), which might interfere with phylogenetic

interpretation. The final data matrix consisted of 71 mitogenomes, each comprising 36 loci (two rRNA, 12 protein-coding and 22 tRNA genes) and averaging 16 kb.

In order to evaluate the impact of ingroup taxon sampling on the results, we performed a phylogenetic analysis using the same analytical parameters and the same outgroups as Botero-Castro et al. (2018), but with an ingroup taxon sampling that significantly exceeds previous analyses. In their study, Botero-Castro et al. (2018) used sequences from 19 phyllostomid species, whereas we used 42 species. We consider this step as our ‘reference analysis’ and the resulting topology as our ‘reference tree’.

To evaluate the influence of partitioning, we analyzed various *a priori* schemes, in addition to new estimated ones. For the *a priori* nucleotide partition schemes, following Botero-Castro et al. (2018), we used one partition, five partitions (two independent partitions for rRNAs and tRNAs combined with three partitions corresponding to each codon position), 14 partitions (two independent partitions for rRNAs and tRNAs plus one partition for each protein-coding gene) and 38 partitions (two independent partitions for rRNAs and for tRNAs plus 36 partitions, one partition for each codon for each gene). For each *a priori* partition scheme, we used a generalized time reversible (GTR) model of substitution rates along with a gamma (G) distribution and a fraction of invariable (I) sites (GTR+G+I). In addition, we also estimated the optimal partition scheme and evolutionary model using PartitionFinder v.2.1.1 (Lanfear et al., 2012) and ModelFinder (Kalyaanamoorthy et al., 2017) as implemented in IQ-TREE 2 (Minh et al., 2020) (Table 2-2). The best partitioning schemes were chosen based on the Akaike information criterion (AIC) (Bozdogan, 1987).

To evaluate the impact of taxon sampling in the outgroups, we compared our reference analysis from the complete sample of 71 sequences (henceforth, ‘full outgroup dataset’) with a new dataset comprising a subset of 48 nucleotide sequences with only the mitochondrial genomes

of Noctilionidae Gray, 1821 and Mormoopidae Saussure, 1860 as outgroups ('reduced outgroup dataset'; Table 2-2). We followed Baker et al. (2003), who repeatedly found these families as outgroups of Phyllostomidae in their higher-level classification analyses with both mitochondrial and nuclear markers. For each dataset, we estimated the best nucleotide substitution models and partitioning schemes (Table 2-2).

Table 2-2. Nucleotide data set used for Phyllostomidae phylogenetic analysis. "Full outgroup dataset" corresponds to the complete sample of 71 sequences, "Reduced outgroup dataset" corresponds to a sub-sample made up of 48 sequences. The partition schemes are described, and the Akaike Information Criterion values are compared. The asterisks (*) denote the best-evaluated partition schemes by this criterion.

Bioinformatics software	Partition scheme	Number of partitions	Number of Parameters	-lnL	AIC
Full outgroup dataset					
RAxML-NG	A single partition	1	149	-334,497.3	669,292.6
RAxML-NG	rRNA + tRNA + protein-coding genes	14	292	-326,799.5	653,985.1
RAxML-NG	rRNA + tRNA + codon positions	5	193	-332,165.0	664,914.0
RAxML-NG	rRNA + tRNA + protein-coding genes + codon positions	38	556	-324,889.2	650,890.4*
PartitionFinder RAxML-NG	Partition by gene	31	436	-330,755.8	662,383.7
IQTREE2	Partition by gene	19	148	-339,430.8	679,157.6
Reduced outgroup dataset					
RAxML-NG	A single partition	1	103	-219,193.7	438,593.4
RAxML-NG	rRNA + tRNA + protein-coding genes	14	246	-217,376.1	435,244.2
RAxML-NG	rRNA + tRNA + codon positions	5	147	-212,155.9	424,605.8
RAxML-NG	rRNA + tRNA + protein-coding genes + codon positions	38	510	-210,734.2	422,488.4*
PartitionFinder RAxML-NG	Partition by gene	28	358	-213,561.8	427,839.7
IQTREE2	Partition by gene	16	254	-211,257.2	423,022.5

*Best-evaluated partition schemes by this criterion.

Abbreviations: AIC, Akaike information criterion; lnL, Log likelihood; rRNA, ribosomal RNA; tRNA, transfer RNA.

We also performed various analyses using our dataset as amino acids. We only considered the coding genes and evaluated the impact of outgroups and partitioning schemes as with the nucleotides. For amino acid datasets, we used one and 12 partitions (one for each protein-coding

gene) as proposed by Botero-Castro et al. (2018), and the resulting best-fitting schemes suggested by PartitionFinder and ModelFinder (Table 2 3). The *a priori* partitions were analysed with the Le and Gascuel amino acid replacement general matrix (LG; Le and Gascuel, 2008) and the Mitochondrial Mammalia amino acid substitution model (mtMAM; Yang et al., 1998) of substitution rates, respectively, along with a gamma distribution and a fraction of invariable sites (LG+G+I; mtMAM+G+I). The best partitioning schemes were chosen based on the AIC. As with nucleotides, we evaluated the impact of outgroups by comparing our reference analysis (71 mitogenomes with full outgroups) with a dataset comprising a subset of 48 amino acids sequences, with only Noctilionidae and Mormoopidae as outgroups.

Table 2-3. Amino acids data set used for Phyllostomidae phylogenetic analysis. “Full outgroup dataset” corresponds to the complete sample of 71 sequences, “Reduced outgroup dataset” corresponds to a sub-sample made up of 48 sequences. The partition schemes are described and the Akaike Information Criterion values are compared. The asterisks (*) denote the best-evaluated partition schemes by this criterion.

Bioinformatic software	Partition scheme	Number of partitions	Number of Parameters	-lnL	AIC
Full outgroup dataset					
RAxML-NG	A single partition	1	141	-75,293.4	150,868.7
RAxML-NG	Protein-coding genes	12	231	-70,132.27	140,726.5
PartitionFinder	Partition by gene	7	178	-70,134.5	140,625.1*
RAxML-NG					
IQTREE2	Partition by gene	4	159	-73617.015	147,552.03
Reduced outgroup dataset					
RAxML-NG	Partition by gene	1	95	-50,664.04	101,518.09
RAxML-NG	Protein-coding genes	12	185	-46,991.45	94,352.90
PartitionFinder	Partition by gene	6	147	-46,976.63	94,247.27*
RAxML-NG					
IQTREE2	Partition by gene	4	113	-49,380.65	98,987.31

*Best-evaluated partition schemes by this criterion.

We performed an additional analysis considering only the coding genes from our full nucleotide dataset using a 36-partition scheme (one partition for each codon for each gene, excluding *nd6*). This analysis was performed to examine the influence of the data type (amino acid vs. the nucleotide) using the same dataset (only the protein-coding genes).

We performed maximum likelihood (ML) analyses using RAxML-NG (Kozlov et al., 2019), starting from ten parsimony trees and ten random trees. Bootstrapping was performed with the

bootstopping option ('bs-trees autoMRE{N}' command). Bootstrap support values were obtained using the classical Felsenstein metric (Felsenstein, 1985) and transfer bootstrap expectation (Lemoine et al., 2018). For comparative reasons, we also inferred the ML tree using IQ-TREE and the edge-linked partition model (Nguyen et al., 2015; Chernomor et al., 2016) and obtained node support with the ultrafast bootstrap (Hoang et al., 2018) by performing 1000 replicates. Bayesian inference (BI) analyses were performed using MrBayes v.3.2.7 (Ronquist et al., 2012). We partitioned the sequences in 38 sets corresponding to two independent partitions for rRNAs and for tRNAs plus 36 partitions, one partition for each codon for each gene, and used the best analytical scheme as evaluated by the AIC. We ran eight Markov Chain Monte Carlo (MCMC) chains for ten million generations, with default heating values. The sampling frequency was set every 1000 generations, and the first 25 000 samples were discarded as burn-in. A consensus tree was built under the majority rule consensus of all trees obtained in the eight runs after the burn-in period. We used the 'sumt' command to produce summary statistics for trees sampled during a Bayesian MCMC analysis. Posterior probabilities of nodes were regarded as estimators of confidence. Finally, trees were visualized and edited in FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). Only the optimal partitioning schemes found under ML were evaluated using BI.

Results

After DNA extraction and sequencing, we obtained a mean of 640 043 reads (SD 329 238) per library (Table 2-4). Among the 32 newly generated mitochondrial genomes, genomes of 23 species have not been published previously and are first reported here. All mitochondrial genomes show the typical circular structure, with an average length of 16 690 bp (SD 152 bp; Table 2.4) and harbour the gene order found in other mammalian genomes (Pumo et al., 1998; López-Wilchis et al., 2017).

Table 2-4. General features of the new bat mitogenome assemblies.

QCAZ	Species	Total reads*	Mean Coverage	mtDNA Reads	mtDNA Reads (%)	mtgenome Length	G+C Content (%) [†]
18085	<i>Lophostoma brasiliense</i>	5,020,095	5,931	630,938	12.57	16,653	40.7
18217	<i>Anoura cultrata</i>	5,196,044	4,675	491,888	9.47	16,552	38.67
18218	<i>Anoura geofroyi</i>	5,387,000	4,910	513,801	9.54	16,605	39.87
18219	<i>Carollia castanea</i>	6,010,339	6,504	733,256	12.2	16,711	41.45
18221	<i>Carollia brevicauda</i>	714,6821	7,043	784,051	10.97	16,711	41.45
18226	<i>Carollia brevicauda</i>	7,153,480	6,414	770,227	10.77	16,711	41.45
18227	<i>Chiroderma salvini</i>	5,376,798	11,000	1,129,92	21.01	16,689	41.36
18228	<i>Artibeus ravus</i>	5,546,264	5,943	634,436	11.44	16,709	37.84
18229	<i>Enchisthenes hartii</i>	5,082,139	6,758	703,230	13.84	16,718	38.22
18230	<i>Glossophaga soricina</i>	5,389,214	3,055	347,814	6.45	16,529	37.35
18233	<i>Choeroniscus minor</i>	6,611,734	217	24,935	0.38	16,637	37.59
18236	<i>Lonchophylla robusta</i>	5,501,494	6,901	720,793	13.1	16,666	43.68
18237	<i>Micronycteris hirsuta</i>	5,244,058	10,723	1,109,69	21.16	16,589	42.85
18238	<i>Platyrrhinus matapalensis</i>	5,362,494	6,572	664,783	12.4	16,541	40.68
18241	<i>Sturnira bakeri</i>	5,818,828	6,939	787,240	13.53	16,637	43.27
18245	<i>Artibeus literatus</i>	4,412,371	943	104,092	2.36	16,709	37.84
18246	<i>Artibeus aequatorialis</i>	5,470,809	3,540	368,764	6.74	16,709	37.84
18273	<i>Lonchophylla concava</i>	4,421,729	2,306	232,980	5.27	16,691	39.65
18274	<i>Lonchophylla concava</i>	3,878,886	780	81,974	2.11	16,692	39.97
18280	<i>Micronycteris megalotis</i>	5,125,473	7,611	790,574	15.42	16,589	42.79
18284	<i>Molossus molossus</i>	4,834,631	3,731	385,460	7.97	16,612	36.26
18287	<i>Molossus molossus</i>	5,164,704	6,877	713,304	13.81	16,615	36.54
18288	<i>Myotis riparius</i>	5,610,421	8,940	994,147	17.72	17,052	35.57
18292	<i>Myotis albescens</i>	4,967,853	3,058	326,789	6.58	17,128	35.58
18293	<i>Myotis riparius</i>	4,441,706	2,722	318,278	7.17	17,186	35.81
18297	<i>Phyllostomus discolor</i>	4,216,226	3,482	359,346	8.52	16,692	41.74
18299	<i>Platyrrhinus nigellus</i>	5,369,804	5,302	553,748	10.31	16,541	40.68
18306	<i>Sturnira bakeri</i>	5,518,196	8,591	914,490	16.57	16,637	43.27
18307	<i>Sturnira bakeri</i>	5,365,597	9,504	995,712	18.56	16,637	43.27
18308	<i>Sturnira bakeri</i>	5,973,325	9,823	1,040,51	17.42	16,637	43.27
18312	<i>Sturnira ludovici</i>	4,528,183	8,222	864,316	19.09	16,646	43.61
18371	<i>Desmodus rotundus</i>	6,115,261	12,311	1,389,88	22.73	16,668	47.68

*Trim/merge/duplicate.

[†] Approximate values on draft genomes.

Reference analysis

We used as reference the phyllostomid phylogeny inferred from all genes under ML using RAxML-NG (Kozlov et al., 2019). Sequence evolution was modelled using a GTR+G+I model and a 38-partition scheme comprising two independent partitions, for rRNAs and for tRNAs, plus one partition per gene and per codon position for each protein-coding gene. We used 71 complete mitogenomes, which included the ‘full outgroup dataset’ (Table 2-2). In essence, this analysis corresponds exactly to the one performed previously by Botero-Castro et al. (2018) except for the ingroup, for which we added 23 new phyllostomid species to the 19 previously used in 2018.

As expected, Phyllostomidae are recovered as monophyletic and divided into 11 clades corresponding to all known subfamilies (Figure 2-1). At deeper nodes (subfamilies), we cannot observe differences between our reference topology and the best evaluated topology obtained by Botero-Castro et al. (2018), but the addition of new mitogenomes shows genus-level relationships not presented by Botero-Castro et al. (2018) that help to clarify the relationships between species, particularly those in nectar-feeding subfamilies. When compared with the other analyses that combine mitochondrial and nuclear markers (Baker, 2000; Baker et al., 2003; Rojas et al., 2016), the main difference in our analysis lies in the position of Lonchorhininae as sister group to Phyllostominae Gray, 1825, not to a clade formed by Lonchophyllinae Griffiths, 1982 and the mainly frugivorous subfamilies.

The phylogenetic relationships between the subfamilies Stenodermatinae Gervais, 1856, Rhinophyllinae Baker, Solari, Cirranello & Simmons, 2016, Carolliinae and Glyphonycterinae Baker, Solari, Cirranello & Simmons, 2016 are recovered with strong support. Stenodermatinae share a common ancestry with Rhinophyllinae after diverging from a clade formed by Carolliinae and Glyphonycterinae. Regarding the nectar-feeding phyllostomids,

Lonchophyllinae appear as sister to a lineage comprising the clades formed by Stenodermatinae + Rhinophyllinae and Carollinae + Glyphonycterinae (Figure 2-2), with strong nodal support [bootstrap support (BS) = 91%, node not recovered with BI] and in agreement with Baker et al. (2003), Rojas et al. (2011) and Rojas et al. (2016).

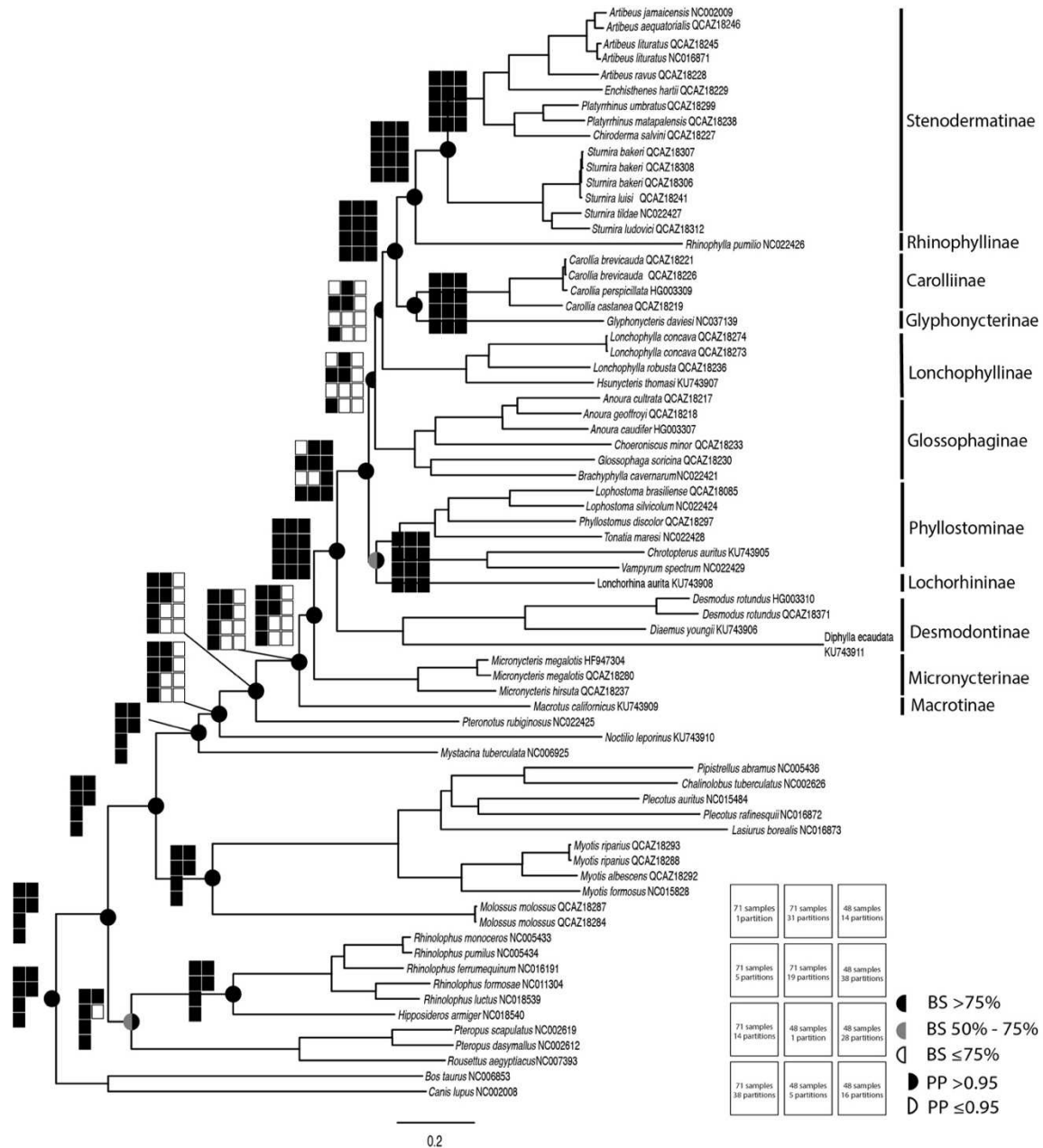


Figure 2-2. Phylogeny of Phyllostomidae. Phylogeny based on complete mitochondrial genomes (nucleotide sequences). The tree represents the best maximum likelihood (ML) phylogeny inferring the relationships of Phyllostomidae and other chiropteran lineages. The tree was reconstructed in RA xML under the GTR+GAMMA+I model using the full outgroup dataset (71 taxa and 14 703 nucleotides). Shaded semicircles on the nodes indicate ML bootstrap support (as a percentage) and Bayesian posterior probabilities (see inset key). The absence of a semicircle on a node indicates that it was not recovered by ML or Bayesian inferences. Each Navajo

rug shows whether the specified node was retrieved (black square) or not (white square) in the different analyses performed (see inset key).

Glossophaginae Bonaparte, 1845 are the nectar-feeding clade that diverged first and comprise two major lineages: one containing Anoura Gray, 1838 and *Choeroniscus* Thomas, 1928 and the other containing *Glossophaga* E. Geoffroy, 1818 and *Brachyphylla* Gray, 1833. In addition, Phyllostominae appear as a sister group to Lonchorhinae with moderate bootstrap support, and vampire bats are monophyletic and defined as a clade composed of three genera (Desmodontinae), with well-resolved relationships. In our reference tree, Macrotinae are recovered as a sister group to all remaining Phyllostomid subfamilies, and Mormoopidae appear as a sister family to Phyllostomidae, also in concordance with previous phylogenetic reconstructions (Baker et al., 2003, 2016; Cirranello et al., 2016).

All phylogenetic relationships between non-phyllostomid families remained consistent with the results obtained in previous studies with mitogenomes except that, for the first time, mitogenomes of the family Molossidae (*Molossus molossus* É. Geoffroy, 1805) are added and appear closely related to Vespertilionidae.

Impact of partitioning schemes

We performed 12 different analyses with various *a priori* partition schemes, in addition to new estimated ones (Table 2-2). We found that a GTR+G+I evolution model along with a 38-partition scheme (thus corresponding to our ‘reference analysis’) yielded the smallest AIC value (Table 2-2). This evolutionary model and partition scheme also yielded the best AIC value in the analysis performed by Botero-Castro et al. (2018). The 11 additional analytical schemes are thus suboptimal in the sense that they yielded higher AIC values than with the optimal partitioning scheme, including those with IQ-TREE performed for comparative reasons. However, this evaluation is particularly relevant because it provides a different view of the stability of our results compared with statistical resampling, such as bootstrap. The presence (or

not) of key clades was thus evaluated with a sensitivity analysis of the various analytical conditions and is represented with a Navajo rug approach (Figure 2-2; Supplementary data 2).

Across the analyses, we were able to identify stable nodes. The phylogenetic relationships between the subfamilies Stenodermatinae, Rhinophyllinae, Carolliinae and Glyphonycterinae are recovered in all analyses. Rhinophyllinae appear consistently as a sister clade to Stenodermatinae, and both subfamilies form a sister clade to another formed by Carolliinae and Glyphonycterinae. Vampire bats (Desmodontinae) consistently appear in the same position in all resulting topologies, and the relationships between the three species are all well resolved. Interestingly, the subfamily Lonchorhininae, which had a poorly supported position in previous studies (Rojas et al., 2016), is stable as sister to Phyllostominae in our analyses.

We identified unstable nodes formed by subfamilies for which phylogenetic relationships varied depending on the number of partitions. Lonchophyllinae as sister to the clade formed by Stenodermatinae + Rhinophyllinae and Carolliinae + Glyphonycterinae appear in only four of the 12 analyses, with bootstrap values ranging from 63 to 96%. Lonchophyllinae are found as sister to a clade composed of Lonchorhininae, Phyllostominae, Glossophaginae, Glyphonycterinae, Carolliinae, Rhinophyllinae and Stenodermatinae, depicting even better bootstrap values in the corresponding analyses (BS = 91–100%). The subfamily is also recovered as sister to Glossophaginae in several analytical schemes, with medium to strong support values (BS = 45–97%). As the position of Lonchophyllinae changes in the topologies, so does that of Glossophaginae and its phylogenetic placement. Macrotinae appear as a sister group of all remaining phyllostomids in 50% of the analyses performed, with absolute support [BS = 100%, posterior probability (PP) = 1], but Micronycterinae are also recovered as sister clade to all remaining phyllostomids (including Macrotinae) in the other 50% of the analyses, also with high support (BS = 100%; PP = 1).

Impact of outgroup taxon sampling

By comparing the topologies obtained with the ‘full outgroup dataset’ and the ‘reduced outgroup dataset’ (Table 2-2), we observe an effect caused by the number of outgroups used. Although most of the phylogenetic relationships using fewer outgroups are consistent with the reference tree obtained from the analysis of 71 sequences, some relationships are less stable. In most of the analyses with the ‘full outgroup dataset’, Lonchophyllinae appear as sister to the nodes formed by Stenodermatinae + Rhinophyllinae and Carolliinae + Glyphonycterinae, but in almost all analyses performed with the ‘reduced outgroup dataset’, Lonchophyllinae are recovered as sister to Glossophaginae, with medium to strong support values (BS = 45–97%). Taking this parameter into account, we again observe that as the position of Lonchophyllinae changes in the topologies, so does that of Glossophaginae and their phylogenetic placement. Unlike what was observed in all analyses with all sequences, with the ‘reduced outgroup dataset’, we found Micronycterinae as a sister clade to all remaining phyllostomids (including Macrochinae; BS = 75–89%), although less supported than Macrochinae as the sister clade to all remaining phyllostomids found with the ‘full outgroup dataset’.

Impact of types of data

The phylogenetic resolution and associated nodal supports across analyses differ significantly according to the types of data (i.e. nucleotides vs. amino acids). For amino acids, we performed eight different analyses (Table 2-3). All resulting topologies were compared, and the presence (or not) of key clades was represented with a Navajo rug approach (Supplementary data 2). For amino acid datasets, the best-evaluated topology was poorly supported and did not agree with the best-evaluated topology of the nucleotide analyses.

Phyllostomidae were recovered as monophyletic and divided into 11 clades corresponding to all known subfamilies. However, in general, we also found more unstable nodes with lower

support than with nucleotides. We found Lonchophyllinae as sister to a clade composed of Lonchorhininae, Phyllostominae, Glossophaginae, Glyphonycterinae, Carolliinae, Rhinophyllinae and Stenodermatinae with strong bootstrap supports (BS = 98%, node not recovered with BI; Supplementary data 2). Also, we found Lonchophyllinae as the nectar-feeding clade that diverged first and as sister to a clade containing Glossophaginae, which then diverged from a lineage containing Glyphonycterinae, Carolliinae, Rhinophyllinae and Stenodermatinae, with poor to strong support values (BS = 40–91%). Finally, we found Lonchophyllinae forming a clade with Glossophaginae. The position of Lonchorhininae appeared unclear when amino acid datasets were analyzed. As with nucleotides, we found Lonchorhininae as sister to Phyllostominae in some analyses, although mostly with poor bootstrap support. In most of the amino acid analyses, Lonchorhininae appeared as sister to clades containing the subfamilies Phyllostominae, Lonchophyllinae, Glossophaginae, Glyphonycterinae, Carolliinae, Rhinophyllinae and Stenodermatinae. Another important variation in relationship to the results we obtained with nucleotide datasets was the position of Micronycterinae: in all the analysis carried out with amino acids, Micronycterinae appeared as the sister lineage to all the other phyllostomids with strong support values (BS = 90–100%).

Our analysis in which only the coding genes from our full nucleotide dataset using a 36-partition scheme were considered yielded a topology that mostly resembled the best-evaluated phylogeny from the amino acid dataset and also did not agree with the best-evaluated topology of the nucleotide analyses (

Supplementary data 3).

Impact of phylogenetic methods

For the BI, we used the same analytical scheme as for ML. We observed that the phylogenetic resolution and associated nodal supports differed depending on the inference method. Even with high bootstrap values, some nodes were not recovered under BI.

Figure 2-2 (for nucleotides) and Supplementary data 2 (for amino acids) summarize and compare the support values of the ML and BI analyses. In the ML and BI trees, it can be observed that not all nodes were recovered under all ML or BI analyses. We found relationships between subfamilies (particularly in the less stable nodes) statistically less likely to occur owing to PPs < 0.5; for example, a monophyletic relationship between Lonchophyllinae and Phyllostominae (PP = 0.47). Also, the relationship between Lonchorhinae and the rest of the subfamilies or those of Micronycterinae or Macrotinae as basal clades of Phyllostomidae are neither clear nor consistent under Bayesian analysis.

Discussion

In this study, we provide newly sequenced complete mitogenomes for 26 bat species, from 16 genera of three families. Our phylogenomic analysis of phyllostomids confirms the relationships so far agreed between the subfamilies with strong supports and is highly congruent with those previously suggested by Baker et al. (2016), Rojas et al. (2016) and Botero-Castro et al. (2018). Phyllostomidae were monophyletic and divided into 11 subfamilies. The phylogenetic relationships between Stenodermatinae, Rhinophyllinae, Carollinae and Glyphonycterinae, in addition to the position of Desmodontinae, were consistent and well supported. Lonchophyllinae evolved independently of Glossophaginae, although inconsistent results across different analyses were evident among nectar-eating subfamilies (see specific discussion

hereafter). Unlike what was found by Rojas et al. (2016), Lonchorhininae appeared consistently as the sister of Phyllostominae. Finally, in our reference tree, Macrochinae were retrieved as a sister group to all remaining phyllostomid subfamilies. Despite high support values in both the ML and BI analyses, our sensitivity analysis highlighted that some relationships were unstable when parameters related to the number of outgroup taxa, number of partitions and types of data (i.e. nucleotides vs. amino acids) were evaluated, and we hereafter discuss further the stability of our results and their implication for evolutionary studies.

The choice of outgroup could have important consequences for the resulting phylogenetic tree (Graybeal, 1998; Schneider & Cannarozzi, 2009). From all possible outgroups, the closest one is the best choice to root a tree, because shorter distances suffer less from statistical error, and the expected number of homoplasies between any ingroup and the outgroup is minimized (Muse & Weir, 1992; Smith, 1994). We expected that by removing all other mammalian families and keeping the closest outgroups (reduced outgroup dataset), the phylogeny of Phyllostomidae could be improved or at least would not be affected. On the contrary, our results showed discrepancy between phylogenies obtained with the full outgroup dataset and the reduced outgroup dataset. We found stronger phylogenetic congruence when all sequences, even those less phylogenetically related, were maintained as outgroups. This was particularly true for the most basal nodes in phyllostomids, related to the phylogenetic placement of Macrochinae and Micronycterinae.

Early debates concentrated on the choice of the appropriate evolutionary model (Kelsey et al., 1999), with the adequacy of the model potentially producing differences between topologies with short internal branches (Schneider & Cannarozzi, 2009). However, it is now well recognized that choosing the most adequate model is unimportant and that using the most complex model provides consistent topologies (Abadi et al., 2019). However, variation in

substitution patterns across genes and sites should still be accommodated, and the partitioning of the dataset can have a strong influence on the topology (Kainer & Lanfear, 2015). Likewise, some of our results proved to be sensitive to the choice of the partitioning scheme. This is particularly visible in the ‘intermediate’ part of the tree (between the shallowest nodes and the deeper nodes), where branch lengths are noticeably short. However, the optimal results were stable because they were retrieved in four of six partition schemes.

Of all the analytical schemes that we evaluated, a strong influence of the type of data (nucleotides vs. amino acids) has been observed. In general, the best-evaluated model for amino acids yielded a topology that was incongruent with the best-evaluated nucleotide topology. Our best-supported result for amino acids (71 samples with a seven-partition scheme; Supplementary data 2) contradicted most previous relevant molecular analyses (Baker, 2000; Baker et al., 2003; Rojas et al., 2011, 2016; Dávalos et al., 2012; Cirranello et al., 2016), although it was congruent with the best-evaluated topology for amino acids by Botero-Castro et al. (2018). Both ML and BI analyses reconstructed a similar topology, but more key clades showed lower support values, if compared with nucleotide datasets. These low phylogenetic resolutions might be attributable to the use of coding genes only, and thus fewer characters. This was demonstrated with the analysis we carried out using only coding genes from the nucleotide dataset (

Supplementary data 3).

In our reference tree, Lonchophyllinae and Glossophaginae were not sister clades. Both ML and BI analyses strongly supported the non-monophyly of these lineages. However, the most notorious incongruities across analyses were those related to the phylogenetic position of the nectar-eating subfamilies. In the case of nectarivores, five of 12 analyses carried out with nucleotides and one of eight analyses carried out with amino acids depicted these two subfamilies as monophyletic. Although these results might agree with morphological phylogenies, such as that of Wetterer et al. (2000) and some of the analyses made by Dávalos et al. (2012), our best-supported results showed neither groups as sister taxa. The homoplasies observed in the aforementioned morphological phylogenies might be the result of morphological similarities associated with ecological adaptations to nectar feeding (Gatesy et al., 1996; Sánchez-Villagra & Williams, 1998; Wiens et al., 2003). However, profound examination of morphological characters, such as the oral muscle complexes (Griffiths, 1982; Datzmann et al., 2010), and recent extensive molecular analyses (Baker et al., 2016; Rojas et al., 2016) strongly support that Lonchophyllinae and Glossophaginae evolved nectarivory independently, as confirmed by our analyses.

Across most of our analyses, the position of Lonchorhinae was stable and well supported as sister to Phyllostominae. Until the beginning of this century, *Lonchorhina* Tomes, 1863 was placed in Phyllostominae (Baker et al., 1989; Koopman, 1994; Wetterer et al., 2000; Jones et al., 2002). Baker et al. (2003) included *Lonchorhina* in its own monogeneric subfamily, Lonchorhinae, using as evidence a Bayesian analysis that combined the nuclear gene *RAG2* and the mitochondrial genes 12S rRNA, Val tRNA and 16S rRNA. In their analysis, *Lonchorhina* diverged from the remainder of the phyllostomids after the divergence of vampires, but before the common ancestor of the remaining subfamilies. Rojas et al. (2011),

analyzed the same genes as Baker et al. (2003), but this resulted in another hypothesis, in which *Lonchorhina* appeared as sister to a clade comprising Glossophaginae, Lonchophyllinae, Carolliinae, Glyphonycterinae, Rhinophyllinae and Stenodermatinae. Later, Rojas et al. (2016), in a comprehensive phylogenetic analysis using combinations of seven nuclear loci and five mitochondrial genes from phyllostomid species, found *Lonchorhina* as sister to a clade that excluded Glossophaginae and Phyllostominae, although with poor support (i.e. bootstrap value < 50%). Our phylogenomic approach supports Lonchorhininae as a sister to Phyllostominae. We herein provide a better resolution of the phylogenetic relationships between these insectivore subfamilies, although we consider that this hypothesis could be evaluated by analyzing molecular data from all species in the genus. The six species included in *Lonchorhina* are essentially insectivores and are characterized by having highly developed and complex ears, tragi and nasal leaves (Solari et al., 2019a). Representatives of *Lonchorhina* depend on caves for roosting, which are, in general, difficult to access, causing specimens to be rare in museum collections (Mantilla-Meluk & Montenegro, 2016). The incomplete taxonomic coverage, both morphological and molecular, has historically prevented an accurate reconstruction of the relationships within *Lonchorhina* and among Lonchorhininae and other subfamilies.

Conclusions

Our mitogenomic analysis yielded a topology that was well supported and highly congruent with previous phylogenetic results. Despite variations across analyses, our optimal topology confirmed the known relationships among most subfamilies: nectarivores evolved independently, and Macrotinae are sister to all other subfamilies. We also propose the hypothesis that Lonchorhininae might be the sister subfamily of Phyllostominae. We found that deeper relationships were sensitive to the outgroup and selection of types of data, and that the intermediate relationships characterized by short internal branches were sensitive to the

partitioning schemes. In adequate analytical conditions, complete mitogenomes proved to be extremely useful for resolving patterns of phylogenetic relationships within phyllostomids. This is of particular interest considering that genome skimming is probably the most efficient method to analyse museum specimens or highly degraded material, which, in turn, might be the only accessible evidence for solving taxonomic conundrums. In addition, future improvements on the phylogeny of phyllostomid bats could come from inclusion of more species and the use of large nuclear datasets, such as transcriptomics, gene capture or full genome sequencing (Tsagkogeorga et al., 2013; Lei & Dong, 2016; McCormack et al., 2016; Potter et al., 2021).

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Data Availability

The data underlying this article are available in the GenBank Nucleotide Database at <https://www.ncbi.nlm.nih.gov/genbank/>, and can be accessed with the unique accession numbers provided in Table 2.1. Additional taxonomic and collection data from specimens are available at: <https://bioweb.bio/faunaweb/mammaliaweb/>

Chapter 3. A polytypic species revisited: Phylogenetic and morphological variation, taxonomic status, and geographical distribution of *Trachops* (Chiroptera: Phyllostomidae)

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M. Alejandra Camacho*^{1,2}, Pablo A. Menéndez-Guerrero³, Balázs Horváth⁴, Dániel Cadar⁴,
Jérôme Murienne¹

¹Centre de Recherche sur la Biodiversité et l'Environnement (CRBE UMR5300), Université de Toulouse, CNRS, IRD, Toulouse INP, Université Toulouse 3 – Paul Sabatier (UT3), Toulouse, France.

²Museo de Zoología, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Quito, Ecuador.

³Laboratorio de Macroecología y Cambio Global, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

⁴WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Bernhard Nocht Institute for Tropical Medicine, 20359 Hamburg, Germany

*Corresponding author: Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre 1076 y Roca. 170525. Email: macamachom@puce.edu.ec

Abstract

The taxonomic status of the neotropical bat genus *Trachops* is reevaluated through an integrated study that incorporates morphological, morphometric, and molecular data across its extensive geographic range. Our research, which included previously unexamined geographical regions, revealed substantial insights into the diversity within *Trachops*. Genetic and morphological results support elevation of *T. cirrhosus ehrhardti*, distributed within the Atlantic Forest of Brazil, to species status due to differences in southeastern Brazil specimens. Conversely, our analysis found insufficient evidence to maintain the subspecific distinction of *T. c. coffini*, which lacks diagnosable morphological characters and is not genetically distinct from *T. c. cirrhosus* across its distribution range. Additionally, our findings challenge a prior notion of latitudinal differentiation in body size in *T. cirrhosus*, because specimens from western South America and northeastern South America exhibit similar sizes to those from Central America. These results underscore the importance of revising the taxonomic framework for this bat genus—contributing to a more precise understanding of its evolutionary relationships—and further enhancing conservation efforts considering potential threats to the newly recognized species in the imperiled Atlantic Forest of Brazil.

Key words: Fringe-lipped Bat, mitochondrial DNA analysis, neotropical phyllostomids, taxonomic evaluation, *Trachops cirrhosus*, *Trachops cirrhosus ehrhardti*, *Trachops* diversity.

Introduction

The Fringe-lipped Bat, *Trachops cirrhosus* (Spix, 1823), is a member of the family Phyllostomidae (Gray, 1825), and prior to this study the only recognized species in the genus (Solari et al., 2019). This species has a wide distribution in the Americas—ranging from southern Mexico to Brazil—and occurs at mid to high elevations on both sides of the Andes Cordillera, across the Amazon, and in the Atlantic Forest (Jones & Carter, 1976; Cramer et al., 2001; Williams & Genoways, 2008; Solari et al., 2019). The species occurs in humid tropical and subtropical forests, and its distribution encompasses primary, secondary, disturbed, and gallery forests at woodland edges and near cultivated areas (Fenton et al., 1992; Ditchfield, 1996; Tirira, 2017; Solari et al., 2019).

Trachops is a monophyletic genus (Ditchfield, 1996; Baker et al., 2003; Camacho et al., 2022) and can be easily distinguished from other bats by its finger-like dermal projections on the chin and lips (Figure 3-1). Historically, *Trachops* taxonomy has undergone numerous changes (Spix, 1823; Schinz, 1844; Gray, 1825, 1847; Felten, 1956a). Initially described by Spix in 1823 within the genus *Vampyrus*, the name was reassigned to what we currently recognize as *Vampyrum* (Williams & Genoways, 2008). Subsequently, taxonomy clarified by (1825) led to delineation of species into new genera, culminating in the establishment of the genus *Trachops* (Gray, 1847). The taxonomic narrative included *Phyllostoma angusticeps* Gervais 1856, and *Tylostoma mexicana* Saussure 1860 as junior synonyms following a reevaluation of their classification. This genus was further refined when *Trachops fuliginosus* Gray 1965, was recognized as a junior synonym of *T. cirrhosus*. Goldman (1925) provided a description of *T. coffini*. Later, Felten (1956a) not only described *T. c. ehrhardti*, but also reevaluated the taxonomic classification of *T. coffini*, designating it as a subspecies of *T. cirrhosus* (Felten, 1956b).



Figure 3-1. Adult *Trachops cirrhosus* captured in Parque Nacional Yasuní, Orellana, Ecuador. Note the conspicuous finger-like dermal projections on chin and lips. Photo: Rubén D. Jarrín.

Current taxonomy was established with 3 recognized subspecies: *T. c. cirrhosus* (Spix, 1823), occurring from Costa Rica to northeastern Brazil; *T. c. coffini* (Goldman, 1925), distributed from Mexico to Nicaragua; and *T. c. ehrhardti* (Felten, 1956b), found only in southeastern Brazil (Figure 3-2 ; Jones & Carter, 1976; Solari et al., 2019).

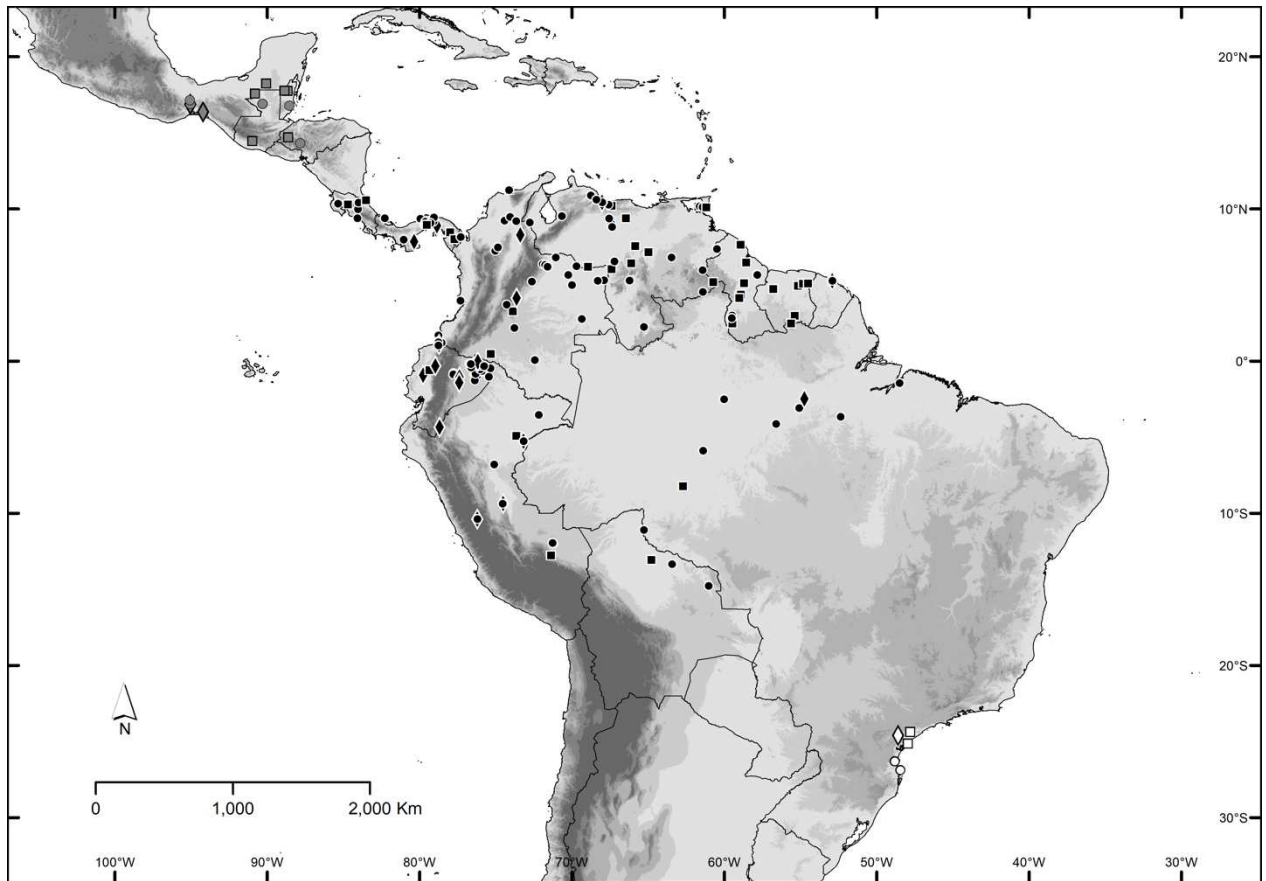


Figure 3-2. Collecting localities of the *Trachops* specimens analyzed in this study. The localities are shown in the Supplementary data 4. Black symbols represent *T. c. cirrhosus*, gray symbols represent *T. c. coffini*, and white symbols represent *T. c. ehrhardti*. Circles are specimens that were measured only, squares are sequenced only, and diamonds are both.

Descriptions of the subspecies of *T. cirrhosus* are brief and lack comparisons with specimens from Central and South America locations, leading to unclear geographic ranges and diagnostic characters. Moreover, only a few recent studies have addressed the systematics of *Trachops* itself. Ditchfield (1996) used partial sequences of the mitochondrial *Cytb* gene and identified 5 distinct haplotypes with an average sequence divergence ranging from 5.5% to 11%, demonstrating strong geographic structuring and limited sharing of haplotypes across distant localities. Later, and despite the high genetic divergences yielded by the mitochondrial marker *COI*, Clare et al. (2007) and Clare (2011) could not find congruence with the topology yielded by the nuclear marker *Dby*. However, they observed many intraspecific groups occupying

sympatric distributions, strongly suggesting ongoing or past speciation events. Most recently, Fonseca (2019) proposed elevating *T. c. ehrhardti* to the species level based on integrative evidence in morphology, ecology, and genetics, while maintaining *T. c. cirrhosus* and *T. c. coffini* as subspecies of *T. cirrhosus*—but these taxonomic changes have not yet been formalized in a peer-reviewed publication, and diagnostic morphological characterization of *T. cirrhosus* subspecies remain unclear.

In this study, we revise the taxonomic composition and phylogenetic structure of the *Trachops* genus based on molecular, morphological, morphometric, and geographic data that includes a significant sample from the West South American region. We aim to address this issue by asking whether: 1) current subspecies of *T. cirrhosus* have valid phylogenetic and morphological justifications; 2) each subspecies is supported by distinct and diagnosable characters; and 3) cryptic species can be inferred in *Trachops* through molecular and morphological analysis. In doing so, we seek to clarify, correctly diagnose, and geographically limit *T. cirrhosus* subspecies. We used a phylogenomic dataset based on the complete mitochondrial genome which has provided clarification of systematics in Phyllostomid bats (Camacho et al., 2022). In addition, we performed extensive taxon and geographic sampling. This diverse geographic sampling encompasses a wide range of habitats and ecological zones where *T. cirrhosus* subspecies are found.

Materials and methods

Specimens, tissue samples, and biorepositories

For morphometric analyses, we measured 238 specimens of *T. cirrhosus*. Only adults were included in order to study sex, age, and reproductive conditions (Kunz et al., 1996). We included representatives from all named subspecies and from their complete geographic distributions in Central and South America. The samples comprised fluid-preserved specimens, study skins, and skulls deposited in the following institutions: American Museum of Natural History, New York, NY, USA (AMNH; $n = 70$); Colección de Mamíferos del Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Colombia (IAvH; $n = 19$); Museo de Historia Natural Gustavo Orcés, Escuela Politécnica Nacional, Quito, Ecuador (MEPN; $n = 9$); Museo de Zoología, División de Mastozoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador (QCAZ; $n = 34$); Senckenberg Naturmuseum Frankfurt, Frankfurt, Germany (SMF; $n = 3$); National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM; $n = 98$); and Colección del Mamíferos de la Universidad del Valle, Cali, Colombia (UV; $n = 5$). The measurements of the specimens of *T. c. ehrhardti* from the SMF were shared by the curator of this collection. We obtained tissue samples from the following collections: AMNH; Field Museum of Natural History, Chicago, IL, USA (FMNH); Museum of Southwestern Biology, Albuquerque, NM, USA (MSB); QCAZ; Royal Ontario Museum, Toronto, Canada (ROM); and SMF (Supplementary data 4).

Genomic DNA isolation, amplification, and sequencing

We gathered a dataset comprising 78 tissue samples, dating from 1899 to 2018. The tissue samples included heart, liver (~40mg), claws (~ 3 mm), and biopsy punch wing snippets (~3 x 3 mm diameter). Mitochondrial DNA sequencing was achieved using a genome skimming procedure following Camacho et al. (2022). Laboratory procedures were carried out at the NGS

Core Facility of the Bernhard Nocht Institute for Tropical Medicine in Hamburg, Germany. The extraction and amplification of archival DNA were performed in a dedicated clean room facility, which was separated from the location where fresh samples and post-PCR products were handled. Stringent contamination prevention protocols and negative controls were also implemented.

The DNA extraction process for various sample types (dried skin, ethanol, or formaldehyde-preserved tissues) involved proteinase K digestion at 55°C using 20 µl of proteinase K and 220 µl of ATL lysis buffer (MinElute Reaction Cleanup kit, Qiagen). Prior to digestion, samples were thoroughly washed with nuclease-free water (Qiagen). The incubation time for proteinase K digestion varied depending on tissue type and sample preservation, ranging from 5 to 24 hours. Following digestion, DNA was extracted and purified using the Qiagen MinElute kit, with each sample eluted to a final volume of 60 µl. DNA concentration was measured using Qubit and Bioanalyzer instruments, to confirm DNA quantification. To estimate DNA size distribution for library preparation, the QIAseq FX DNA Library Kit (Qiagen) was used, with double index barcode labelling according to the manufacturer's instructions. DNA fragmentation was often encountered due to the high degradation of nucleic acid (DNA \leq 500 bp) material and low concentration (\leq 3 ng/µl). The HiFi PCR Master Mix from the QIAseq FX kit was utilized to amplify DNA regions with varying GC contents, minimizing sequencing bias caused by PCR, such as nucleotide misincorporations from cytosine deamination. Subsequently, the libraries underwent quality control to determine fragment size using the Agilent 2100 Bioanalyzer, and concentration was assessed using a Qubit 2.0 Fluorometer. After normalization, samples and negative controls were pooled and subjected to sequencing on the NextSeq 2000 platform (2 × 100 cycles) (Illumina, San Diego, CA, USA).

Mitogenomes assembly and annotations

Raw reads were first subjected to a qualitative assessment, followed by removal of adaptor sequences and filtration of polyclonal and low-quality reads (<55 bases long) using CLC Workbench (Qiagen). Overlapping paired-end (PE) reads were merged to improve quality, while non-overlapping pairs and orphan reads were left unchanged. Deduplication was performed with an assumed 100% identity using BBTools (Bushnell, 2014), expanding the length of contigs produced during de novo assembly. Custom assembly was conducted using Megahit (Li et al., 2016) and Spades (Prjibelski et al., 2020) applications. A specialized Chiroptera mitochondrial database and BLASTN were employed to identify potential bat mitochondrial genomes in the resulting contigs. This process was followed by remapping and visual validation of circularization using CLC Genomics Workbench 22. All assembled mitochondrial genomes were annotated using the MITOS2 metazoan pipeline (Bernt et al., 2013; Al Arab et al., 2017), followed by manual adjustment in Geneious v.9.0.5 (<https://www.geneious.com>). The mitochondrial DNA sequences obtained in this study have been deposited in GenBank (Appendix 1).

Phylogenetic analysis

Ribosomal RNA (rRNA) and transfer RNA (tRNA) loci were aligned using MUSCLE (Edgar, 2004), while protein-coding genes sequences were aligned using TranslatorX (Abascal et al., 2010). As outgroups, we selected sequences from *Lophostoma brasiliense*, *L. silvicola*, *Phylloderma stenops*, *Phyllostomus hastatus*, *Macrophyllum macrophyllum*, and *Tonatia maresi* shown as closely related in previous phylogenetic studies (Botero-Castro et al., 2018; Camacho et al., 2022).

We performed a Maximum Likelihood (ML) analysis using RAxML-NG (Kozlov et al., 2019), starting from 10 parsimony trees and 10 random trees. We used the optimal partitioning scheme

of 38 partitions (2 independent partitions for rRNAs and 36 partitions for tRNAs, 1 partition for each codon for each gene) and a Generalized Time Reversible (GTR) model of substitution rates along with a gamma (G) distribution and a fraction of invariable (I) sites (GTR+G+I).

Bootstrap support values were obtained using the classical Felsenstein metric (Felsenstein, 1985) and transfer bootstrap expectation (Lemoine et al., 2018). Bayesian inference (BI) analyses were performed using MrBayes v.3.2.7 (Ronquist et al., 2012). We partitioned sequences in 38 sets corresponding to 2 independent partitions for rRNAs and for tRNAs plus 36 partitions—1 partition for each codon for each gene—and used the best analytical scheme as evaluated by the Akaike's Information Criterion (AIC). We ran 8 Markov Chain Monte Carlo (MCMC) chains for 10 million generations, with default heating values. The sampling frequency was set every 1000 generations, and the first 25,000 samples were discarded as burn-in. A consensus tree was built under the majority rule consensus of all trees obtained in the 8 runs after the burn-in period. We used the 'sumt' command to produce summary statistics for trees sampled during a Bayesian MCMC analysis. Posterior probabilities of nodes were regarded as estimators of confidence. Trees were visualized and edited in FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). We adopted the nodal support criteria established by Moratelli et al. (2017) in the ML analysis: robust support is considered when bootstrap values exceed or are equal to 75%; support is deemed moderate when values range from 50% to 75%; and values equal to or below 50% are indicative of negligible support. We calculated uncorrected pairwise (p) distances within and among samples of *T. cirrhosus* using MEGA 11 (Stecher et al., 2020; Tamura et al., 2021).

Morphological and morphometric analyses

A total of 27 measurements were taken. Among these, 9 cranial measurements and 4 external measurements were selected for statistical analyses, as indicated by asterisks (*) in Table 3-1. The remaining measurements were employed for descriptive purposes.

External and osteological characteristics were based on, but not limited to the guidelines proposed by Velazco (2005), Tavares et al. (2014), Molinari et al. (2017), and Garbino et al. (2020). Dental nomenclature follows Miller (1907), Freeman (1998), Garbino and Tavares (2018), and Garbino et al. (2020). Skull, dentition, and external characters were measured with digital calipers (to the nearest 0.01 mm). Total length, tail, hindfoot, ear, and body mass were recorded from skin labels and were only used for descriptive assessments (mean, range, and standard deviation).

Table 3-1. Cranio-dental and external measurement definitions. The asterisks (*) indicate the measurements that were used in the statistical analyzes of morphometry.

Measurement (abbreviation)	Definition
Calcar length (CL)	From the joint with the ankle to the calcar tip.
Ear length (E)	Intertragic notch of the ear to the outer tip
Forearm length (FA) *	Distance from the elbow (tip of the olecranon process) to the wrist (including the carpals). This measurement is taken with partially folded wings.
Hindfoot length (HF)	Distance from the ankle to the tip of the claw.
Metacarpal III (MET-III) *	Distance from the joint of the wrist (carpal bones) with the 3rd metacarpal to the metacarpophalangeal joint of 3rd digit.
Metacarpal IV (MET-IV) *	Distance from the joint of the wrist (carpal bones) with the 4th metacarpal to the metacarpophalangeal joint of 4th digit.
Metacarpal V (MET-V) *	Distance from the joint of the wrist (carpal bones) with the 5th metacarpal to the metacarpophalangeal joint of 5th digit.
Tail length (T)	Distance from dorsal flexure at base of the tail to the tip of the last caudal vertebra.
Tibia length (TiL)	Length from the proximal end of the tibia to the distal base of the calcar.
Total length (TL)	Head and body length excluding tail.
Weight (W)	Mass in grams.

Measurement (abbreviation)	Definition
Braincase height (BCH)	Height of the braincase, posterior to the auditory bullae from the basioccipital to the sagittal crest.
Breadth across upper molars (M2-M2) *	Greatest width of palate across labial margins of the alveoli of M2s.
Breadth of brain case (BB) *	Greatest breadth of the globular part of the braincase, excluding mastoid and paraoccipital processes.
Condyl canine length (CCL)	Distance from the occipital condyles to the anterior border of the of the upper canines.
Condyl incisive length (CIL) *	Distance between a line connecting the posteriormost margins of the occipital condyles and the anteriormost point on the upper incisors.
Coronoid height (COH)	Perpendicular height from the ventral margin of mandible to the tip of coronoid process.
Dentary length (DENL)	Distance from midpoint of condyle to the anteriormost point of the dentary.
Greatest length of skull (GLS) *	Greatest distance from the occiput to the anteriormost point on the premaxilla (including the incisors).
Mandibular toothrow length (MANDL)	Distance from the anteriormost surface of the lower canine to the posteriormost surface of m3.
Mastoid (process) breadth (MPW) *	Greatest breadth across skull, including mastoid processes.
Maxillary toothrow (MTRL)	Distance from the anteriormost surface of the upper canine to the posteriormost surface of the crown of M3.
Molariform toothrow (MLTRL) *	Distance from the anteriormost surface of P3 to the posteriormost surface of the crown of M3.
Palatal width at canines (C-C) *	Distance between the outermost extremities of the cinguli of upper canines.
Palatal length (PL)	Distance from the posterior palatal notch to the anteriormost border of the incisive alveoli.
Post orbital constriction breadth (PB) *	Least breadth at the postorbital constriction.
Zygomatic breadth (ZB) *	Greatest breadth across the zygomatic arches.

We log-transformed the variables and ensured normality via Kolmogorov-Smirnov tests (Sokal & Rohlf, 1995). We then performed two Analyses of Variances (ANOVAs) with the 13 morphological variables to determine sex-based differences within subspecies. With no significant differences found (all $P < 0.05$), we combined data from males and females for further analysis. We used Principal Component Analysis (PCA) as a unified statistical approach to investigate morphological variation in *T. cirrhosus*, applying it in 2 distinct contexts. First,

we applied the PCA focusing on taxonomic identification and current distribution ranges to assess morphometric divergence among recognized *Trachops* subspecies. This involved summarizing key characteristics of the dataset to interpret complex relationships and trends in morphological traits (Sokal & Rohlf, 1995). Subsequently, PCA scores were subjected to a multivariate analysis of variance (MANOVA) and Post-hoc multiple comparisons (Holm-Bonferroni correction) to detect significant morphometric differences among subspecies (Rice, 1989; Irschick & Shaffer, 1997). Second, we used PCA to understand patterns of geographic variation. Here, the same PCA was employed, but with the variable being a more specific geographic distribution area rather than country boundaries. Six geographic areas were established as follows, based on Molinari et al. (2023): 1) CAM - Central America from Mexico to Panama, delimited by the Atrato-San Juan Depression to the south in Colombia; 2) WSAm - West South America defined as the western slopes of the northern Andes including the Colombian Western Cordillera, the Central Colombian Cordillera, and the Pacific coasts of Colombia, Ecuador, and Peru, to the Central Andes, delimited by the Atrato-San Juan Depression to the north and the Southern Andes to the south; 3) ESAm - East South America demarcated as the upper forested slopes on the eastern side of the Andes and the low Amazon of Colombia, Ecuador, Peru, and Bolivia; and the entire Amazon drainage of Brazil, including northeastern Bolivia, and the Orinoco drainages of Colombia and Venezuela; 4) NEC/NWV, NE Colombia and NW Venezuela comprising the Orinoco llanos and Andean piedmont plus the Venezuelan Coast range including the Guajira peninsula and the Maracaibo depression (Ferrer-Pérez et al., 2009); 5) GS - the Guyana Shield including the Venezuelan states of Bolivar and Amazonas, and a portion of Delta Amacuro, the entire territories of Guyana, Surinam, and French Guyana, and parts of northern Brazil; and 6) AF - Atlantic Forest confined to the southernmost eastern region of Brazil including the Mata Atlántica ecosystem.

Results

Specimens, tissue samples, and biorepositories

Altogether, we measured 238 specimens of *T. cirrhosus* from all named subspecies (i.e., 202 *T. c. cirrhosus*, 31 *T. c. coffini*, and 5 *T. c. ehrhardti*), sourced from 7 scientific collections (Supplementary data 4). Additionally, out of the 78 tissue samples, we successfully sequenced a total of 54 complete mitochondrial genomes (i.e., 47 *T. c. cirrhosus*; 5 *T. c. coffini*; 2 *T. c. ehrhardti*; Supplementary data 4, **Appendix 1**). The remaining 24 individuals that could not be sequenced corresponded, mostly, to samples of ancient tissues preserved in formalin. Twenty-two specimens were both measured and sequenced. To analyze geographic data, we used 173 unique collection locations (Figure 3-2).

Phylogenetic analysis

Monophyly of *Trachops* was recovered with strong support from both the ML analysis (bootstrap support [BS] = 100%), and from the Bayesian analysis (Bayesian posterior probability [BPP] = 1). There is broad agreement between analysis methods (ML and BI) for this dataset. We found 2 well-supported and genetically distant clades: 1 containing the subspecies *T. c. coffini* nested within *T. c. cirrhosus* (Clade 1; Figure 3-3) while the other contained the subspecies *T. c. ehrhardti* (Clade 2; Figure 3-3).

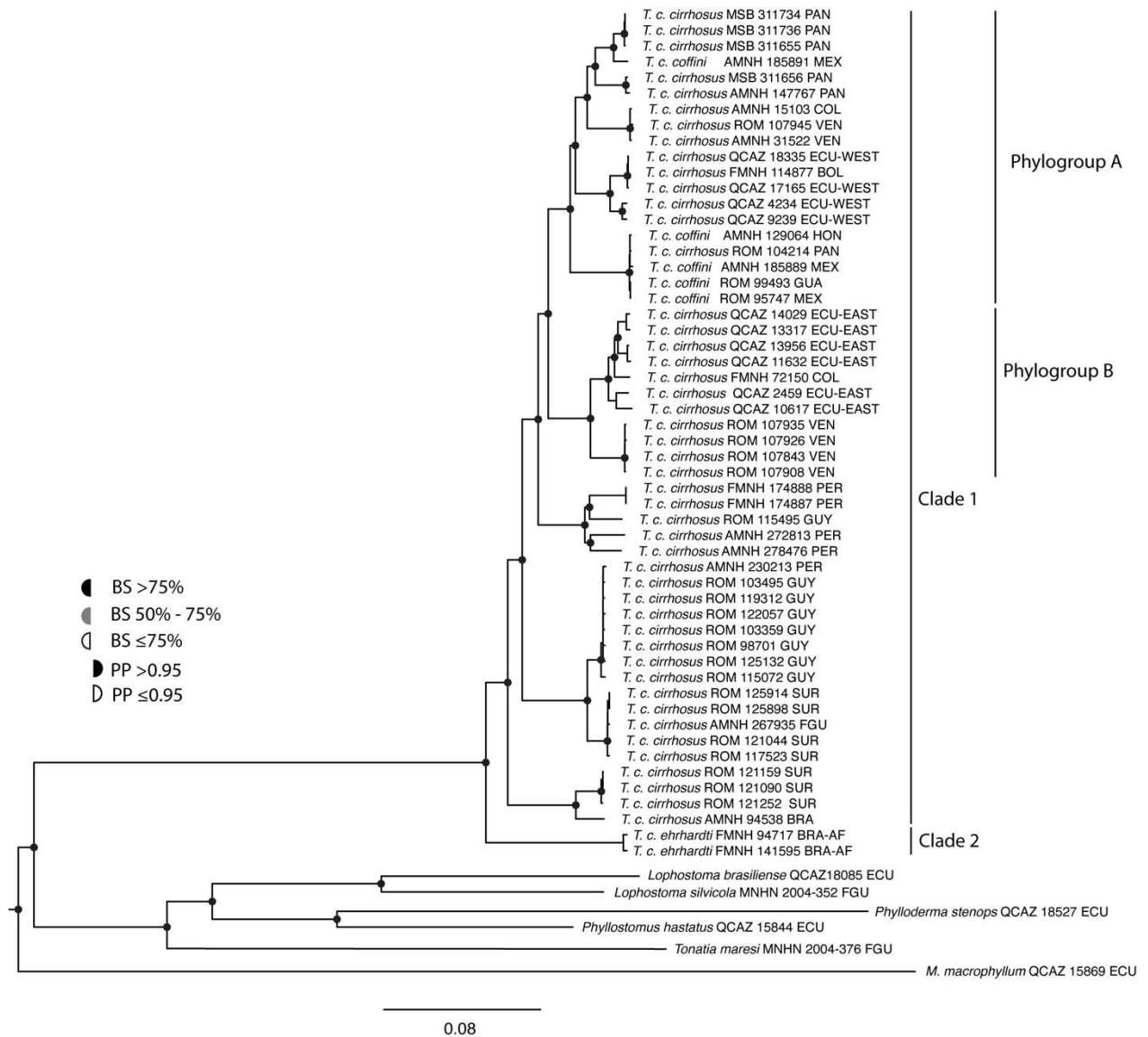


Figure 3-3. Maximum Likelihood phylogeny of *Trachops*. Phylogeny based on complete mitochondrial genomes (nucleotide sequences). The tree was reconstructed in RAxML under the GTR+G+I model using 60 complete mitogenomes including outgroups. Shaded semicircles on the nodes indicate ML bootstrap support (as a percentage) and Bayesian posterior probabilities from a congruent Bayesian Inference Analysis (see insert key).

In Clade 1 we recovered 2 clear phylogroups with Phylogroup A comprising samples from Mexico, Guatemala, Honduras, Panama, western Colombia, northeastern Colombia, northwestern Venezuela, and western Ecuador. According to the geographical groups analyzed in the morphometric analyses (see Methodology and following section of Results), all of these sequences correspond to the geographic groups CAM (Central America), WSA (West South America), and NEC/NWV (NE Colombia and NW Venezuela), except for 1 sequence from

Bolivia (FMNH 114877, classified in our geographical analysis as belonging to the ESAm - East South America group), that in the ML topology appears nested with individuals from western Ecuador. Within this phylogroup, there is a sequence from the province of El Petén, Guatemala that is located 93 km away from the type locality of *T. c. coffini* (Guyo, Petén, Guatemala). The subspecies do not align with geographically distinct monophyletic groups, and furthermore, they do not match the geographical distributions historically associated with *T. c. cirrhosus* and *T. c. coffini*. Mean pairwise uncorrected sequence distances between what has been named as *T. c. cirrhosus* and *T. c. coffini* is 5.7% (Table 3-2), which is not different from the computed intraspecific divergence values of 5.5% in *T. c. cirrhosus* (Table 3-3).

Table 3-2. Estimates of evolutionary divergence across sequence pairs between groups. The number of base differences per site, calculated by averaging over all sequence pairs between groups, is depicted below the diagonal and scaled as percentages.

	<i>T. c.</i> <i>cirrhosus</i>	<i>T. c.</i> <i>coffini</i>	<i>T. c.</i> <i>ehrharti</i>	<i>L.</i> <i>brasiliense</i>	<i>P.</i> <i>hastatus</i>	<i>M.</i> <i>macrophyllum</i>	<i>P.</i> <i>stenops</i>	<i>L.</i> <i>silvicola</i>	<i>T.</i> <i>maresi</i>
<i>Trachops c.</i> <i>cirrhosus</i>	-	0.001	0.002	0.003	0.003	0.003	0.003	0.003	0.003
<i>Trachops c.</i> <i>coffini</i>	5.7	-	0.002	0.002	0.003	0.003	0.003	0.004	0.004
<i>Trachops c.</i> <i>ehrharti</i>	8.2	8.6	-	0.003	0.003	0.003	0.003	0.004	0.004
<i>Lophostoma</i> <i>brasiliense</i>	16.9	17.2	17.2	-	0.003	0.003	0.003	0.003	0.003
<i>Phyllostomus</i> <i>hastatus</i>	16.6	16.8	16.8	14.5	-	0.003	0.003	0.004	0.004
<i>Macrophyllum</i> <i>macrophyllum</i>	18.0	18.1	18.2	17.8	17.9	-	0.003	0.004	0.004
<i>Phylloderma</i> <i>stenops</i>	18.4	18.8	18.6	16.3	14.6	19.3	-	0.004	0.004
<i>Lophostoma</i> <i>silvicola</i>	18.6	19.0	19.0	12.7	15.2	19.6	16.9	-	0.004
<i>Tonatia</i> <i>maresi</i>	18.9	19.1	18.7	16.7	16.2	20.1	18.1	16.5	-

Table 3-3. Estimates of evolutionary divergence across sequence pairs within analyzed groups. Standard error estimate(s) are shown in the second column and were obtained by a bootstrap procedure (1000 replicates).

	Distance %	S.E. (%)
<i>Trachops c. cirrhosus</i>	5.5	0.0
<i>Trachops c. coffini</i>	1.9	0.0
<i>Trachops c. ehrhardti</i>	0.0	0.0

We have identified a sister clade to Phylogroup A that we named as Phylogroup B (Figure 3-3), comprised of individuals from Eastern Ecuador and Eastern Venezuela (Orinoco Basin) and corresponding to the geographical group ESAm (East South America) as identified in our morphometric analyses. Besides these 2 strongly supported phylogroups, no further distinct phylogenetic relationships were found within Clade 1, although there is a noticeable clustering of samples from the northeastern region of South America corresponding to the Guyana Shield (see Discussion section).

According to the concordant phylogenies of ML and BI analyses, Clade 2 forms a monophyletic clade containing specimens from the southeastern region of São Paulo in Brazil, which is geographically close to the state of Santa Catarina (type locality of *T. c. ehrhardti*). According to geographical groups analyzed in the morphometric analyses (see Materials and methods and the following Results section), these sequences correspond to the geographic group AF (Atlantic Forest; Figure 3-3).

Morphologic and morphometric variation

Our examination of *Trachops* subspecies highlights morphological congruence—particularly in qualitative traits—between *T. c. cirrhosus* and *T. c. coffini*, contrasting with the distinct morphology of *T. c. ehrhardti*. Our scrutiny failed to discern marked morphological divergences between *T. c. cirrhosus* and *T. c. coffini*, challenging their current classification as distinct taxa. The attributes previously considered distinctive including skull shape, rostrum morphology,

dimensions of premolars and molars, and mandible features, displayed uniformity across these 2 subspecies in our geographical sampling (Figure 3-4). Table 3-4—illustrating variable ranges among the *Trachops* subspecies—suggests a considerable overlap, rendering these variables ineffective as discrete discriminators among the 3 subspecies.

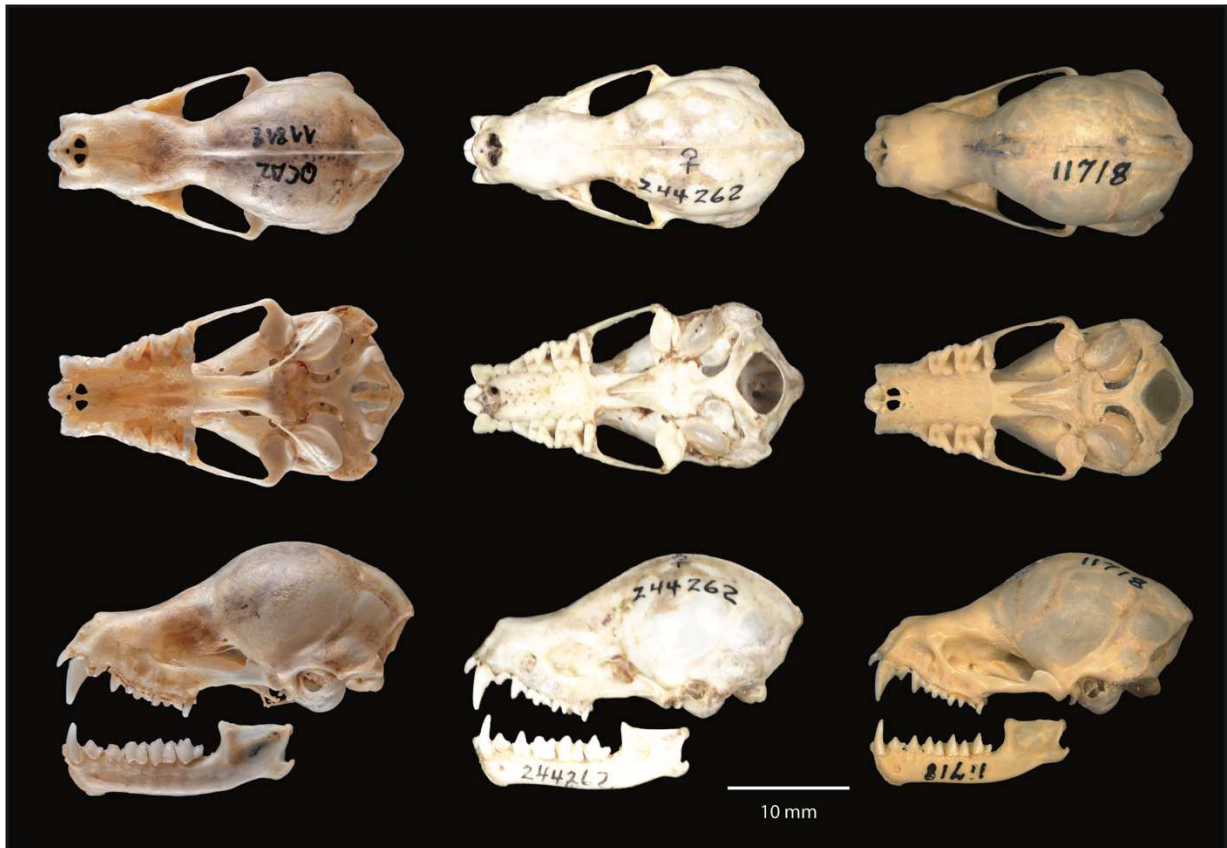


Figure 3-4. Skulls of *Trachops cirrhosus cirrhosus*, *T. c. coffini* and *T. c. ehrhardti*. Dorsal, ventral, and lateral views of the skulls and lateral view of the mandibles of *T. c. cirrhosus* (left); *T. c. coffini* (middle), and *T. c. ehrhardti* (right) Photos: Rubén D. Jarrín (QCAZ 11818); M. Alejandra Camacho (NMNH 244262); Anika Vogel (SMF 11718).

Table 3-4. Measurements (mm) and body masses (g) of *Trachops* subspecies. Descriptive measures: minimum–maximum; arithmetic mean (standard deviation).

Variable	<i>Trachops c. cirrhosus</i> (n = 202)	<i>Trachops c. coffini</i> (n = 31)	<i>Trachops c. ehrhdarti</i> (n = 5)
Breadth of brain case (BB)	11.07–12.97; 11.60 (0.28)	10.33–12.03; 11.49 (0.26)	11.30–11.50; 11.40 (0.10)
Palatal width at canines (C-C)	5.72–6.85; 6.22 (0.21)	5.26–6.68; 5.98 (0.26)	5.4–5.80; 5.63 (0.21)
Condyllocanine length (CCL)	22.23–26.79; 25.01 (0.71)	20.93–26.12; 24.17 (0.86)	
Condylolincisive length (CIL)	23.30–27.95; 26.03 (0.69)	21.87–26.78; 25.10 (0.79)	23.90–24.50; 24.20 (0.30)
Coronoid height (COH)	4.97–6.80; 5.85 (0.30)	4.79–6.28; 5.42 (0.31)	
Dentary length (DENL)	17.41–20.48; 18.97 (0.60)	16.11–19.93; 18.15 (0.67)	
Greatest length of skull (GLS)	26.13–31.24; 29.21 (0.79)	24.70–30.02; 28.27 (0.88)	26.90–27.70; 27.20 (0.44)
Breadth across upper molars (M2-M2)	9.15–11.03; 10.14 (0.32)	8.34–10.86; 9.77 (0.38)	9.80
Mandibular toothrow length (MANDL)	10.71–12.40; 11.42 (0.34)	7.61–11.78; 10.92 (0.54)	
Molariform toothrow (MLTRL)	6.68–7.81; 7.22 (0.21)	6.07–7.67; 6.96 (0.26)	
Mastoid (process) breadth (MPW)	12.78–14.96; 13.70 (0.41)	11.40–14.31; 13.21 (0.46)	13–13.40; 13.20 (0.20)
Maxillary toothrow (MTRL)	9.50–11.33; 10.51 (0.32)	8.57–10.93; 10.11 (0.40)	9.60–10; 9.8 (0.20)
Post orbital constriction breadth (PB)	4.85–5.76; 5.34 (0.17)	4.66–5.71; 5.11 (0.18)	5
Palatal length (PL)	10.12–12.72; 11.40 (0.47)	8.92–12.42; 11.00 (0.52)	
Zygomatic breadth (ZB)	12.46–15.97; 14.53 (0.48)	12.06–15.70; 13.96 (0.58)	13.3–14.10; 13.77 (0.42)
Braincase height (BCH)	9.24–12.20; 10.84 (0.47)	9.39–12.25; 10.63 (0.42)	
Calcar length (CL)	9.19–18.21; 13.29 (1.52)	10.84–16.55; 13.64 (1.30)	11.62
Forearm length (FA)	53.36–64.70; 59.23 (2.25)	49.57–62.11; 57.88 (2.51)	53.99–60; 57.29 (2.43)
Hindfoot length (HF)	12.40–23; 17.38 (2.35)	13.92–22; 17.66 (2.04)	11.47–16.01; 13.74 (3.21)
Metacarpal III (MET-III)	35.31–52.88; 48.18 (2.49)	37.56–53.40; 47.63 (2.27)	39.45–52.30; 47.56 (4.91)
Metacarpal IV (MET-IV)	37.12–55.48; 50.24 (2.56)	37.98–54.43; 49.34 (2.36)	40.57–53.30; 49.51 (5.18)

Variable	<i>Trachops c. cirrhosus</i> (n = 202)	<i>Trachops c. coffini</i> (n = 31)	<i>Trachops c. ehrhdarti</i> (n = 5)
Metacarpal V (MET-V)	39.04–58.86; 53.53 (2.73)	41.15–58.13; 53.25 (2.20)	42.85–55.60; 51.79 (5.2)
Tibia length (TiL)	16.82–29.69; 25.90 (1.84)	20.38–28.90; 24.94 (1.45)	20.61–25.16; 23.09 (1.66)
Tail length (T)	10–28; 16.93 (3.25)	10–22; 16.11 (2.75)	14.38
Total length (TL)	59.70–128; 96.16 (11.35)	56.25–107; 91.09 (15.94)	
Ear length (E)	23.07–39; 31.10 (4.06)	20–39; 31 (4.06)	27.65–31; 29.33 (2.37)
Weight (W)	16–55; 34.48 (7.74)	22–46; 33.19 (6.99)	

In the PCA, 2 principal components cumulatively accounted for 73% of the total variance in our log-transformed dataset (Table 3-5). The PCA plot (Figure 3-5) reveals substantial overlap in the 2D morpho-space among subspecies, attributed to their size and shape congruities. Notably, *T. c. cirrhosus* is positioned towards the right on PC1, indicating its relatively larger size compared to *T. c. coffini*. However, this positioning does not demarcate distinct groupings. PC1 predominantly shows size variations, influenced by skull length metrics such as Condyle-Incise Length (CIL), Greatest Length of Skull (GLS), Maxillary Palatal Width (MPW), and Maxillary Toothrow Length (MTRL), each with factor loadings exceeding 0.8. Conversely, PC2 appears to reflect shape variations, primarily influenced by Palatal Breadth (PB).

Table 3-5. Principal components analysis loadings for adult specimens of *Trachops*. Loadings, eigenvalues, and percentage of variance for 2 from a PCA of the 13 linear measurements.

Variables	PC1	PC2
Breadth of brain case (BB)	.554	.280
Palatal width at canines (C-C)	.778	.314
Condylolincisive length (CIL)	.919	.068
Greatest length of skull (GLS)	.919	.071
Breadth across upper molars (M2-M2)	.756	.293
Mastoid (process) breadth (MPW)	.820	.212
Molariform toothrow (MLTRL)	.872	.067
Post orbital constriction breadth (PB)	.383	.698
Zygomatic breadth (ZB)	.864	.128
Forearm length (FA)	.743	-.180
Metacarpal III (MET-III)	.690	-.555
Metacarpal IV (MET-IV)	.800	-.515
Metacarpal V (MET-V)	.786	-.546
Eigenvalues	7.787	1.731
% of explained variance	59.9	13.3
% of cumulative variance	59.9	73.2

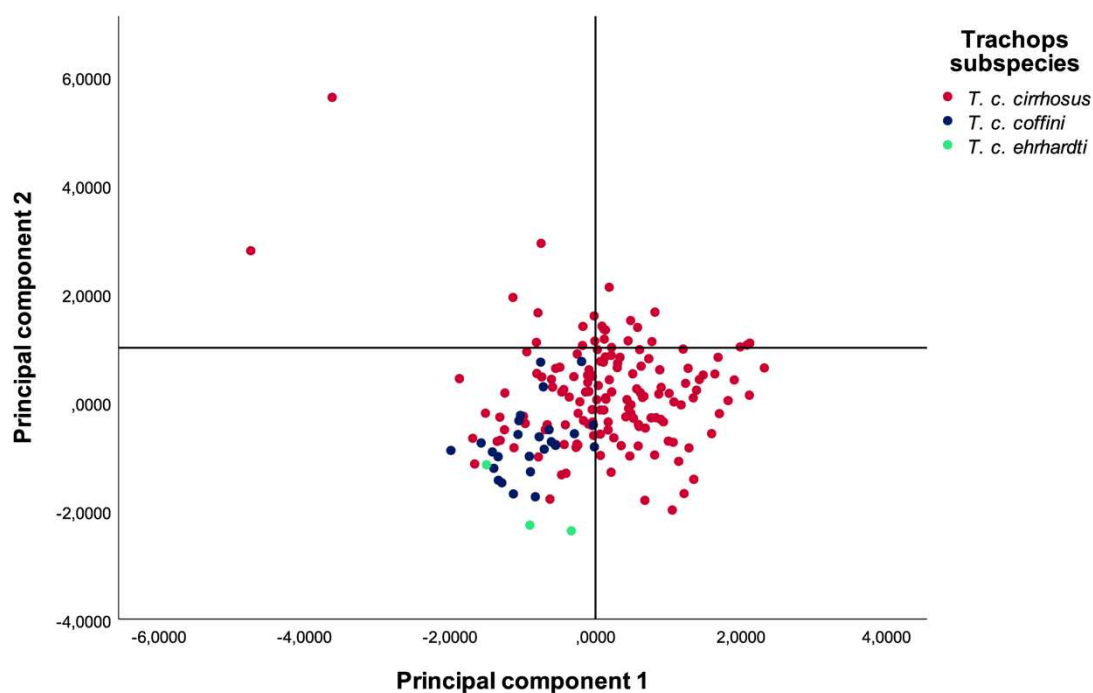


Figure 3-5. Principal Component Analysis of *Trachops* subspecies. Principal components (PC's) from a PCA based on 13 measurements from 238 individuals. Samples: *Trachops cirrhosus cirrhosus* (red circles); *T. c. coffini* (blue circles); and *T. c. ehrhardti* (green circles).

Despite the apparent overlap in morpho-space depicted in Figure 3-5, our multivariate analysis of variance (MANOVA) employing Pillai's Trace test identified statistically significant differences among the subspecies on both PC1 and PC2 ($F = 16.373$, $P < 0.01$). Further post-hoc multiple comparison analyses delineated a statistical distinction between *T. c. cirrhosus* and *T. c. coffini* in terms of size on PC1 ($P < 0.01$), but not between *T. c. cirrhosus* and *T. c. ehrhardti* ($P > 0.05$). Moreover, no statistical differences were noted between *T. c. coffini* and *T. c. ehrhardti* ($P > 0.05$). These statistical disparities might be influenced by the limited sample size for *T. c. ehrhardti*, which does not sufficiently echo in morphological differentiation. The observed statistical differences, particularly between *T. c. cirrhosus* and *T. c. coffini*, are evident in the context of size, as indicated by PC1. However, the overarching morphological congruence, especially considering the overlap in the PCA plot, suggests that despite some statistical variance, these subspecies share a degree of morphological commonality.

Geographic variation

There is a noteworthy overlap in the 2D morpho-space between geographic-based groups (Figure 3-6). The multivariate analysis of variance suggested that there are differences between the geographic groups on PC1 and PC2 as suggested by Pillai's Trace test ($F = 17.629$, $P < 0.01$). Post-hoc multiple comparison analyses unveiled variation in size (PC1) among samples. Specifically, there was a size difference between samples from CAM compared to those from ESAm, GS, and NEC/NWV, but not when compared to WSAm samples. The plot illustrates that both CAM and WSAm samples cluster to the left along PC1, denoting their smaller size, but they do not end up forming a separate cluster. Conversely, AF specimens were notably smaller in size compared to GS samples, although not significantly different from the rest of the regions. In fact, the GS sample exhibited the largest size relative to all other groupings. Notably, NEC/NWV specimens did not display a clear grouping pattern. Regarding the PC2,

AF specimens exhibited notable differences, particularly in variables such as palate width, in comparison to ESAm specimens. Additionally, a highly significant difference was observed between CAm and ESAm specimens.

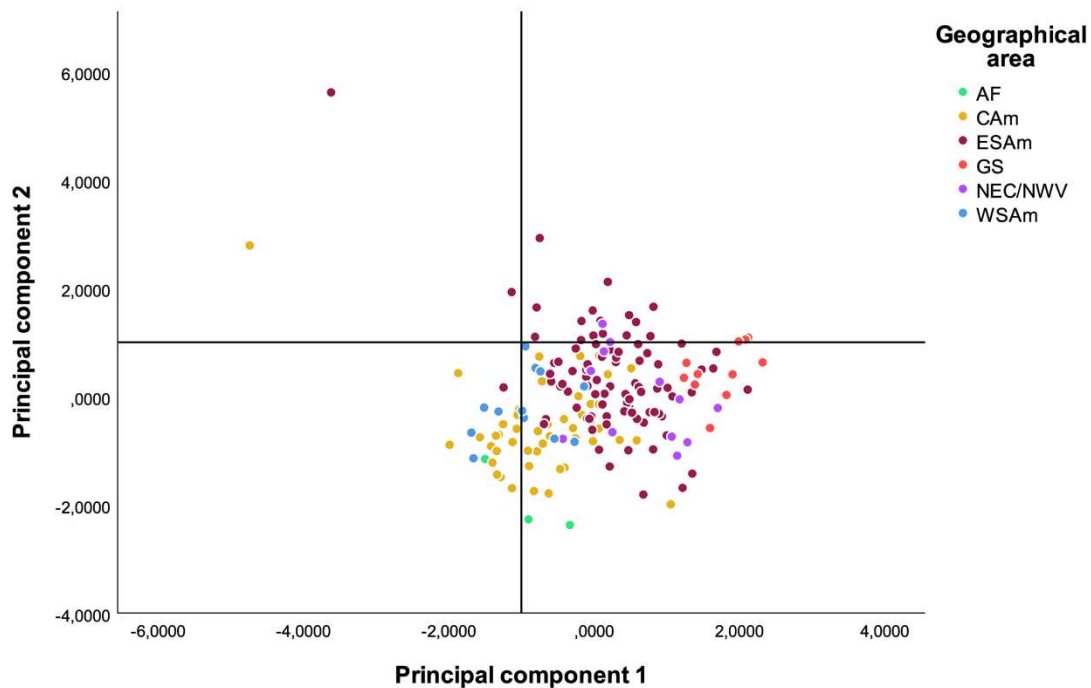


Figure 3-6. Principal Component Analysis of *Trachops* geographic groups. The analysis includes 6 geographic groups and 238 specimens of *Trachops* of the 13 cranial measurements considered in the morphometric analysis. Meaning of geographic abbreviation areas: **CAm** = Central America; **WSAm** = West South America; **ESAm** = East South America; **NEC/NWV** = NE Colombia and NW Venezuela; **GS** = the Guiana Shield; and **AF** = Atlantic Forest.

Both qualitative and quantitative assessments reveal a noticeable degree of morphological and genetic similarity between the traditionally recognized subspecies *T. c. cirrhosus* and *T. c. coffini*. Moreover, our geographical analysis reveals that the distribution of morphological variation among different regions does not align with the historical classification of subspecies. These findings strongly support their consideration as part of the same taxonomic group. Additionally, our investigation provides definitive evidence for elevating *T. c. ehrhardti* to the status of a distinct species due to significant genetic and morphological disparities observed in

specimens from the southeastern region of Brazil. We present a revised description of the genus *Trachops* and the subspecies *T. c. cirrhosus* and *T. c. ehrhardti* below.

Taxonomy

The genus *Trachops* can be easily recognized by their highly specialized warty outgrowths or protuberances on the lips and chin. The margin of the nose leaf is finely serrated on the edge, anteriorly connected to the upper lip (Spix, 1823). Ears are large and anteriorly covered with hairs projecting beyond anterior margins (Goldman, 1925); folds inside the pinnae are well marked. The tail is short and appears on the upper side of a broad interfemoral membrane (Goodwin & Greenhall, 1961). The ventral fur is characterized by a pale brown coloration (Spix, 1823). Dorsal fur is cinnamon-brown, varying to darker shades in some specimens (Goodwin & Greenhall, 1961). The base of hairs is always white, and the tips are ashy. Underparts are dull brownish tinged with grayish brown (Goodwin & Greenhall, 1961).

Family Phyllostomidae Gray, 1825

Subfamily Phyllostominae Gray, 1825

Trachops Gray, 1847

***Trachops cirrhosus* Gray, 1847**

Vampyrus cirrhosus Spix, 1823:64. No type locality is stated in Spix's description, but on page 53 Spix said the bats were collected in Brazil. Type locality restricted to Para, Brazil, by Husson (1962:115).

Ph[yllostoma]. cirrhosum Fischer, 1829:126. Name combination.

Vampyris cirrhosum Gray, 1847:481. Emendation of *Vampyrus cirrhosus* Spix

Phyllostoma angusticeps Gervais, 1856:47. Type locality "province de Bahia, au Brasil."
Trachops cirrhosus (Spix, 1823) as determined by Arroyo-Cabrales et al. (2016:4)

Tylostoma mexicanum (de Saussure, 1860:484) [72 in the reprint]. Type locality "les régions chaudes du Mexique." [Restricted to Veracruz by Gardner and Ramírez-Pulido (2020:5) = *Trachops cirrhosus mexicanus* (de Saussure, 1860)]

Trachops fuliginosus Gray, 1865:14. Type locality "Pernambuco," Brazil (= *Vampyrus cirrhosus* Spix).

Trachyops cirrhosus Dobson, 1878:481. First use of name combination and incorrect subsequent spelling of *Trachops* Gray, 1847.

Trachops coffini Goldman, 1925:23. Type locality "Guyo, Petén, Guatemala." Type locality restricted to "El Gallo, 8 mi. west Yaxhá, on the Remate-El Cayo trail, Petén, Guatemala" by de la Torre (1956:189).

Trachops cirrhosus coffini Felten, 1956a:189. Type locality restricted to Para, Brazil, by Husson (1962:115). First use of name combination assigning subspecific status to *coffini*.

This species is monotypic.

Amended distribution and habitat. This species is widely distributed in eastern and southern Mexico, extending from Veracruz, in the southeast, southeastward through Central America, into South America, including Colombia, Venezuela, the Guyanas, Ecuador, northern and central Peru, Bolivia, Trinidad, as well as northern, southern, and central Brazil (Figure 3-7). This species occurs at elevations from sea level (e.g., Belem, Para, Brazil) up to 1800 m (Las Tolas, Pichincha, Ecuador; Arcos et al., 2007). Records of *T. cirrhosus* are predominantly associated with habitats in humid tropical forests, as well as sub-montane and montane forests.

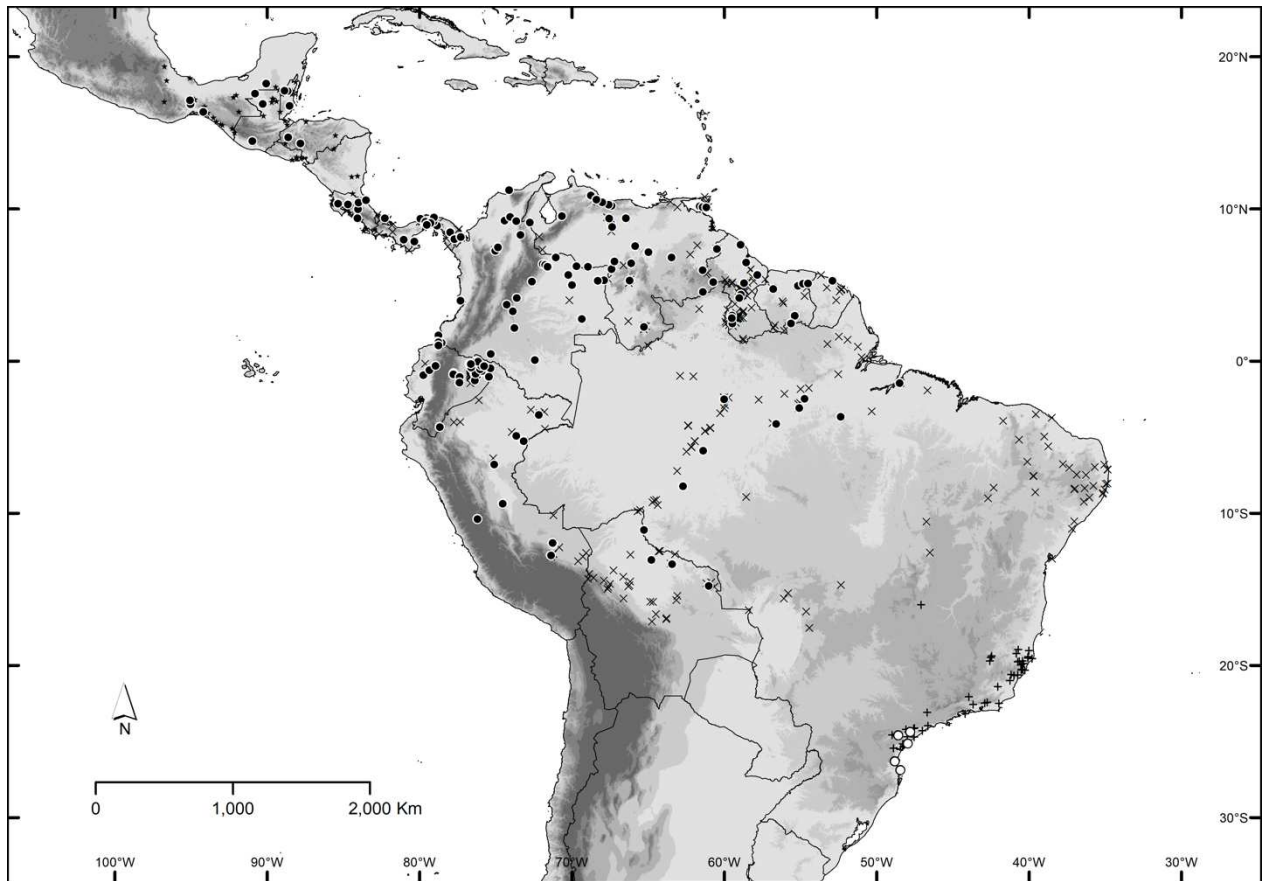


Figure 3-7. Emended distributions of *Trachops cirrhosus* and *Trachops ehrhardti*. Black circles and crosses (• and x) represent the recorded locations of *Trachops cirrhosus*. White circles and plus signs (o and +) correspond to the recorded locations of *Trachops ehrhardti*. The crosses and plus signs are the data collected by Fonseca (2019), sourced from <https://github.com/bsf07/Defesa.git>.

Amended diagnosis and comparison. *Trachops cirrhosus* is a relatively robust (Spix, 1823), medium-size bat (FA 49.57–64.70 mm; GLS 24.70–31.24 mm). Overall size is larger than *Trachops ehrhardti* (Table 3-6). The skull is large and elongated. The elevated braincase above the rostrum was previously believed to be a distinguishing characteristic, potentially separating the subspecies *cirrhosus* and *coffini*. The latter was described as having a smaller and slenderer shape compared to *T. c. cirrhosus*, although similar to *T. c. ehrhardti*. This characteristic is not diagnostic; rather, it displays variability across the entire species distribution. The sagittal crest may or may not be developed in males and females, contrary to the well-developed sagittal crest character mentioned by Goodwin and Greenhall (1961). Poorly developed sagittal crest is

common in most specimens reviewed for the entire area of distribution. The faint notch in the cutting edge of the upper incisors (Goldman, 1925) is variable. Inferior premolar 1 (p1) is taller compared to the same tooth in *T. c. ehrhardti* which is wider overall. The first lower premolar is three-fourths the height of the third premolar but wider in cross-section (Dobson, 1878). Upper and lower premolars in *T. cirrhosus* are relatively narrower than in *T. ehrhardti*. *Trachops cirrhosus* presents a contracted premolar tooththrow and an expanded molar tooththrow. The mandible is broad and robust; the last molar is in close proximity to the fulcrum, a high coronoid process, and an expanded angular process (Figure 3-4).

Table 3-6. Morphological data of *Trachops cirrhosus* and *T. ehrhardti*. Measurements (mm) and body masses (g) of *Trachops cirrhosus* and *T. ehrhardti*. Descriptive measures: minimum–maximum; arithmetic mean (standard deviation).

<i>Variable</i>	<i>T. cirrhosus</i> <i>n</i> = 233	<i>T. ehrhardti</i> <i>n</i> = 5
Breadth of brain case (BB)	10.33–12.97; 11.56 (0.28)	11.30–11.50; 11.40 (0.10)
Palatal width at canines (C-C)	5.26–6.85; 6.13 (0.26)	5.40–5.80; 5.63 (0.21)
Condyllocanine length (CCL)	20.93–26.79; 24.70 (0.87)	—
Condylolincisive length (CIL)	21.87–27.95; 25.67 (0.85)	23.90–24.50; 24.20 (0.30)
Coronoid height (COH)	4.79–6.80; 5.71 (0.36)	—
Dentary length (DENL)	16.11–20.48; 18.70 (0.72)	—
Greatest length of skull (GLS)	24.70–31.24; 28.86 (0.94)	26.90–27.70; 27.20 (0.44)
Breadth across upper molars (M2-M2)	8.34–11.03; 10 (0.39)	9.80
Mandibular tooththrow length (MANDL)	10.06–12.40; 11.05 (0.40)	—
Molariform tooththrow (MLTRL)	6.07–7.81; 7.12 (0.27)	—
Mastoid (process) breadth (MPW)	11.40–14.96; 13.51 (0.49)	13–13.40; 13.20 (0.20)
Maxillary tooththrow (MTRL)	8.57–11.33; 10.36 (0.40)	9.60–10; 9.8 (0.20)
Post orbital constriction breadth (PB)	4.66–5.76; 5.25 (0.21)	5
Palatal length (PL)	8.92–12.72; 11.25 (0.53)	—
Zygomatic breadth (ZB)	12.06–15.97; 14.31	13.3–14.10; 13.77

<i>Variable</i>	<i>T. cirrhosus</i> <i>n</i> = 233	<i>T. ehrharti</i> <i>n</i> = 5
	(0.59)	(0.42)
Braincase height (BCH)	9.24–12.25; 10.76	—
	(0.46)	—
Calcar length (CL)	9.19–18.21; 13.41	11.62
	(1.44)	
Forearm length (FA)	49.57–64.70; 58.71	53.99–60; 57.29
	(2.43)	(2.43)
Hindfoot length (HF)	12.40–23; 17.49	11.47–16.01; 13.74
	(2.24)	(3.21)
Metacarpal III (MET-III)	35.31–53.40; 47.93	39.45–52.30; 47.56
	(2.41)	(4.91)
Metacarpal IV (MET-IV)	37.12–55.48; 49.93	40.57–53.30; 49.51
	(2.51)	(5.18)
Metacarpal V (MET-V)	39.04–58.86; 53.42	42.85–55.60; 51.79
	(2.54)	(5.2)
Tibia length (TiL)	16.82–29.69; 25.57	20.61–25.16; 23.09
	(1.76)	(1.66)
Tail length (T)	10–28; 16.61 (3.08)	14.38
Total length (TL)	56.25–128; 94.13	—
	(11.39)	—
Ear length (E)	20–39; 31.81 (3.61)	27.65–31; 29.33
		(2.37)
Weight (W)	16–55; 34.07 (7.48)	

Remarks. The character of m1 with a less developed paraconid in *T. c. cirrhosus* than in *T. coffini* mentioned by Dobson (1878) was not evident as a diagnostic character.

Natural history. *Trachops cirrhosus* is a large-eared gleaner bat that hunts by listening for prey-generated sounds (Obrist et al., 1993). It roosts in caves, hollow trees, road culverts, sewer systems, and buildings in groups of up to 50 individuals (Hall & Dalquest, 1963; Fleming et al., 1972; Alvarez-Castañeda & Álvarez, 1991; Kalko et al., 1999; Halczok et al., 2018). The relatively sedentary foraging behavior of these gleaners is reflected in their wing morphology, a characteristic that influences their use of small foraging areas and short commuting distances. They have been observed to engage in 2 distinct flight patterns while foraging: long flights that extend for several uninterrupted minutes and short sally flights lasting less than 1 minute (Cramer et al., 2001). These bats possess relatively short and broad wings, an adaptation that

enhances their maneuverability, particularly in environments filled with obstacles (Marinello & Bernard, 2014). However, this wing morphology comes with a trade-off because it makes continuous flight over extended distances energetically costly (Norberg & Rayner, 1987; Kalko et al., 1999).

Trachops cirrhosus hunts for prey in continuous flight, presumably depending on prey availability. Prey is usually taken from the substrate (gleaning mode) in a brief landing or may be caught occasionally on the wing (aerial mode; Kalko et al., 1999). *Trachops cirrhosus* is a carnivorous bat that feeds on a wide variety of prey species including insects, frogs, lizards, and other small vertebrates (Gardner, 1977; Pine & Anderson, 1979; Barclay et al., 1981; Tuttle & Ryan, 1981; Bonato & Facure, 2000; Bonato et al., 2004; Giannini & Kalko, 2005; Page & Jones, 2016). Additionally, it has been observed consuming fruits and seeds (Whitaker & Findley, 1980; Humphrey et al., 1983; Cramer et al., 2001). Foraging areas cover from 3 to 12 ha and commuting distances between roost and foraging areas are < 2 km (Kalko et al., 1999). The emergence time and activity peak of *T. cirrhosus* coincided with the maximum calling activity of Tungara frogs (Leptodactylidae; Tuttle & Ryan, 1981; Ryan et al., 1983). The number and duration of long flights reduce as the frog calling declines; giving way to an increased frequency of short flights, which might also indicate a switch from frogs to other prey such as insects (Belwood, 1990; Kalko et al., 1996, 1999). This bat is very flexible in its responses to prey calls by updating acoustic information with echo acoustic and gustatory cues as it approaches potential prey, enabling bats to avoid potentially lethal mistakes (Page & Jones, 2016). Captive studies show that cues can also be learned socially and transmitted across individuals (Jones et al., 2013; Page & Jones, 2016; Flores et al., 2020).

Very little is known about the reproductive biology of *T. c. cirrhosus*. Females give birth to 1 offspring at a time coinciding with the start of the rainy season (Flores & Page, 2017), but the

gestation period length is unknown. Females of this species have been reported to be pregnant in March, April and December (Villa-R., 1967; Alvarez-Castañeda & Álvarez, 1991). In Brazil, Trajano (1984) suggested a polyestrous reproductive pattern, with 2 annual birth peaks, one before and the other after August. In Trinidad and Tobago, Goodwin and Greenhall (1961) found a colony of *T. cirrhosus* composed of 6 individuals of both sexes, including pregnant females in March (Bredt et al., 1999). The social structure of *T. cirrhosus* is still not fully understood, but a recent 6-year study in Panama showed evidence of female philopatry and preferred co-roosting associations in both sexes; kin-biased associations were also detected among pairs of females but not males (Flores et al., 2020).

During the mating season, reproductive males have enlarged testes and create an odorous substance called forearm crust that is smeared on their forearm. Flores and Page (2017) and Flores et al. (2019) discovered that fringe-lipped males scratch their body with 1 hind claw, including the area around a prominent mid-ventral chest gland, insert the same hind claw into the mouth, and then repeatedly lick the forearm. Apparently, this substance is not related to female preferences since two-thirds of females selected the scent of a male without forearm crust, but it does play a fundamental role in male-male interactions (Flores et al., 2019).

Family Phyllostomidae Gray, 1825
Subfamily Phyllostominae Gray, 1825
Trachops Gray, 1847
***Trachops ehrhardti* Felten, 1956**
Ehrhardt's Fringe-lipped Bat

Holotype. An adult female (SMF 11716) collected by W. Ehrhardt in 1908. The specimen is preserved in ethanol with the skull removed and cleaned, housed at the Senckenberg Museum of Natural History, Frankfurt, Germany.

Type locality. "Joinville, Sta. Catarina, Brazil."

Paratypes. One adult female (SMF 11717) and 1 adult male (SMF 11718) collected by W. Ehrhardt in 1908 in the type locality. Both specimens are preserved in ethanol, with the skulls removed and cleaned, housed at the Senckenberg Museum of Natural History, Frankfurt, Germany.

Etymology. The species (prior *Trachops cirrhosus ehrhardti*) was named in honor of Mr. Wilhelm Ehrhardt, a prominent collector and taxidermists born in British Guiana. He operated in Brazil in the early 20th century where he collected thousands of specimens of various animal groups that he supplied to several museums and scientific institutions.

Distribution and habitat. Southeastern Brazil. Specimens are known from humid environments of the Atlantic Forest of the states of Santa Catarina, Parana, Sao Paulo, Minas Gerais, Rio de Janeiro and Espirito Santo (Figure 3-7).

Diagnosis. Medium-size bat (FA 54–60 mm; GLS 26.90–27.70 mm), smaller than *Trachops cirrhosus* (Table 3-6). Ventral fur from the base of hair shows a lighter shade of brown that gradually transitions to an ashy color at the tips. Dorsal fur is characterized by a reddish-brown to cinnamon-brown hue. The base of hair appears whitish, creating a contrast with the slightly

ashy tone at the tips. Underparts are light brownish with a grayish tint. This coloration is attributed to the presence of light-colored tips on the hairs, and there are white-tipped hairs specifically found on the underparts. The pinnae are hairy, with marked folds. Lips and chin are ornamented with wart-like protuberances and ears are large and clothed with hairs projecting conspicuously beyond anterior margins as in *T. cirrhosus* (Goldman, 1925). Skull is noticeably smaller in comparison to *T. cirrhosus*. Braincase is smaller, forming an angle with a less pronounced rostrum than in *T. cirrhosus*, however, this character shows more degree of variation in the latter. Sagittal crest is not developed. In skulls analyzed, which belong to the type series, it is observed that the lingual edges of the w-shaped stylar shelf are elongated. The molar crowns are relatively elongated and in the dorsal view, the parastyle, mesostyle, and metastyle of the second and third upper molars are farther away from the maxillary bone. The formed cusps are farther apart than in *T. cirrhosus*, giving them a broader appearance. These characters could be important to identify individuals from the eastern areas of Brazil where there could be contact between *T. cirrhosus* and *T. ehrhardti* (Figure 3-4).

Comparisons. The size and shape of the skull of the 2 species are similar, but in *T. ehrhardti* it is smaller on average. In *T. cirrhosus*, the skull is larger and more elongated than in *T. ehrhardti* and the braincase is more elevated above the rostrum. However, these characters show a degree of variation. *Trachops cirrhosus* presents a faint notch in the cutting edge of the upper incisors; in contrast, the notch is broader in *T. ehrhardti* featuring an open groove on the cutting edge, but this characteristic is also variable. The first lower premolar 1 (p1) in *T. ehrhardti* is wider overall compared to the same teeth in *T. cirrhosus* which is taller than wider. The molariform toothrow in *T. cirrhosus* is longer than in *T. ehrhardti*. In *T. cirrhosus* the mandible is broader and more robust than in *T. ehrhardti*. In *T. cirrhosus* the dentary is tall and has a contracted premolar toothrow, and an expanded molar toothrow, with the last molar being closer to the fulcrum, a high coronoid process, and an expanded angular process, which could be associated

with a higher bite force (Nogueira et al., 2009). Regarding the width of the cranium, 3 measurements are broader in *T. cirrhosus* than *T. ehrhardti* (i.e. Breadth of brain case, Mastoid process breadth, and Zygomatic breadth).

Natural history. To the best of our knowledge, precise data on the natural history of this population are currently lacking, although expected to share similarities with *T. cirrhosus*. This species inhabits the Atlantic Forest, which encompasses both lowland and montane systems along the Atlantic coast of southeastern Brazil, as well as the contiguous moist-subtropical forest of the Parana basin (Pavan et al., 2016). Similar to *T. cirrhosus*, it is likely that they seek refuge in caves, hollow trees, culverts, and buildings, often in small groups comprising a few tens of individuals. The species might prey on insects, frogs, lizards, and other small vertebrates. Currently, no information is available regarding their reproductive behavior.

Remarks. The species was described by Felten (1956b) based on 3 specimens collected in 1908 by W. Ehrhardt at Joinville in the Brazilian state of Santa Catarina, considerably expanding the southern limit of distribution of the species in South America. The only distinctive characteristic of the subspecies mentioned by Felten (1956b) is its size, notably smaller in relation to animals from the northern part of South America and similar in size to the former subspecies *T. c. coffini*. In a degree dissertation from the Federal University of Espírito Santo (see Fonseca, 2019), it was proposed based on an integrative approach to morphological, ecological, and phylogenetic evidence, that *T. cirrhosus ehrhardti* should be elevated to the species category; however, the species was not formally described until now.

Discussion

Our investigation of *Trachops*—which integrates data from morphology, morphometrics, and molecular analysis encompassing almost the totality of its geographic distribution—has

provided clarification into the taxonomic status of the species. Our main findings include the recognition of specific status of *T. ehrhardti*, and *T. c. coffini* as a junior synonym of *T. cirrhosus*. Incorporating *T. cirrhosus* specimens from Colombia, Ecuador, and Peru was crucial to our classification because it represented the first taxonomic exploration of the species in the West South American region. We not only expanded the geographical scope of the analysis, but also shed light on previously unexplored genetic and morphological variation within *T. cirrhosus* populations.

***Trachops* in phylogenetic context**

Mitochondrial DNA diversity in *T. cirrhosus* is significant, as evidenced by 3 previous studies. First, Ditchfield (1996) identified 5 clades using 10 partial sequences of the mitochondrial gene *Cytb* from Guatemala, Panama, French Guyana, and Brazil. These findings suggested that current subspecies classifications may underestimate haplotypic diversity, indicating that *Trachops* should be considered a multi-species complex. In this topology, *T. c. coffini* is not monophyletic unlike *T. c. cirrhosus*, and the author attributed this haplotypic diversity to reduced mobility or increased philopatry in *Trachops* across its geographic range, although these hypotheses remain unconfirmed (Ditchfield, 1996).

Second, Clare (2011) investigated pattern congruence between 2 independently evolving genetic regions (mitochondrial genome and Y chromosome), finding significant mitochondrial haplotypic divergence, but not in the *Dby* 7th intron region. Despite using more samples than Ditchfield (1996), the author did not include specimens from West South America. In this topology, *T. c. coffini* once again was not a monophyletic clade distinct from *T. c. cirrhosus*, lacking geographical congruence to support its separation as a subspecies.

Finally, Fonseca (2019) proposed a more integrative approach with a greater number of sequences using a dataset composed of 2,341 bp of *Cytb*, *COI*, *D-loop* (mitochondrial DNA)

and *STAT5A* (nuclear DNA) from 129 tissues. Samples from western South America—particularly Colombia, Ecuador, and Peru—were not included in this study. Consistent with Clare et al. (2011), the ML phylogeny did not render *T. c. coffini* as an independent clade of *T. c. cirrhosus*, with little genetic differentiation (Fonseca, 2019). The author proposed that *Trachops* should be divided into two lineages recognized at the species level: a monotypic *T. ehrhardti*; and *T. cirrhosus* with 2 subspecies (*T. c. cirrhosus* and *T. c. coffini*). However, the phylogenetic justification for maintaining *T. c. coffini* as a subspecies was unclear, without a well-delineated geographic distribution.

In this study, we included an extensive taxon sampling with new localities from Colombia, Ecuador, and Peru, as well as information from complete mitochondrial genomes. Our findings revealed 2 reciprocally monophyletic clades, each exhibiting significant genetic divergence. The first clade, *T. cirrhosus*, notably includes sequences from northern Central America (*T. c. coffini*), challenging the classical notion of *T. c. coffini* as a distinct subspecies and suggesting instead a more cohesive genetic identity within the *T. cirrhosus* clade. The second clade, *T. ehrhardti*, stands as a monophyletic group, sister to *T. cirrhosus*. These results prompt a reconsideration of taxonomic structure within *Trachops*, particularly the delineation of *T. ehrhardti* and *T. cirrhosus*, and the subspecies status of *T. c. coffini* within this framework.

From the phylogeny, we identified a phylogroup predominantly formed by samples from the Guyana Shield, which encompasses a significant geographical region including Guyana, French Guyana, Suriname, and parts of Brazil and Venezuela (Lim & Engstrom, 2001; Lim et al., 2005). This region, being one of the oldest land formations on Earth, has provided relatively unchanged habitats over millions of years due to its tectonic stability, allowing the preservation of ancient genetic lineages and promoting processes of isolated speciation (Lim, 2018). This interpretation is supported by geological and biogeographic studies highlighting the role of the

Guyana Shield as a refuge for biological diversity during significant environmental changes, such as those occurring in the Miocene (Hoorn et al., 2010). Moreover, phylogenetic evidence might suggest that the identified group within the Guyana Shield represents an early divergence within the species *T. cirrhosus*. Organisms inhabiting this region underwent adaptive shifts and range dynamics in response to the diverse environments that emerged during the Late Miocene and Pliocene. These changes were influenced by tectonic uplift, temperature fluctuations, sea level variations, the formation of inland seaways, and shifts in vegetation composition (Harrington, 1962; Hoorn et al., 1995; Janis, 1993; Miller et al., 2005). Therefore, understanding biodiversity within ancient genetic lineages on the Guyana Shield is critical for understanding patterns of diversity and distribution in the Neotropics.

In light of the paraphyly of clades containing samples from the Guyana Shield, we refrain from naming them as discreet phylogroups. However, we emphasize the significance of this region as observed in other mammalian groups (Leite et al., 2015; Lim, 2018; Voss et al., 2013). Studies like those of Lim and Lee (2018) also have noted genetic structuring in *T. cirrhosus* and distinct lineages comprising individuals from the Guyana Shield. Furthermore, Zarate-Martinez et al. (2018) proposed that diversification in South America, including the Amazon, may follow a north-south pattern. Voss et al. (2013) mentioned that Guyanese mammals are thought to be more closely related to lowland Amazonia, so this could explain why individuals from Peru are closely related to individuals from Guyana, as found by Lim and Lee (2018) and this study. Additional biogeographic analyses and further sampling would be beneficial for evaluating these diversification scenarios. that have occurred.

***Trachops* in the morphometric context**

When *T. coffini* was described as a separate species by Goldman (1925), he reported larger measurements for *T. cirrhosus*. The author revised 18 specimens from the type locality of *T.*

coffini with others from Venezuela, Colombia, and Panama that he assumed represented typical *T. cirrhosus* without specifying quantity or localities. However, his analysis, based on a limited geographical range and a small sample size, raised questions about the robustness of these early findings. Felten (1956a) and Felten (1956b) revisited this classification, incorporating a broader geographic range that included Central America. His work, which suggested classifying the Central American forms as *T. c. coffini* subspecies, highlighted potential intraspecific variation within the species. However, Felten's conclusions—particularly regarding dental morphology and size differences—were largely inferential and lacking quantitative rigor. The author proposed maintaining the names *T. c. cirrhosus* and *T. c. coffini* as subspecies and added a third, *T. c. ehrhardti* based on 3 specimens from southeastern Brazil but with no apparent difference other than the smaller size compared to samples from Colombia (reviewed by Hershkovitz 1949) and similar in size to the samples from El Salvador (Felten, 1956a).

No other significant morphological studies of *Trachops* were conducted until 6 decades later, when Fonseca (2019) revised specimens mostly from localities in Brazil. The quantitative analysis revealed a substantial overlap in morphometric space across the 3 subspecies studied, notably between *T. c. cirrhosus* and *T. c. coffini*, challenging the notion of distinct morphometric differentiation between these subspecies. This finding suggested a morphometric continuum rather than discrete categories. Qualitatively, Fonseca (2019) observed that *T. c. coffini* exhibited a pronounced angle between the rostrum and braincase in lateral view, a characteristic less evident in *T. c. cirrhosus*. In dental morphology, Fonseca (2019) noted that the first lower premolar (p1) in *T. c. coffini* possesses a unique shape distinct to this subspecies, although the specifics of this form were not delineated. Additionally, she reported that the m1 tooth in *T. c. coffini* demonstrates a more developed paraconid compared to *T. c. cirrhosus*. The potential displacement of p3 to the labial surface in *T. c. coffini*, as opposed to the lingual surface in *T. c. cirrhosus*, suggests further morphological variability. Notably, Fonseca (2019) noted that

specimens from Costa Rica displayed morphological traits and cranial sizes more similar to *T. c. coffini*, diverging from the expected distribution patterns. Furthermore, individuals from Panama exhibited traits from both *T. c. coffini* and *T. c. cirrhosus* in equal frequencies, leading Fonseca to propose a clinal variation in size across the range of the species, with smaller individuals observed in Central America and becoming larger in South America, positioning Panama as a zone of intergradation.

In our study, encompassing specimens from the western region of the Andes and northern South America, we did not observe the diagnostic characters reported by Fonseca (2019). Specifically, our samples from Central America did not exhibit morphological differences between the northern and southern regions as previously proposed. These discrepancies—particularly regarding the distinct cranial angles in *T. c. coffini* and the unique shape of p1—underscore continuous and widespread morphological variation within the *Trachops* genus, challenging the notion of these characters as discrete or diagnostic for the species. Our findings highlight that morphological diversity in *T. cirrhosus* exhibits a broad spectrum of variation across its entire range. Even though the PCA revealed size variation on PC1—predominantly influenced by skull length metrics—the plot depicted considerable overlap in morpho-space indicating a high degree of morphological congruence. This overlap challenges the current subspecies classification based on size and shape traits. Contrary to the proposed latitudinal cline in size, our data indicate that specimens from western South America (WSAm)—encompassing the Pacific regions of Colombia and Ecuador—and NEC/NWV are similar in size to those from Central America. This finding is consistent with zoogeographical studies that have identified a close affinity between the bat fauna of the western Andes and Central America (Koopman, 1976, 1978, 1982; Hoffmann & Baker, 2003; Clare, 2011), suggesting a strong influence of historical events, ecological processes, and landscape heterogeneity (Manel et al., 2003) in shaping the distribution and morphology of bat species, including *Trachops*. The role of

heterogeneity of habitats and environmental conditions as causes of morphological and genetic divergence (Turmelle et al., 2011; Lindsey & Ammerman, 2016) is also evident in our findings.

On the subspecific status of *Trachops cirrhosus coffini*.

Despite previous assertions, notably by Fonseca (2019), our findings indicate that *T. c. cirrhosus* and *T. c. coffini* lack distinct morphological synapomorphies and do not exhibit reciprocal monophyly. This observation aligns with the criteria outlined by Patten (2015), who mentions that a subspecies should be morphologically distinct and geographically circumscribed, yet not necessarily forming a distinct genetic clade. According to Molinari (2023), subspecies designation is appropriate for populations with significant and heritable morphological differences, even if genetic differentiation is insufficient for species-level recognition. Our analyses, however, reveal that morphological distinctions traditionally used to separate *T. c. coffini* and *T. c. cirrhosus* do not meet these standards. The lack of clear geographical circumscription, combined with our findings that these groups are not morphologically diagnosable—but principally nor genetically distinct—challenges the validity of their current subspecific status. Stevens (2023) offers a compelling ecological perspective on the interpretation of phenotypic variation within subspecies, which reinforces our findings that morphological distinctions between *T. c. coffini* and *T. c. cirrhosus* reflect ecological rather than subspecific variations. Similar to *Chrotopterus auritus* as revised by Stevens (2023), morphological differences noted by Goldman (1925) in *T. coffini* were possibly not truly distinctive but rather a product of limited sampling across distant localities, a concept Stevens called the 'Merriamian shortfall.' While Fonseca (2019) acknowledges clinal size variation yet supports the subspecies distinction, our study does not demonstrate clinal variation. Instead, we found that *T. cirrhosus* displays phenotypic variability throughout its geographic distribution, from the perspective of qualitative external and quantitative morphometric characteristics. This

ecological understanding of phenotypic plasticity challenges the traditional reliance on subspecies categorization, where such distinctions may not reflect genetic or evolutionary significance. Thus, our study advocates for a reevaluation of the subspecific classification of *T. c. coffini*, proposing an approach that acknowledges the continuous nature of environmental and morphological variation.

Reliance on size-only differences in recognizing subspecies is contentious. Molinari (2023) underscores that diagnostic morphological characters should not be attributable to phenotypic plasticity. Considering studies indicating size variation as a source of morphological plasticity in bats (McLellan, 1984; Jarrín-V. et al., 2010; Jarrín-V. & Menéndez-Guerrero, 2011; López-Aguirre et al., 2015), the use of body size as a sole criterion for taxonomic decisions becomes problematic. Geographic variation in body size, influenced by a multitude of factors including genetic adaptations and environmental conditions affecting growth rates, further complicates its reliability as a distinguishing feature (McLellan, 1984; Berry et al., 1987; Ebenhard, 1990; Jarrín-V. et al., 2010; Jarrín-V. & Menéndez-Guerrero, 2011; López-Aguirre et al., 2015). Additionally, methodological inconsistencies—including instrumental and human errors—can lead to data discrepancies (Fox et al., 2020), casting doubt on the robustness of size-based differentiation. Our study, therefore, suggests that the current classification of *T. c. coffini* as a subspecies of *T. cirrhosus* lacks support, both morphologically and genetically.

On the specific status of *T. ehrhardti*

Trachops ehrhardti should be elevated to species status based on the presence of clearly diagnosable morphological characters, geographically circumscription within the Atlantic forests of South Brazil (Mata Atlántica), and substantial molecular divergence. Broader cranial measurements (BB, MPW, ZB) allow *T. cirrhosus* to be separated from *T. ehrhardti* and both species form reciprocally monophyletic clades separated by a genetic divergence of over 8%.

Geographic isolation would primarily imply loss of ecological exchangeability, and possibly mating impediments with *T. cirrhosus*. Originally proposed by Haffer (1969), the Refuge Theory for the Neotropics suggests that Pleistocene glaciation cycles created contraction and subsequent expansion of forested areas that, in turn, would create allopatry between populations of the same forest-dwelling species, leading to intraspecific differentiation and subsequently to speciation. Paleopalynological research supports the high dynamism of Neotropical forested regions: the Atlantic Forest and the Amazon were connected in the past (Vivo, 1997) but separating as increasing aridity in the Tertiary triggered the formation of the belt of xeromorphic formations between them (Martins et al., 2009). There was likely a predominantly arboreal vegetation during most of the Pleistocene, with typical Amazonia and Atlantic Forest tree species found in what it is now the dry diagonal that separates these 2 biomes. This continuous portion of dry vegetation that extends diagonally across a third of the South American continent comprises mainly deciduous forests and open formations including ecosystems such as the Cerrado, Caatinga, and Chaco (Werneck, 2011; Turchetto-Zolet et al., 2013). Historical climatic oscillations, particularly those induced by Pleistocene glaciations (Rocha et al., 2020), may have influenced the distribution and diversification of *Trachops* because they may have led to habitat contractions and expansions within the Atlantic Forest, fostering periods of isolation and allopatric speciation among populations. The dry diagonal serves as a secondary barrier to gene flow, even for species presumed to have high dispersal capabilities like bats and birds. Phylogeographic studies on lizards and geckos (Fonseca et al., 2018; Werneck, 2012) and birds (Moura et al., 2018; Rocha et al., 2020) in this region have identified distinct genetic lineages tied to different biomes, illustrating its role as a significant physical and ecological barrier that divides the humid forests of the Amazon from the Atlantic Forest, thus limiting ecological exchangeability and potentially introducing mating barriers due to geographical isolation. The extent to which these climatic fluctuations and associated vegetation changes affected patterns

of distribution and diversification of the fauna remains a central question in understanding the evolution of forest-associated taxa.

Conservation implications

Recognized bat diversity has increased due to new species descriptions and taxa raised from subspecific level or synonymy (Burgin et al., 2018), but also as a result of clarification of cryptic species within several genera, e.g., *Platyrrhinus* (Velazco et al., 2023), *Glossophaga* (Calahorra-Oliart et al., 2021), and *Sturnira* (Yáñez-Fernández et al., 2023). These changes in taxonomy may have an impact on the conservation of species. The genus *Trachops* is composed of 2 monotypic species: *T. cirrhosus* maintains its “Least Concern” conservation status at the global level, with no major threats known throughout its range (Miller et al., 2015). However, it is important to revise the conservation status of *T. ehrhardti* because its current range is restricted to southeastern Brazil. The Atlantic Forest is one of the leading biodiversity 'hotspots'—areas possessing the highest concentrations of endemic species, and often are the most severely threatened by habitat loss (Baptista & Rudel, 2006). In the Atlantic Forest biome, the predominant land cover (476,000 km²) comprises 9% of the land area of Brazil (Souza et al., 2020), but is highly fragmented by roads and urban centers, and immersed in a large agriculture matrix, reducing old secondary forest cover being reduced to 12% of its original expanse over the past 30 or more years (de Rezende et al., 2015; Souza et al., 2020). If this scenario remains unchanged, then elevating *T. ehrhardti* to a species should trigger immediate protection efforts.

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Chapter 4. Insights into the diversity of *Phylloderma* and *Macrophyllum* (Phyllostomidae) with the description of a new cryptic species from Central America

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M. Alejandra Camacho*^{1,2}, Santiago F. Burneo², Balázs Horváth³, Dániel Cadar³, Jérôme Murienne¹



Adult *Phylloderma stenops*. Ecuador. Photo: Rubén D. Jarrín.

¹Centre de Recherche sur la Biodiversité et l'Environnement (CRBE UMR5300), Université de Toulouse, CNRS, IRD, Toulouse INP, Université Toulouse 3 – Paul Sabatier (UT3), Toulouse, France.

²Museo de Zoología, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Quito, Ecuador.

³WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Bernhard Nocht Institute for Tropical Medicine, 20359 Hamburg, Germany

*Corresponding author

Abstract

Based on morphological analyses and complete mitochondrial genomes, this study assesses taxonomic diversity within two Neotropical bat species, *Phylloderma stenops* and *Macrophyllum macrophyllum* (Chiroptera, Phyllostomidae), across South and Central South America, with special emphasis on probing cryptic speciation in the latter region. Our phylogenetic analyses reveal the existence of highly supported clades showing significant genetic divergences, suggesting the presence of unrecognized species. In the case of *Phylloderma*, these findings lead us to propose elevating to species level, under the pre-existing name *P. septentrionalis*, the populations from northern Central America. In the case of *Macrophyllum*, the few names available refer to Brazilian populations, thus we assign the Central American populations to a new species, *Macrophyllum* sp. nov., which we describe. The genetic data delineate distinct northern and southern clades within each genus, separated by geological barriers such as the Hess Escarpment and the Guanacaste Cordillera, which restrict gene flow and foster genetic divergence between populations. These findings emphasize the need to integrate molecular data with geological and biogeographic insights to elucidate speciation processes across such barriers. The genome skimming approach used here effectively reveals significant genetic differentiations that are correlated with geographic and geological boundaries, thus enhancing our understanding of the evolutionary dynamics of Neotropical bats and highlighting the critical role of combining morphological and molecular data to discover hidden diversity.

Keywords: Central America, Cryptic speciation, *Macrophyllum*, Mitochondrial genomes, *Phylloderma*, Phyllostomidae, Phylogenetics.

Introduction

The Neotropical region hosts diverse ecosystems in terms of habitats and serves as home to an exceptional biodiversity (Rojas et al., 2016). Despite its relatively small area, amounting to about 0.4% of the Earth's terrestrial surface, Central America is home to the Mesoamerican Biodiversity Hotspot (Mittermeier et al., 2011), thus harbors an important richness of bat species. The biodiversity of Central America has been underestimated due to factors such as cryptic diversity (Arteaga et al., 2012; Fouquet et al., 2007; Navarro et al., 2001), uneven research efforts across countries, and logistical challenges arising from political instability (Castellanos, 2019; Crawford et al., 2007). However, the current species count for the region is on the rise, with several species highlighting endemism and biogeographical patterns (Garbino et al., 2024; López-Cuamatzi et al., 2024).

The diversity and high endemism of Central America have been attributed to a complex biogeographical history resulting from geological transformations that have created barriers to organismic dispersal, such as high elevations and topographic depressions (Briggs, 1994; Escalante & Morrone, 2020; Marshall, 2007; Patterson, 2020). Among these barriers are the Talamanca Cordillera, which uplifted during the Pliocene and runs the length of Central America; the lowland Isthmus of Tehuantepec separating the Yucatan Peninsula from the rest of Mexico; the Motagua-Polochic Fault that forms highland areas in Guatemala and Honduras (uplifted during the Late Miocene); the lowland Nicaraguan Depression (which was partially inundated during the Miocene-Pliocene) bisecting Central America; the Central Panamanian Lowlands; and the Darien Highlands (Coates et al., 2004; Marshall, 2007; Molinari et al., 2023). Moreover, historical exchanges of species between North and South America, along with the climatic cycles of the Plio-Pleistocene era, have contributed to the formation of distinct and diverse plant and animal communities within Central America (Suárez-Atilano et al., 2014;

Woodburne, 2010). This complex history has resulted in a significant portion of Central American biodiversity being tightly dependent on altitude, where mid- to high-elevation regions have served as temperate refugia during periods of climatic change, and have latter become hotspots for species diversification (Castellanos, 2019; Hernández-Canchola & León-Paniagua, 2017; McCain, 2007; Smith et al., 2007).

The bat family Phyllostomidae, which is restricted to the tropics and subtropics of the New World, represents the most diverse chiropteran group of the Neotropical Region. It currently includes 61 genera and 227 species that occupy a very wide range of habitats (MDD, 2024; Solari et al., 2019). Within Phyllostomidae, the subfamily Phyllostominae includes 10 genera, namely *Chrotopterus*, *Gardnerycteris*, *Lophostoma*, *Macrophyllum*, *Mimon*, *Phylloderma*, *Phyllostomus*, *Trachops*, *Tonatia*, and *Vampyrum* (Baker et al., 2016); all of which are represented in Central America by one or more species.

Phylloderma is one of the monotypic genera within Phyllostominae. Known as the pale-faced bat, *P. stenops* (Peters, 1865) occupies a wide distributional range in the Neotropics, adapting to various forest habitats; yet its ecological role, behavioral patterns, and morphological and genetic diversity have not been fully studied (Carter et al., 1966; Martínez-Cerón et al., 2019; Solari et al., 2019; Williams & Genoways, 2008). The species can be easily distinguished from other members of Phyllostominae by a unique combination of characters, namely the possession of two pairs of lower incisors, three lower premolars, a rostrum shorter than the braincase, and pale wing tips (Williams & Genoways, 2008). Though it is similar in appearance to *Phyllostomus*, *P. stenops* can be recognized by its nose-leaf, which is fused to the upper lip and rectilinear at the base, as opposed to the free and semicircular in *Phyllostomus*; the presence of three lower premolars, as opposed to two in *Phyllostomus*; and a lighter facial color compared to *Phyllostomus* (Bomfim et al., 2017; de Souza et al., 2022; Trujillo & Albuja, 2005).

Phylloderma also possesses gular glands that are well-developed in reproductive males and produce a musky odor that may act as a chemical signal conveying information about the mating status and identity of the individual (Adams et al., 2018; Muñoz-Romo et al., 2021). The species is omnivorous, and its diet primarily consists of fruit, adult insects, and larvae and pupae taken from the nests of social wasps (Giannini & Kalko, 2004; Jeanne, 1970).

The taxonomy of this species has remained unrevised since 1979, with three recognized subspecies whose descriptions were based solely on morphometric data and distributional records. The nominal subspecies *P. s. stenops* (Peters, 1865), has an ample distribution ranging from Panamá to southeastern Brazil; *P. s. septentrionalis* (Goodwin, 1940) is distributed throughout Central America, from Mexico to Costa Rica; and *P. s. boliviensis* (Barquez & Ojeda, 1979) is endemic distributed to southeastern Bolivia. Recent evidence suggests that *Phylloderma stenops* shows geographic variation across the Andes (Martínez-Cerón et al., 2019) highlighting the need for research aimed to expand our understanding of the evolutionary patterns within the species.

Another monotypic genus within the subfamily Phyllostominae is *Macrophyllum* with its nominal species, *M. macrophyllum* (Schinz, 1821) known as the Long-legged bat. The species was initially described as *Phyllostoma macrophyllum* by Schinz in 1821 with the type locality stated as "In the forests of Brazil". The species was reassigned to the genus *Macrophyllum* by Gray (1838), under the new name *Macrophyllum nieuwiedii* and "Brazil" as the type locality. A subsequent name change includes *Dolichophyllum macrophyllum* by Allen (1900). Finally, the species was named *Macrophyllum macrophyllum* by Nelson (1912), which is the currently valid name. Despite its broad distribution, no subspecific variation has been described yet for *M. macrophyllum* (Smith, 2008). The species is found across a wide range of environments, from Mexico to Argentina, and adapts to seemingly diverse habitats, including rainforests and arid

shrublands (Feijó et al., 2015). The Long-legged bat shows special anatomical adaptations, such as long and powerful legs and feet, and a remarkable interfemoral membrane that is lined with rows of dermal denticles (Harrison, 1975). These adaptations are thought to be specialized for foraging, specifically for skimming the surfaces of bodies of water, as the bulldog bats *Noctilio* spp. (Noctilionidae), the proboscis bat *Rhynchonycteris naso* (Emballonuridae), and some evening bats *Myotis* spp. (Vespertilionidae) do (Gardner, 1977; Harrison & Pendleton, 1974; Weinbeer et al., 2006). This species possesses an insectivorous diet, as often seen in Phyllostominae; its hunting strategy includes a combination of gleaning and aerial hawking (Harrison, 1975; Weinbeer & Kalko, 2007).

The high biodiversity of Central America invites the study of evolutionary patterns and speciation. Despite recent advances in the research on the diversity, biogeography, and evolution of mammals across this region, the distribution and drivers of bat diversity remain insufficiently understood (Castellanos, 2019). This study aims to address this matter by examining the phylogenetic relationships, taxonomic uncertainties, and potential cryptic diversity within *Phylloderma* and *Macrophyllum*, through the application of an integrative taxonomy approach, combining molecular and morphological data. Morphological analyses were conducted on craniodental traits, while molecular data were obtained through genome skimming—a method that provides shallow-pass genome sequence to recover highly repetitive regions of the genome, such as the complete mitochondrial genome (Straub et al., 2012). In the case of *Phylloderma*, we examined specimens of all the named subspecies. Our taxonomic decisions are founded on the general definition of species proposed by Simpson (1951) and modified by Molinari (2023) to include operational criteria, according to which a species is a phyletic lineage (ancestral-descendent sequence of populations) evolving independently of others, and diagnosably distinct based on heritable morphological characters, genetic markers, or both.

Materials and methods

Biological samples and natural history repositories

We included representatives from most of the geographical distribution of *Phylloderma stenops* and *Macrophyllum macrophyllum* from Central and South America (Figure 4-1). Samples of all the named subspecies of *P. stenops* were included in the morphological analysis, although no DNA sequences could be retrieved for *P. s. boliviensis*.

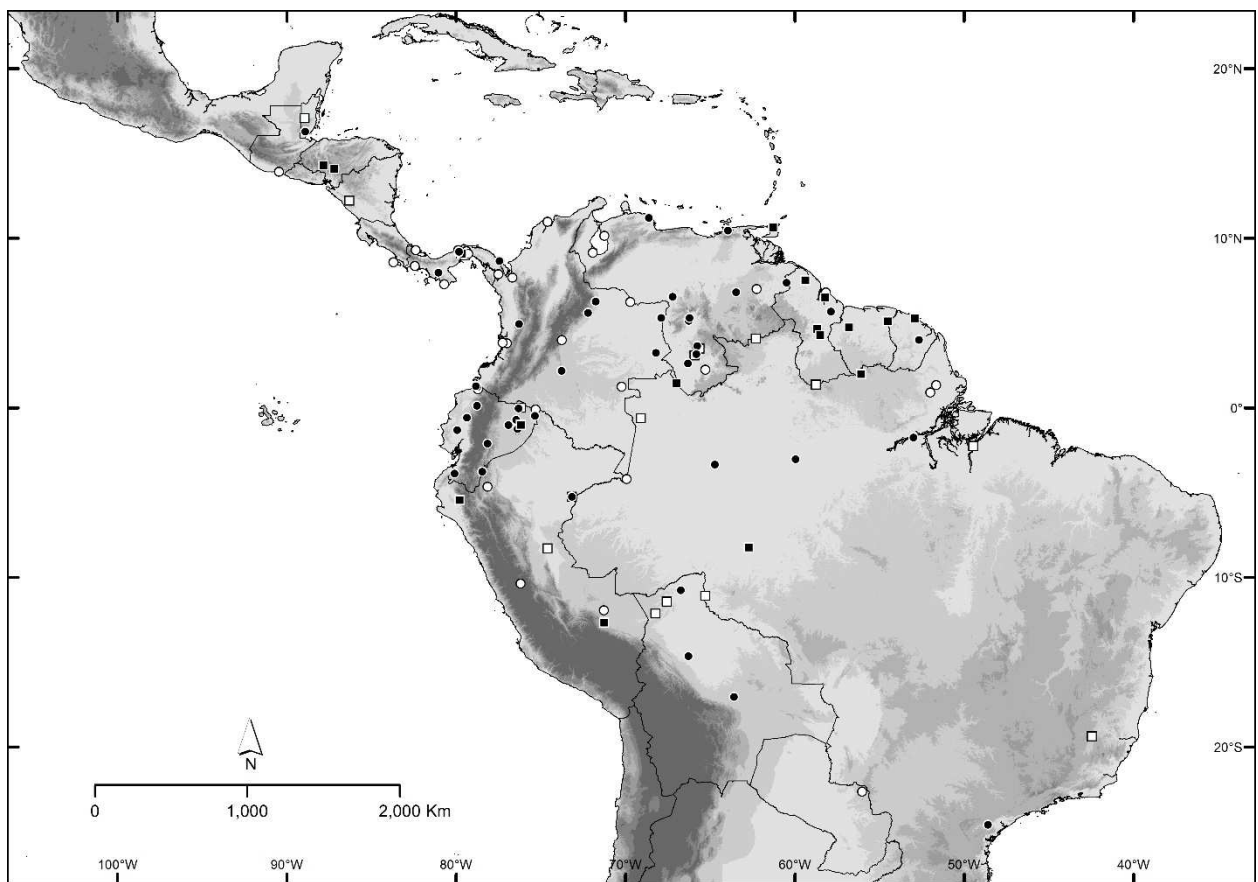


Figure 4-1. Collecting localities *Phylloderma* and *Macrophyllum*. The locality details, analyzed in this study, are shown in Supplementary data 5. Black symbols represent *Phylloderma* and white symbols represent *Macrophyllum*. Squares are sequenced specimens. Circles are measured-only specimens.

For the morphometric analyses, we examined 58 specimens of *Phylloderma* and 84 of *Macrophyllum* housed in the following collections: AMNH, American Museum of Natural History, New York, NY, USA; FML, Colección de Mamíferos, Fundación Miguel Lillo, 104

Tucumán, Argentina; IAvH, Colección de Mamíferos del Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Colombia; MEPN, Museo de Historia Natural Gustavo Orcés, Escuela Politécnica Nacional, Quito, Ecuador; QCAZ, Museo de Zoología, División de Mastozoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador; USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; UV, Colección del Mamíferos de la Universidad del Valle, Cali, Colombia (Supplementary data 5). The measurements of *P. stenops boliviensis* specimens in the FML ($N = 2$) were shared with us by the curator of this collection.

We obtained tissue samples from 18 *Phylloderma* specimens and 38 *Macrophyllum* specimens that are housed at the QCAZ, or were loaned from the AMNH; FMNH, Field Museum of Natural History, Chicago, IL, USA; MSB, Museum of Southwestern Biology, Albuquerque, NM, USA; and ROM, Royal Ontario Museum, Toronto, Canada (Supplementary data 5).

Lastly, we included the locality data, and measurements, if available, listed in Martínez-Cerón (2019), Sánchez-Palomino et al. (1993), Esbérard and Faria (2006), Bomfim et al. (2017), Verde et al. (2021), Trujillo and Albuja (2005), Medina-Fitoria et al. (2015), Santos-Moreno and Gallardo-Sipriano (2014), and Medellín et al. (1986).

Molecular data

We gathered a tissue set comprising 60 samples (18 of *Phylloderma*, 38 of *Macrophyllum* and 4 of other species). These included heart or liver (~40mg), claws (~ 3 mm), and biopsy punch wing snippets (~3 x 3 mm diameter). Mitochondrial DNA sequencing was performed using a genome skimming procedure as recently performed for other Phyllostomids (Camacho et al., 2022). Laboratory procedures were carried out at the NGS Core Facility of the Bernhard Nocht Institute for Tropical Medicine in Hamburg. The extraction and amplification of DNA were performed in a dedicated clean room facility, separated from the area where samples and post-

PCR products were handled. Stringent contamination prevention protocols and negative controls were also implemented. The DNA extraction process for various sample types (dried skin, ethanol, or formaldehyde-preserved tissues) involved proteinase K digestion at 55°C using 20 µl of proteinase K and 220 µl of ATL lysis buffer (MinElute Reaction Cleanup kit, Qiagen). Prior to digestion, the samples were thoroughly washed with nuclease-free water (Qiagen). The incubation time for proteinase K digestion varied depending on the tissue type and sample preservation, ranging from 5 to 24 hours. Following digestion, DNA was extracted and purified using the Qiagen MinElute kit, with each sample eluted to a final volume of 60 µl. DNA concentration was measured using Qubit and Bioanalyzer instruments. For library preparation, the QIAseq FX DNA Library Kit (Qiagen) was used, with double index barcode labelling according to the manufacturer's instructions. DNA fragmentation was often avoided due to the high degradation of nucleic acid ($\text{DNA} \leq 500 \text{ bp}$) material and low concentration ($\leq 3 \text{ ng}/\mu\text{l}$). The HiFi PCR Master Mix from the QIAseq FX kit was utilized to amplify DNA regions with varying GC contents, minimizing sequencing bias caused by PCR, such as nucleotide misincorporations from cytosine deamination. The libraries underwent quality control to determine fragment size by using the Agilent 2100 Bioanalyzer, and the concentration was assessed using a Qubit 2.0 Fluorometer. After normalization, the samples and negative controls were pooled and subjected to sequencing on the NextSeq 2000 platform (2×100 cycles) (Illumina, San Diego, CA, USA).

Raw reads were first subjected to a qualitative assessment, followed by the removal of adaptor sequences and the filtration of polyclonal and low-quality reads (<55 bases long) using CLC Workbench (Qiagen). Overlapping paired-end (PE) reads were merged to improve quality, while non-overlapping pairs and orphan reads were left unchanged. Deduplication was performed with an assumed 100% identity using BBTools (Bushnell, 2014), expanding the length of contigs produced during *de novo* assembly. Custom assembly was conducted using

Megahit (Li et al., 2016) and Spades (Prjibelski et al., 2020) applications. All the assembled mitochondrial genomes were annotated using the MITOS2 metazoan pipeline (Al Arab et al., 2017; Bernt et al., 2013), followed by visual inspection and manual adjustment in Geneious v.9.0.5 (<https://www.geneious.com>; (Kearse et al., 2012). We added the following complete mitogenome sequences from Genbank to also analyze them as outgroups in the subsequent phylogenetic analysis, following the results of Camacho et al. (2022): *Trachops cirrhosus* (PP410143), *Lonchorhina aurita* (KU743908), *Glossophaga soricina* (ON321893), *Lochophylla robusta* (ON357721), *Lophostoma brasiliense* (ON310506), and *Phyllostomus discolor* (ON357733)

Ribosomal RNA (rRNA) and transfer RNA (tRNA) loci were aligned using MUSCLE (Edgar, 2004), while protein-coding genes sequences were aligned using TranslatorX (Abascal et al., 2010). Analytical conditions for mitochondrial genomes can vary greatly and have been previously carefully evaluated (Botero-Castro et al., 2013, 2018). We here used the optimal partitioning schemes and evolutionary models as previously estimated by Camacho et al. (2022). We conducted the phylogenetic analysis with a partitioning scheme of 38 partitions (2 independent partitions for rRNAs and 36 partitions for tRNAs, one partition for each codon for each gene) and a Generalized Time Reversible (GTR) model of substitution rates along with a gamma (G) distribution and a fraction of invariable (I) sites (GTR+G+I). We performed a Maximum Likelihood (ML) analysis using RAxML-NG (Kozlov et al., 2019), starting from 10 parsimony trees and 10 random trees. Bootstrap support values were obtained using the classical Felsenstein metric (Felsenstein, 1985) and transfer bootstrap expectation (Lemoine et al., 2018). Trees were visualized and edited in FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). For the analysis, bootstrap values of 75% or higher were considered to denote robust support. Moderate support was recognized for bootstrap values ranging between 50% and 75%. Bootstrap values at or below 50% were interpreted as providing negligible support within our

analytical framework (Moratelli et al., 2017). Finally, we calculated uncorrected pairwise (p) distances among samples from each genus using MEGA 11 (Stecher et al., 2020; Tamura et al., 2021).

From the tissue samples, 15 *Phylloderma* and 22 *Macrophyllum* complete mitogenomes were successfully sequenced (Supplementary data 5), along with the complete mitochondrial genome from the following additional Phyllostominae species: *Gardenycteris crenulata* (QCAZ18512), *Tonatia bakeri* (QCAZ18699), *Chrotopterus auritus* (QCAZ17594), and *Vampyrum spectrum* (QCAZ18135) as outgroups. A total of 41 complete mitogenomes sequences newly generated in this study have been deposited in GenBank (Appendix 2).

Morphological data

The total sample included 58 specimens of *Phylloderma* and 84 of *Macrophyllum* from which craniomandibular and external measurements were obtained. To control for developmental variability, only mature specimens were included, identified by the complete ossification of cranial sutures and metacarpal epiphyses, following the criteria established by Kunz et al. (1996) and Novaes et al. (2021). For each specimen, 26 metrics were recorded, encompassing 16 craniomandibular and 10 external dimensions. Total Length (TL), Tail Length (T), Hind Foot (HF), and Ear Length (E), and Mass (W) were obtained from specimen tags when available. We used digital calipers with a precision of 0.01 mm for the acquisition of measurements. Only craniomandibular metrics were used in subsequent quantitative analyses, while the remaining measurements were considered for descriptive purposes. Cranial and dental structures follow the nomenclature of Velazco (2005), Tavares et al. (2014), and Garbino et al. (2020). We followed Miller (1907) and Cirranello et al. (2016) in recognizing the three lower premolars in bats as p2, p3, p4, with p1 missing. We used standard abbreviations for tooth position, with upper-case letters for upper teeth and lower-case letters for lower teeth: I/i, upper/lower

incisors; C/c, upper/lower canines, P/p, upper/lower premolars; M/m, upper/lower molars; and tooth positions indicated by numbers.

External and craniomandibular measurements and abbreviations are defined as follows: *calcar length (CL)*, from the joint with the ankle to the calcar tip; *ear length (E)*, from intertragic notch of the ear to the outer tip; *forearm length (FA)*, distance from the elbow (tip of the olecranon process) to the wrist (including the carpals); *hindfoot length (HF)*, distance from the ankle to the tip of the claw; *metacarpal III (MET-III)*, distance from the joint of the wrist (carpal bones) with the 3rd metacarpal to the metacarpophalangeal joint of 3rd digit; *metacarpal IV (MET-IV)*, distance from the joint of the wrist (carpal bones) with the 4th metacarpal to the metacarpophalangeal joint of 4th digit; *metacarpal V (MET-V)*, distance from the joint of the wrist (carpal bones) with the 5th metacarpal to the metacarpophalangeal joint of 5th digit; *tail length (T)*, distance from dorsal flexure at base of the tail to the tip of the last caudal vertebra; *tibia length (TiL)*, length from the proximal end of the tibia to the distal base of the calcar; *total length (TL)*, head and body length excluding tail; *weight (W)*, mass in grams; *braincase height (BCH)*, height of the braincase, posterior to the auditory bullae from the basioccipital to the sagittal crest; *breadth across upper molars (M2-M2)*, greatest width of palate across labial margins of the alveoli of M2s; *breadth of brain case (BB)*, greatest breadth of the globular part of the braincase, excluding mastoid and paraoccipital processes; *condylocanine length (CCL)*, distance from the occipital condyles to the anterior border of the of the upper canines; *condyloincisive length (CIL)*, distance between a line connecting the posteriormost margins of the occipital condyles and the anteriormost point of the upper incisors; *coronoid height (COH)*, perpendicular height from the ventral margin of mandible to the tip of coronoid process; *dentary length (DENL)*, distance from midpoint of condyle to the anteriormost point of the dentary; *greatest length of skull (GLS)*, greatest distance from the occiput to the anteriormost point on the premaxilla (including the incisors); *mandibular toothrow length (MANDL)*, distance from

the anteriormost surface of the lower canine to the posteriormost surface of m3; *mastoid (process) breadth (MPW)*, greatest breadth across skull, including mastoid processes; *maxillary tooththrow (MTRL)*, distance from the anteriormost surface of the upper canine to the posteriormost surface of the crown of M3; *molariform tooththrow (MLTRL)*, distance from the anteriormost surface of P3 to the posteriormost surface of the crown of M3; *palatal width at canines (C-C)*, distance between the outermost extremities of the cinguli of upper canines; *palatal length (PL)*, distance from the posterior palatal notch to the anteriormost border of the incisive alveoli; *postorbital constriction breadth (PB)*, least breadth at the postorbital constriction; *zygomatic breadth (ZB)*, greatest breadth across the zygomatic arches.

To address the issue of missing values—due to fractures and loss of parts of the skull—which accounted for 3% of measurements in *Phylloderma* and 4.4% in *Macrophyllum*, we employed the multiple imputation method available in SPSS (IBM Corp., 2011). Data normality was assessed through logarithmic transformation, followed by the Shapiro-Wilk test which is particularly potent for detecting normality deviations in small to moderate sample sizes (Sokal & Rohlf, 1995). Molecular findings guided the subsequent exploratory ANOVA to assess size differences across sexes within phylogroups derived from phylogenetic analyses: in *Phylloderma*, the Panamanian-South American form was treated as distinct from the Honduran form, which would correspond to the known subspecies *P. s. septentrionalis*. *P. stenops boliviensis* was excluded from these analyses and subsequent multivariate tests due to being represented by only two specimens. For *M. macrophyllum*, the ANOVA explored variation between the Panamanian-South American form and the Nicaraguan form. Both exploratory analyses (*Phylloderma* and *Macrophyllum*) focused solely on the greatest length of the skull (GLS) as the dependent variable. Due to a lack of significant sex-based size differences within both genera, males and females were pooled together for the morphometric analysis that followed.

Subsequently, using the same phylogroups in each genus, morphometric variation was tested to determine whether the population samples differed in the 16 craniomandibular dimensions. Given the strictly two groups per analysis, we conducted an independent *t*-test to evaluate statistical differences in group means, incorporating the Levene's test for equality of variances and *t*-corrected *P*-values. The Levene's test assesses the homogeneity of variances, with the null hypothesis positing equal variances across groups (Sokal & Rohlf, 1995). A significance value greater than 0.05 suggests no significant variance differences, allowing the use of equal variance assumptions in the *t*-test.

Finally, a Principal Component Analysis (PCA) was performed on log-transformed craniodental measurements by using the variance–covariance matrix within SPSS to identify general trends in cranial size and shape variations, and to compare morphologically the taxa.

Results

Phylogenetic findings

We found the same evolutionary pattern for the *P. stenops* and *M. macrophyllum* datasets. For *Phylloderma*, the phylogenetic analysis recovered two highly supported clades, one containing individuals distributed from Panama to South America that would correspond to the current subspecies *P. s. stenops* (Clade 1, Figure 4-2) and another from Honduras (the North Central American form) that would correspond to what is currently known as *P. s. septentrionalis* (Clade 2, Figure 4-2).

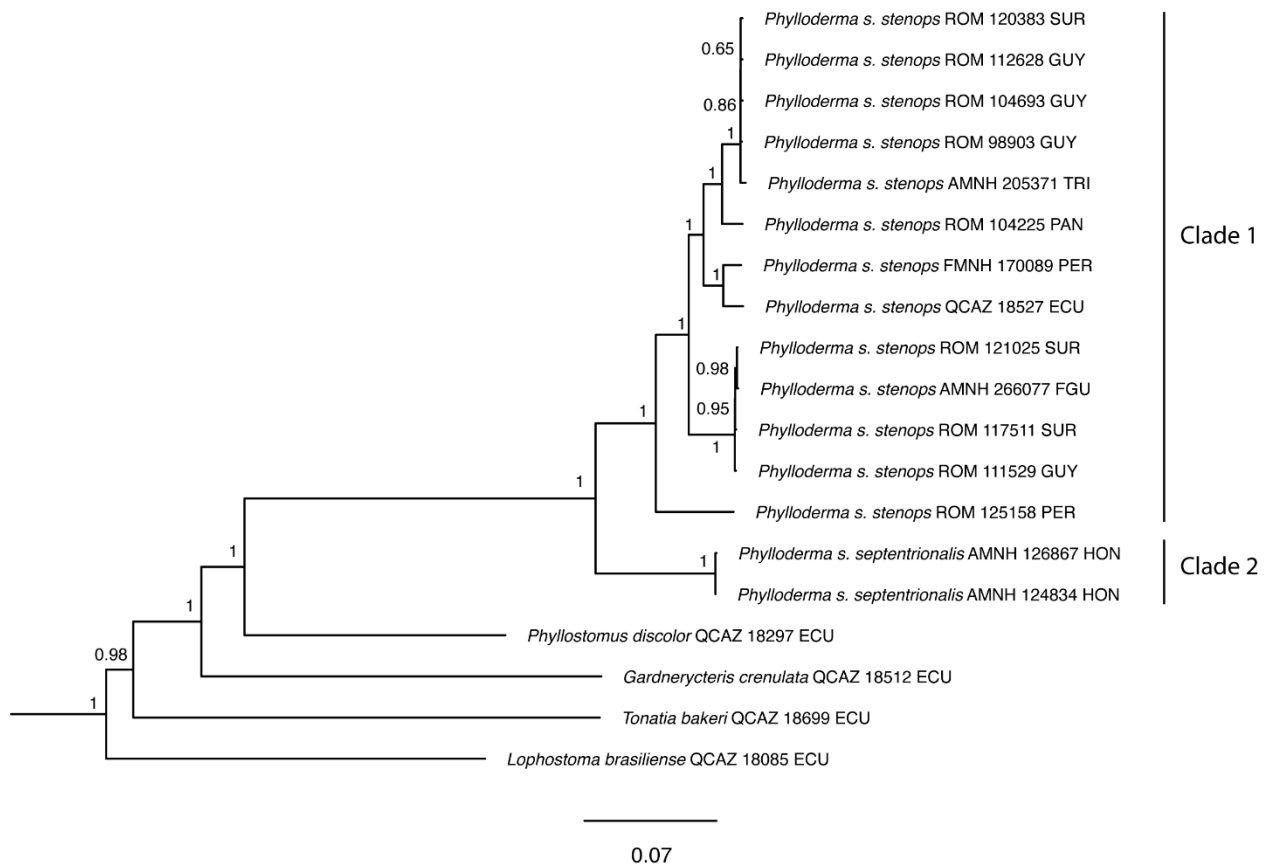


Figure 4-2. Evolutionary relationships of *Phylloderma*. Selected portion of the phylogenetic tree showing *Phylloderma* evolutionary relationships. The complete phylogeny was reconstructed in RAxML under the GTR+G+I model using 47 complete mitogenomes including outgroups and is shown in Supplementary data 6. The numbers in the nodes indicate ML bootstrap supports: BS values of 0.75 or higher were considered as robust supported; bootstrap values ranging between 0.50 and 0.75 are of moderate support; and bootstrap values at or below 0.50 were considered as low supported.

Estimates of evolutionary divergence over sequence pairs between these clades, as defined by the analysis of the complete mitogenomes, showed an 8.60% genetic distance between the Panamanian-South American form (*P. s. stenops*), and the North Central American form (*P. s. septentrionalis*; Table 4-1). For *M. macrophyllum*, the corresponding phylogenetic analysis also recovered two highly supported clades, one containing individuals from Nicaragua (the North Central American form), and another containing individuals distributed from Panama to South America (Figure 4-3). The estimates of evolutionary divergence over sequence pairs between clades was 8.36 % (Table 4-1).

Table 4-1. Estimates of evolutionary divergence between analyzed sequence pairs. The number of base differences per site are the result of averaging all sequence pairs between groups. The of rate variation among sites was modeled with a gamma distribution.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	<i>M. macrophyllum</i> Panamanian-South American form	-													
2	<i>M. macrophyllum</i> North Central American form	8.36%	-												
3	<i>Trachops cirrhosus</i>	18.12%	18.26%	-											
4	<i>Phylloderma s.</i> <i>stenops</i>	19.38%	19.24%	18.76%	-										
5	<i>P. s. septentrionalis</i>	19.52%	19.19%	18.70%	8.60%	-									
6	<i>Phyllostomus</i> <i>discolor</i>	18.23%	18.14%	17.20%	15.26%	15.21%	-								
7	<i>Gardenycteris</i> <i>crenulatum</i>	18.10%	18.15%	17.06%	16.47%	16.51%	14.53%	-							
8	<i>Tonatia bakeri</i>	18.23%	18.17%	17.04%	16.61%	16.59%	15.20%	15.86%	-						
9	<i>Lophostoma</i> <i>brasiliense</i>	17.82%	17.94%	16.93%	16.49%	16.53%	15.02%	15.50%	15.53%	-					
10	<i>Vampyrum</i> <i>spectrum</i>	19.52%	19.62%	19.64%	18.42%	18.53%	17.59%	18.47%	17.77%	17.82%	-				
11	<i>Chrotopterus</i> <i>auritus</i>	20.56%	20.60%	20.39%	19.59%	19.70%	18.89%	19.84%	19.71%	18.98%	16.31%	-			
12	<i>Lonchorhina aurita</i>	18.94%	19.10%	18.09%	18.78%	19.19%	17.77%	17.89%	17.56%	17.67%	18.71%	20.04%	-		
13	<i>Glossophaga</i> <i>soricina</i>	19.01%	19.30%	18.06%	20.18%	20.43%	18.34%	18.42%	18.40%	18.11%	20.29%	21.15%	18.80%	-	
14	<i>Lonchophylla</i> <i>robusta</i>	19.83%	19.48%	18.88%	18.94%	18.94%	17.82%	18.41%	18.23%	17.87%	17.72%	19.46%	18.27%	18.72%	-

Morphometric findings

At first glance, in *Phylloderma*, the descriptive measurements of the phylogroups (Table 4-2) show that *P. s. septentrionalis* is larger, both in craniomandibular and external measurements, than the South American subspecies (*P. s. stenops* and *P. s. boliviensis*). Measurements of both phylogroups were normally distributed (Shapiro-Wilk significance values >0.05 ; not shown); and the ANOVA to test differences between sexes, using GLS as the dependent variable, showed that males and females do not differ significantly within subspecies regarding corrected size ($P > 0.05$).

The Levene's test to assess the equality of variances in the independent *t*-test showed non-significant values ($P > 0.05$), so we accepted the null hypothesis of equality of population variances and refer the significance value of the *t*-test assuming equal variances. For *P. s. stenops* and *P. s. septentrionalis*, in all the variables examined, there were highly significant differences between the population variances ($P < 0.001$,

Supplementary data 7), which shows that, in terms of the metric variables, and taking into account that the subspecies *septentrionalis* is larger, this analysis is consistent with what was observed in the phylogeny. The presence of significantly different variances between variables, based on an independent *t*-test, suggests that craniometric measurements vary significantly between subspecies, supporting the notion that these measurements may be useful to differentiate subspecies.

Table 4-2. Morphological data of *Phylloderma stenops* subspecies. Measurements are given in millimeters. Descriptive measures of *P. s. septentrionalis* and *P. s. stenops*: minimum–maximum; arithmetic mean (standard deviation). Measurements of *P. s. boliviensis* correspond to the two specimens of the type series FML 383 and FML 413, respectively.

Measurement	<i>P. s. septentrionalis</i> (<i>N</i> = 6)	<i>P. s. stenops</i> (<i>N</i> = 50)	<i>P. s. boliviensis</i> (<i>N</i> = 2)
Breadth of brain case (BB)	13.80, 13.57–13.97 (0.18)	12.96, 12.19–13.67 (0.35)	12.78; 13.22
Palatal width at canines (C-C)	6.97, 6.77–7.09 (0.13)	6.40, 5.87–7.13 (0.36)	6.32; 6.19
Condyllocanine length (CCL)	29.32, 29.08–29.64 (0.25)	26.98, 24.93–29.06 (1.10)	27.01; 27.01
Condylolincisive length (CIL)	30.61, 30.47–30.88 (0.2)	28.24, 26.09–30.34 (1.14)	28.8; 27.3
Coronoid height (COH)	8.66, 8.55–8.79 (0.09)	8.02, 7.07–9.19 (0.54)	8.67; 8.48
Dentary length (DENL)	22.04, 21.59–22.39 (0.29)	20.14, 18.31–21.83 (0.95)	19.97; 20.34
Greatest length of skull (GLS)	34.55, 34.13–35.3 (0.50)	31.67, 29.64–33.81 (1.11)	30.95; 31.33
Breadth across upper molars (M2-M2)	10.66, 10.4–10.82 (0.15)	9.75, 8.66–10.74 (0.51)	9.63; 9.28
Mandibular tooththrow length (MANDL)	12.2, 11.67–13.63 (0.72)	11.28, 10.4–11.96 (0.41)	11.54; 11.45
Molariform tooththrow (MLTRL)	6.93, 6.8–7.07 (0.11)	6.63, 6.18–7.1 (0.23)	8.32; 8.26
Mastoid (process) breadth (MPW)	15.83, 15.77–15.98 (0.10)	14.11, 13.09–15.78 (0.56)	14.52; 14.35
Maxillary tooththrow (MTRL)	11.31, 10.91–12.73 (0.7)	10.34, 9.5–11.04 (0.39)	10.56; 10.17
Post orbital constriction breadth (PB)	9.50, 9.29–9.73 (0.20)	8.98, 8.3–9.47 (0.26)	8.68; 8.77
Palatal length (PL)	15.21, 15.04–15.3 (0.15)	13.60, 12.05–15.18 (0.77)	13.37; 13.76
Zygomatic breadth (ZB)	17.38, 16.73–17.69 (0.34)	15.52, 14.21–16.85 (0.69)	15.25; 15.9
Braincase height (BCH)	11.96, 11.56–12.54 (0.42)	11.31, 10.11–13.56 (0.57)	12.83; 13.51
Calcar length (CL)	18.25	14.35, 11.05–17.73 (1.64)	14.14; 13.08
Forearm length (FA)	76.28, 65.43–81.64 (5.59)	71.57, 63.72–77.77 (3.29)	73.2; 73.2
Hindfoot length (HF)	20.07, 11.4–23 (4.31)	19.51, 14–23 (2.17)	20.12; 18.04
Metacarpal III (MET-III)	78.33, 75.04–81.6 (2.6)	67.48, 61.7–73.8 (3.03)	65.34; 66.81
Metacarpal IV (MET-IV)	76.9, 73.4–80.33 (2.48)	66.82, 58.73–72.96 (3.30)	64.88; 65.42
Metacarpal V (MET-V)	80.08, 75.96–83.05 (2.67)	69.25, 63.28–75.4 (3.27)	66.72; 67.83
Tibia length (TiL)	31.08, 29.5–32.73 (1.62)	28.84, 25.83–32.98 (1.97)	29.62; 28.46

Measurement	<i>P. s. septentrionalis</i> (<i>N</i> = 6)	<i>P. s. stenops</i> (<i>N</i> = 50)	<i>P. s. boliviensis</i> (<i>N</i> = 2)
Tail length (T)	16.88, 11.49–21 (4.45)	18.39, 7.44–25 (3.9)	14.65; 15.15
Total length (TL)	110.77, 100–130 (11.15)	110.90, 65–134 (13.04)	140; 140
Ear length (E)	26.69, 22.11–31 (3.36)	26.44, 15–32 (3.20)	21.7; 23.2

Regarding the Principal Component Analysis (PCA), the loadings table shows how each craniometric measurement contributes to the first three components (Appendix 3). Component 1 explains a large fraction of the variability (72.85%), with significant contributions from variables such as CCL and GLS. The high loadings on this component suggest that these measurements are particularly important to differentiate between individuals in the data set. Component 2, although explaining much less of the variance (7.93%), has a very high contribution of BCH, indicating that this measure is relevant to understanding differences between subspecies (shape component). Component 3, explaining 6.83% of variance, has a high negative loading for MLTRL, which could represent another pattern of variability in craniometric measurements. The PCA scatter plot shows the distribution of the two subspecies in the first two principal components. A trend towards a separation between the two subspecies is observed along the first component (horizontal axis), suggesting that it captures relevant variability that may be related to differences between subspecies. However, there is considerable overlap, indicating that the separation is not complete (or is only partly evolved; Figure 4-4).

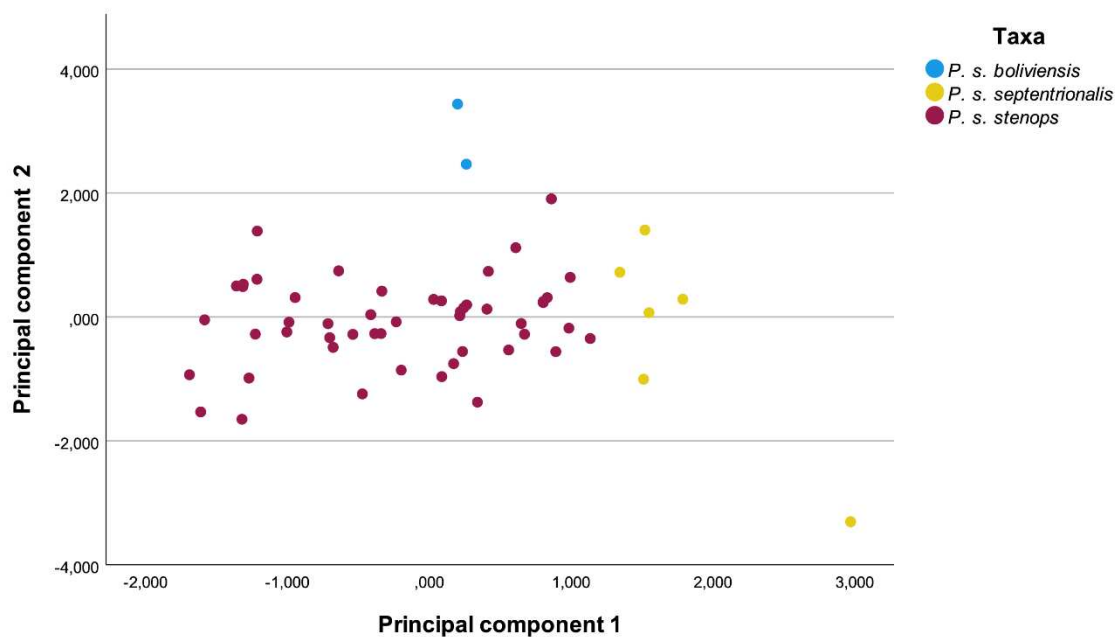


Figure 4-4. PCA scatter plot of *Phylloderma*. The analysis is based on 16 log-transformed craniodental measurements of 58 *Phylloderma* specimens.

On the other hand, *M. macrophyllum* does not present significant morphometric differences between the phylogroups or clades obtained in phylogenetic analyses, hereafter called the Panamanian-South American form and the North Central American form. From the descriptive statistics, it is observed that both attributed populations overlap in all their measurements (Table 4-3). Measurements of both groups or forms were normally distributed (Shapiro-Wilk significance values >0.05 ; not shown), and the ANOVA to test differences between sexes, using GLS as the dependent variable, showed that there were no differences within samples of the Panamanian-South American form, but that there were differences within samples of the North Central American form sample ($P < 0.05$); however, we attribute this last result to an artifact due to the sample size ($N = 10$).

Table 4-3. Morphological data of *Macrophyllum* forms. Measurements are given in millimeters. Descriptive measurements: arithmetic mean, minimum–maximum and (standard deviation).

Measurement	<i>Macrophyllum</i> Panamenian-South American form (N = 74)	<i>Macrophyllum</i> North Central American form (N = 10)
Breadth of brain case (BB)	8.03, 7.09–8.81(0.29)	8.09, 7.95–8.25 (0.1)
Palatal width at canines (C-C)	3.47, 2.96–3.88 (0.17)	3.4, 3.24–3.68 (0.12)
Condyl canine length (CCL)	14.18, 12.84–15.19 (0.37)	14.24, 13.89–14.51 (0.2)
Condyl incisive length (CIL)	14.81, 13.49–15.86 (0.38)	14.77, 14.32–15.07 (0.21)
Coronoid height (COH)	3.58, 2.39–4.07 (0.24)	3.53, 3.39–3.77 (0.12)
Dentary length (DENL)	10.59, 9.76–11.26 (0.27)	10.47, 10.08–10.81 (0.24)
Greatest length of skull (GLS)	17.09, 15.56–18.11 (0.42)	17.08, 16.78–17.38 (0.18)
Breadth across upper molars (M2-M2)	6.35, 5.73–6.91 (0.23)	6.24, 5.95–6.39 (0.14)
Mandibular toothrow length (MANDL)	6.1, 5.54–6.5 (0.19)	5.9, 5.85–6.23 (0.11)
Molariform toothrow (MLTRL)	4.18, 3.84–4.45 (0.13)	4.02, 3.9–4.29 (0.11)
Mastoid (process) breadth (MPW)	8.80, 7.92–9.22 (0.28)	8.9, 8.78–9.21 (0.14)
Maxillary toothrow (MTRL)	5.64, 5.07–6.12 (0.19)	5.59, 5.41–5.91 (0.14)
Post orbital constriction breadth (PB)	3.29, 2.92–3.57 (0.14)	3.24, 3.04–3.55 (0.17)
Palatal length (PL)	5.78, 3.47–6.51 (0.38)	5.83, 5.43–6.18 (0.22)
Zygomatic breadth (ZB)	9.46, 7.89–10.16 (0.43)	9.39, 9.2–9.56 (0.15)
Braincase height (BCH)	6.73, 5.6–7.52 (0.34)	6.52, 6.18–7.29 (0.35)
Calcar length (CL)	19.30, 16.53–24.15 (1.53)	19.27, 18.38–20.06 (0.76)
Forearm length (FA)	34.87, 27.52–38.35 (1.95)	35.9, 33.45–37.5 (1.33)
Hindfoot length (HF)	13.49, 10.24–16 (1.53)	12.4, 10–14 (1.26)
Metacarpal III (MET-III)	34.77, 27.85–39.94 (1.94)	35.86, 33.29–37.62 (1.41)
Metacarpal IV (MET-IV)	33.22, 26.67–38.16 (1.73)	33.7, 31.5–35.52 (1.40)
Metacarpal V (MET-V)	34.47, 27.4–39.88 (1.79)	35, 30.87–37.03 (1.80)
Tibia length (TiL)	15.02, 13.12–18.26 (0.9)	15.40, 14.43–16.1 (0.72)
Tail length (T)	40.49, 30–49 (3.78)	44.3, 37–53 (4.45)
Total length (TL)	89.9, 73–102 (5.68)	92.7, 90–97 (2.06)
Ear length (E)	17.82, 11.38–21 (1.83)	17.9, 17–19 (0.74)

The Levene's test to assess the equality of variances in the independent *t*-test showed non-significant values ($P > 0.05$), so we accept the null hypothesis of equality of population variances and refer to the significance value of the *t*-test assuming equal variances. For the Panamanian-South American form and the North Central American form, almost all variables examined showed that there are no significant differences between the population variances ($P > 0.05$, Supplementary data 8); which shows that, in terms of the metric variables, both groups are equal in size. The Principal Component Analysis is consistent with these results: the analysis extracted four components; however, it failed to reduce the dataset in informative variables, as well as to find general trends in cranial size and shape (Appendix 3). The Principal Component Analysis scatter plot represents the first two principal components of the data set, that is, linear combinations of the original variables that capture the greatest possible variability in the data set (Figure 4-5). Most of the points are concentrated around the center of the graph, with some outliers scattered farther away from the center. This suggests that there is considerable variability in the two components among all individuals in both populations. However, there does not appear to be a clear separation between the two *M. macrophyllum* forms, because the points overlap substantially. This indicates that the populations are similar in craniometric measurements or that more components are needed to clearly distinguish them. An analysis of the loadings of the original variables on the first four components shows specifically that in component 1 loadings are large and positive, suggesting that it summarizes size. The highest loadings in this component correspond to COH, PL, and to a lesser extent to ZB and BCH (Appendix 3). This indicates that these measurements contribute more to the variation captured by the first component; however, no pattern or correlation is observed between the original variables and the co-components. There are three seemingly outliers of the point cloud that correspond to specimens from Beni, Bolivia (AMNH209320); Darien, Panama (USNM

306547); and Valle del Cauca, Colombia (UV 13630), all from the Panamanian-South American form of *M. macrophyllum*.

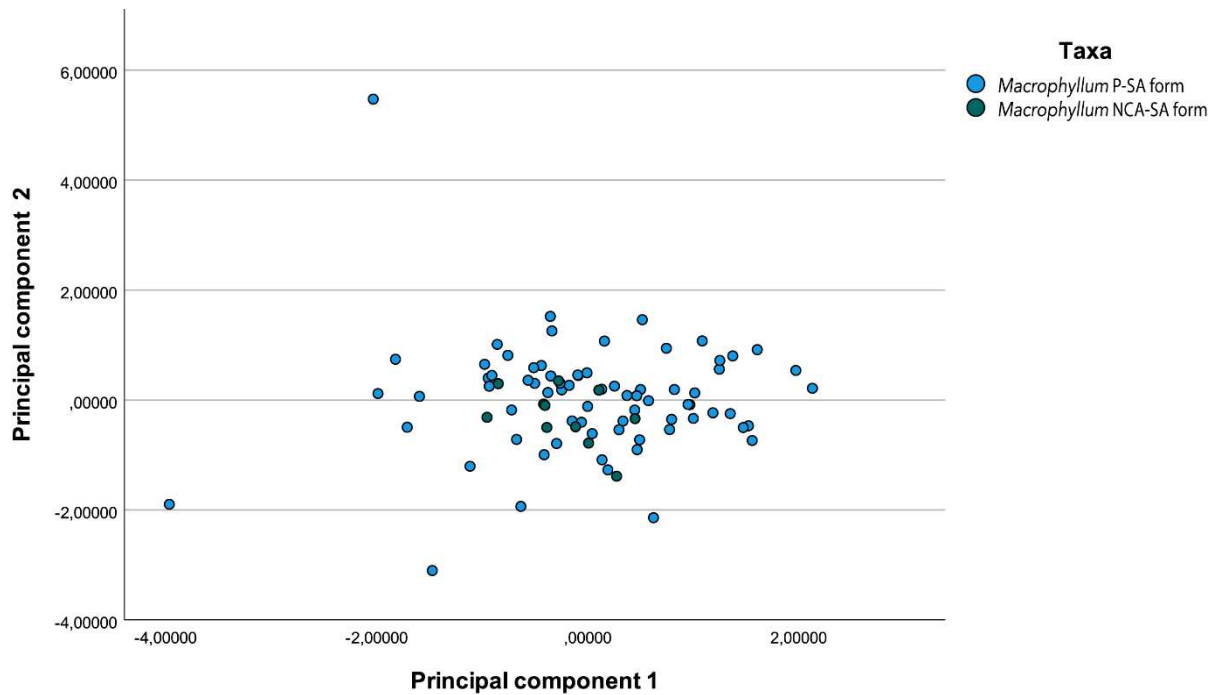


Figure 4-5. PCA scatter plot of *Macrophyllum*. The analysis is based on 16 log-transformed craniodental measurements of 84 *Macrophyllum* specimens.

We present a revision of the genus *Phylloderma* and the amended descriptions of *P. s. septentrionalis* and *P. s. stenops* below.

Taxonomy

Family Phyllostomidae Gray, 1825

Subfamily Phyllostominae Gray, 1825

Genus *Phylloderma* Peters, 1865

***Phylloderma septentrionalis* Goodwin 1940**

Northern Pale-faced Bat

Phylloderma stenops septentrionalis Handley 1966:762

Holotype. Adult female; AMNH 126868; collector Cecil F. Underwood (Collector number 2033). Skin with skull removed (Goodwin, 1940). The specimen is housed in the American Museum of Natural History, New York, USA.

Type locality. Las Pilas, 6 miles north of Marcala, about 1200 m of elevation; Department of La Paz, Honduras

Paratypes. Two adult females from La Flor Archaga, Honduras (Goodwin, 1940); museum numbers AMNH 126867 and AMNH 126869. The sequence of the complete mitogenomes of the specimen AMNH 126867 are available in the Genbank (Accession number *pending*).

Distribution and habitat. *Phylloderma septentrionalis* occurs from southern Mexico (Oaxaca and Chiapas) to Nicaragua, presumably with the Hess Escarpment and the Talamanca Cordillera as the limit of its distribution (Figure 4-6). This species is considered rare and inhabits primarily lowland and humid forest, although it has been recorded from a variety of forested and agricultural habitats (Carter et al., 1966; Santos-Moreno & Gallardo, 2014; Timm et al., 1989).

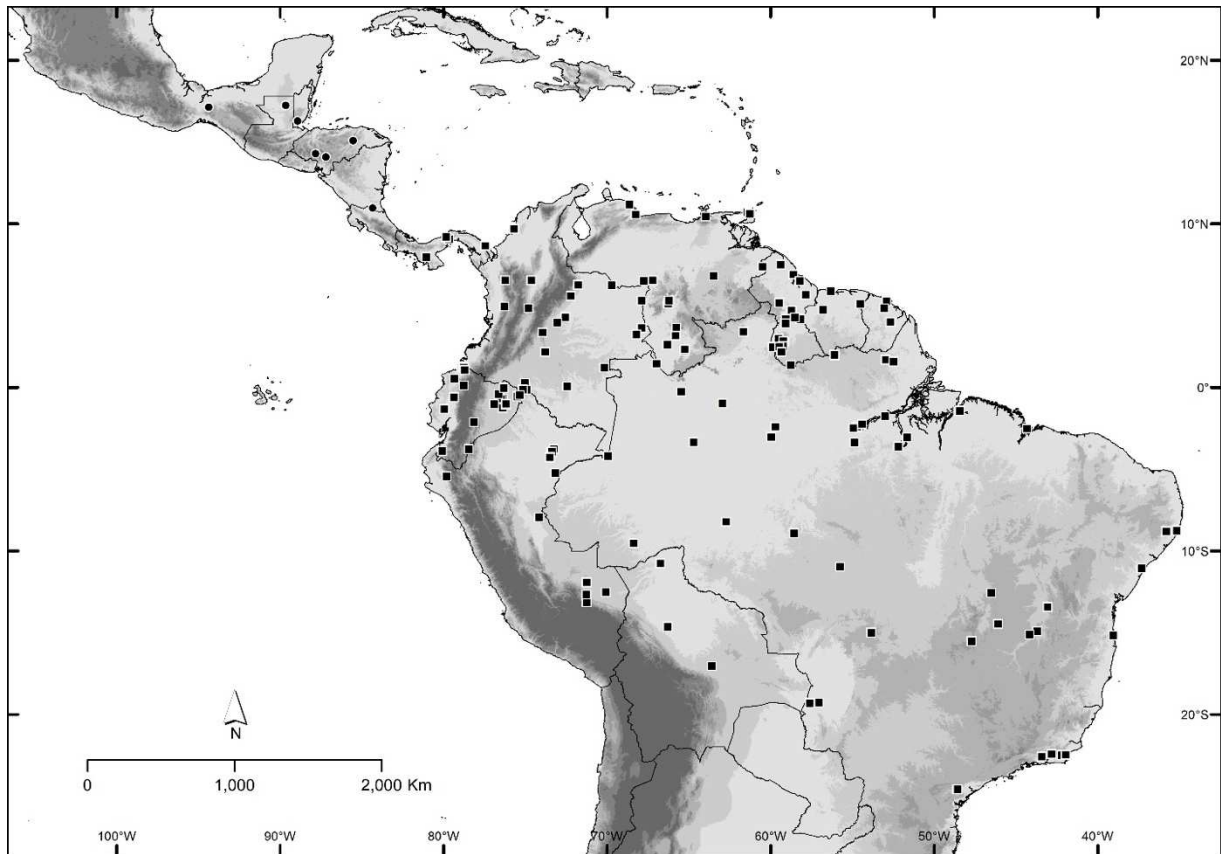


Figure 4-6. Distribution of *Phylloderma septentrionalis* and *Phylloderma stenops*. Circles: distribution of *Phylloderma septentrionalis*; squares: emended distribution of *Phylloderma stenops*.

Diagnosis and description. *Phylloderma septentrionalis* is a large and robust species. Reported measurements for this species include Total length (TL), 100–137 mm, forearm length (FA), 65.4–81.6 mm; and greatest skull length (GSL), 34.1–35.3 mm. Externally it is similar to *P. stenops*, but larger and differing in cranial characters (Goodwin, 1940). The chin has a V-shaped naked cushion margined by round papillae. The nose leaf is slender. The hair is short. The upperparts are brown with lighter bases; the underparts are lighter in color that extend on the shoulders and sides of neck; the wing membranes are blackish brown (Goodwin, 1940; Hall & Kelson, 1959). The ears are large and pointed, with slight undulations on their posterior half; the tragus is long and narrow. The tail is visible from the upper side of the uropatagium.

The upper inner incisors are bilobed; the upper external incisors are small, without evident lobulation, although the cutting edges are not uniform. The lower external incisors have irregular cutting edges, but not evidently bilobed and somewhat smaller than the internal ones. Goodwin (1940) noted that the inner lower incisors are twice as wide as the outer; however, they are not wider, but longer. The anterior face of the upper canine lacks longitudinal grooves. The lower canine has a cingulum on its inner side rising less above level of incisors when viewed from the front. The anterior upper premolar (P1) is in contact with canine anteriorly and with the posterior upper premolar (P3) posteriorly, but not mounted on it. The second lower premolar (p3) is tiny and is located between the other two premolars. The main cusp of the third lower premolar (p4) lacks a point. The upper molars have shallow metacones and paracones and the metastylar and parastylar shelves are relatively shorter, with a distinct W-shaped pattern. The lower molars have trigonids with sharp and serrated cuspids (Goodwin, 1940). The braincase is relatively high and rounded.

The rostrum is shorter than the braincase, with no depression between the orbits. It is also rather wide, giving a robust appearance to the skull. The zygomatic arches are rounded and complete (Goodwin, 1940; Figure 4-6).

Comparisons. The averages of the craniodental and external measurements analyzed in this study were higher for *P. septentrionalis* than for *P. stenops*. In the cases of GLS, MET III, METIV and METV, the measurements do not overlap and are always greater in the North Central American species. The skull differs in size, but not in shape, although there are some differences in the teeth worth noting: the upper inner incisors are bilobed in *P. septentrionalis*, but with smooth or weakly bifid cutting edges in *P. stenops* (Barquez & Ojeda, 1979); the main cusp of the lower third premolar (p4) is not pointed in *P. septentrionalis*, but the contrary in *P. stenops*; the metacones and paracones are rather shallow, and the metastylar and parastylar

shelves are shorter in *P. septentrionalis* compared to *P. stenops*. The ears of *P. septentrionalis* are pointed, with less visible striations, while in *P. stenops* they are rounded with more marked inner striations (Barquez & Ojeda, 1979; Figure 4-7).



Figure 4-7. Skull morphology of *Phylloderma septentrionalis* and *Phylloderma stenops*. Dorsal, ventral, and lateral views of the skulls and lateral view of the mandibles of the holotype of *Phylloderma septentrionalis* (left), and *Phylloderma stenops* (right). Photos: M. Alejandra Camacho (AMNH 126868) and Rubén D. Jarrín (QCAZ 18826).

Natural history. This species consumes fruits, other plant materials, and insects (Laval, 1977; McCarthy, 1982; York, 2008). York (2008) suggested that it may be an effective seed disperser for the Annonaceae and Cucurbitaceae; however, the details of dietary breath in the species remain unknown. Little is known about the reproductive cycle: LaVal (1977) reported capturing a pregnant female with a single embryo during the dry season (early February) in Costa Rica.

Conservation status. The International Union for Conservation of Nature (IUCN) classifies this species as 'Least Concern'. However, the Mesoamerican taxon (formerly *Phylloderma s. septentrionalis*) is listed as 'Threatened' within the Conservation Initiatives for the Mammals of Oaxaca, Mexico (Briones-Salas et al., 2016), and is deemed 'Critically Endangered' in Nicaragua (Medina-Fitoria, 2014; Nassar et al., 2020). In Honduras, it has not been categorized under any risk or threat category (Turcios-Casco et al., 2020), and its conservation status remains unstudied in Guatemala, Belize, and El Salvador. Given its rarity in Central America, and its restriction to well-preserved habitats, its vulnerability increases with threats to ecosystems. Hopefully, recognition of *P. septentrionalis* at the species level will catalyze targeted conservation efforts and activities that safeguard it against imminent risks in Central America, such as deforestation and climate change.

Family Phyllostomidae Gray, 1825

Subfamily Phyllostominae Gray, 1825

***Phylloderma stenops* Peters, 1865**

Southern Pale-faced Bat

Ph[ylostoma (Phylloderma)] stenops W. Peters 1865. RNH 16843: adult, probably male; mounted skin, skull removed; “Cayenne”, French Guiana.

G[uandira] cayanensis Gray 1866. Locality “Cayenne,” French Guiana.

Phylloderma stenops Dobson 1878. Recognize *Phylloderma* as a genus and invalids *Guandira cayanensis*

Phylloderma stenops Handley 1966. Pg 762. Synonymized *septentrionalis* under *stenops* but recognized both as subspecies.

Phyllostomus stenops Baker et al. 1988: 13

Phylloderma stenops returns to the use of *Phylloderma* pg. 46 Timm et al 1989.

Two subspecies are recognized: *Phylloderma stenops stenops* and *Phylloderma stenops boliviensis*

Type. An adult, probably male. Skin with skull removed (RNH 16843), collector and date of capture unknown. The specimen might be in the Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands (Carter & Dolan, 1978).

Type locality. Cayenne, French Guiana (Peters, 1865).

Amended distribution and habitat. The species is distributed from the south of the Central Cordillera in Panama to South America, including the Guianas, Venezuela, Trinidad and Tobago, Colombia, Ecuador, Perú, Bolivia, and Brazil (Figure 4-8). The species is considered rare, and primarily inhabits lowland and humid vegetation types, such as evergreen, mesic or riparian forests, inundated floodplain forests, deciduous forests, also extending into drier regions such as dry and xeric shrublands (Bomfim et al., 2017; Carrera et al., 2010; Ramírez-Fráncel et al., 2015; Salas et al., 2014; Williams & Genoways, 2008). Salas et al. (2014) recorded the species in a place heavily disrupted by livestock, with small remnants of riparian vegetation.

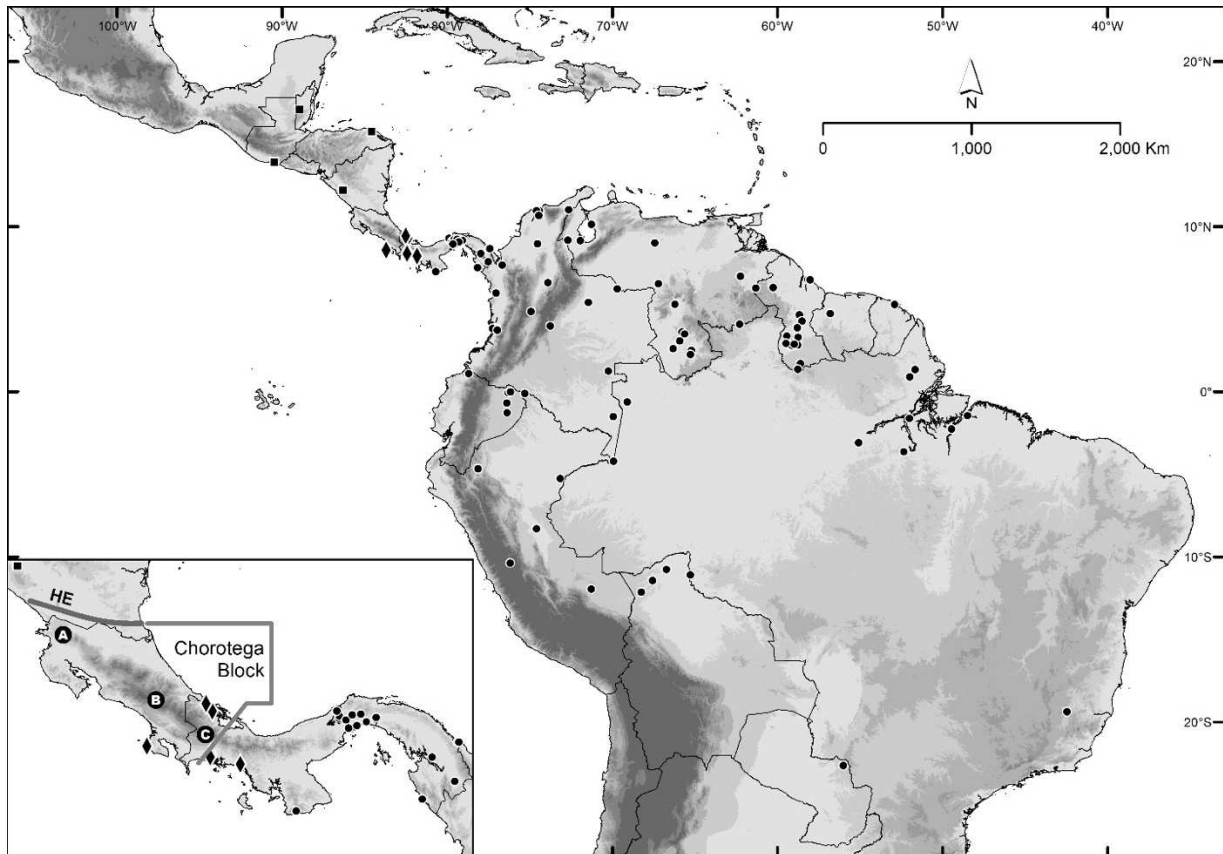


Figure 4-8. Distribution of *Macrophyllum macrophyllum* and *Macrophyllum* sp. nov. Circles: *Macrophyllum macrophyllum*; squares: *Macrophyllum* sp. nov. Insert shows area of uncertain specimens that need a molecular revision (diamonds). A, Guanacaste Cordillera; B, Central Cordillera of Costa Rica; C, Talamanca Cordillera; HE, Hess Escarpment (Gutiérrez-García & Vázquez-Domínguez, 2013; Marshall, 2007).

Amended diagnosis and description. *Phylloderma stenops* can be characterized as a large and robust bat. Reported measurements for this species include total length (TL), 65–140 mm; forearm length (FA), 63.7–81 mm; and greatest skull length (GSL), 29–33.8 mm. Apart from the general characteristics of its genus, this species is distinguished by a robust skull, and a low (relatively flat) facial profile. The chin is bare, V-shaped, and encircled by a cushion bordered by a row of rounded or elongated papillae (Trujillo & Albuja, 2005). The nose leaf is wide at the base and has a pointed tip. The fur is short, with dorsal hairs exhibiting a reddish-brown color. The wing membranes attach high on the back, creating a partially nude appearance; the wingtips are whitish. The ears are well separated, moderately large, and rounded; the tragus is long and slender. The calcar is approximately equal to or shorter than the length of the foot, and

the tail extends only halfway inside the uropatagium, protruding slightly from the dorsal surface (Barquez & Ojeda, 1979; Díaz et al., 2011; Emmons & Feer, 1999; Husson, 1962; Martínez-Cerón et al., 2019).

The upper inner incisors are relatively long compared to the external ones, with smooth or weakly bifid cutting edges, and usually converging distally. The upper external incisors are bilobed and slightly less than half the size of the internal ones. Barquez & Ojeda (1979), referring to *Phylloderma stenops boliviensis*, pointed out that its upper internal incisors are not bilobed; although it may be a characteristic of the subspecies, or of the two individuals used for the description; this cannot be considered a diagnostic character because there is variability in the sample studied. The lower inner incisors are bilobed, or at least a medial notch is clearly visible which denotes that they are weakly lobed (Figure 4-6). The lower external incisors have cutting edges that are irregular, but are not evidently lobed, are smaller than the internal ones, and are covered by the cingula of the canines. Barquez & Ojeda (1979) also pointed out that the four lower incisors are even, probably referring to the fact that they do not have lobes; but, again, although it may be a characteristic of the subspecies, or of the two individuals used for the description of *P. s. boliviensis*, this is not a diagnostic character of the species. The upper canines are subtriangular, the anterior face of upper canine lacks longitudinal grooves. There is a prominent cingulum on the anterolingual side, in contact with or covering the back of the upper external incisors. Lower canines rise prominently above level of incisors if viewed from the front. The anterior upper premolar (P1) is in contact with the canine and with posterior upper premolar (P3), and the posterior cingulum is mounted on the labial side. P3 is caniform, with its posterior cusp in contact with M1. The anterior lower premolar (p2) rests on the canine. The second premolar (p3) is minuscule and enclosed between the other premolars and is not displaced lingually from the toothrow. The main cusp of the third premolar (p4) is pointed. The upper molars are large, with a tall anterior paracone and a posterior metacone, and with radiating

crest with a distinct W-shaped pattern. The metastylar and parastylar shelves are relatively large and give the impression of tall crowns. M3 has a postparacrista only and is V-shaped. The trigonids of the lower molars have a large buccal protoconid flanked anterolingually by the paraconid, and posterolingually by the metaconid; when viewed laterally the dental arcade of the lower molars is sharp and serrated.

The braincase is relatively high and rounded. The rostrum is shorter than the braincase and has a dorsal profile that is not convex and does not have a depression between the orbits. The zygomatic arches are rounded and complete.

The diploid number is 32, and the fundamental number is 58 (de Souza et al., 2022).

Comparisons. On the average, all craniodental and external measurements are smaller in *P. stenops* than in *P. septentrionalis*, with overlapping ranges except for GLS, MET III, MET IV, and MET V. The skull differs in size but not in shape. The upper inner incisors have smooth or weakly bifid cutting edges in *P. stenops*, as opposed to being clearly bifid in *P. septentrionalis*. The main cusp of the third premolar (p4) is more pointed in *P. stenops* than in *P. septentrionalis*. Metacones and paracones are not as deep, and the metastylar and parastylar shelves are relatively shorter in *P. septentrionalis* than in *P. stenops*. The ears of *P. stenops* are rounded with distinct marginal striations on the inner surface, whereas in *P. septentrionalis* the ears are more pointed with less visible striations (Barquez & Ojeda, 1979; Figure 4-7).

A comparison of the two South American subspecies reveals that *P. s. boliviensis* is larger than *P. s. stenops*, although this conclusion is based on the only two specimens reported to date. The lower incisors of *P. s. stenops* are even and unlobed, whereas in *P. s. boliviensis* the middle incisors are longer than the outer ones and bilobed (as in *P. septentrionalis*). The dorsal fur of *P. s. stenops* is distributed uniformly, whereas in *P. s. boliviensis* there is a clearer zone at the neck level (Barquez & Ojeda, 1979).

Natural history. There is limited knowledge regarding the natural history of the genus *Phylloderma*. Apparently, *P. stenops* is omnivorous. Its diet primarily consists of fruits of the families Annonaceae, Cucurbitaceae, Myrtaceae, and Cecropiaceae; and has been documented consuming insects, insect larvae, and pupae from an active nest of a social wasp (Giannini & Kalko, 2004; Jeanne, 1970), as well as lizards (Pye, 1967), and possibly other small vertebrates (Esbérard & Faria, 2006; Jeanne, 1970). The species typically inhabits tropical forests below 1000 m (Carrera et al., 2010), and seems to prefer foraging in natural clearings beneath the dense canopy of pristine lowland forests (Koopman, 1976). *Phylloderma stenops* commonly roosts in caves (Trajano, 1982; Bredt et al., 1999; Esbérard et al., 2005). Based on observations in captivity in Brazil, Esberard (2012) affirmed that *P. stenops* shows a polyestric strategy, with seasonal reproduction; and presents birth synchrony and postpartum oestrus; also the gestational period does not exceed 167 days, and sexual maturity is reached around the age of 241–285 days. The bat fly, *Strebla christinae*, has been reported from this species (Handley, 1966).

Remarks. Peters (1865) originally described the species based on a mounted specimen, without taking measurements, providing only a description of dental number and shape, as well as of the form of the skull, noting its similarity to that of *Phyllostomus*. He mentioned size and shape to be comparable to those of *P. hastatus*, species from which it is differentiated by a narrower shoulder girdle (Peters, 1865). Handley (1966) observed specimens from Panama to exhibit mixed traits of both nominal species of *Phylloderma* (*P. stenops* and *P. septentrionalis*) yet agreeing more with the former. Consequently, he referred to the Panamanian individuals as *P. s. stenops*, thus acknowledging subspecific differentiation, and synonymized *septentrionalis* with *stenops*.

Herein, we recognize two well supported mitochondrial clades of *Macrophyllum macrophyllum*. In doing so, we consider the North Central American form to be specifically and cryptically distinct from the Panamanian-South American form. Thus, we describe it as a new species.

Family Phyllostomidae Gray, 1825

Subfamily Phyllostominae Gray, 1825

Genus *Macrophyllum* Gray, 1838

***Macrophyllum* sp. nov. Camacho & Burneo**

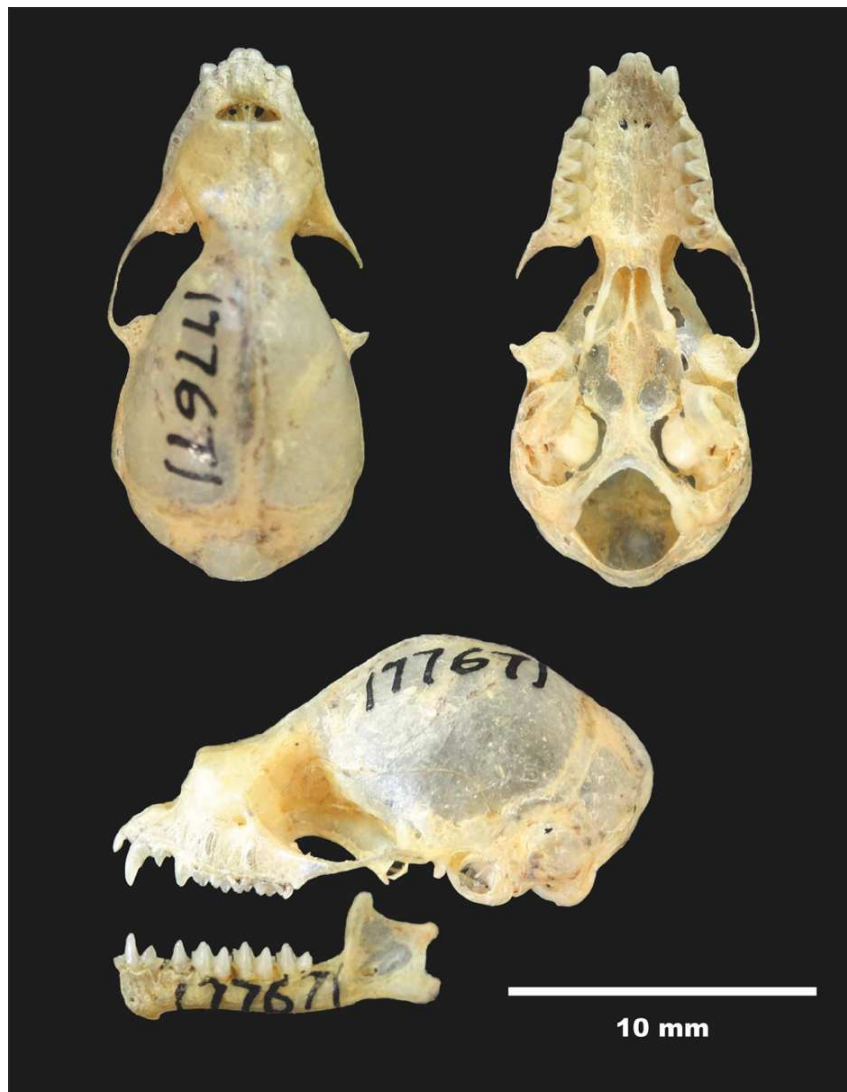


Figure 4-9. Skull morphology of *Macrophyllum* sp. nov. Dorsal, ventral, and lateral views of the skull and lateral view of the mandible of a specimen of *Macrophyllum* sp. nov. (AMNH 177671, locality Lake Jilola, Managua, Nicaragua).

Holotype. An adult female preserved as skin and skull, housed at the American Museum of Natural History, New York, USA (AMNH 177671, Figure 4-9). The specimen was collected by Charles R. Lewis on February 4, 1958 (original field number 1136). The sequence of the complete mitogenome of the specimen AMNH 177671 has been uploaded to the Genbank (Accession number *pending*).

Type locality. Lake Jiloa, Managua, Nicaragua (12.22°, -86.32°).

Paratypes. Two specimens housed at the American Museum of Natural History, New York, USA: AMNH 177664, an adult male collected by Rodney Atkins on February 3, 1958 (original field number 871), and AMNH 177665; an adult female also collected by Rodney Atkins on February 3, 1958 (original field number 872). Both specimens are preserved as skin and skull. The sequences of the complete mitogenomes were uploaded to the Genbank (Accession numbers *pending*).

Distribution. Currently known from southeastern Mexico (Tabasco and Chiapas) to Nicaragua. Possibly Costa Rica (Figure 4-8).

Diagnosis and comparisons. *Macrophyllum* sp. nov. (Figure 4-9) is diagnosable mainly based on geographic distribution and mitochondrial data. This cryptic species is recognized as a genetically highly distinct group based on complete mitochondrial genomes. *Macrophyllum* sp. nov. is a small and slender bat: TL, 90–97 mm; forearm, 33.5–37.5 mm; GLS, 16.8–17.4 mm. These measurements overlap with those of *M. macrophyllum*. In external appearance, it is similar to and is almost indistinguishable from *M. macrophyllum*, which is its Panamanian–South American counterpart. However, the coronoid height (COH) and palatal length (PL) show jaw shape to be the main source of variation and might indicate an incipient geographical differentiation. As its congener, *Macrophyllum* sp. nov. is easily recognized and separable from bats of other genera based on its elongated legs, feet, and claws, and on its long interfemoral

membrane, supported by the tail, which extends to its margin, and the calcanea, which are longer than the tibiae. It is also distinguishable because of the ventral appearance of the interfemoral membrane, which is ornamented with longitudinal rows of projecting saw-like dermal denticles (Goodwin, 1946; Handley, 1957; Harrison, 1975), and because the nose leaf is prominent and lanceolate, with a median ridge (Harrison, 1975). The fur is long, dense, and light brown to reddish, with bases lighter than tips. Felten (1955b) had already noted a much lighter and redder coloration in specimens from El Salvador than in those from Guiana reported by Goodwin (1946).

Measurements. The ranges of 16 craniomandibular measurements and 10 external measurements are provided in Table 4-3.

Ecology. The species occurs in rainforests, particularly near watercourses (Harrison, 1975; Harrison & Pendleton, 1974; Williams & Genoways, 2008). Observations indicate that individuals often congregate in small groups within sea caves, vaulted caverns, and rock crevices near water bodies (Bloedel, 1955; Hill & Bown, 1963; Linares, 1966). Reports from Nicaragua indicate that specimens were captured shortly after dusk in a mist net stretched across bodies of water, where bats were flying close to the bank and probably not more than a foot above the water (Davis et al., 1964; Valdez & LaVal, 1971). *Macrophyllum* sp. nov. is insectivorous. Detailed analysis of stomach contents from specimens in El Salvador confirmed a diet predominantly composed of aerial insects of the orders Lepidoptera and Diptera (Harrison, 1975; Harrison & Pendleton, 1974). Davis et al. (1964) proposed aquatic hunting as the foraging method. Roosting groups may exhibit a harem-like system, inferred from the male to female ratio heavily skewed towards females (Seymour & Dickerman, 1982). Furthermore, findings of all-male roosts suggest that non-harem males roost communally. The absence of subadult males could indicate either earlier dispersal, or exclusion from the group by a dominant

male (Felten, 1955; Wilson & Gamarra de Fox, 1991) or that females use alternate roosts (Seymour & Dickerman, 1982). Pregnant females have been observed in both wet and dry seasons in El Salvador (Felten, 1955; Seymour & Dickerman, 1982; Wilson, 1979); there is a report of pregnancy in April (23 mm embryo) in Chiapas, Mexico (Medellin et al., 1986). From their observations in El Salvador, Harrison and Pendleton (1974) reported the embryos to possess disproportionately long feet and claws, similar in size to the forearms and nearly half the crown-rump length, as well as the species-specific dermal denticles on the interfemoral membrane distinctively present at early stages of embryonic development (Harrison & Pendleton, 1974). This species has been reported living in association with *Carollia subrufa* and *Glossophaga sp.* (Seymour & Dickerman, 1982).

Conservation. Conservation is hampered by the lack of information. Future efforts must focus on monitoring populations, identifying key habitats, and understanding the species' role in ecosystem functioning. The development of management strategies should be guided by research exploring the effects of deforestation, habitat fragmentation, and climate change.

Discussion

In this study, we have examined phylogenetic relationships and cryptic diversity within *Phylloderma* and *Macrophyllum*, using both morphological and phylogenetic approaches. We employed complete mitochondrial genomes, and additionally generated sequences for outgroups that were sequenced for the first time for science. Some of these samples are ancient, dating from 1912 to 2019. It has already been demonstrated that under adequate analytical conditions, complete mitogenomes prove to be useful for resolving patterns of phylogenetic relationships within phyllostomids. In cases such as that of this study, it is particularly interesting to consider that genome skimming is likely the most efficient method to analyze museum specimens, or highly degraded material (Camacho et al., 2022).

***Phylloderma*: cleaning up a taxonomic disarray**

The morphometric patterns found in this study, particularly for the larger-sized *Phylloderma s. septentrionalis* (compared to *P. s. stenops* and *P. s. boliviensis*), are consistent with the previous findings of Peters (Peters, 1865), Goodwin (1940), and Barquez & Ojeda (1979). Our results confirm that *P. s. septentrionalis* exhibits greater dimensions, both in craniomandibular and external measurements, compared to its South American counterparts, *P. s. stenops* and *P. s. boliviensis*. A deeper analysis of population variability between *P. s. stenops* and *P. s. septentrionalis* across all measurements showed highly significant differences, and the PCA accounted for a significant portion of the variance (72.85%) in component 1, with notable contributions from measurements such as CCL and GLS, which were thus important in distinguishing among individuals within the dataset. We identified two highly supported clades separating Honduran individuals (*P. s. septentrionalis*) from Panamanian and South American ones (*P. s. stenops*), with a clear genetic separation amounting to by an 8.60% genetic divergence.

The findings of our analysis for *Phylloderma* revealed two facts: first, the morphological evidence strongly supports the classification of *P. s. septentrionalis* as a distinct taxonomic unit, distinguished by its larger size. Across all measurements, the North Central American form consistently shows greater averages than the Panamanian-South American form, despite some overlap in size. Notably, no overlap exists with respect to GLS, MET III, MET IV, and MET V, which are measurements showing *P. s. septentrionalis* to be consistently larger. The skull differs in size, but not in shape; however, noteworthy dental differences exist (see Taxonomy section). Although specimens from the type series of *P. s. boliviensis* were not included in the statistical analysis owing to sample size limitations, descriptive data suggest that this Bolivian endemic subspecies falls within the intermediate size range. Secondly, mitochondrial DNA (mtDNA)

data reveals restricted gene flow among these subspecies. This evidence points to the uniqueness of the North Central American pale-faced bat's population, suggesting that it represents a distinct species, for which we propose formal elevation to the species rank.

P. septentrionalis would thus have a distribution limited from southern Mexico (Oaxaca and Chiapas) to Costa Rica (Carter et al., 1966; Santos-Moreno & Gallardo, 2014; Timm et al., 1989). *P. stenops* would occur from Southern Panama to South America. We here amend the distribution proposed by Solari et al. (2019), who suggested the range of *P. s. septentrionalis* to be from Southern Mexico and Central America to northwestern South America (western Colombia, western Ecuador, and northwestern Peru). This distribution pattern is similar to that observed in other species, e.g., *Uroderma convexum*, *Tonatia bakeri*, *Lophostoma occidentale* (Basantes et al., 2020; Mantilla-Meluk, 2014; Paúl M. Velazco & Cadenillas, 2011). Furthermore, Martínez-Cerón et al. (2019) suggested a trans-Andean clade for *Phylloderma*, distinct from a cis-Andean clade more closely related to specimens from eastern Ecuador and the Guiana Shield; however, this trans-Andean clade, composed of samples from Colombia and Panama, appears nested in the larger cis-Andean clade, thus not supporting the existence of a trans-Andean clade. Additionally, this study did not include samples from North Central America, leaving the phylogeographic limits unclear.

***Macrophyllum*: unveiling cryptic diversity**

The phylogenetic analysis for *M. macrophyllum* also showed two distinct clades, both with robust support. One phylogroup correspond to individuals from Nicaragua (North Central American form) and the other, to individuals from Panama and South America, with a remarkable genetic divergence of 8.36 %. This findings within *M. macrophyllum* might suggests congruent evolutionary processes to those observed in *Phylloderma*.

For *M. macrophyllum*, morphometric analyses were unable to differentiate the populations under study; however, phylogenetic analyses revealed that the North Central American form and the Panamanian-South American form are genetically distinct, with geographic boundaries aligning with significant geographical features in Central America (see Biogeography section). The mitochondrial divergence within the North Central American form of *Macrophyllum* indicates that it constitutes a distinct albeit cryptic species, meaning that morphology fails to delimit it (Jörger & Schrödl, 2013).

For now, and until more data from regional collections in Central American countries are available, molecular datasets are currently the unequivocal method to discriminate between the two evolutionary lineages. It is documented that speciation events in bats could occur without noticeable morphological changes, as demonstrated for *Rhogeessa genowaysi* by Baker (1984), *Eumops wilsoni* by Baker et al. (2009), and species of *Uroderma* (Mantilla-Meluk, 2014). As in the cases of these species, our results show that traditional taxonomic characters are insufficient to delineate species boundaries within *Macrophyllum*; however, the divergent North Central American lineage is strongly supported by phylogenetic analyses. The divergence value observed between the phylogroups of *M. macrophyllum* (> 8%) falls within the range typically observed in comparisons between congeneric chiropteran species (2–11%, *Cytb*), as shown by Bradley and Baker (2001); and is even greater than in other species, such as the recently described *Vampyressa villai*, which diverges from its sister species *V. thyone* by 4.66% (Garbino et al., 2024), a genetic distance and is comparable to the mean mitochondrial divergence found between congeneric species of other phyllostomids: 7% for *Sturnira*, 9% for *Artibeus*, 7.5% for *Carollia*, 5.5% for *Chiroderma*, 10% for *Dermanura*, and 7.2% for *Platyrrhinus* (Bradley & Baker, 2001; Molinari et al., 2017; Velazco, 2013; Velazco & Patterson, 2008).

In the mentioned cases, as in our study, we acknowledge that the species described are defined primarily on mtDNA, and that reliance only on mtDNA is not ideal because a phylogeny does not necessarily represent a species tree (Andersen & Light, 2012; Ballard & Whitlock, 2004; Maddison, 1997). However, mtDNA data are adequate to address phylogeographic questions, especially when the goal of the study is to examine taxonomic or geographic limits of recently evolved species (Andersen & Light, 2012). Advocates of a morphology-based taxonomy might argue that we have not pursued a fully integrative approach, given that we have not included diagnostic characters for describing the new *Macrophyllum* species, and it could be debated that we should have waited to gather a larger sample, invoking a principle of caution. Our results align with case 7 of the “Mutually Exclusive Combinations of Morphological and Genetic Differentiation” proposed by Molinari (2023) to assign populations to different evolutionary categories, based on the primacy of genetics over morphology to infer phylogenetic relationships. Thus, no matter if a set of populations is poorly (cryptic species) differentiated morphologically, it must be recognized as a species if genetics mandate so (Oyston et al., 2022).

The absence of clear morphological diagnostic characters does not necessarily indicate a lack of biological or genetic barriers between populations, nor does it mean that these populations are indistinguishable in terms of ecology, behavior, or evolutionary history (Delić et al., 2017). The challenge of identifying species through traditional morphology should not prevent us from recognizing the evolutionary histories that have resulted in forms too similar to be differentiated morphologically, or that have led lineages to vary in many attributes other than their physical appearance (Delić et al., 2017). In most cases, molecular methods delineate species with greater precision than traditional morphological approaches, and even offer statistical support for species hypotheses (Fujita et al., 2012; Oyston et al., 2022). Neglecting the description of cryptic species could potentially lead to issues, such as a failure to assess true diversity for conservation planning. More studies on the genus *Macrophyllum* are needed: we believe that it

is essential to prioritize the collection and field study of this and other rare or neglected species. The discovery of cryptic species is crucial to our understanding of evolutionary processes, historical biogeography, ecology, and is also integral to conservation strategies (Delić et al., 2017).

In the case of *Macrophyllum*, while statistical analyses failed to find highly significant differences in the measurements that we analysed, we were able to identify slight trends towards a larger size in jaw measurements. The highest loadings on the first principal component of our analysis were coronoid height (COH) and palatal length (PL), indicating that jaw shape was the main source of variation, and suggesting the potential for incipient geographical variation in jaw anatomy between the two major monophyletic groups of the genus. This same pattern of variation in mandible shape has been previously observed in species complexes, such as *Glossophaga soricina* (Calahorra-Oliart et al., 2021), and *Tonatia maresi* and *T. bakeri* (Basantes et al., 2020).

The shape and size of the mandible, and particularly the correlation between the proportions of the coronoid and condylar processes and the size of the mastication muscles, account for part of the bite force (Freeman, 1998; Popowics & Herring, 2006). Generally, more pronounced coronoids positioned further from the condylar process, and thicker dental bones, are indicators of a stronger bite, which would allow the consumption of hard-shelled prey; whereas coronoids close to the condylar process and thinner dentaries suggest the consumption of softer prey (López-Aguirre et al., 2015; López-Cuamatzi et al., 2024).

The diet of *Macrophyllum* has not been studied in detail. It is known that it is predominantly composed of aerial insects such as Lepidoptera and Diptera (Harrison, 1975; Harrison & Pendleton, 1974). As mentioned before, cranial morphology and morphometry could suggest a certain degree of specialization in the type of food consumed; however, what a species eats

most likely varies according to the relative abundance of insects, and competition with other species, among other factors (Freeman, 1981). This is why it is important to conduct further studies to determine the feeding habits of the Mesoamerican and Panamanian-South American clades of *Macrophyllum*.

Geological and biogeographic considerations

The tectonic and geological history of Central America plays an important role in shaping the speciation and distribution patterns of bat species, including *Phylloderma* and *Macrophyllum*. The geological features of the region have not only structured the landscape, but have also acted as major barriers to gene flow (Mann, 2007; Marshall, 2007). This is crucial to understand species diversification through vicariance and dispersal in the region (Patterson, 2020; Sanmartín et al., 2001). The genetic distinctions between the northern and southern clades of *Phylloderma* and *Macrophyllum* strongly correlate with the geological and topographical divisions imposed by the Central Cordillera of Panama and the Hess Escarpment. The latter, which is a steep slope marking the boundary of the Eastern Pacific Rise, has been proven to be important as a barrier separating related species in several taxonomic groups, such as insects (Halffter, 1987), amphibians (Smith et al., 2007), reptiles (Jadin et al., 2012; Suárez-Atilano et al., 2014), birds (Navarro et al., 2001; Rocha-Méndez et al., 2019), and bats (Hernández-Canchola & León-Paniagua, 2017).

The varied ecological zones created by different altitudes along the Talamanca Range—the only region in southern Central America exceeding 4,000 meters in elevation— contribute to environmental heterogeneity influencing bat diversity and distribution. This is evident by the alignment of species across distribution zones, as previously described by Ortega and Arita (1998), with the geological and ecological divisions of the region. For instance, all of Costa

Rica and Panama exhibit species compositions that are distinct from those of the northern transition zone, which includes parts of Nicaragua.

A meta-analysis by Gutiérrez-García and Vázquez-Domínguez (2013) which combined systematic studies, population genetics, and phylogeography of various taxa (including Chiroptera) identified the area south of Nicaragua as the "Panamanian clade", a distinct genetic assemblage of species influenced by the formation of the Isthmus of Panama, which disperse towards Costa Rica along the Chorotega block, a significant tectonic feature extending from Costa Rica to the Panamanian highlands (Giunta & Oliveri, 2009). This highlights the significant impact of geological events on the evolutionary trajectories of species, with the Hess Escarpment playing a fundamental role both in defining the boundaries of tectonic activity and influencing species distribution.

Regarding *Phylloderma*, our findings corroborate these boundaries, with specimens from Mexico, Belize, Guatemala, Honduras, and Nicaragua forming a North Central American clade. Unfortunately, the absence of Costa Rican specimens in our analysis precludes confirmation that they belong to the Panamanian-South American clade. Our results for *Macrophyllum* are similar, mostly consistent with the Hess Escarpment as a potential limit between the northern and southern clades. However, the influence of the Guanacaste, Central, and Talamanca cordilleras could not be conclusively established due to the lack of distinct morphological characters between the clades. Additional genetic data are required to determine if all Costa Rican and Panamanian specimens would join the Panamanian-South American clade (currently including the Canal Area of Panama), or if the specimens distributed both north and south of the Talamanca Cordillera, and Western Panamanian specimens, would join the northern clade. Finally, our phylogenetic analyses of *Phylloderma stenops* and *Macrophyllum macrophyllum* revealed a remarkable evolutionary parallelism between them. *P. stenops* and *M. macrophyllum*

are rare species, thus are not well represented in scientific collections; however, both are widely distributed in the Neotropics and span large environmental gradients, which makes them good subjects for cryptic diversity examination (Stevens, 2023). Our findings reveal that not only the North Central American populations of both genera are distinct, but also that there is an underlying biogeographic element influencing their diversification. This pattern is not unique to our studied species but is evident in other bats such as *Lophostoma evotis* (Davis & Carter, 1978), *Uroderma davisii* (Baker & McDaniel, 1972; Mantilla-Meluk, 2014), *Sturnira parvidens* E. A. Goldman, 1917, *Glossophaga leachii* (Gray, 1844) as well as in nonvolant mammals (Álvarez-Catañeda et al., 2019), birds (Navarro et al., 2001; Rocha-Méndez et al., 2019), reptiles (Jadin et al., 2012), and fishes (Calixto-Rojas et al., 2021).

Central America is a biodiversity hotspot with a vast species richness, despite its relatively small area (Mittermeier et al., 2011); so it is not surprising that access to more information uncovers the existence of new species, many of them endemic to Central America. Examples include *Glossophaga morenoi* Martínez y Villa-R. 1938, and the recently described *Vampyressa villali* (Garbino et al., 2024) and *Corynorhinus leonpaniaguae* (López-Cuamatzi et al., 2024). Nevertheless, there is likely still an underestimation of the biodiversity of Central America (Castellanos et al., 2019), with limited number of recent specimens in scientific collections within the countries of this region (Nassar et al., 2020), along with challenges in obtaining or accessing fresh or adequately preserved tissue samples for genetic analysis.

Conclusions

Accurate taxonomic classification is central to the fields of biogeography, ecology, conservation, among others, serving as a basis for decisions aimed at protecting biodiversity at various scales. The precise identification and classification of species and subspecies are critical to understand biodiversity, but more importantly to identify biodiversity hotspots and

prioritizing conservation efforts. Furthermore, the identification and description of cryptic diversity, especially in regions as biodiverse and ecologically significant as Central America, poses important challenges due to the complexity of the ecosystems and the need for multidisciplinary approaches to accurately delineate species boundaries.

Morphological and molecular evidence using an extensive set of complete mitogenomes enabled us to distinguish *P. septentrionalis* from *P. stenops*. Our findings reveal that the newly recognized species is not merely a subspecies glorified by the use of the phylogenetic species concept, and that it has a high conservation value due to being endemic to Central America. Likewise, our research suggests *M. macrophyllum* to represent a cryptic species complex consisting of two distinct taxonomic entities. *Macrophyllum* sp. nov. is a cryptic species, also endemic to Central America. We recommend conducting further field studies involving the collection and proper storage of specimens in local museums in Central America, particularly in countries with limited collections, such as El Salvador. Similar efforts should be devoted in South America to the subspecies *P. s. boliviensis*, for which owing to the limited number of specimens (only two since its description in 1979) a comprehensive assessment of its status remains elusive.

As we further discuss, the implications of our results for each species makes it clear that the evolutionary narratives of *Phylloderma* and *Macrophyllum* are complex and influenced by both historical biogeography and ecological factors. Our study contributes to a more detailed understanding of bat diversity in Central America, highlighting the need for comprehensive taxonomic and evolutionary studies to protect biodiversity in this crucial region.

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Chapter 5. Synthesis and conclusions

In this synthetic and concluding chapter, I first discuss how we have enriched taxonomic knowledge of Phyllostomidae, detailing new findings that challenge and refine previous classifications. Second, I discuss how the use of mitogenomes marks a significant advancement in the study of evolutionary biology, providing a more precise tool for resolving phylogenetic ambiguities among Leaf-nosed bats. Third, while acknowledging the methodological strengths, I also address the limitations and future perspectives of using mitochondrial DNA for phylogenetic studies.

A robust picture of the evolutionary relationships within Phyllostomidae

The understanding of bat systematics in the Neotropics has experienced substantial advancement through the integration of various approaches and methodologies merging molecular and morphological data analyses to construct more robust phylogenies, particularly for the Phyllostomidae family (Baker et al., 2003; Cirranello et al., 2016; Dávalos et al., 2014). Historically, the molecular phylogenies of Phyllostomidae relied on a limited set of markers, combining mitochondrial and nuclear sequences (Baker et al., 2003; Baker, 2000; Baker et al., 2012; Rojas et al., 2016). These approaches have significantly contributed to clarifying the evolutionary relationships among the major clades within Phyllostomidae, particularly, elucidating the paraphyletic nature of the subfamily Phyllostominae, leading to the delineation of Micronycterinae, Glyphonycterinae, and Lonchorhininae as separate subfamilies, although the precise relationships among these and other subfamilies within different phylogenies remained ambiguous (Rojas et al., 2011; Cirranello et al., 2016).

To contribute to the understanding of Phyllostomidae systematics and considering the shift towards phylogenomics due to its increased accessibility and cost-effectiveness (see the

Introduction section), our study has incorporated new sequences of complete mitochondrial genomes. Prior to our investigation, comprehensive analyses employing complete genomes had been solely conducted by Botero-Castro et al. (2013, 2018). Our analysis partly corroborates, but also clarifies, the relationships identified in these studies, which are also congruent with findings previously suggested by Cirranello et al. (2016), Baker et al. (2016), and Rojas et al. (2016).

In our comparative analyses, which utilized a variety of analytical parameters, the phylogenetic relationships between the subfamilies Stenodermatinae, Rhinophyllinae, Carolliinae, and Glyphonycterinae, as well as the position of Desmodontinae, were consistently supported. Our topologies revealed that Lonchophyllinae and Glossophaginae acquired nectarivory independently; and that Lonchorhininae consistently appeared as the sister group to Phyllostominae, contrasting with findings by Rojas et al. (2016). Moreover, Macrochinae was identified as a sister group to all other phyllostomid subfamilies (Camacho et al., 2022).

Furthermore, this investigation explored the interdependence of methodological choices—partition schemes, outgroup selection, data types, and phylogenetic methods—revealing their collective impact on phylogenetic resolution and associated nodal supports across topologies. Notably, we could observe that the best evaluated and best supported phylogenies were those performed with the complete data matrix of nucleotides, with complex and highly parameterized substitution models and with complete partition schemes with independent partitions for rRNAs, for tRNAs, and for each gene and per codon position.

Interestingly, we observed increased congruence in phylogenies with the use of a larger outgroup dataset, challenging the conventional expectation that a reduced, closely related outgroup set would improve tree resolution (Graybeal, 1998; Schneider & Cannarozzi, 2009). Rubinoff and Holland (2005) suggested that a qualitative measure of phylogenetic robustness

can be gleaned by comparing topologies produced using different analytical parameters; moreover, their assertion that cohesive evolutionary patterns across various independent data partitions enhance confidence in datasets representing the same evolutionary history was also evident in our sensitivity analysis to evaluate Maximum Likelihood and Bayesian Inferences along with different partition schemes.

As the analysis at the subfamily level was enriched with the addition of samples, so was the analysis of genera, particularly of *Trachops*, *Phylloderma*, and *Macrophyllum* of the Phyllostominae subfamily.

The comprehensive investigation of the genus *Trachops*, encompassing morphology, morphometrics, and molecular data across its distribution, has advanced our understanding of its taxonomic and evolutionary dynamics. Notably, the inclusion of complete mitochondrial genomes in our analysis has been important in resolving longstanding taxonomic ambiguities within the genus, culminating in the recognition of two distinct species, *Trachops cirrhosus* and *Trachops ehrhardti*, and the reclassification of *T. c. coffini* as a synonym of *T. cirrhosus*.

In addition, our phylogenetic analyses have revealed the presence of distinct clades within both *Phylloderma stenops* and *Macrophyllum macrophyllum*, suggesting a deeper evolutionary divergence than previously recognized. This divergence is substantiated by significant morphometric differences among *Phylloderma stenops* and *Phylloderma septentrionalis*, the Panamanian-South American form and the North Central American form, respectively. Not so in *Macrophyllum macrophyllum* species complex where we have recognized a cryptic species, *Macrophyllum* sp. nov., that is diagnosable mainly on genetic and geographic distribution data.

Our findings underscore the important role of Central America in bat diversity and speciation. Notably, the distinct clades identified within the taxa under study align with the major geological structures of the area. This might suggest a possible historical biogeographical

imprint on the evolutionary history of these species. While our study did not directly address the processes of vicariance and dispersal, the observed phylogenetic patterns invite further investigation into these evolutionary dynamics.

The species count of the Phyllostomidae family has therefore increased from 227 to 230 species, and the three analyzed genera should no longer be considered monotypic. Furthermore, two of the species recognized here, *Phylloderma septentrionalis* and *Macrophyllum* sp. nov., are endemic to Mesoamerica with limited distribution ranges. Their conservation status warrants assessment due to these restricted distributions.

The contributions of complete mitogenomic data

In recent decades, genetic data have been increasingly used to delimit new taxa, and the number of studies that rely primarily on this evidence to make taxonomic arguments for mammals such as bats, rodents, or cetaceans have been particularly important because morphology-based taxonomic descriptions for those groups are difficult (Taylor et al., 2017). Particularly with bats, comparative studies using mitochondrial DNA (mtDNA) sequences have proven efficient in revealing that diversity has been underestimated and that some sampled specimens may belong to species complexes (Fučíková & Lahr, 2016; Mota et al., 2022).

Fast-evolving mitochondrial genes such as COI and Cytb, have served as a taxonomic tool for use in bats differentiation and identification. The COI or fragments of this gene have proven to be useful because they are conserved enough to be targeted with generic primer sets, while variable enough to provide variation at the interspecific level, hence they are used as barcode loci (Bohmann et al., 2020). However, the extensive generation and use of these barcodes has also shown limitations. One primary limitation is the taxonomic resolution that traditional barcoding can achieve. Although designed to distinguish species, this method may not perform

as effectively at other levels. For instance, it might be less accurate at the population level or when identifying broader phylogenetic categories such as subfamilies, families, or orders due mainly to sequence saturation (Chambers & Hebert, 2016). Another possible limitation, inherent to the relatively short length, is that often, by themselves, single mitochondrial markers are not adequate to resolve phylogenies with high statistical support, and their signal can be confounded due to phenomena such as hybridization and introgression (Bohmann et al., 2020). In addition to this, and in operational terms, obtaining small portions of intact DNA through PCR amplification could pose challenges because the DNA content of many museum specimens of interest is often heavily degraded due to age, storage conditions, or chemical treatment (Orlando et al., 2015).

The several cost-effective advantages of NGS technologies (see Introduction section) have provided partial solutions to these limitations. The high-throughput sequencing (HTS) methods, as the one used in this thesis, have facilitated the sequencing of complete mitochondrial genomes, with the genome skimming approach emerging as a particularly effective method for this purpose (Coissac et al., 2016; Straub et al., 2012). Genome skimming, as outlined in the Introduction section, is a low coverage sequencing approach that, while capable of sequencing traditional barcode loci, is effective in its ability to sequence and assemble complete organellar genomes (Gillett et al., 2014). This technique allows for the sequencing of multiple samples simultaneously through library indexing, which means that a single sequencing run could produce dozens or even hundreds of mitochondrial genomes. Besides, genome skimming has shown great effectiveness for acquiring mitogenomes from museum specimens, as we have demonstrated here, managing to obtain complete sequences of specimens preserved as dry skins and in ethanol or formalin for over a century. Notable examples of the application of these HTS techniques include investigations into the evolutionary history of rare species where access to fresh specimens is limited but can be accessed from scientific collections, such as New Guinea

crowned pigeons of the genus *Goura* (Besnard et al., 2016) or for critically endangered species such as the Puebla deer mouse, *Peromyscus mekisturus* (Castañeda-Rico et al., 2020). Furthermore, genome analysis has been instrumental in assessing the taxonomic identity of historical specimens from poorly documented or recently extinct species, such as the blue antelope (*Hippotragus leucophaeus*), whose entire mitogenome has been recovered from a museum specimen whose identification was uncertain (Plaxton et al., 2023) or for the study of Pleistocene extinct species such as the American mastodon (*Mammot americanum*), where complete mitochondrial genomes were extracted from fossil tooth pieces, revealing insights into the impact of glacial and interglacial cycles on distribution dynamics and the phylogeography of this species (Karpinski et al., 2023).

Although numerous studies have previously explored the geographical and genetic diversity of bats using fragments or complete sequences of mitochondrial genes (e.g., Baker et al., 2003; Calahorra-Oliart et al., 2021; Clare, 2011; Rojas et al., 2016), the genome skimming technique has uncovered more profound insights as it has significantly enhanced our understanding of the genetic variation and large-scale phylogeographic patterns within bat populations by analyzing extensive segments of mitochondrial DNA (Botero-Castro et al., 2018; du Toit et al., 2017)

Analysis of complete mitochondrial genomes instead of short mtDNA sequences has markedly improved the resolution of phylogeographic and phylogenetic analyses across diverse mammalian taxa. For instance, research leveraging entire mitochondrial genomes of brown bears (*Ursus arctos*) has elucidated novel patterns of population structure and phylogeographic processes (Keis et al., 2013). Similarly, in-depth phylogenetic analyses using complete mitochondrial DNA from killer whales (*Orcinus orca*) have facilitated the recognition of three distinct ecotypes as phylogenetically discrete and well-supported clades, meriting their classification as separate species (Morin et al., 2010). Such studies underscore the enhanced

analytical power of using full mitogenomes to discern phylogenetic relationships and geographic population structures.

In the specific case of phyllostomine bats, the use of individual mitochondrial genes alone or together with nuclear genes has revealed somewhat unresolved conclusions about the position of Macrotoninae and Lonchorrhinae with respect to the rest of the Phyllostomidae subfamilies (Rojas et al., 2016). In this sense, and at this intermediate level in the phylogenetic relationships of Phyllostomidae, complete mitogenome data has been especially useful as it allows us to achieve a high resolution and to gain a deeper insight into the phylogeny of the family, to conclude that Lonchorrhinae is the sister subfamily of Phyllostominae and Macrotoninae is sister to all other subfamilies (Camacho et al., 2022).

Mitogenomic data have demonstrated their utility in resolving previously ambiguous phylogenies and in uncovering genetic differentiation as well as increased intrageneric diversity, as evidenced in genera such as *Trachops*, *Phylloderma*, and *Macrophyllum*, clarifying the relationships within phylogenetically difficult groups where rapid radiations made other markers, such as nuclear, less effective (Kern et al., 2020).

It is only logical to think that in the future, the desirable end goal would be to generate fully assembled nuclear genomes. However, and particularly for developing countries, nuclear genomes are still expensive to generate as they require sequencing to high depths of coverage and the assembly is constrained by depth of sequencing and the repeat structure of the genome (Bohmann et al., 2020). Complete mitochondrial genomes are more affordable and, operationally, their size is large enough to contain an important number of informative characters yet small enough to be analyzed using standard computing resources (Kern et al., 2020).

In this thesis only mitochondrial information has been used, however, far from being incomplete, it is a contribution to the evolutionary tree of mammals, which is rapidly being resolved with important agreement between nuclear and mitochondrial data sets (Lin et al., 2002). We consider that the findings of this work have contributed to the resolution of existing phylogenetic controversies, the construction of robust phylogenetic trees, and the detection of cryptic species diversity.

In addition to enriching the taxonomic count as previously mentioned, a significant contribution of this work is the increase of the number of complete mitochondrial genomes examined, surpassing the number used by Botero-Castro et al. (2018) by 42%. This enhancement in the phylogenomic dataset of Phyllostomidae includes the introduction of complete mitochondrial genomes for 26 species across 16 genera, as detailed in Chapter 1. Furthermore, our study incorporates 96 sequences (54 from *Trachops*, 15 from *Phylloderma*, 22 from *Macrophyllum*, and 5 new outgroup sequences), as discussed in Chapters 2 and 3.

Delimiting species and understanding biodiversity

Species delimitation, the detection of cryptic species diversity, the resolution of existing phylogenetic controversies, and the construction of robust phylogenetic trees remains an integrative field that depends on increasingly diverse data types, which has benefited from cost-effective genetic sequencing techniques and the accumulation of that information generated. This dynamism observed in the last two decades in bats reflects a vibrant field whose ultimate goal is the achievement of a stable classification (Fujita et al., 2012).

A stable taxonomy is particularly important for any field that relies on accurate measures of biodiversity, including ecology and conservation, as well as for research projects dedicated to understanding the evolution of organismal traits, including developmental biology, comparative

biology, and genomics (Mace, 2004). In this sense, and in an effort to reduce the possible subjectivity, there has recently been a tendency in the literature to pursue an “integrative taxonomy” which attempts to make use of many different sources of data (molecular, morphological, behavioral, or ecological data) to delimit species in a stable and transparent manner (Padial et al., 2010).

The integration of evidence goes hand in hand with the understanding on how we define a species. Precisely, from the integrative and operational aspects proposed by de Queiroz (1998), there seems to be an agreement that species are “separately evolving metapopulation lineages” and that the numerous evidence applied in species delimitation are considered as operational criteria. These criteria include, among other aspects, the reproductive isolation, ecological divergence, *or* differences in molecular genetic characters, which we use as separate lines of delimiters, ideally, in an integrative way (Hawlitschek et al., 2012). In this thesis, we integrate genetic, morphological, and geographical evidence and the taxonomic decisions are founded on the general definition of species proposed by Simpson (1951) and reworded by Molinari (2023) in which a species is a phyletic lineage (ancestral-descendent sequence of populations) evolving independently of others, and diagnosably distinct based on heritable morphological characters, genetic markers, or both.

However, and no matter how many lines of evidence we want to use as operational criteria to recognize species, the increasing utilization of molecular data has reinforced the conclusion that morphological evolution is often cryptic, as has been seen in amphibians (Borkin et al., 2004; Ortega et al., 2022), reptiles (Yáñez-Muñoz et al., 2018), birds (Forcina et al., 2023), and mammals (Clare, 2011; Esquivel et al., 2022), which in the first instances leads to a still underestimation of the number of species (Borkin et al., 2004). In bats, cryptic diversity could be due to three main reasons: recent divergence, phylogenetic niche conservatism or

morphological stasis, and morphological convergence (Fišer et al., 2018). In addition, despite their ability to fly, not all bat species can disperse over large distances, so genetic interchange in a population is therefore conditional on geographical proximity, with populations that inhabit isolated lands (such as islands or mountains) being more susceptible to genetic divergence and speciation (López-Cuamatzi et al., 2024; Zhang et al., 2019).

For the reasons mentioned, the identification of cryptic species in bats and the application of various types of data for species delimitation can present challenges. However, efforts must be made, and it is through these studies and interdisciplinary collaboration that it is possible to recognize, classify and protect the biodiversity of bats, considering that the identification and description of cryptic species could even have important implications for the conservation and the protection and management of natural resources (Delić et al., 2017). Preventing habitat loss is perhaps the greatest challenge to global biodiversity conservation and this often requires estimation of species richness and endemism. The discovery of geographical and habitat-related patterns in distribution of cryptic species could therefore uncover as-yet-unknown zones of endemism and diversity that might warrant reconsideration for habitats or sites for conservation. Molecular evidence has revealed that several already endangered species are cryptic species complexes, making them a collection of even more critically endangered species with fewer numbers and smaller distributions (Bickford et al., 2007).

Limitations and future perspectives

Several next-generation sequencing technologies have been developed, each offering a distinct set of advantages and limitations critical to project design, especially for those aimed at assessing biodiversity. The genome skimming method used in this research, utilizing the Illumina platform, has enabled the sequencing of samples primarily from specimens in scientific collections, yielding a significant amount of data. The cost-effectiveness of this

technique has improved access to mitochondrial genome sequencing and facilitated a rapid expansion in data generation capabilities, allowing for genome-scale sequencing from minimally processed organismal materials—such as direct samples from blood or skin snippets (Van Den Bussche & Lack, 2013).

Despite these advantages, whole mitogenome sequencing remains rare and underutilized in developing regions, including many countries in South and Central America, for various reasons such as lack of appropriate infrastructure, lack of financial resources, and poorly trained personnel (Colella et al., 2021). Furthermore, as the cost of data generation decreases and performance increases, data storage is also likely to become an issue in this region (Schadt et al., 2010); and although storage and manipulation problems can be resolved, the tasks of assembling, aligning, annotating, , and analyzing complete mammalian genomes of numerous taxa present similar difficulties (Van Den Bussche & Lack, 2013). To solve, at least in part, these challenges, it is crucial to increase collaboration between institutions, particularly in countries with limited resources in biodiversity areas. Collaborative projects have improved molecular systematics, evolutionary biology, and biodiversity conservation capabilities, highlighting the transformative results of molecular techniques such as genome skimming.

Regarding the use of mitochondrial sequences, these have resolved phylogenies more deeply than originally expected (see Botero-Castro 2013, 2018; Camacho et al., 2022). However, their utility as markers for highly divergent lineages remains a matter of debate. To validate these mitochondrial-derived phylogenies, further examination of nuclear markers is recommended (Curole & Kocher, 1999), as both mitochondrial and nuclear DNA can reveal different phylogenetic patterns (Griffiths et al., 2004). The presence of incongruence between mitochondrial DNA and nuclear DNA does not inherently dictate a preference for one phylogenetic tree over another. Ideally, datasets that show discrepancies between mtDNA and

nDNA should be regarded as potentially more informative than those that are strictly congruent (Rubinoff & Holland, 2005).

Consequently, this thesis does not advocate for the exclusive use of mtDNA. Instead, it advocates for the future integration and comparison of multiple data sources, including nDNA, morphology, behavior, cytology, or ecology and thus achieve a comprehensive understanding of the evolutionary history of the species under study. As noted earlier, the phylogenies presented in this thesis are consistent with others that included nDNA markers at deeper levels. However, there is a need for comparative research at the genus and species levels, especially for under-studied genera such as *Phylloderma* and *Macrophyllum*. This dissertation opens several paths for future research, such as investigating the ecological niches and behaviors of the newly recognized species, further genetic studies to explore intraspecific variation, and expanded surveys to enhance our understanding of these species' distributions. This is particularly critical for Central America.

An important aspect highlighted throughout the chapters of this dissertation is the use of natural history collections. These repositories are cumulative records of biodiversity that expand over time through the addition of new specimens. Increasingly, researchers are using these specimens for a variety of scientific inquiries that extend beyond the initial scope of tissue samples, skeletal remains, and geographical data (Cook & Light, 2019). Such applications include genetic or biogeographic studies, climate change research, or ecological modeling, thereby enhancing our understanding of biodiversity and ecosystem dynamics. Furthermore, these archives are increasingly serving as elements for a proactive and predictive approach to zoonotic spillover, risk assessment, and threat mitigation (DiEuliis et al., 2016).

Unfortunately, while museum biorepositories are established in nearly all countries in Central and South America, access to specimens is not always available. Tissue collections, particularly

in biodiverse regions, are often underdeveloped due to insufficient financial resources to support infrastructure, collections growth, database management, and permanent staff (Colella et al., 2021). Furthermore, sampling restrictions arising from permitting issues, political instability, or high costs further complicate this issue (Malaney & Cook, 2018), especially in Central America (Castellanos, 2019). Many Central American mammals have not been recently sampled throughout their range, and some countries, such as Nicaragua and El Salvador, have been historically under sampled. This scarcity of data is evident in the biogeographic literature, which lacks comprehensive investigations into many widespread species (Castellanos, 2019; Feijó & Brandão, 2022). Currently, the primary sources for obtaining research specimens are large museums in countries like the United States. Although the techniques used in this research have proven effective in extracting high-quality genetic material of ancient specimens from these collections, there remains a crucial need for strategic investments in biorepository infrastructure.

In chapters 3 and 4 of this thesis, I utilized an integrative dataset of molecular, morphological, and geographical data from natural history collections to investigate the biogeographic patterns of *Trachops*, *Phylloderma*, and *Macrophyllum*. The analysis of mitochondrial genomes of Central American specimens of *Phylloderma septentrionalis* and *Macrophyllum* sp. nov. led to the recognition of these two species. However, additional genetic information is needed, as well as the integration of data from lesser-explored localities. Regional analyses of biodiversity are crucial for identifying potential drivers of diversification and pinpointing areas in greater need of conservation (Rosauer et al., 2016). Our study, along with others in the region (see Álvarez-Catañeda et al., 2019; Arteaga et al., 2012), highlights several key areas in Central America important for mammal biodiversity across several orders, such as the Guanacaste, Central, and Talamanca cordilleras and surrounding regions. These findings hint at the need for further

research into the patterns of important environmental variables in Central America, a region shaped by complex geologic and climatic processes (Marshall, 2007).

Finally, the reclassification within the genus *Trachops* and *Phylloderma*, as well as the description of the new species of *Macrophyllum*, require a reevaluation of conservation strategies and priorities. The elevation of *T. ehrhardti* and *P. septentrionalis* to species status, considering its restricted range and the threatened status of their habitats, underscores the urgency of targeted conservation efforts. These cases exemplify how revised taxonomies can reshape conservation priorities and actions, emphasizing the critical role of accurate taxonomic identification in biodiversity preservation.

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Appendices

Appendix 1. General features of the mitogenome sequences of *Trachops*. Specimen information includes voucher identification, GenBank accession number, and taxonomic information.

Museum number	GenBank Accession number	Species	N° of reads	Mean coverage	N° of mtDNA reads	mtDNA length	GC content (%)
AMNH-15103	PP393701	<i>T. cirrhosus</i>	4,324,942	104.79	29,909	16,584	37.58
AMNH-31522	PP391757	<i>T. cirrhosus</i>	2,236,998	37.01	10,356	16,601	37.58
AMNH-94538	PP393700	<i>T. cirrhosus</i>	2,151,243	23.34	5,747	16,591	37.37
AMNH-129064	PP391758	<i>T. cirrhosus</i>	2,740,862	79.92	22,336	16,595	37.58
AMNH-147767	PP393699	<i>T. cirrhosus</i>	2,789,364	7.54	1,891	16,598	37.58
AMNH-185889	PP391759	<i>T. cirrhosus</i>	3,336,996	17.44	4,212	16,596	37.25
AMNH-185891	PP393698	<i>T. cirrhosus</i>	4,357,962	101.30	22,996	16,625	37.3
AMNH-230213	PP393697	<i>T. cirrhosus</i>	4,526,938	399.14	90,418	16,622	37.66
AMNH-267935	PP393696	<i>T. cirrhosus</i>	3,494,936	164.35	37,044	16,621	37.53
AMNH-272813	PP393695	<i>T. cirrhosus</i>	5,430,224	147.99	33,493	16,655	37.58
AMNH-278476	PP391760	<i>T. cirrhosus</i>	5,304,394	57.21	12,932	16,653	37.59
FMNH-72150	PP391761	<i>T. cirrhosus</i>	7,428,142	7.64	1,773	16,578	37.35
FMNH-114877	PP391763	<i>T. cirrhosus</i>	7,529,998	82.99	18,741	16,598	37.36
FMNH-174887	PP391765	<i>T. cirrhosus</i>	5,776,246	1,237.67	282,126	16,743	37.7
FMNH-174888	PP391766	<i>T. cirrhosus</i>	5,550,234	282.11	64,164	16,713	37.68
MSB-311655	PP391767	<i>T. cirrhosus</i>	4,271,430	68.59	15,555	16,600	37.58
MSB-311656	PP391768	<i>T. cirrhosus</i>	4,543,408	261.75	59,681	16,602	37.58
MSB-311734	PP391769	<i>T. cirrhosus</i>	4,944,460	169.89	38,553	16,603	37.37
MSB-311736	PP410133	<i>T. cirrhosus</i>	5,215,900	62.19	14,095	16,603	37.37
QCAZ-2459	PP410134	<i>T. cirrhosus</i>	3,690,346	54.62	12,560	16,630	37.46
QCAZ-4234	PP410135	<i>T. cirrhosus</i>	1,393,255	4,840.69	504,106	16,822	37.6
QCAZ-9239	PP410136	<i>T. cirrhosus</i>	3,751,390	157.88	35,638	16,623	37.47
QCAZ-10617	PP410137	<i>T. cirrhosus</i>	5,240,730	7.99	1,829	16,620	37.47
QCAZ-11632	PP410138	<i>T. cirrhosus</i>	6,075,978	111.02	25,064	16,571	37.31
QCAZ-13317	PP410139	<i>T. cirrhosus</i>	2,001,147	17.4	1,800	16,619	37.5
QCAZ-13956	PP410140	<i>T. cirrhosus</i>	5,352,738	29.08	6,658	16,621	37.35
QCAZ-14029	PP410141	<i>T. cirrhosus</i>	6,361,566	104.18	23,674	16,619	37.42
QCAZ-17165	PP410142	<i>T. cirrhosus</i>	6,480,538	20.02	4,522	16,614	37.34
QCAZ-18335	PP410143	<i>T. cirrhosus</i>	4,985,186	83.00	18,723	16,579	37.35
ROM-95747	PP410144	<i>T. cirrhosus</i>	5,305,082	299.46	67,789	16,624	37.32
ROM-98701	PP410145	<i>T. cirrhosus</i>	5,550,346	43.24	9,792	16,612	37.67
ROM-99493	PP440246	<i>T. cirrhosus</i>	3,211,086	79.60	18,085	16,621	37.31
ROM-103359	PP440254	<i>T. cirrhosus</i>	4,896,612	14.83	3,326	16,317	36.24
ROM-103495	PP440255	<i>T. cirrhosus</i>	4,853,726	522.78	118,097	16,621	37.59
ROM-104214	PP440247	<i>T. cirrhosus</i>	4,148,188	39.36	9,909	16,582	37.3
ROM-107843	PP440257	<i>T. cirrhosus</i>	4,250,814	17.87	4,049	16,617	37.41

Museum number	GenBank Accession number	Species	N° of reads	Mean coverage	N° of mtDNA reads	mtDNA length	GC content (%)
ROM-107908	PP440248	<i>T. cirrhosus</i>	3,634,508	67.38	15,210	16,593	37.4
ROM-107926	PP440249	<i>T. cirrhosus</i>	3,176,256	169.41	38,250	16,602	37.41
ROM-107935	PP440250	<i>T. cirrhosus</i>	3,613,554	260.21	58,765	16,607	37.39
ROM-107945	PP440251	<i>T. cirrhosus</i>	4,380,050	281.34	63,704	16,637	37.65
ROM-115072	PP440252	<i>T. cirrhosus</i>	6,091,173	10.53	2,211	16,577	37.29
ROM-115495	PP440253	<i>T. cirrhosus</i>	7,436,128	21.57	4,895	16,624	37.42
ROM-117523	PP440256	<i>T. cirrhosus</i>	1,990,214	180.74	18,148	16,749	37.6
ROM-119312	PP461476	<i>T. cirrhosus</i>	4,225,778	167.44	37,808	16,611	37.67
ROM-121044	PP461477	<i>T. cirrhosus</i>	4,673,024	426.56	96,388	16,613	37.56
ROM-121090	PP461478	<i>T. cirrhosus</i>	4,051,452	46.35	10,501	16,622	37.65
ROM-121159	PP461479	<i>T. cirrhosus</i>	6,174,610	1,006.61	227,410	16,594	37.62
ROM-121252	PP461480	<i>T. cirrhosus</i>	4,190,554	197.92	44,724	16,615	37.69
ROM-122057	PP461481	<i>T. cirrhosus</i>	4,946,730	215.59	49,150	16,622	37.67
ROM-125132	PP461482	<i>T. cirrhosus</i>	5,841,756	217.79	48,522	16,631	37.65
ROM-125898	PP461483	<i>T. cirrhosus</i>	5,053,904	26.77	6,039	16,589	37.51
ROM-125914	PP461484	<i>T. cirrhosus</i>	5,364,036	132.02	29,962	16,606	37.53
FMNH-94717	PP391762	<i>T. ehrhardti</i>	3,597,194	8.72	1,996	16,618	37.03
FMNH-141595	PP391764	<i>T. ehrhardti</i>	4,767,214	20.62	4,716	16,619	37.3

Appendix 2. General features of the mitogenome sequences of *Phylloderma* and *Macrophyllum*. Specimen information includes voucher identification, GenBank accession number, and taxonomic information.

Museum number	Genbank accession number	Species	N° of reads	Mean coverage	N° mtDNA reads	mtDNA length	GC content (%)
AMNH-177663	Pending	<i>Macrophyllum</i> sp. nov.	3,640,723	10.6	2,264	16,611	38.9
AMNH-177664	Pending	<i>Macrophyllum</i> sp. nov.	7,204,232	8.89	2,110	16,598	38.9
AMNH-177665	Pending	<i>Macrophyllum</i> sp. nov.	3,231,313	7.73	1,682	16,607	39
AMNH-209320	Pending	<i>M. macrophyllum</i>	4,798,588	94.08	21,407	16,603	38.8
AMNH-266039	Pending	<i>M. macrophyllum</i>	4,470,336	366.09	83,755	16,638	38.9
AMNH-273075	Pending	<i>M. macrophyllum</i>	5,545,494	244.08	55,249	16,579	38.8
AMNH-78412	Pending	<i>M. macrophyllum</i>	1,467,458	15.55	3,954	16,559	38.8
AMNH-78416	Pending	<i>M. macrophyllum</i>	2,442,357	37.08	10,542	16,561	38.8
AMNH-78534	Pending	<i>M. macrophyllum</i>	2,781,994	23.8	6,478	16,564	38.8
AMNH-96019	Pending	<i>M. macrophyllum</i>	2,964,066	127.47	38,743	16,557	39.6
AMNH-97252	Pending	<i>M. macrophyllum</i>	3,444,588	59.06	14,648	16,630	38.9
FMNH-19225	Pending	<i>M. macrophyllum</i>	3,079,446	16.17	3,862	16,598	39
FMNH-62153	Pending	<i>M. macrophyllum</i>	3,780,594	6.22	1,429	16,600	38.7
MSB-211941	Pending	<i>M. macrophyllum</i>	4,569,656	113.61	25,645	16,603	38.9
MSB-57010	Pending	<i>M. macrophyllum</i>	2,001,147	17.4	1,800	16,614	38.9
QCAZ-15869	Pending	<i>M. macrophyllum</i>	8,974,593	17.21	3,773	16,785	38.9
ROM-104199	Pending	<i>M. macrophyllum</i>	4,236,066	293.13	66,423	16,561	39
ROM-104200	Pending	<i>M. macrophyllum</i>	3,980,630	117.59	26,589	16,588	39
ROM-106563	Pending	<i>M. macrophyllum</i>	5,096,230	83.34	19,161	16,765	38.9
ROM-106564	Pending	<i>M. macrophyllum</i>	4,962,514	197.18	45,312	16,779	38.9
ROM-115718	Pending	<i>M. macrophyllum</i>	4,608,668	107.84	24,420	16,592	38.8
ROM-117379	Pending	<i>M. macrophyllum</i>	4,453,672	218.67	49,539	16,594	38.8
AMNH-124834	Pending	<i>P. septentrionalis</i>	2,964,066	127.47	38,743	16,557	42.6
AMNH-126867	Pending	<i>P. septentrionalis</i>	3,067,808	24.39	7,079	16,557	42.6
AMNH-205371	Pending	<i>P. stenops</i>	3,504,881	8.34	2,136	16,609	43
AMNH-266077	Pending	<i>P. stenops</i>	3,344,494	9.17	2,126	16,563	42.7
FMNH-170089	Pending	<i>P. stenops</i>	2,633,696	142.23	32,106	16,623	43.1
QCAZ-18527	Pending	<i>P. stenops</i>	5,861,338	226.13	51,189	16,601	43.1
ROM-104225	Pending	<i>P. stenops</i>	3,784,894	281.51	63,841	16,653	42.9
ROM-104693	Pending	<i>P. stenops</i>	4,363,446	607.41	137,905	16,633	43
ROM-111529	Pending	<i>P. stenops</i>	4,110,102	528.52	119,842	16,616	42.6
ROM-112628	Pending	<i>P. stenops</i>	3,935,428	199.94	45,227	16,602	43
ROM-117511	Pending	<i>P. stenops</i>	3,427,954	215.72	48,996	16,690	42.8
ROM-120383	Pending	<i>P. stenops</i>	4,354,592	276.36	62,585	16,604	43
ROM-121025	Pending	<i>P. stenops</i>	11,472,012	55.02	12,482	16,598	42.6
ROM-125158	Pending	<i>P. stenops</i>	4,481,174	10.77	2,480	16,590	42.7
ROM-98903	Pending	<i>P. stenops</i>	3,507,790	143.38	32,561	16,607	43
QCAZ-18512	Pending	<i>Gardenycteris crenulata</i>	7,311,686	213.03	48,192	16,580	39.7
QCAZ-18699	Pending	<i>Tonatia bakeri</i>	6,534,148	14.19	3,220	16,634	39.9
QCAZ-18135	Pending	<i>Vampyrum spectrum</i>	5,479,148	63.66	14,449	16,674	45.8
QCAZ-17594	Pending	<i>Chrotopterus auritus</i>	6,074,722	56.17	12,811	16,779	45.9

Appendix 3. Results of Principal Component Analysis of *Phylloderma* and *Machophyllum*. Loadings and the percentage of variance explained by the first principal components derived from a PCA of 16 linear measurements from adult specimens.

<i>Phylloderma</i>	Component		
	1	2	3
Breadth of brain case (BB)	0.180	0.083	0.137
Palatal width at canines (C-C)	0.115	-0.183	-0.048
Condyl canine length (CCL)	0.700	-0.056	0.039
Condyl incisive length (CIL)	0.066	-0.069	0.049
Coronoid height (COH)	0.128	0.067	-0.162
Dentary length (DENL)	0.091	-0.109	0.009
Greatest length of skull (GLS)	0.510	-0.032	0.098
Breadth across upper molars (M2-M2)	0.094	-0.086	0.185
Mandibular tooththrow length (MANDL)	0.053	-0.098	-0.143
Molariform tooththrow (MLTRL)	0.076	0.179	-0.794
Mastoid (process) breadth (MPW)	0.045	0.286	0.303
Maxillary tooththrow (MTRL)	0.065	-0.143	-0.117
Post orbital constriction breadth (PB)	0.180	0.009	0.143
Palatal length (PL)	0.137	-0.161	0.039
Zygomatic breadth (ZB)	0.081	0.113	0.292
Braincase height (BCH)	0.044	0.783	-0.123
SUMQ	0.90	0.9	0.96
Proportion of variance	72.85%	7.93%	6.83%

<i>Macrophyllum</i>	Component			
	1	2	3	4
Breadth of brain case (BB)	0.130	-0.002	0.078	-0.130
Palatal width at canines (C-C)	0.281	0.052	0.300	0.197
Condyl canine length (CCL)	0.104	0.003	0.021	0.046
Condyl incisive length (CIL)	0.094	0.006	0.024	0.040
Coronoid height (COH)	0.513	0.446	-0.732	0.164
Dentary length (DENL)	0.102	0.007	0.035	0.054
Greatest length of skull (GLS)	0.085	0.008	0.026	0.029
Breadth across upper molars (M2-M2)	0.160	-0.013	0.083	0.149
Mandibular tooththrow length (MANDL)	0.128	0.009	0.005	0.154
Molariform tooththrow (MLTRL)	0.134	0.004	0.041	0.096
Mastoid (process) breadth (MPW)	0.147	0.030	0.050	-0.138
Maxillary tooththrow (MTRL)	0.132	-0.014	0.018	0.188
Post orbital constriction breadth (PB)	0.128	-0.038	0.277	-0.351
Palatal length (PL)	0.526	-0.874	-0.449	-0.093
Zygomatic breadth (ZB)	0.362	0.054	0.192	-0.041
Braincase height (BCH)	0.285	0.132	-0.125	-0.744
SUMQ	1.0	0.99	0.98	0.9
Proportion of variance	39.39%	18.55%	12.96%	8.31%

Supplementary data

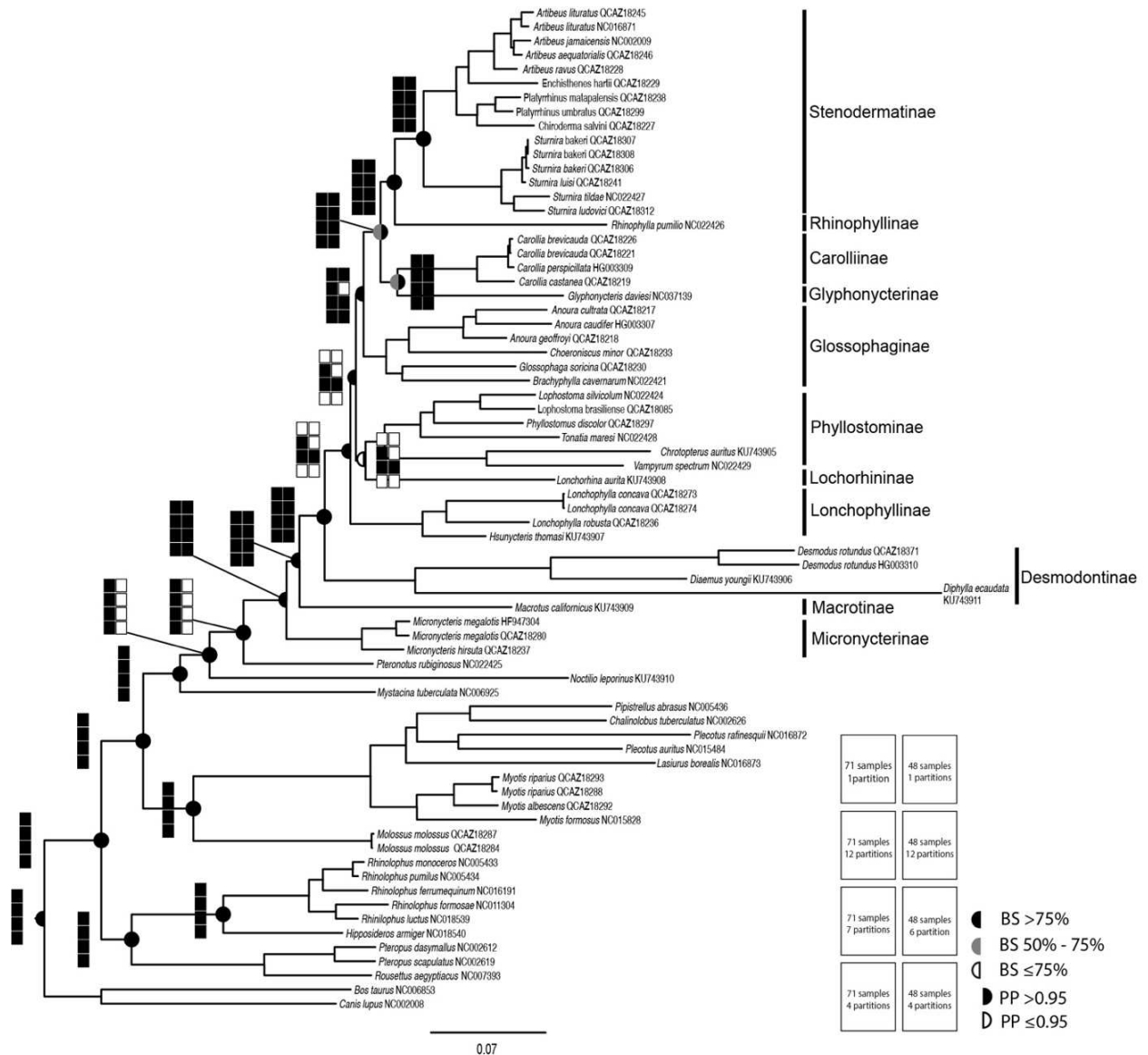
Supplementary data - Chapter 2

Supplementary data 1. Taxonomic and collection data from Ecuadorian specimens. Specimens analyzed in this study whose mitogenome sequences were generated (for GenBank accession numbers, see Table 2-1), including elevation (in meters), weight (in grams), standard external measurements (in millimeters) and GPS coordinates (in decimal degrees).

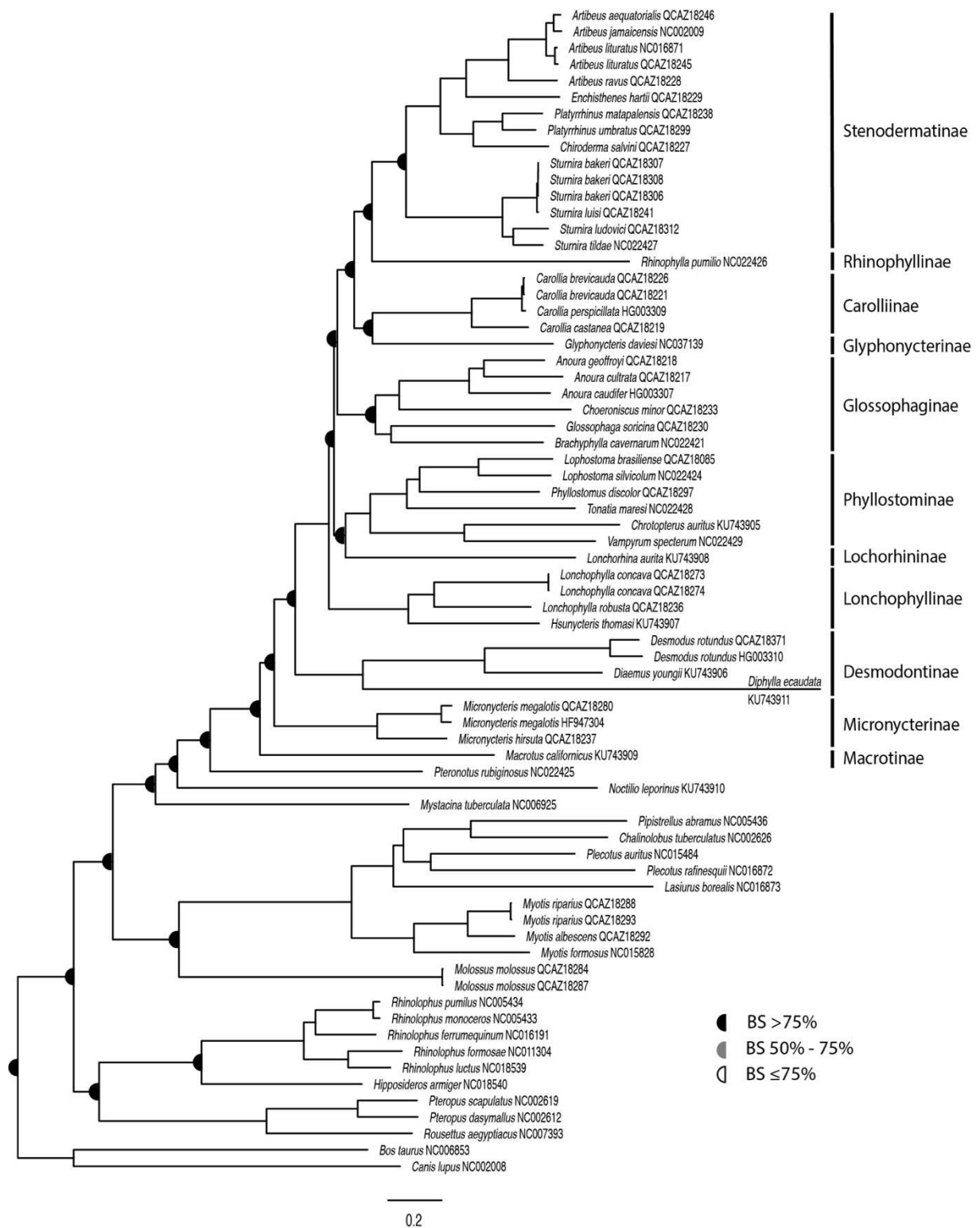
Museum number	Field number	Genus	Species	Sex	Province	Precise locality	Longitude	Latitude	Altitude	Weight	Forearm	Head-body length	Tail length	Ear length	Hind foot length
QCAZ18085	NK298731	<i>Lophostoma</i>	<i>brasiliense</i>	F	Santo Domingo	La Lorena	-79.139733	-0.2747998	577	8	34	53.7	8	20.2	8.6
QCAZ18217	QKM54786	<i>Anoura</i>	<i>cultrata</i>	F	Cotopaxi	San Cristóbal	-79.1532668	-0.8607	517	18.5	41.9	53.7	-	10.8	10.3
QCAZ18218	QKM54793	<i>Anoura</i>	<i>geoffroyi</i>	M	Cotopaxi	Jardín de los sueños	-79.2045668	-0.8372	621	13	41.7	68.8	-	17.3	11.4
QCAZ18219	QKM54798	<i>Carollia</i>	<i>castanea</i>	M	Cotopaxi	San Cristóbal	-79.1532668	-0.8607	460	10.5	34.4	47.5	12	16.5	11
QCAZ18221	QKM54776	<i>Carollia</i>	<i>brevicauda</i>	M	Cotopaxi	Jardín de los sueños	-79.2045668	-0.8372	400	14.5	39.2	47.3	10.2	17.2	10.7
QCAZ18226	QKM54797	<i>Carollia</i>	<i>brevicauda</i>	F	Cotopaxi	San Cristóbal	-79.1532668	-0.8607	462	14	41.6	62	10.2	9	12
QCAZ18227	QKM54794	<i>Chiroderma</i>	<i>salvini</i>	F	Cotopaxi	Jardín de los sueños	-79.2045668	-0.8372	621	27	49.5	65.5	-	14.8	12.8
QCAZ18228	QKM54800	<i>Artibeus</i>	<i>ravus</i>	M	Cotopaxi	Manguilita El Triunfo	-79.20735	-0.9117498	312	10.5	38.5	45.9	-	15.7	11
QCAZ18229	QKM54795	<i>Enchisthenes</i>	<i>hartii</i>	F	Cotopaxi	Jardín de los sueños	-79.2045668	-0.8372	573	18	40.5	51.3	-	15.4	9.3
QCAZ18230	QKM54777	<i>Glossophaga</i>	<i>soricina</i>	F	Cotopaxi	Jardín de los sueños	-79.2045668	-0.8372	395	9	35	44	5.4	13.1	9
QCAZ18233	QKM54807	<i>Choeroniscus</i>	<i>minor</i>	F	Cotopaxi	Jardín de los sueños	-79.2045668	-0.8372	495	8	35.1	41.4	5.5	10.5	9.3
QCAZ18236	QKM54804	<i>Lonchophylla</i>	<i>robusta</i>	M	Cotopaxi	Manguilita El Triunfo	-79.20735	-0.9117498	285	15	43.7	52.5	12.8	17	13.7
QCAZ18237	QKM54805	<i>Micronycteris</i>	<i>hirsuta</i>	F	Cotopaxi	Jardín de los sueños	-79.2045668	-0.8372	473	13.5	43.2	51	13.2	22.7	12.9

Museum number	Field number	Genus	Species	Sex	Province	Precise locality	Longitude	Latitude	Altitude	Weight	Forearm	Head-body length	Tail length	Ear length	Hind foot length
QCAZ18238	QKM54801	<i>Platyrrhinus</i>	<i>matapalensis</i>	M	Cotopaxi	Manguilita El Triunfo	-79.20735	-0.9117498	285	14	32.9	52.3	-	17	11.7
QCAZ18241	QKM54792	<i>Sturnira</i>	<i>luisi</i>	M	Cotopaxi	Guasaganda	-79.1468667	-0.7798167	481	21.5	41.8	56.6	-	15.9	10.8
QCAZ18245	NK298728	<i>Artibeus</i>	<i>literatus</i>	F	Santo Domingo	La Lorena	-79.139733	-0.2747998	577	80	74.2	105.5	-	20.4	17.8
QCAZ18246	NK298686	<i>Artibeus</i>	<i>aequatorialis</i>	M	Santo Domingo	Reserva Otongachi	-78.9518828	-0.3212998	856	46	65	89	-	22	14
QCAZ18273	NK298727	<i>Lonchophylla</i>	<i>concava</i>	F	Santo Domingo	La Lorena	-79.139733	-0.2747998	577	6.5	33.2	56.9	9.5	11.5	8.2
QCAZ18274	NK298732	<i>Lonchophylla</i>	<i>concava</i>	F	Santo Domingo	La Lorena	-79.139733	-0.2747998	577	7	34	58.6	11.3	14.4	10.4
QCAZ18280	NK298706	<i>Micronycteris</i>	<i>megalotis</i>	M	Santo Domingo	La Lorena	-79.139733	-0.2747998	577	5.5	36	49	12	20	10
QCAZ18284	NK298713	<i>Molossus</i>	<i>molossus</i>	M	Santo Domingo	La Lorena	-79.139733	-0.2747998	577	17	39	65	44	14	11
QCAZ18287	NK298712	<i>Molossus</i>	<i>molossus</i>	F	Santo Domingo	La Lorena	-79.139733	-0.2747998	577	22	42	69	44	15	12
QCAZ18288	NK298683	<i>Myotis</i>	<i>riparius</i>	M	Santo Domingo	Reserva Otongachi	-78.9518828	-0.3212998	856	4.5	35	46	35	14	7
QCAZ18292	NK298716	<i>Myotis</i>	<i>albescens</i>	F	Santo Domingo	Río Mulaute	-78.993183	-0.131917	549	5	37	52	33	15	9
QCAZ18293	NK298651	<i>Myotis</i>	<i>riparius</i>	F	Pichincha	Alambi	-78.680733	-0.030217	1479	5	36	46	37	14	7
QCAZ18297	NK298644	<i>Phyllostomus</i>	<i>discolor</i>	M	Pichincha	Alambi	-78.680733	-0.030217	1496	45	64	94	10	24	19
QCAZ18299	NK298679	<i>Platyrrhinus</i>	<i>umbratus</i>	M	Pichincha	Alambi	-78.680733	-0.030217	1492	28.5	79	75	-	18	14
QCAZ18306	NK298688	<i>Sturnira</i>	<i>bakeri</i>	F	Santo Domingo	Reserva Otongachi	-78.9518828	-0.3212998	856	14.5	42	61	-	16	13
QCAZ18307	NK298689	<i>Sturnira</i>	<i>bakeri</i>	M	Santo Domingo	Reserva Otongachi	-78.9518828	-0.3212998	856	22	43	66	-	18	12
QCAZ18308	NK298692	<i>Sturnira</i>	<i>bakeri</i>	M	Santo Domingo	Hacienda Tinalandia	-79.054433	-0.2979668	685	21.5	44	64	-	17	12
QCAZ18312	NK298640	<i>Sturnira</i>	<i>ludovici</i>	M	Pichincha	Alambi	-78.680733	-0.030217	1496	25	47	75	-	19	15
QCAZ18371	QKM54671	<i>Desmodus</i>	<i>rotundus</i>	F	Manabí	Las Tunas	-80.8152861	-1.6621917	11	36	60.5	68.8	-	20.3	15.6

Supplementary data 2. Phylogeny of Phyllostomidae based on mitochondrial genomes. The tree represents the best maximum likelihood (ML) phylogeny inferring Phyllostomidae and other relationships of chiropteran lineages based on amino acid sequences. The tree was reconstructed in RA under the mtMAM and mtREV+I+G+F models using the full outgroup dataset (71 taxa and 3606 amino acids). Colour-filled semicircles on the nodes indicate ML bootstrap support (as a percentage) and Bayesian posterior probabilities (see inset key). The absence of a semicircle on the node indicates that it was not recovered by ML or Bayesian inferences. Each Navajo rug shows whether the specified node was retrieved (black square) or not (white square) in different analyses performed (see inset key).



Supplementary data 3. Phylogeny of Phyllostomidae based on coding DNA sequences. The tree is based on coding DNA sequences (CDS) from our full nucleotide dataset using a 36-partition scheme, reconstructed in RA under the GTR+GAMMA+I model. Colour-filled semicircles on the nodes indicate maximum likelihood (ML) bootstrap support (as a percentage; see inset key).



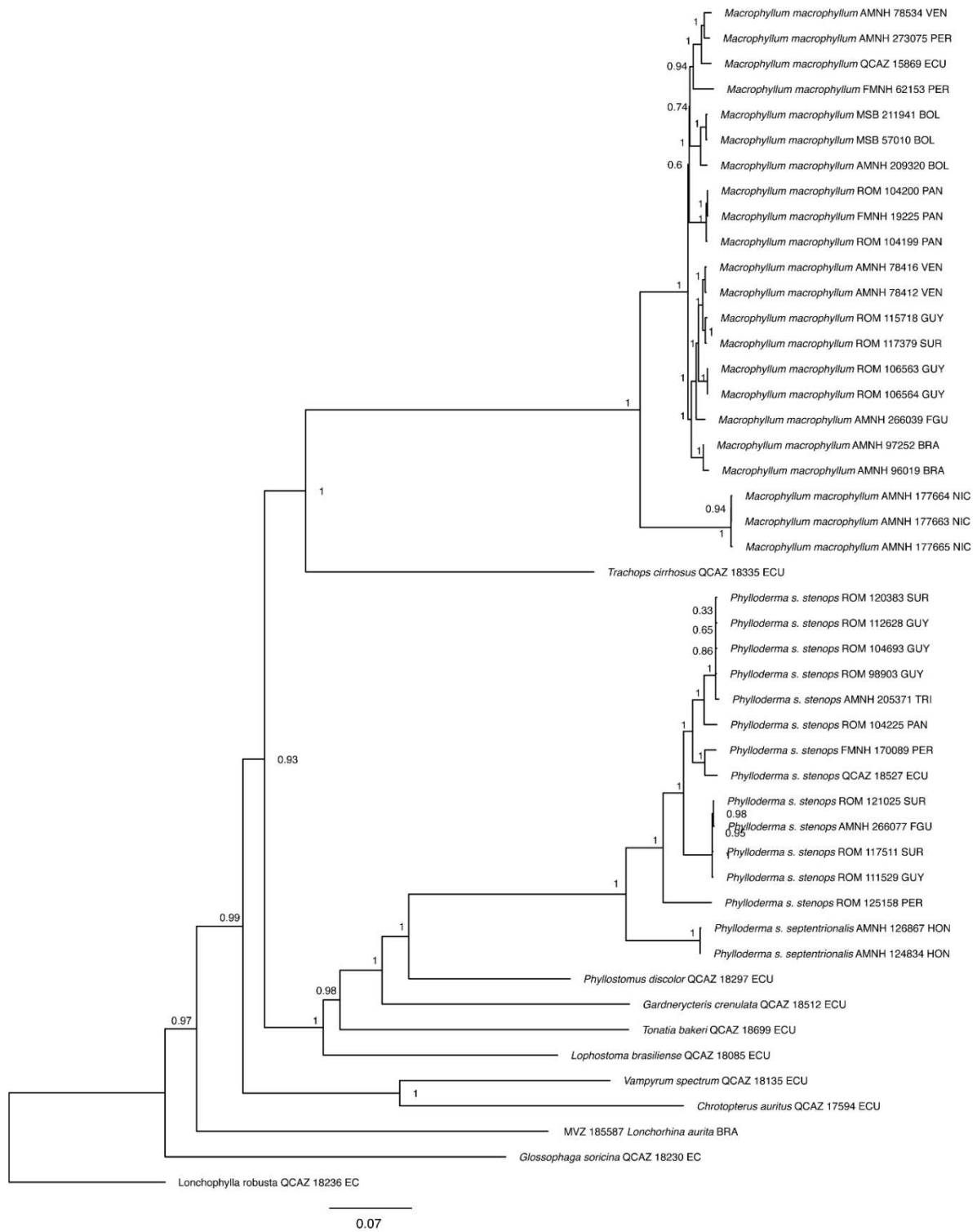
Supplemental data - Chapter 3

Supplementary data 4. Detailed list of *Trachops* specimens used in the research. Includes voucher number, species, locations, including specimens that were measured, sequenced or both. This supporting Information may be found in the online version of the preprint version of the article at bioRxiv web-site: <https://www.biorxiv.org/content/10.1101/2024.01.04.574068v1.supplementary-material>

Supplemental data - Chapter 4

Supplementary data 5. Detailed list of specimens of *Phylloderma* and *Macrophyllum*. The table includes the voucher number, species name, precise locations where each specimen was collected, and indicates whether specimens were measured, sequenced, or subjected to both types of analysis. This supporting Information may be found in: <https://shorturl.at/iHLM8>

Supplementary data 6. Maximum Likelihood phylogeny of *Phylloderma* and *Macrophyllum*. The phylogeny is based on complete mitochondrial genomes and was reconstructed in RAxML under the GTR+G+I model using 47 complete mitogenomes including outgroups. The numbers in the nodes indicate ML bootstrap supports: BS values of 0.75 or higher were considered as robust supported; bootstrap values ranging between 0.50 and 0.75 are of moderate support; and bootstrap values at or below 0.50 were considered as low supported.



Supplementary data 7. Independent samples t-test of *Phylloderma*. Tests comparing measurements of *Phylloderma stenops* and *Phylloderma septentrionalis*. Presented data include the F-statistic (F) and significance (Sig.) from Levene's Test, the t-statistic (t), degrees of freedom (df), p-value (Sig. bilateral), mean difference, standard error of the difference, and the 95% confidence interval for the mean difference. Results are provided for assumptions of both equal and unequal variances.

		Independent Samples Test								
		Levene's test for equality of variances		t test for equality of means						
		F	Sig.	t	df	Sig. (bilateral)	Mean difference	Standard error difference	95% confidence interval of the difference	
									Inferior	Superior
BB	Equal variances are assumed	.076	.784	-4.306	54	.000	-.65223	.15147	-95591	-.34854
	Equal variances are not assumed			-4.107	6.116	.006	-.65223	.15880	-1.03901	-.26544
C-C	Equal variances are assumed	.027	.870	-4.734	54	.000	-.75474	.15943	-1.07438	-.43509
	Equal variances are not assumed			-3.840	5.732	.009	-.75474	.19654	-1.24117	-.26830
CCL	Equal variances are assumed	1.297	.260	-6.011	54	.000	-2.77632	.46185	-3.70228	-1.85036
	Equal variances are not assumed			-6.831	6.773	.000	-2.77632	.40642	-3.74393	-1.80872
CIL	Equal variances are assumed	1.443	.235	-5.891	54	.000	-2.78750	.47320	-3.73621	-1.83879
	Equal variances are not assumed			-7.136	7.110	.000	-2.78750	.39064	-3.70835	-1.86665
COH	Equal variances are assumed	1.354	.250	-3.591	54	.001	-.80106	.22307	-1.24828	-.35384
	Equal variances are not assumed			-4.535	7.369	.002	-.80106	.17664	-1.21455	-.38757
DENL	Equal variances are assumed	.409	.525	-5.609	54	.000	-2.29399	.40899	-3.11396	-1.47402
	Equal variances are not assumed			-5.208	6.041	.002	-2.29399	.44044	-3.36995	-1.21803
GLS	Equal variances are assumed	3.941	.052	-6.470	54	.000	-2.93375	.45346	-3.84287	-2.02462
	Equal variances are not assumed			-12.02	13.30	.000	-2.93375	.24397	-3.45957	-2.40793
M2-M2	Equal variances are assumed	2.614	.112	-4.898	54	.000	-1.06214	.21685	-1.49689	-.62739
	Equal variances are not assumed			-6.191	7.375	.000	-1.06214	.17156	-1.46366	-.66062
MANDL	Equal variances are assumed	1.537	.220	-4.774	54	.000	-.91320	.19127	-1.29667	-.52973
	Equal variances are not assumed			-3.029	5.376	.027	-.91320	.30152	-1.67223	-.15416
MLTRL	Equal variances are assumed	9.146	.004	-4.365	54	.000	-.55193	.12644	-.80543	-.29843
	Equal variances are not assumed			-2.134	5.166	.084	-.55193	.25865	-1.21046	.10660
MPW	Equal variances are assumed	2.764	.102	-4.635	54	.000	-1.20488	.25994	-1.72604	-.68372
	Equal variances are not assumed			-3.144	5.454	.023	-1.20488	.38325	-2.16591	-.24385
MTRL	Equal variances are assumed	1.738	.193	-5.198	54	.000	-.96948	.18652	-1.34342	-.59554
	Equal variances are not assumed			-3.334	5.388	.018	-.96948	.29083	-1.70117	-.23779
PB	Equal variances are assumed	.147	.703	-4.402	54	.000	-.48044	.10913	-.69923	-.26165
	Equal variances are not assumed			-5.299	7.072	.001	-.48044	.09067	-.69440	-.26648
PL	Equal variances are assumed	.512	.477	-6.375	54	.000	-2.09878	.32924	-2.75886	-1.43869
	Equal variances are not assumed			-6.764	6.476	.000	-2.09878	.31027	-2.84465	-1.35291
ZB	Equal variances are assumed	4.830	.032	-6.534	54	.000	-1.88185	.28803	-2.45931	-1.30439
	Equal variances are not assumed			-11.13	11.011	.000	-1.88185	.16904	-2.25386	-1.50984

Independent Samples Test

		Levene's test for equality of variances		t test for equality of means						
		F	Sig.	t	gl	Sig. (bilateral)	Mean difference	Standard error difference	95% confidence interval of the difference	
									Inferior	Superior
BCH	Equal variances are assumed	.292	.591	-1.147	54	.256	-.29319	.25555	-.80554	.21916
	Equal variances are not assumed			-1.030	5.953	.343	-.29319	.28466	-.99105	.40467

Supplementary data 8. Independent samples t-test of *Macrophyllum*. Tests comparing measurements of *Macrophyllum macrophyllum* vs. *Macrophyllum* sp. nov. Presented data include the F-statistic (F) and significance (Sig.) from Levene's Test, the t-statistic (t), degrees of freedom (df), p-value (Sig. bilateral), mean difference, standard error of the difference, and the 95% confidence interval for the mean difference. Results are provided for assumptions of both equal and unequal variances.

		Levene's test for equality of variances		t test for equality of means							
		F	Sig.	t	gl	Sig. (bilateral)	Mean difference	Standard error difference	95% confidence interval of the difference		
										Inferior	Superior
BB	Equal variances are assumed	3.752	.056	-.659	82	.512	-.06024	.09148	-.24222	.12173	
	Equal variances are not assumed			-1.312	34.424	.198	-.06024	.04592	-.15352	.03303	
C-C	Equal variances are assumed	1.638	.204	1.289	82	.201	.07216	.05600	-.03924	.18357	
	Equal variances are not assumed			1.640	14.068	.123	.07216	.04400	-.02216	.16649	
CCL	Equal variances are assumed	3.318	.072	-.616	82	.539	-.07556	.12261	-.31947	.16834	
	Equal variances are not assumed			-.985	19.476	.337	-.07556	.07674	-.23592	.08479	
CIL	Equal variances are assumed	3.686	.058	.036	82	.971	.00444	.12155	-.23736	.24623	
	Equal variances are not assumed			.056	18.227	.956	.00444	.07893	-.16124	.17011	
COH	Equal variances are assumed	1.509	.223	.528	82	.599	.04065	.07700	-.11252	.19383	
	Equal variances are not assumed			.870	20.676	.395	.04065	.04675	-.05667	.13797	
DENL	Equal variances are assumed	.340	.562	1.034	82	.304	.09875	.09552	-.09127	.28877	
	Equal variances are not assumed			1.181	12.711	.259	.09875	.08364	-.08236	.27986	
GLS	Equal variances are assumed	5.095	.027	-.102	82	.919	-.01377	.13438	-.28110	.25356	
	Equal variances are not assumed			-.184	25.193	.856	-.01377	.07490	-.16796	.14042	
M2-M2	Equal variances are assumed	4.159	.045	1.551	82	.125	.11281	.07273	-.03187	.25750	
	Equal variances are not assumed			2.254	16.620	.038	.11281	.05005	.00704	.21859	
MANDL	Equal variances are assumed	4.216	.043	1.694	82	.094	.10418	.06151	-.01817	.22653	
	Equal variances are not assumed			2.456	16.563	.025	.10418	.04242	.01450	.19386	
MLTRL	Equal variances are assumed	2.325	.131	3.746	82	.000	.15989	.04269	.07498	.24481	
	Equal variances are not assumed			4.353	12.896	.001	.15989	.03674	.08047	.23932	
MPW	Equal variances are assumed	3.809	.054	-2.391	82	.019	-.23013	.09624	-.42157	-.03868	
	Equal variances are not assumed			-4.130	22.946	.000	-.23013	.05572	-.34540	-.11486	
MTRL	Equal variances are assumed	1.173	.282	.834	82	.407	.05084	.06094	-.07040	.17208	
	Equal variances are not assumed			1.036	13.721	.318	.05084	.04906	-.05458	.15626	
PB	Equal variances are assumed	.470	.495	.758	82	.451	.03770	.04976	-.06129	.13669	
	Equal variances are not assumed			.676	10.887	.513	.03770	.05576	-.08518	.16058	
PL	Equal variances are assumed	.198	.658	-.759	82	.450	-.10097	.13305	-.36564	.16371	
	Equal variances are not assumed			-.950	13.830	.358	-.10097	.10626	-.32914	.12721	
ZB	Equal variances are assumed	6.830	.011	.536	82	.593	.08045	.15012	-.21820	.37909	
	Equal variances are not assumed			1.060	33.556	.297	.08045	.07593	-.07393	.23483	

		Levene's test for equality of variances		t test for equality of means						
		F	Sig.	t	gl	Sig. (bilateral)	Mean difference	Standard error difference	95% confidence interval of the difference	
									Inferior	Superior
BCH	Equal variances are assumed	.018	.895	1.630	82	.107	.19015	.11663	-.04186	.42217
	Equal variances are not assumed			1.624	11.544	.131	.19015	.11708	-.06606	.44637

Titre : Systématique moléculaire et morphologique des chauves-souris Phyllostomidae : aperçus à partir des génomes mitochondriaux complets

Mots clés : Chauves-souris, Systématique, Phyllostomidae, Trachops, Phylloderma, Macrophyllum

Résumé : Cette thèse explore la systématique moléculaire et morphologique des chauves-souris Phyllostomidées, utilisant des génomes mitochondriaux complets pour éclairer les relations évolutives, le statut taxonomique et la spéciation cryptique au sein de cette famille diverse. Dans le premier chapitre, je présente une phylogénie révisée des Phyllostomidae basée sur des génomes mitochondriaux complets de 26 espèces, dont 23 ont été nouvellement séquencées pour cette étude. Grâce à une analyse minutieuse, j'ai établi un cadre évolutif robuste qui clarifie les relations auparavant incertaines parmi les sous-familles, notamment celles des Lonchorhininae et des Macrotinae. Ce travail pose les bases pour une classification plus précise au sein de la famille. Le deuxième chapitre se concentre sur le genre *Trachops*, réévaluant son statut taxonomique à travers une approche intégrée combinant des données morphologiques, morphométriques et moléculaires. Nos découvertes suggèrent l'élévation de *T. cirrhosus ehrhardti* au rang d'espèce, reconnaissant sa distinction génétique et morphologique dans le sud-est du Brésil. En plus, je ne trouve aucun soutien pour maintenir *T. c. coffini* comme une sous-espèce distincte. Le genre *Trachops* comprendrait alors deux espèces monotypiques, *T. cirrhosus* et *T. ehrhardti*. Le troisième chapitre enquête sur la biodiversité et la spéciation cryptique potentielle au sein des genres *Phylloderma* et *Macrophyllum*, avec un accent particulier sur la révélation d'une nouvelle espèce cryptique d'Amérique centrale. Les analyses complètes indiquent la présence de deux clades fortement soutenus au sein de *Phylloderma stenops* et *Macrophyllum macrophyllum*, menant à la proposition d'élever la population d'Amérique centrale du Nord de *Phylloderma*, *P. s. septentrionalis*, au rang d'espèce, et l'introduction de *Macrophyllum* sp. nov., une nouvelle espèce endémique de la région. Cette thèse souligne la complexité de la diversité des chauves-souris Phyllostomidées et le rôle crucial des analyses moléculaires et morphologiques intégrées pour découvrir la diversité cachée. Cette contribution fournit une compréhension plus claire des processus de spéciation chez les chauves-souris tropicales, soulignant l'importance d'une classification taxonomique précise pour des stratégies de conservation efficaces.

Title: Molecular and Morphological Systematics of Phyllostomid Bats: Insights from complete mitochondrial genomes

Key words: Bats, Systematics, Phyllostomidae, Trachops, Phylloderma, Macrophyllum

Abstract: This thesis explores the molecular and morphological systematics of Phyllostomid bats, utilizing complete mitochondrial genomes to illuminate the evolutionary relationships, taxonomic status, and cryptic speciation within this diverse family. In the first chapter, I present a revised phylogeny of Phyllostomidae based on complete mitochondrial genomes from 26 species, 23 of which are newly sequenced for this study. Through meticulous analysis, I achieved a robust evolutionary framework that clarifies previously uncertain relationships among subfamilies, notably those of Lonchorhininae and Macrotinae. This work sets the stage for a more accurate classification within the family. The second chapter focuses on the genus *Trachops*, reassessing its taxonomic status through an integrated approach that combines morphological, morphometric, and molecular data. Our findings suggest the elevation of *T. cirrhosus ehrhardti* to species status, recognizing its genetic and morphological distinctiveness in southeastern Brazil. Additionally, I find no support for maintaining *T. c. coffini* as a distinct subspecies. The genus *Trachops* would then consist of two monotypic species, *T. cirrhosus*, and *T. ehrhardti*. Chapter three investigates the biodiversity and potential cryptic speciation within the genera *Phylloderma* and *Macrophyllum*, with a particular emphasis on revealing a new cryptic species from Central America. Our comprehensive analyses indicate the presence of two highly supported clades within both *Phylloderma stenops* and *Macrophyllum macrophyllum*, leading to the proposal of elevating the North Central American population of *Phylloderma*, *P. s. septentrionalis*, to species status, and the introduction of *Macrophyllum* sp. nov., a new species endemic to the region. This thesis underscores the complexity of Phyllostomid bat diversity and the critical role of integrated molecular and morphological analyses in uncovering hidden diversity. This contribution provides a clearer understanding of speciation processes in tropical bats, emphasizing the importance of accurate taxonomic classification for effective conservation strategies.