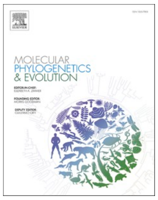
Molecular Phylogenetics and Evolution 201 (2024) 108219

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev



Phylogenomic insights into the worldwide evolutionary relationships of the stingless bees (Apidae, Meliponini)

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ARTICLE INFO

Keywords:
Apoidea
CURE
Gene-genealogy interrogation
Museomics
Partitioning
Phylogeny
UCEs

ABSTRACT

Stingless bees (tribe Meliponini) are remarkable for their characteristically large social colonies, their capacity to produce honey and other useful products, and their morphological and behavioral diversity. They have a disjunct pan-tropical distribution, primarily occurring in warm and humid environments in the Neotropical, Afrotropical, and Indo-Australasian regions. Even though phylogenetic hypotheses have been proposed for Meliponini based on morphology and molecular data, many questions are still unsolved regarding the evolutionary relationships and systematics of the tribe. In this contribution, we present a large phylogenomic dataset comprising over 2500 ultra-conserved element (UCE) loci sequenced for 153 species of Meliponini, representing all known genera of stingless bees. The genera *Camargoia*, *Paratrigonoides*, *Plectoplebeia, Cleptotrigona*, *Ebaiotrigona*, *Papuatrigona*, *Pariotrigona*, *Platytrigona*, and *Sahulotrigona* were included in molecular phylogenetic analyses for the first time. Concatenated and species-tree analyses were performed using different partitioning strategies and summary methods. We performed gene-genealogy interrogation (GGI) on several recalcitrant nodes to resolve discordances among recovered tree topologies. Results were mostly consistent among analyses, recovering three main lineages of Meliponini congruent with the biogeographic domains to which they are associated. Within major clades, discordances were found in relation to previous works. The genus *Frieseomelitta* was recovered as paraphyletic in relation to *Trichotrigona*, and the genus *Lepidotrigona* was revealed to be composed of two independent lineages. Even though concatenated and weighted ASTRAL analyses were mostly effective in recovering the relationships favored by GGI, they retrieved different results in relation to the phylogenetic placements of *Oxytrigona* and *Cephalotrigona*. The most favored hypothesis in GGI analyses was not found in any other analyses, being more congruent with morphological evidence and highlighting the relevance of exploring the support given to alternative hypotheses through topological tests. Recent advances in our capacity to generate molecular sequences from old specimens using modern sequencing methods allowed for unparalleled sampling across genera, yielding a backbone for the phylogenetic relationships of stingless bees, which will further investigations into their systematics and evolution.

1. Introduction

General interest in bees has increased due to alarm about the decline in species in the face of human modification of the environment. Stingless bees (Apidae: Meliponini) are an important group of bees to research, given their diversity, characteristically large social colonies, and capacity to produce honey and other products that are useful for us (Michener, 2007; Grüter, 2020). They are one of the most abundant groups in tropical and subtropical environments, with an intriguing disjunct pantropical distribution that includes most of the Neotropical, Afrotropical, and Oriental regions and humid parts of Australia (Kerr and Maule, 1964; Michener, 1990, 2007). There are over 600 species currently described, with about 75 % of them being found in the Neotropical region, where the Amazon rainforest represents a major

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https://doi.org/10.1016/j.ympev.2024.108219
Received 20 June 2024; Received in revised form 7 October 2024; Accepted 13 October 2024

center of diversity (Grüter, 2020; Melo, 2020). The diversity of the tribe is not yet fully known, with many species waiting for proper recognition and formal description (Roubik, 2018).

Connections between stingless bees and human societies have been recorded for dozens of centuries in the regions where they occur naturally (Crane, 1999). Stingless bees provide many valuable products to humans, such as honey, propolis, resin, wax, and pollen (Nogueira-Neto, 1997). Initially, these products were obtained by hunting colonies in the wild and later by farming through beekeeping (Jones, 2012). The husbandry of stingless bees, conventionally called meliponiculture, has developed from rearing colonies in chopped trunks to specialized wooden boxes that can be opened and closed again to manage the products inside. The earliest records of meliponiculture are those related to Mesoamerican people, who started the practice as early as 1400–1900 years ago (Quezada-Euán, 2018). The topic has an extensive literature, which evolved in consonance with discoveries about stingless bee behavior and ecology (Nogueira-Neto, 1997). In this sense, knowing the identity of stingless bee species is imperative for better preserving them, for instance, by avoiding occasional introductions and consequent generation of hybrid forms, as well as facilitating documentation of such cases (Eardley and Kwapong, 2013; Graf et al., 2020).

Meliponini is one of the four extant tribes included within the group of the corbiculate bees. These bees are characterized by a corbicula, a concavity on the outer surface of the metatibia used to carry pollen and other floral resources (Michener, 1990). Other corbiculate bees and, therefore, close relatives of the stingless bees, are the Apini (honey bees), Bombini (bumble bees), Euglossini (orchid bees), and a handful of fossil lineages (Engel, 2001). The corbiculates represent a key group for the understanding of animal societies, as all Apini, Bombini, and Meliponini, as well as Euglossini to some extent, exhibit eusocial behaviors (Michener, 1969, 1990; see Wilson, 1971 for a definition of eusociality). Besides being the most species-rich tribe among the extant corbiculates, stingless bees are also unique in lacking a functional sting apparatus (Kerr and de Lello, 1962; Michener, 1990; Grüter, 2020). This has led the group to develop defense mechanisms that, coupled with their eusociality, gave rise to an unparalleled diversity of behaviors.

Understanding the evolutionary relationships within corbiculate lineages is paramount for our interpretation of the origins and evolution of eusociality (Almeida and Porto, 2014). Due to their complex colonies, the clade composed of stingless bees, bumble bees, and honey bees represents one of the rare events of animal evolution into superorganismality (Boomsma and Gawne, 2018). Stingless bees share similar biological traits with honey bees, such as the high morphological and physiological divergence among castes, maintenance of perennial colonies, and food storage strategies (Moure et al., 1958; Michener, 1990; Grüter, 2020). On the other hand, phylogenetic hypotheses based on molecular data highly support bumble bees as the closest living relatives of stingless bees (Cameron, 1993; Koulianos et al., 1999; Cameron and Mardulyn, 2001; Lockhart and Cameron, 2001; Kawakita et al., 2008; Cardinal and Danforth, 2011, 2013; Hedtke et al., 2013; Martins et al., 2014; Romiguier et al., 2016; Bossert et al., 2017; 2019; Branstetter et al., 2017; Almeida et al., 2023). This has suggested that the eusocial traits observed in Apini and Meliponini also reflected in their morphology (Porto and Almeida, 2021), could result from evolutionary convergence among both groups. Alternatively, an underexplored hypothesis is that bumble bees developed different eusocial behaviors by modifying the traits present in the common ancestor of the three tribes (Melo, 2020).

1.1. Diversity and classification of stingless bees

Substantial behavioral diversity can also be found within the stingless bees. Species may have different foraging habits (Hubbell and Johnson, 1978; Hrncir and Maia-Silva, 2012); diets, including tear-drinking and obligate necrophagy (Bänziger et al., 2009; Noll, 1997); defensive mechanisms, including acid-smypitting (Schwarz, 1948); and usurpation behavior (Cunningham et al., 2014). More interestingly, a few lineages evolved as robbers, depending solely on the pillaging of other stingless bee nests (Schwarz, 1948, see also Grüter, 2020 for a revision on Meliponini behavior). In response to this behavior, specific defensive strategies have evolved in different groups (Grüter et al., 2012; Rech et al., 2013). Another noticeable characteristic of stingless bees is their nest architecture diversity (Roubik, 2020). Nests are commonly established within preexisting cavities, such as hollow trunks, which the bees adapt by building muddy walls to isolate their contents from outside elements (Schwarz, 1948; Wille and Michener, 1973). Alternatively, nests can be constructed in the ground, within active or inactive nests of other eusocial insects (i.e., ants and termites), exposed on trees and slanting walls, or in different situations that those bees can opportunistically explore (Wheeler, 1913; Schwarz, 1948). The entrances of the nests also show astonishing diversity, often useful for taxonomic purposes (e.g., Camargo and Pedro, 2003a). Internally, nests can vary in many attributes, with species having particular brood cell arrangements, shapes and sizes of storage pots, kinds of materials composing internal structures, and presence of additional chambers, among other traits (Wille and Michener, 1973; Nogueira-Neto, 1997; Roubik, 2006). Such a range of behavioral diversity is accompanied by remarkable morphological disparities (Fig. 1) since workers of some species can be as tiny as 2 mm; in contrast, others are larger than honey bees (Nogueira-Neto, 1951; Melo, 2020)

The classification of Meliponini has undergone multiple modifications throughout its history. The first genus-level names proposed were *Melipona* Illiger, 1806 and *Trigona* Jurine, 1807, often the only names considered by early contributions (Smith, 1854, 1863; Schwarz, 1938; Michener, 1944). Father Jesus S. Moure proposed a classification with more genera, some of them further divided into subgenera (Moure, 1946, 1950, 1951, 1961). This treatment was used as a basis for many subsequent contributions (Camargo and Pedro, 1992b, 2003a, 2009; Eardley, 2004; Rasmussen, 2008; Pedro and Camargo, 2009; Rasmussen et al., 2017; Grüter, 2020; Camargo et al., 2023), and the naturalness of Moure's proposal has largely been confirmed by molecular phylogenies (Rasmussen and Camargo, 2008; Rasmussen and Cameron, 2010). Concomitantly, a classification with fewer genera was maintained in use by some authors (Schwarz, 1948; Wille, 1979; Michener, 1990, 2007), and intermediate hypotheses were also applied (e.g., Sakagami, 1975; Ayala, 1999). Most recent classifications proposed for the Eastern Hemisphere Meliponini follow Moure's system (Eardley, 2004; Rasmussen, 2008; Grüter, 2020), with additions of supraspecific names and lumping of genera (see Engel et al., 2021, 2023). For the Neotropical taxa, Camargo et al. (2023) recognized subgenera only within the genus *Melipona*, while Engel et al. (2023) lowered many genera to subgenus level.

1.2. Stingless bee diversity from a phylogenetic perspective

Investigations of phylogenetic relationships among stingless bee lineages have an extensive background, which was, for a long time, based on morphological evidence (Schwarz, 1948; Wille, 1979; Michener, 1990; Camargo and Pedro, 1992a, 1992b, 1996; Roubik et al., 1997; Oliveira, 2002; Camargo and Roubik, 2005). More recently, a few attempts have been made to apply molecular data to this task (Costa et al., 2003; Ramírez et al., 2010; Rasmussen and Camargo, 2008; Rasmussen and Cameron, 2007, 2010)

Among the available phylogenetic hypotheses, the most comprehensive to date is that by Rasmussen and Cameron (2010), which used five molecular markers sequenced for 186 species and additional molecular data for selected taxa in order to confirm deeper relationships. Their results were, in many aspects, highly discordant with previous hypotheses based on morphology, having a crucial impact on the systematics of the group. Their results also yielded important insights into the biogeography of Meliponini, which could be divided into two major clades: one comprising all Neotropical genera and the other including all

Fig. 1. Diversity of stingless bees. A) *Dactylurina staudingeri* (Gribodo, 1893) from the Democratic Republic of the Congo; B) *Meliponula bocandei* (Spinola, 1851) from Kenya; C) *Tetragonula carbonaria* (Smith, 1854) from Australia; D) *Geniotrigona thoracica* (Smith, 1857) from Malaysia; E) *Lepidotrigona terminata* (Smith, 1878) from Indonesia; F) *Melipona colimana* Ayala, 1999 from Mexico; G) *Nannotrigona mellaria* (Smith, 1862) from Costa Rica; H) *Oxytrigona cagafogo* (Muller, 1874) from Brazil; I) *Paratrigona subnuda* (Moure, 1947) from Brazil; J) *Scaura amazonica* Melo, 2004 from Brazil; K) *Tetragona clavipes* (Fabricius, 1804) from Brazil; L) *Trigonisca* sp. from Brazil. Photograph credits: A: Nicolas Vereecken and Alain Pauly; B: Subramanian Sevgan; C: Graham Stott; D: Fiora Li; E: Mark Chao; F: Omar R. Martín; G: Jürg Sommerhalder; H, J, and L: Adriana Tiba and Julio Pupim; I and K: Felipe B. Fraga.

taxa from the Eastern Hemisphere (Rasmussen and Cameron, 2010). The latter can be further subdivided into an exclusively Indo-Malayan and Australasian lineage (formerly included in the genus *Trigona* by e.g., Michener, 2007) and a primarily Afrotropical lineage, which also includes some minute forms from Southeastern Asia and the Australian and New Guinean genus *Austroplebeia* Moure, 1961 (Rasmussen and Cameron, 2007, 2010). Divergence times were estimated for the origin of stingless bees to the second half of the Cretaceous, allowing for a better understanding of their biogeography. When considering the Neotropical, Afrotropical, Indo-Malayan, and Australasian areas, stingless bee biogeography was marked by ancient vicariant events, with dispersal playing a secondary role (Rasmussen and Cameron, 2007). Nevertheless, the exact order of biogeographic events and the connections indicated by the known fossil record are still unclear.

Although the results of Rasmussen and Cameron (2007, 2010) became the basis for the current meliponine classification, nearly no comprehensive phylogenetic investigations using alternative datasets have been conducted since then. Additionally, it is still unclear how morphological novelties, complex social traits, and nesting preferences, among other biological features, evolved throughout the natural history of Meliponini. Additional important contributions include studies on the evolution of size-dimorphism (Quezada-Euán et al., 2019), changes in chromosome numbers (Travenzoli et al., 2019), and comparative morphology (Porto and Almeida, 2021; Porto et al., 2021), which, although focused primarily on corbiculate bee relationships, included a representative sampling of the major stingless bee lineages. With the increasing popularization of genome-scale datasets, the application of phylogenomic methods to uncoverand increase confidence in relationships among bee lineages has become increasingly common (Romiguier et al., 2016; Bossert et al., 2017, 2019; Branstetter et al., 2017, 2021; Freitas et al., 2021, 2023; Almeida, Bossert et al., 2023). Phylogenomics, in opposition to traditional phylogenetic analyses based on Sanger sequencing, makes use of large amounts of genomic data for establishing evolutionary kinship (Pennisi, 2008). Besides greatly increasing the amount of data available for inferences, NGS-based phylogenomic approaches have enabled “museomics”, other sequencing of very old museum specimens, thus broadening our capacity to sample rare species (Blaimer et al., 2016; Ruane and Austin, 2017; Freitas et al., 2023). Consequently, this has improved taxonomic classifications and increased our knowledge about evolutionary processes, such as biogeography. Currently, the most popular phylogenomic markers for bees are the ultraconserved elements (UCEs). These markers have highly conserved cores that are flanked by increasingly divergent regions (i.e., with more mutations away from the core) when comparing among taxa (Bejerano et al., 2004; Faircloth et al., 2012). Due to this property, UCEs presumably have information useful for inferring relationships in many depths of the tree of life (Faircloth et al., 2012).

Given that fourteen years have passed since the publication of the broad investigation of Rasmussen and Cameron (2010), in the present study, we provide a novel phylogeny for the Meliponini, including representatives of every known genera. We consider that another global phylogenetic analysis of Meliponini addresses major questions raised by previous contributions, such as (1) the confirmation or dissonance of clades retrieved using an independent source of data; (2) the interrogation of nodes that showed low support; and (3) the positioning of taxa not yet included in any phylogenetic analysis of the tribe. For this purpose, we use a newly generated phylogenomic dataset comprising over 2500 UCE loci sequenced for all genera currently accepted for the tribe. We also analyze the generated dataset using concatenate and species-tree approaches and explore critical areas of incongruence using gene-tree based methods.

2. Material and methods

2.1. Classification and taxon sampling

For Neotropical taxa, we follow the classification of Camargo et al. (2023). Although *Dolichotrigona* Moure, 1950 clearly renders *Trigonisca* Moure, 1950 paraphyletic (Rasmussen and Cameron, 2010), we opted to maintain the use of the name of the former until a proper revision of the group's systematics is carried out. For Afrotropical taxa, we follow the classification proposed by Moure (1961), except for the use of *Cleptotrigona* Moure, 1961, here treated as a separate genus and not as a subgenus of *Lestrimelitta* Friese, 1903. The subgenera considered for the genus *Hypotrigona* Cockerell, 1934 by Moure (1961) are also treated as genera, following Eardley (2004). For Indo-Malayan and Australasian taxa we follow Rasmussen (2008), which is largely based on Moure (1961), with the addition of *Wallacetrigona* Engel and Rasmussen, 2017, *Ebaiotrigona* Engel and Nguyen, 2022, and species described subsequently. Grüter (2020) considers *Sahulotrigona* Engel and Rasmussen, 2017 as a genus, an opinion followed herein based on the results of preliminary analyses. In summary, subgenera are only applied to *Melipona,* following Camargo et al. (2023). This classification is roughly the same as that adopted by Grüter (2020), with few modifications. Table 1 summarizes the classification adopted in this work, which will be properly accounted for in the Discussion. A summary of the sampling of Meliponini is also provided in Table 1, which can be complemented with information in Table S1 (online Supplementary Materials).

A dataset of ultraconserved elements (UCEs) was assembled comprising 169 bee taxa, including 16 species from the tribes Centridini, Euglossini, Bombini, and Apini, and 153 species of Meliponini. The outgroup sampling aimed to represent all currently valid extant corbiculate bee genera and the closely related apine tribe Centridini, as consistently recovered in previous works (Cardinal et al., 2010; Hedtke et al., 2013; Martins et al., 2014; Bossert et al., 2019; Porto and Almeida, 2021; Almeida et al., 2023). Therefore, the root was placed between *Centris analis* and all remaining taxa in the resulting trees. For all outgroup terminals and several Meliponini species, we retrieved UCEs from datasets available from previous publications (Branstetter et al., 2017; Bossert et al., 2019; Grab et al., 2019; Hereward et al., 2020; Sun et al., 2021). Since the monophyly of the four outgroup tribes and their included genera has been confirmed in previous studies, we did not

Table 1
Genus-level classification of extant Meliponini (Apidae), organized by geographic region. Taxa in bold represent genera included for the first time in a molecular phylogenetic study. The column 'Richness [Sampling]' summarizes the number of valid species according to Grüter (2020), Camargo et al. (2023), and Engel et al. (2023), complemented by recently published papers / the number of species sampled for this work between brackets. For additional information about the taxon sampling (including outgroup taxa), refer to Table S1 (online Supplementary Information).

DISTRIBUTION	GENUS	RICHNESS [SAMPLING]
Neotropical	*Aparatrigona* Moure, 1951	2 species [1]
	Camargoia Moure, 1989	3 species [2]
	Celetrigona Moure, 1950	4 species [2]
	Cephalotrigona Schwarz, 1940	5 species [2]
	Dolichotrigona Moure, 1950	10 species [3]
	Duckeola Moure, 1944	2 species [1]
	Friesella Moure, 1946	1 species [1]
	Frieseomelitta Ihering, 1912	14 species [4]
	Geotrigona Moure, 1943	22 species [3]
	Lestrimelitta Friese, 1903	26 species [3]
	Leurotrigona Friese, 1903	4 species [2]
	Melipona (Eomelipona) Moure, 1992	9 species [3]
	Melipona (Melikerria) Moure, 1992	10 species [3]
	Melipona (Melipona) Illiger, 1806	13 species [2]
	Melipona (Meliponiella) Melo, 2021	4 species [1]
	Melipona (Michmelia) Moure, 1975	36 species [3]
	Meliwillea Roubik, Lobo & Camargo, 1997	1 species [1]
	Mourella Schwarz, 1946	1 species [1]
	Nannotrigona Cockerell, 1922	14 species [3]
	Nogueirapis Moure, 1953	6 species [2]
	Oxytrigona Cockerell, 1917	14 species [5]
	Parapartamona Schwarz, 1948	7 species [1]
	Paratrigona Moure, 1951	33 species [3]
	Paratrigonoides Camargo & Roubik, 2005	1 species [1]
	Partamona Schwarz, 1938	32 species [4]
	Plebeia Schwarz, 1938	54 species [7]
	Plectoplebeia Melo, 2016	1 species [1]
	Ptilotrigona Moure, 1951	3 species [2]
	Scaptotrigona Moure, 1942	51 species [5]
	Scaura Schwarz, 1938	7 species [3]
	Schwarziana Moure, 1943	4 species [3]
	Schwarzula Moure, 1946	2 species [2]
	Tetragona Lepeletier & Serville, 1828	15 species [2]
	Tetragonisca Moure, 1946	4 species [3]
	Trichotrigona Camargo & Moure, 1983	2 species [2]
	Trigona Jurine, 1807	36 species [11]
	Trigonisca Moure, 1950	28 species [8]
Afrotropical	*Apotrigona* Moure, 1961	1 species [1]
	Axestotrigona Moure, 1961	5 species [2]
	Cleptotrigona Moure, 1961	2 species [1]
	Dactylurina Cockerell, 1934	2 species [1]
	Hypotrigona Cockerell, 1934	4 species [2]
	Liotrigona Moure, 1961	13 species [2]
	Meliplebeia Moure, 1961	4 species [2]
	Meliponula Cockerell, 1934	1 species [1]
	Plebeiella Moure, 1961	2 species [1]
	Plebeina Moure, 1961	2 species [2]
Indo-Australasian	*Austroplebeia* Moure, 1961	5 species [4]
	Ebaiotrigona Engel & Nguyen, 2022	1 species [1]
	Geniotrigona Moure, 1961	2 species [1]
	Heterotrigona Schwarz, 1939	4 species [2]
	Homotrigona Moure, 1961	4 species [1]
	Lepidotrigona Schwarz, 1939	17 species [4]
	Lisotrigona Moure, 1961	2 species [2]
	Lophotrigona Moure, 1961	1 species [1]
	Odontotrigona Moure, 1961	1 species [1]

(*continued on next page*)

Table 1 (*continued*)

DISTRIBUTION	GENUS	RICHNESS [SAMPLING]
	Papuatrigona Michener & Sakagami, 1990	1 species [1]
	Pariotrigona Moure, 1961	1 species [1]
	Platytrigona Moure, 1961*	4 species [1]
	Sahulotrigona Engel & Rasmussen, 2017	3 species [1]
	Sundatrigona Inoue & Sakagami, 1993	2 species [1]
	Tetragonilla Moure, 1961	4 species [2]
	Tetragonula Moure, 1961	40 species [5]
	Tetrigona Moure, 1961	5 species [2]
	Wallacetrigona Engel & Rasmussen, 2017	1 species [1]
TOTAL	61 genera and 5 subgenera	612 species **[153]**

*Although *Trigona hobbyi* Schwarz, 1937 had been sampled by Rasmussen & Cameron (2007, 2010) as a species of *Platytrigona*, it was recently transferred to *Heterotrigona* Schwarz by Engel (2019). For this reason, *Platytrigona* is listed as a genus included for the first time in a molecular phylogenetic analysis.

sample them extensively. Specimens used for DNA sequencing are deposited at the following institutions (see Table S1 for further information on voucher specimens): Division of Invertebrate Zoology, American Museum of Natural History, New York, USA (AMNH); U.S. National Pollinating Insects Collection, Logan, USA (NPIC); Coleção Entomológica Pe. J. S. Moure, Curitiba, Paraná, Brazil (DZUP); and Coleção Entomológica Prof. "J.M.F.Camargo", Ribeirão Preto, Brazil (RPSP). We provide information on voucher specimens and sampling in Table S1 (online Supplementary Materials).

2.2. DNA extraction

For newly sequenced terminals, we extracted DNA using Zymo Quick-DNA Miniprep Plus kits (Irvine, CA, U.S.A.) by destructively sampling the fore or mid legs of the specimens, except for minute bees, for which the whole bodies were non-destructively soaked in proteinase K for extraction following Branstetter et al. (2021). Most specimens were dry and pinned prior to extraction, some were preserved in 70–99 % EtOH at DZUP and RPSP. For each extraction, we followed the manufacturer's extraction protocol, except for the modifications noted in Branstetter et al. (2021). The extracted DNA was quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and visualized using TapeStation 4150 (Agilent, Santa Clara, CA., U.S.A.). After extraction, specimens were pinned and labeled with voucher numbers, which are provided in Supplementary Table 1.

2.3. UCE data preparation and sequencing

Library preparation and UCE enrichment closely followed the methods reported in Branstetter et al. (2021). Briefly, high-quality DNA samples were sheared to around 400–600 bp using a QSonica Q800 R3 acoustic sonicator (Qsonica, Newtown, CT, U.S.A.), while low-quality DNA extractions were already at or below the required fragmentation, meaning no shearing was required. Sheared DNA was input into a modified library preparation procedure that used KAPA HyperPrep Kits (Roche Diagnostics, Indianapolis, IN, U.S.A.) and custom, 8 bp dual-indexing adapters with adjustments to manufacturer guidelines to run end repair, A-tailing, and ligation reactions at quarter volumes with 12 rounds of PCR. Post-PCR libraries were quantified using a Qubit fluorometer, and library samples below a concentration threshold of 10 ng/ul underwent additional PCR cycles. Unique dual-indexing was used to avoid adapter hopping. Libraries were pooled, and pools of 8–11 samples were standardized to a concentration of 72 ng/ul for enrichment. Enrichment was performed using a modified protocol in which we followed Arbor Biosciences v3.02 protocol for enrichment day 1 (more efficient than the standard protocol), and a standard UCE protocol (enrichment protocol v1.5 available at ultraconserved.org) for day 2. This procedure produces good results for UCE capture, plus results in the sequencing of mitochondrial markers as bycatch. Pools were enriched using a bee-ant specific UCE probe set ('hym-v2-bee-ant-specific' Daicel Arbor Biosciences, Ann Arbor, MI, U.S.A.; Grab et al., 2019). This bait set is a subset of the principal Hymenoptera bait set (Branstetter et al., 2017) and targets 2545 UCE loci, plus an additional 7 'legacy' markers. Post enrichment was precisely quantified using KAPA qPCR Library Quantification Complete kit (Roche Diagnostics, Indianapolis, IN, U.S.A.) to pool equimolarly for final sequencing. Following UCE enrichment, pools were combined into batches of up to 110 individual libraries and submitted for Illumina HiSeq X (2 × 150) lane sequencing at Novogene Inc. (Sacramento, CA). All newly generated data are available on NCBI (BioProject#PRJNA1169349).

2.4. Bioinformatic data processing

The raw sequence data were demultiplexed using bbmap (Bushnell, 2014), allowing for one sequencing error in the index reads. Most of the following bioinformatic steps were carried with PHYLUCE v. 1.7.3 (Faircloth, 2016). Raw reads were demultiplexed and trimmed with Illumiprocessor v. 2.0 (Faircloth, 2013), which uses the Trimmomatic package (Bolger et al., 2014), and sequences were then assembled into contigs using SPAdes v. 3.5.5 (Bankevich et al., 2012). Statistics of the post-assembly contigs are provided in Table S2 (online Supplementary Materials). The contigs were then matched to the UCE probes ('hym-v2-bee-ant-specific'; Grab et al., 2019) with minimum coverage and minimum identity parameters set to 80. Descriptive statistics about the UCE contigs were obtained and are provided in Table S3 (online Supplementary Materials). The contigs matching UCEs were aligned with MAFFT v. 7.471, adding the "-no-trim" flag (Katoh and Standley, 2013). Alignment trimming was conducted using GBLOCKS (Castresana, 2000; Talavera and Castresana, 2007), with default parameters. In the final matrix, we selected only loci that included at least 80 % of the terminals (80p matrix). A preliminary maximum likelihood tree was then generated with IQ-TREE v. 2.2.2.7 (Minh et al., 2020) to visualize anomalous branch lengths so further trimming on poorly aligned portions could be performed with Spruceup (Borowiec, 2019) using the Jukes-Cantor distance method, window size of 20 positions, overlap of 15, lognormal criterion and general cutoffs of 0.98. Manual cutoffs were performed for specific taxa recovered with artefactually long branches after additional IQ-TREE analyses, using the following values: *Austroplebeia symei*: 0.08; *Homotrigona fimbriata*: 0.045; *Lepidotrigona nitidiventris*: 0.045; *Lepidotrigona ventralis*: 0.07; *Leurotrigona gracilis*: 0.07; *Liotrigona bottegoi*: 0.06; *Melipona (Meliponiella) illustris*: 0.08; *Meliwillea bivea*: 0.06; *Oxytrigona mediorufa*: 0.08; *Parapartamona vittigera*: 0.08; *Platytrigona lamingtonia*: 0.08; *Plebeiella lendliana*: 0.06; *Sahulotrigona paradisaea*: 0.045; *Tetragonisca buchwaldi*: 0.09; *Trigona dimidiata*: 0.04; *Trigona pallens*: 0.09; *Trigonisca* aff. *dobzhanskyi*: 0.07; *Trigonisca nataliae*: 0.07; and *Trigonisca pediculana*: 0.08.

Partitioning of the dataset for phylogenetic analyses was performed using CURE (Freitas et al., 2023). This pipeline implements the entropy-based sliding-window site (SWSC-EN) method for identifying core and flanking regions of each UCE (Tagliacollo and Lanfear, 2018). CURE also allows for the identification and merging of UCEs that belong to the same gene using a reference genome and annotates sequences as being exonic, intronic, or intergenic. Data partitioning aims to accommodate the heterogeneity of sequence evolution in genomic datasets, allowing for the application of different models to regions in the DNA with distinct rates of evolution. The partitioning of UCEs is based on the fact that core regions are highly conserved, while flanking regions exhibit higher degree of variation across species (Faircloth et al., 2012). Concatenated analyses were conducted using the complete set of taxa with sequences partitioned by (1) UCE locus, and (2) UCE regions (core, left flank, and right flank). An additional unpartitioned analysis was conducted to search for effects on branch lengths and clade supports.

2.5. Phylogenetic inference

Concatenated analyses were performed on IQ-TREE v. 2.2.2.7 using the −MFP + MERGE and −rclusterf 10 flags, which implements tree search together with partition merging and model selection using ModelFinder2 (Kalyaanamoorthy et al., 2017). To address clade support, we performed UFBoot (Minh et al., 2013; Hoang et al., 2018) and SH-like approximate likelihood ratio test (Guindon et al., 2010) with 1000 replicates each. For species tree analyses, we ranked taxa by number of UCE loci recovered and removed all those with less than 2000 loci. This selection aimed to avoid effects of missing data over downstream analyses and the resulting species trees, since some species were represented by a low number of UCE loci. The resulting dataset (>2000 matrix) was processed in Phyluce and Spruceup using the same pipeline described above, except that manual cutoffs were not performed. We then generated individual gene trees for each locus using IQ-TREE. Two approaches were applied to retrieve the gene trees: (1) individual trees were generated for each UCE partitioned in core and flanking regions, and (2) individual trees were generated for each gene partitioned by genic regions (introns and exons). The grouping of UCEs belonging to the same genes is based on the assumption of non-independent evolution of portions within genes and the observation that longer alignments tend to produce higher-quality gene trees (Van Dam et al., 2021). Although the hym-v2 probe set was designed to select UCE loci that are at least 1 kb apart, some loci have been observed to overlap within the same gene. Accounting for this can improve phylogenetic inference methods that correct for gene tree discordance due to independent lineage sorting effects. Gene trees were used as input for species-tree estimation in ASTER v. 1.15. This program allows for the generation of species trees from unrooted gene trees through the multi-species coalescent model, taking into consideration branch-support and branch-lengths of the individual gene trees as weights (Zhang and Mirarab, 2022). Both unweighted (ASTRAL) and weighted (ASTRAL-hybrid) analyses were performed over each set of trees recovered using different partitioning of loci. The derived trees were visualized in FigTree v. 1.4.4 (Rambaut, 2018).

2.6. Topological tests

We performed tree topological tests to evaluate the support given to conflicting hypotheses by individual loci, taking advantage of the large number of loci in our dataset (>2000). We selected four nodes in the Neotropical clade showing discordances among results (i.e., concatenated, ASTRAL, ASTRAL-hybrid). This selection resulted in 16 different topological hypotheses corresponding to the combinations of both configurations for the four nodes investigated. Over the 16 hypotheses, we performed gene-genealogy interrogation (GGI) analyses following the procedures described by Arcila et al. (2017). This approach is based on the evaluation of the support given to each alternative hypothesis locus-by-locus. Site likelihoods are calculated for each hypothesis through constrained analyses in IQ-TREE, then tested against each other using the approximately unbiased (AU) test, which yields *P*-values used to evaluate the significance of the support given to one tree over the alternative hypotheses. The hypotheses are ranked according to their *P*-values, allowing the identification of the tree favored by each set of UCEs and the summary of the number of loci supporting each of the hypotheses evaluated.

GGI analyses were conducted by using UCEs as individual loci and also by grouping UCEs belonging to the same gene. The 16 trees were generated by hand in Mesquite v. 3.81 (Maddison and Maddison, 2007) and used as input in IQ-TREE as topological constraints. We then generated individual-loci trees congruent with each of the hypotheses being evaluated using constrained analyses. Site-specific likelihoods for each hypothesis were estimated in IQ-TREE based on UCEs grouped by genes and on each of the individual UCEs in separate sets of GGI analyses (Arcila et al., 2017; Freitas et al., 2021). Topological tests were then conducted for each locus in CONSEL v. 0.1 (Shimodaira and Hasegawa, 2001) using the approximately unbiased (AU) test, which yielded the *P*-values posteriorly used to evaluate the significance of the support given to one tree over alternative hypotheses. Ranking of hypotheses and visualization of the results were then performed using R v. 4.3.3 (R Core Team, 2020).

3. Results

The present investigation allowed for the construction of the largest phylogenomic matrix available to date for stingless bees, comprising 153 species distributed in 61 genera. UCE data were generated for the first time for 144 species of Meliponini, with nearly one-half of those species included in a molecular phylogeny for the first time. This sampling was complemented by 16 species of outgroup taxa representing the other four closely related tribes of Apinae (Apini, Bombini, Centridini, and Euglossini), and all genera of corbiculate bees (i.e., *Aglae, Apis, Bombus, Eufriesea, Euglossa, Eulaema*, and *Exaerete*). The analyses included all genera of stingless bees currently accepted, in addition to the five subgenera of *Melipona* (*sensu* Camargo et al., 2023). Sequences of the following genera were made available for the first time: *Camargoia*, *Cleptotrigona*, *Ebaiotrigona*, *Papuatrigona*, *Paratrigonoides*, *Pariotrigona*, *Platytrigona*, *Plectoplebeia,* and *Sahulotrigona*.

A total of 2522 loci were captured across all samples. The 80p matrix based on the complete dataset comprised 1993 loci, resulting in an alignment 665,521 bp long. Some taxa were represented by only a few UCEs (see Table S3), which could influence the reliability of species trees estimated from the whole dataset (Figures S1–S3). To overcome the effects of missing data, we estimated species trees using the '>2000′ dataset, which included 146 of the 153 species of the complete matrix (Figures S4–S7). After filtering the >2000 dataset to include only loci represented in more than 80 % of the taxa, 2113 UCE-loci were retrieved, resulting in an alignment with 780,456 bp. The increased length of the latter dataset in relation to the complete 80p matrix is derived from the removal of taxa containing a large amount of missing data. This way, more UCEs were retrieved as present in at least 80 % of the taxa, which now represent those with the best phylogenomic coverage. Using CURE, UCEs were assigned to a total of 1183 gene loci using the *Apis mellifera* reference genome (13 UCEs were interpreted as belonging to intergenic regions, which were therefore excluded from downstream gene-tree inferences). These analyses allowed us to visualize the effect of taxon and gene sampling on the inferences of evolutionary relationships among stingless bees.

Phylogenetic trees resulting from concatenated analyses are shown with branch lengths and supports in Figures S1–S3 (online Supplementary Materials). All concatenated analyses, using different partitioning schemes, recovered identical tree topologies with maximum support (UFBoot and SH-like approximate likelihood ratio test) for most nodes. The resulting phylogeny is shown in Fig. 2. Bombini was consistently recovered as the sister group of the stingless bees in all analyses, with Apini being recovered as the sister of this clade, and Euglossini as sister to all other corbiculate bees. Meliponini was consistently recovered as comprising three main lineages marked by their geographic affinities. All Neotropical species were recovered as a clade sister to all Eastern Hemisphere lineages (i.e., Afrotropical and Indo-Australasian taxa). Most Indo-Australasian genera were grouped into a clade exclusive to that region, and a primarily Afrotropical clade grouped all genera distributed in Africa plus three Indo-Australasian genera: *Austroplebeia*, *Ebaiotrigona*, and *Lisotrigona* (Fig. 2).

The absolute majority of meliponine genera were recovered as monophyletic. Exceptions include *Frieseomelitta*, *Lepidotrigona*, *Plebeia*, *Scaura*, and *Trigonisca*. *Frieseomelitta* was recovered as paraphyletic in relation to *Trichotrigona*, with *Frieseomelitta flavicornis* and *F. nigra*

Fig. 2. Maximum likelihood phylogenetic relationships among 153 stingless bee species (Apidae: Meliponini) and 16 outgroups representing the remaining four tribes of Apinae. Phylogenetic results retrieved with IQ-Tree using 1993 UCE loci (80p matrix) and partitioning UCEs in core/flank regions with SWSC-EN. Support values on nodes indicate SH-aLRT/UFBoot scores for values below 100/100. Branch lengths proportional to the expected number of character-state changes per site, as indicated by the scale bar (branch lengths linking corbiculate tribes not to scale: in gray); map illustrating the distribution of stingless bees based on Grüter (2020).

forming the sister group of the two recognized species of the cleptobiotic genus *Trichotrigona: T. extranea* and *T. camargoiana*. *Lepidotrigona nitidiventris*, the type species of the genus, was recovered as sister of *Heterotrigona*, with the remaining *Lepidotrigona* forming the sister clade of *Wallacetrigona incisa*. *Plebeia* was recovered as paraphyletic in relation to *Friesella* and *Lestrimelitta*, with part of the species being recovered as sister group of *Friesella* + *Lestrimellitta* + the remaining species. The genus *Scaura* was recovered as paraphyletic in relation to *Schwarzula*, with *Scaura argyrea* being recovered as the sister group to the two recognized species of the latter genus. Finally, *Trigonisca* was recovered as paraphyletic in relation to *Dolichotrigona*, being split in at least four independent lineages.

Among all concatenated analyses, the only clade to receive low support included the taxa *Sahulotrigona paradisaea* and *Papuatrigona atricornis*, with SH-like values between 20.7 and 28.5, and UFBoot values between 39 and 67 (Fig. 2). Despite the low support, the clade was recovered in all concatenated analyses, and its grouping with *Platytrigona keyensis* had maximum support from both metrics. On the concatenated and species-tree analyses using the >2000 matrix (which does not include *S. paradisaea* and *P. atricornis*), *P. keyensis* grouped with *Pariotrigona pendleburyi*, forming a clade sister to *Tetragonilla* + *Tetragonula* (Fig. 5). This result is fully congruent with the results based on the 80p matrix (Figures S1-S3).

Species trees based on individual UCE gene-trees showed few differences in relation to each other, regarding the use of ASTRAL (ASTRAL-UCE) or ASTRAL-hybrid (h-UCE). In contrast, species trees derived from analyses of UCEs grouped according to gene region (ASTRAL-genes and h-genes henceforward) showed differences regarding the summary methods. The h-UCE analysis retrieved topologies that were most similar to concatenated analyses. In the other analyses, differences were noted, especially in the Neotropical clade that comprises *Camargoia, Frieseomelitta*, *Nannotrigona*, *Plebeia*, *Trigona* and allied genera (see below). In the ASTRAL-genes summary tree *Oxytrigona* was recovered as the sister group of *Scaptotrigona*, while in remaining analyses *Oxytrigona* was recovered as sister to *Trigona* and related genera, with *Scaptotrigona* as the sister group to this clade. Differences were also noted in the position of *Cephalotrigona*, which was recovered as sister to *Geotrigona* in ASTRAL-genes and h-genes analyses, while it was recovered as sister to *Tetragona* + *Ptilotrigona* + *Camargoia* in the remaining analyses. Other differences could be noted in the position of *Dactylurina staudingeri* and *Geniotrigona thoracica.* Complete species-trees with branch supports are given in Figures S4–S7 (online Supplementary Materials).

We evaluated support given to each clade recovered in different configurations according to the partitioning strategy and summary method to explore incongruences between species-tree analyses. A total of 16 different hypotheses were evaluated (H1–H16), as illustrated in Fig. 3. For the constrained hypotheses, we assumed four major sets of taxa as monophyletic, based on the results of the concatenated and species-tree analyses: *Frieseomelitta* + *Duckeola* + *Scaura* + *Schwarzula* + *Tetragonisca* + *Trichotrigona* (the "*Frieseomelitta* clade"); *Nannotrigona* + *Mourella* + *Schwarziana* (the "*Nannotrigona* clade"); *Plebeia* + *Friesella* + *Lestrimelitta* (the "*Plebeia* clade"); and *Trigona* + *Camargoia* + *Cephalotrigona* + *Geotrigona* + *Oxytrigona* + *Ptilotrigona* + *Scaptotrigona* + *Tetragona* (the "*Trigona* clade"). Hypotheses were drawn based on (1) the four configurations recovered for the relationships among these four clades, (2) the closest relatives of *Oxytrigona*, and (3) the placement of *Cephalotrigona* in the *Trigona* clade. These hypotheses were ranked according to site-likelihoods calculated through constrained analyses in IQ-TREE for UCEs grouped by gene and individually for each UCE.

Topological tests supported a hypothesis different from that recovered by concatenated analyses (Fig. 4). The most likely result favors (a)

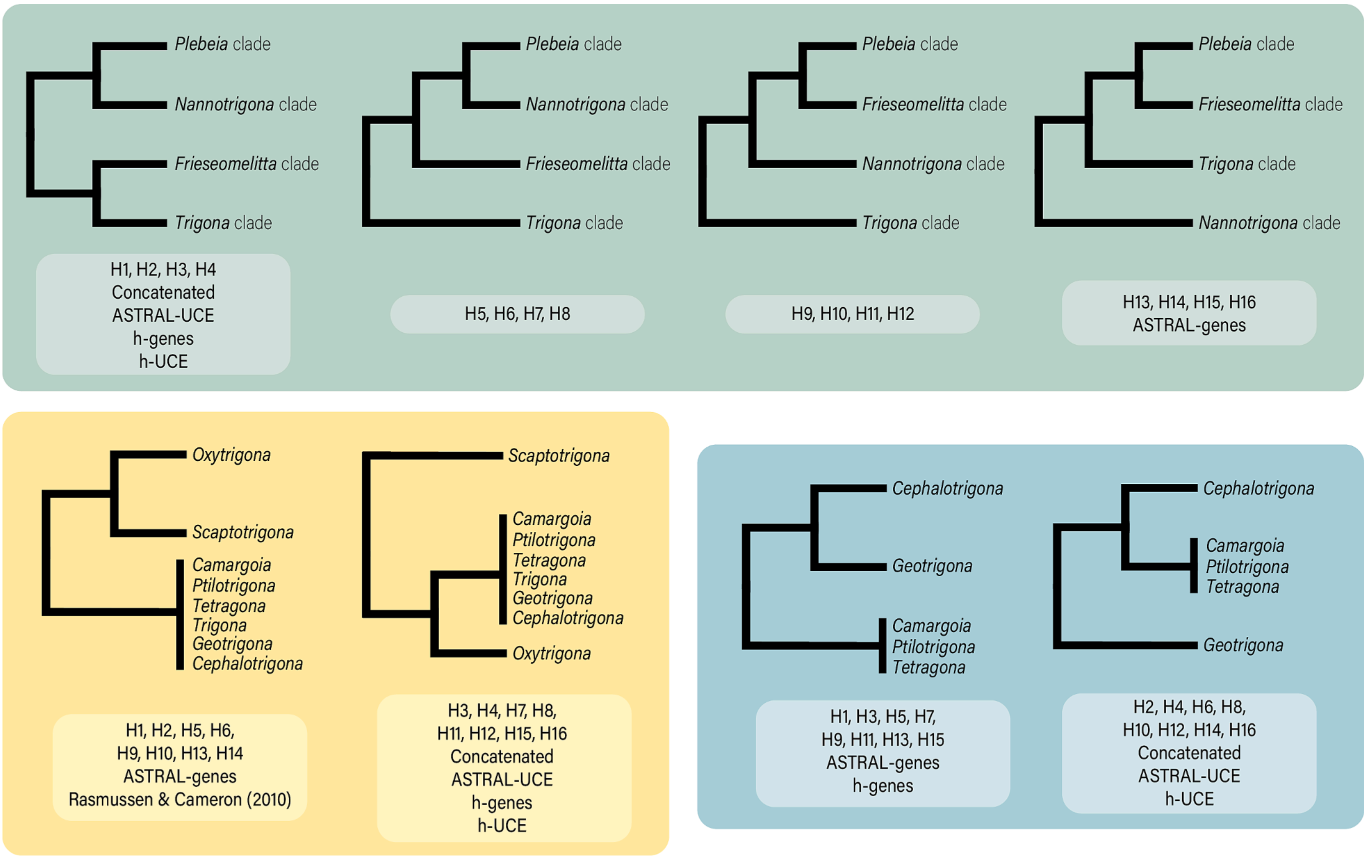

Fig. 3. Summary of the nodes tested in gene-genealogy interrogation (GGI) analyses. Each of the 16 hypotheses tested is composed of a combination of relationships depicted in the color squares.

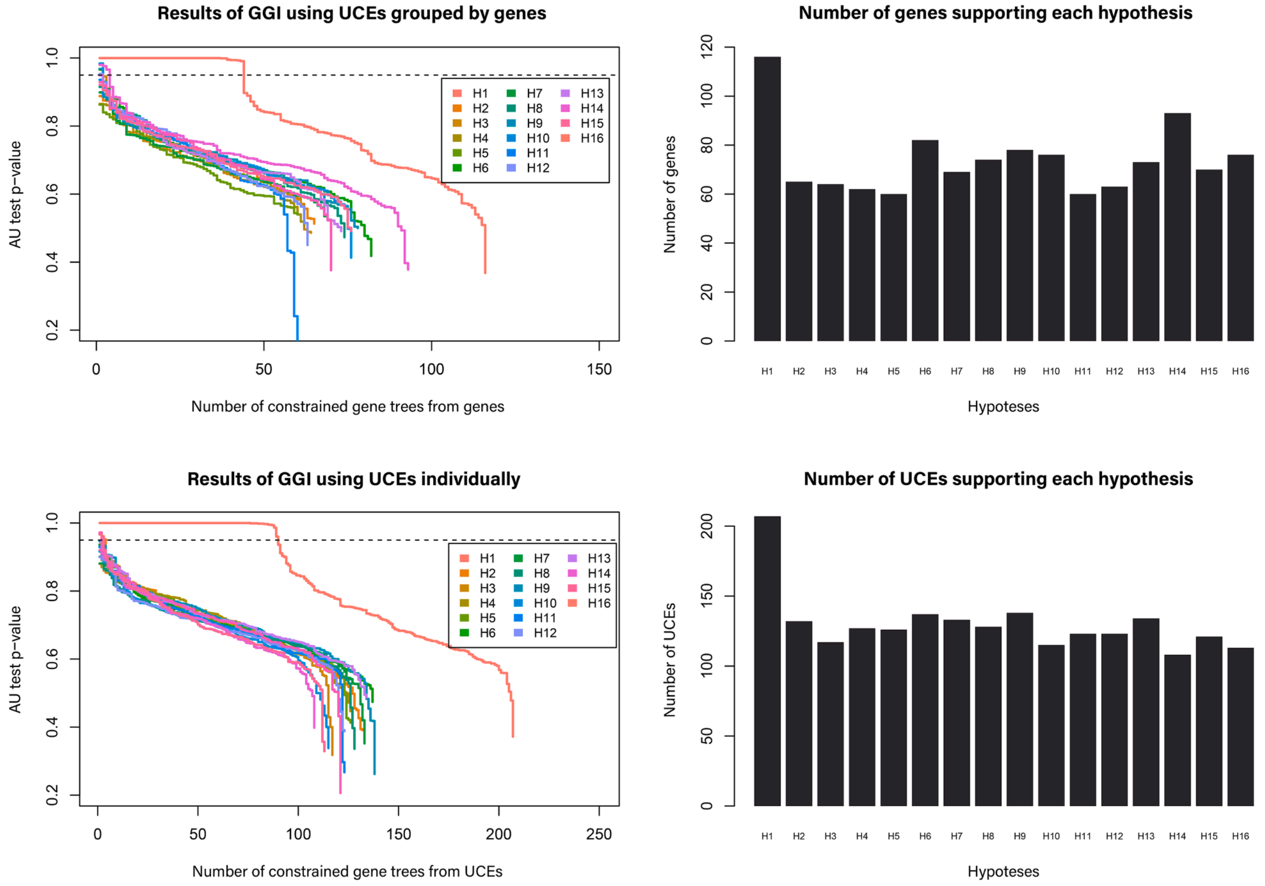

Fig. 4. Results of gene-genealogy interrogation (GGI) analyses of the > 2000 locus dataset. **A.** Linear plot of the *P-value* for the most favored hypothesis in relation to the number of genes. **B.** Number of genes supporting each of the sixteen hypotheses as the most favored in an approximately unbiased (AU) test. **C.** Linear plot of the *P-value* for the most favored hypothesis in relation to the number of individual UCEs. **B.** Number of individual UCEs supporting each of the sixteen hypotheses as the most favored in an approximately unbiased (AU) test.

the *Plebeia* clade forming a monophyletic group with the *Nannotrigona* clade and (b) the *Frieseomelitta* clade grouping with the *Trigona* clade (Fig. 4: H1; Fig. 5: nodes 1 and 2, respectively). The genus *Oxytrigona* was supported as the sister group of *Scaptotrigona* (node 3 in Fig. 5), since *Meliwillea bivea* was absent from the >2000 matrix, and *Cephalotrigona* was supported as the sister group of *Geotrigona* (node 4 in Fig. 5). Both the GGI analyses of the 1183 genes and 2113 UCEs in the >2000 matrix favored H1 as the phylogenetic hypothesis for Neotropical lineages of stingless bees. The tree topology shown in Fig. 5 was the most frequently favored hypothesis regarding site likelihoods, although not recovered in any of the concatenated or species-tree analyses. H1 was supported by 116 genes, with all other hypotheses being favored by 93 genes each or less. Regarding individual UCEs, H1 was supported by 207 loci, with other hypotheses being favored by 137 loci each or fewer. Following this statistic, H2–H16 performed similarly, although clearly less supported than H1. When taking *P*-values into consideration, H1 is unquestionably favored over alternative hypotheses, being the single hypothesis ranked first with a $P > 0.95$ threshold for a significative number of UCEs and genes. In total, 43 genes and 89 UCEs supported this topology, rejecting all the alternative hypotheses, which were never supported by more than 3 genes or UCEs (see Table S4 for a complete summary of the GGI results). This indicates that only H1 was consistently supported as the most likely hypothesis when it was ranked first for a given locus throughout the dataset.

In summary, although most clades were consistently recovered across analytical procedures, a small portion of the relationships exhibited sensitivity to species-tree analyses regarding the merging of UCEs by gene and by the particular summary method used. Analyses using the ASTRAL-hybrid algorithm, which weights clades in individual locus-trees according to branch length and node support, were fully congruent with the results from concatenated analyses (Fig. 2). In contrast, unweighted ASTRAL analyses were discordant, and these were further explored using GGI to evaluate the effect of gene-tree incongruence. Our results indicate that concatenated and weighted-ASTRAL analyses recovered the most supported tree topology for Meliponini, even though the positions of *Cephalotrigona* and *Oxytrigona* might have been influenced by biological drivers of topological conflicts, such as incomplete lineage sorting. Gene-tree incongruence is pervasive in many studies, and here, we showed that for stingless bees, GGI was able to increase confidence at difficult nodes of the meliponine tree. H1 was supported by more loci than all other hypotheses (Fig. 4), a result which is exacerbated when considering significance thresholds. Even though alternative hypotheses received support from a considerable number of loci, the phylogenetic signal might be rather a result of random processes that do not hold in the face of statistical tests. Given this, most discussions are based on the favored hypothesis from GGI analyses.

4. Discussion

4.1. Evolutionary relationships of the stingless bees

In the present contribution, we gathered a representative phylogenomic matrix to explore the evolution of stingless bees worldwide, thus promoting advances in their systematics. The relevance of museomics for properly exploring phylogenetic relationships among biological groups is highlighted in this research. Thanks to the availability of

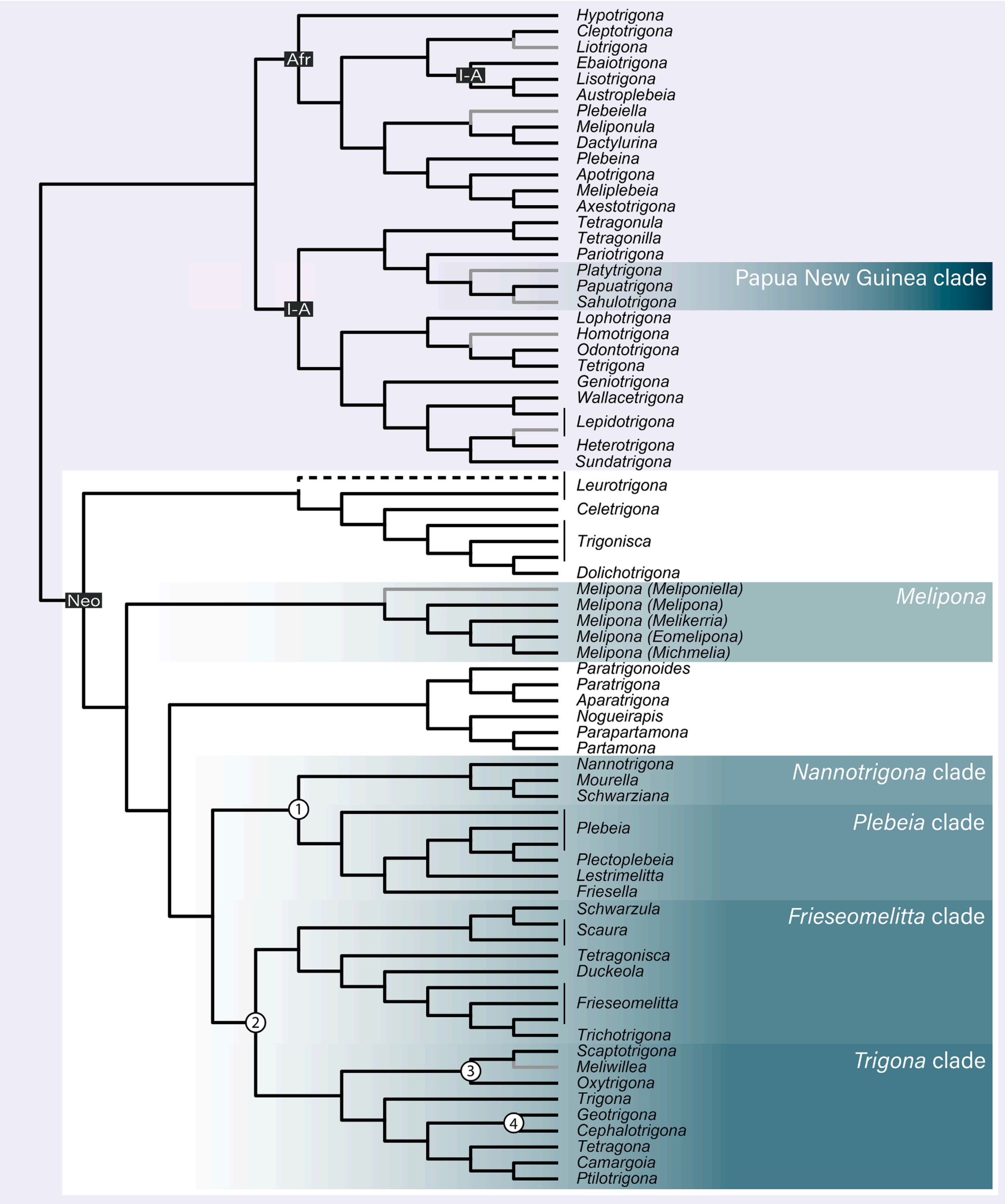

Fig. 5. Summary of phylogenetic relationships among all currently accepted 61 genera of extant Meliponini according to the results of GGI analyses (white box). Numbered clades were appended to the tree topology obtained in concatenated analyses (see Fig. 1). Placement of taxa not included in the > 2000 locus dataset (shaded in grey) based on concatenated analyses; the paraphyly of *Leurotrigona* is based on the putative position of *Leurotrigona pusilla* (dashed line), as recovered by Rasmussen & Cameron (2010). Black rectangles indicate geographic regions: Afrotropical (Afr), Indo-Australasian (I-A), and Neotropical (Neo). Numbers in white circles refer to nodes recovered in different configurations that were tested by GGI analyses (see text).

museum specimens, ultra-conserved element sequences were successfully generated event for species of very rare meliponine genera. Our analyses yielded a fully resolved and well-supported phylogenetic hypothesis for stingless bees, highlighting recalcitrant nodes and addressing possible solutions. We retrieved a backbone phylogeny for stingless bees, also providing a phylogenomic dataset that can be used to further investigate relationships among and within genera. These results are of paramount relevance for a group with such cultural and historical relevance to humans as the stingless bees (Jones, 2012; Quezada-Euán, 2018; Grüter, 2020), which also provides many model species for the study of insect sociality and genetics.

The history of phylogenetic research of Meliponini has been marked by strong incongruence between proposed hypotheses, especially among those based on morphological datasets (summarized in Almeida and

Porto, 2014). Molecular phylogenetic hypotheses, particularly since Rasmussen and Cameron (2007, 2010), have largely agreed with each other (Cardinal et al., 2010; Cardinal and Danforth, 2011; Martins et al., 2014; Bossert et al., 2019; Quezada-Euán et al., 2019; Almeida et al., 2023; Cueva del Castillo et al., 2023; Karuppaiah et al., 2023). Major lineages within Meliponini can be split into three clades, which are congruent with their geographic distribution. Neotropical genera form the sister-clade of the remaining Meliponini, while Eastern Hemisphere genera can be split into two main clades: one comprising only Indo-Australasian species and another with all Afrotropical species plus a few Indo-Malayan and Australasian species. These results were highly supported by Rasmussen and Cameron (2010), including in extended datasets. Our results agree with their general three-lineage pattern (Fig. 2) and allow further exploration of the relationships within each clade, as detailed in the sections below. The origin of the extant lineages can be traced back to events involving four major biogeographic regions that comprise most of the present-day tropical and subtropical humid environments in the Neotropical, Afrotropical, Oriental, and Australian regions. However, the timing and sequence of those events are not yet fully understood, besides recent advances (Rasmussen and Cameron, 2007, 2010; Grüter, 2020). In addition, the relationships between Holarctic fossils (i.e., those in the genera †*Cretotrigona* Engel, 2000, †*Exebotrigona* Engel and Michener, 2000, †*Kelneriapis* Sakagami, 1978, †*Liotrigonopsis* Engel, 2001, and †*Meliponorytes* Tosi, 1896) and the extant fauna remain uncertain (Rasmussen and Cameron, 2010; Engel and Michener, 2013).

Although the bulk of evolutionary relationships found in our results agree with previous inferences based on molecular data, a few conflicts are identified. Given that the most taxonomic representative and data-sufficient investigation of Meliponini relationships was that of Rasmussen and Cameron (2010), most of the discussion in this section will be carried out in comparison to their results. Based on five molecular markers sequenced for over two hundred terminals, Rasmussen and Cameron (2010) retrieved a phylogenetic hypothesis for the major clades of stingless bees, providing better resolution than previous works (Costa et al., 2003; Rasmussen and Cameron, 2007). With the implementation of high-throughput sequencing, this investigation has yielded further insights into exploring the phylogeny of the tribe. Overall, clades previously recovered with relatively low Bayesian posterior probabilities (Rasmussen and Cameron, 2010) are now repositioned in the tree and with greater support (Fig. 2). Our results highlight the relevance of increasing the amount of data to resolve relationships in Meliponini, as well as exploring putative drivers of incongruence. Some differences between the current results and previously published phylogenies are of evolutionary and/or systematic significance and should be noted.

4.2. Eastern Hemisphere stingless bees

With the inclusion of genera that were unavailable for previous molecular phylogenetic investigations, we could evaluate their identity and affinity with other stingless bee groups. Within the Indo-Australasian subclade among Afrotropical lineages, *Ebaiotrigona carpenteri* Engel, 2000, from southeast Asia, was recovered as the sister group to a clade composed of *Austroplebeia* and *Lisotrigona* (Figs. 2, 5). This is in accordance with the decision of Engel et al. (2022) to erect a new genus for the species, which had originally been placed in *Lisotrigona*. *Ebaiotrigona carpenteri* was considered enigmatic due to the resemblance of its male genitalia to those found in species of *Austroplebeia* and for the presence of yellow markings in the head and mesosoma, a feature absent in other *Lisotrigona* but present in *Austroplebeia*. These characters were used to hypothesize a close relationship between the latter and *Ebaiotrigona* (Engel et al., 2022), which was not supported by our results. Nevertheless, we support the adoption of *Ebaiotrigona* as a separate genus, including only the type species so far.

A novel grouping of Australasian stingless bees was recovered in all analyses (Figs. 2, 5): a clade composed of the genera *Papuatrigona*, *Pariotrigona* (both monotypic), and the species *Platytrigona keyensis* (Friese, 1901) and *Sahulotrigona paradisaea* (Engel and Rasmussen, 2017). This clade is geographically distributed in Indonesia, Malaysia, Papua-New Guinea, and nearby islands and was recovered as sister to the clade *Tetragonilla* + *Tetragonula*. These results are partly in conflict with taxonomic arrangements recently proposed for the Indo-Australasian genera (Engel and Rasmussen, 2017; Engel et al., 2023). According to the results of Rasmussen and Cameron (2010), *Tetragonilla* + *Tetragonula* were interpreted as the sister-group of all other species of the Indo-Australasian clade. However, the genera *Papuatrigona*, *Pariotrigona, Platytrigona*, and *Sahulotrigona* had not been included in that research. Given the distinctiveness of *Tetragonilla* and *Tetragonula*, the close relationship between them and the above-mentioned genera of the Indo-Australasian clade makes this result surprising. *Papuatrigona*, *Platytrigona*, and *Sahulotrigona* include mostly *Trigona*-like bees of medium size, while *Pariotrigona* are represented by tiny bees, superficially more similar to *Lisotrigona*, whereas *Tetragonilla* + *Tetragonula* form a clade diagnosed by the overarching scutellum and short malar space (Rasmussen et al., 2017). These results offer important biogeographic insights regarding the Indo-Australasian clade of stingless bees, because *Papuatrigona*, *Platytrigona*, and *Sahulotrigona* are all restricted to Papua-New Guinea, being the only stingless bees present in the island, in addition to *Austroplebeia* and *Tetragonula* (Engel, 2019). Our results indicate that the common ancestor of these three genera must have lived in Papua-New Guinea. The close relationship between the clade and the Indo-Australasian *Tetragonula* also calls attention, but the occupation of New Guinea and Australia by *Tetragonula* must represent an independent event, as evidenced by its close relationship to the Southeast Asian *Tetragonilla*.

Within the New Guinean clade, *Papuatrigona atricornis* (Smith, 1865) is the only species recognized in its genus, previously hypothesized to be closely related to *Lepidotrigona* (Engel et al., 2023), which was not confirmed by our results. Michener (1990) remarked the similarity between *Lepidotrigona* and *Papuatrigona* for their lack of a basal sericeous area on the hind basitarsus and of plumose setae along the entire dorsal margin of the metatibia. The closest relative of *Papuatrigona* in the present investigation is *Sahulotrigona paradisaea* (Fig. 2), originally described as *Heterotrigona (Sahulotrigona)*. Our results show that *S. paradisaea* is distantly related to other species of *Heterotrigona*, supporting Grüter's (2020) position of considering them as separated genera. *Sahulotrigona* also includes two other species that share with *S. paradisaea* the distal insertion of vein M in relation to 1cu-a and the presence of small patches of setae on the posterior surface of the propodeum (Engel, 2019). Finally, the sister group of *Sahulotrigona* + *Papuatrigona* in our analyses is *Platytrigona keyensis* (Figs. 2, 5). Currently, *Platytrigona* also includes three other species, all from New Guinea (Engel, 2019). The Bornean *Heterotrigona hobbyi* (Schwarz, 1937), treated as a *Platytrigona* until recently (Rasmussen, 2008), has been transferred to *Heterotrigona* under the monotypic subgenus *Heterotrigona (Borneotrigona)* by Engel (2019). This decision was based on the position of *H. hobbyi* (as *Platytrigona hobbyi*) as closely related to other species of *Heterotrigona* (the *Heterotrigona* s. str. of Engel (2019)) in the analyses of Rasmussen and Cameron (2010). Even though our analyses did not include *Platytrigona planifrons* (Smith, 1865), the type species of the genus, our results based on *P. keyensis* support the recognition of this genus as a separate taxon (the species currently included in *Platytrigona* are rather homogeneous, differing mainly in color).

The distinctive genus *Lepidotrigona* was not recovered as monophyletic, with *L. nitidiventris* (Smith, 1857) being more closely related to *Heterotrigona* (Fig. 2). Species currently included in *Lepidotrigona* are characterized by the presence of whitish to yellowish scale-like setae bordering the mesoscutum (as in Fig. 1E), besides the tesselate integument, enlarged corbicula and the lack of plumose setae along the dorsal margin of the metatibia, which are commonly found in other genera of the Indo-Australasian clade. The distinctiveness of species groups within *Lepidotrigona* has already been noted by Moure (1961), who identified three main groups. Even though Rasmussen and Cameron (2010) included two species in their sampling, neither of them is from the *L. nitidiventris* group, both grouping with *Wallacetrigona incisa* (Sakagami and Inoue, 1989). Accordingly, in the present results, the three species of *Lepidotrigona* (besides *L. nitidiventris*) are supported as forming a clade with *W. incisa*. These results suggest that the characteristic morphology found in species of *Lepidotrigona* is convergent, and that species in the *L. ventralis* and *L. terminata* groups should be assigned to a new genus (to be proposed in a forthcoming contribution).

In the the Afrotropical clade, *Hypotrigona* was confirmed as the sister group of the remaining Afrotropical Indo-Malayan and Australasian genera (Figs. 2, 5). In previous analyses, the phylogenetic affinities of the genus *Hypotrigona* were not fully resolved, with its species being recovered either as the sister group to *Liotrigona* or to all other stingless bees (Costa et al., 2003; Rasmussen and Cameron, 2007, 2010). The present result is consistent with the Bayesian analysis results of Rasmussen and Cameron (2010). Regarding other Afrotropical genera, our results indicate slightly different relationships as compared to those recovered by Rasmussen and Cameron (2010), but confirming the paraphyly of the genus *Meliponula* Cockerell, 1934, as used by some authors (e.g., Eardley, 2004; Eardley and Urban, 2010; Kiatoko et al., 2023). *Meliponula bocandei* (Spinola, 1861) was herein recovered as sister to *Dactylurina*, forming a very odd clade from the perspective of worker morphology. On the other hand, both genera share the lack of the characteristic brood cell combs (Michener, 1964; Grüter, 2020). Therefore, our results retrieved *M. bocandei* as the single species in the genus, as originally proposed by Moure (1961) and followed by Grüter (2020). *Plebeiella* was recovered as the sister to this clade, even though support metrics were not maximum in all analyses. This position is not supported by the arrangement of brood cells, as *Plebeiella lendliana* build regular combs (Portugal-Araujo, 1963). If confirmed, the close relationship of *Plebeiella* to other African Meliponini groups, as favored by Rasmussen and Cameron (2010), would indicate that the lack of combs in *Dactylurina* and *Meliponula* is plesiomorphic, being retained from the ancestor of the tribe. Finally, the genus *Meliplebeia* formed a clade with *Axestotrigona*, with *Apotrigona* as their sister group. Thus, the use of *Apotrigona* as a subgenus of *Meliplebeia* (as in Engel et al., 2021, 2023) is not supported.

In general, taxa endemic to the Eastern Hemisphere are better accounted for by the classification proposal of Moure (1961) with recent updates (e.g., Grüter, 2020). Even though we aimed to assemble a representative genus-level sampling for the Indo-Australasian stingless bees, we consider that further efforts are needed to delineate a stabler classification of Meliponini in this part of the world. Presumably, improvements in taxon sampling highlighted the need for revisiting the systematics of a few groupings. The Indo-Australasian region is rich in warm and humid environments which, coupled with its complex biogeographic history, served as terrain for the evolution of intriguing stingless bee fauna. Stingless bees provide an interesting case to unveil processes that shaped the region's biological diversity.

4.3. Body size and behavioral evolution in light of the new phylogenetic hypothesis

Pariotrigona pendleburyi (Schwarz, 1939) was recovered as closely related to the New Guinean subclade within the Indo-Australasian clade (Fig. 2). *Pariotrigona* is a genus of tiny bees found from peninsular Malaysia and Thailand in the west to Borneo in the east (Rasmussen, 2008), being differentiated from other Eastern-Hemisphere minute bees by the elongated malar space and the inner orbits only weakly converging ventrally (Moure, 1961). This genus has been traditionally considered as closely related to *Lisotrigona* or *Hypotrigona* (Michener, 1990; Engel et al., 2023), minute forms from southeast Asia and Africa, respectively. The positioning of *Pariotrigona* recovered herein with maximum support implies that minute stingless bees are present in each of the three major clades of the tribe. Congruently to previous phylogenies (Michener, 1990; Rasmussen and Cameron, 2010), the Neotropical lineages (i.e., *Celetrigona*, *Dolichotrigona*, *Leurotrigona*, and *Trigonisca*) are recovered in a clade distantly related to minute bees of the Eastern Hemisphere; *Ebaiotrigona*, *Liotrigona*, and *Lisotrigona* form a clade with the genus *Austroplebeia* (Figs. 2, 5). The latter clade comprises robust bees with extensive yellow markings on the body, sharing with the former genera the weakened wing venation (Michener, 1990). *Hypotrigona* represents a fourth lineage, consistently recovered as the sister group of the clade including the Afrotropical genera (Rasmussen and Cameron, 2010). In the past, the morphological similarity shared among the smallest stingless bees led authors to group them into the same genus, even though assuming geographically disjunct taxa (Moure, 1950; Moure et al., 1958; Wille, 1979). Traditionally, this morphology is interpreted as linked to subsequent miniaturization events from a larger ancestor (Moure, 1961; Michener, 1990, 2001; Camargo and Pedro, 1992a). However, the recovery of these bees in clades representing ancient splits in relation to other groups may indicate that the ancestor of stingless bees exhibited a set of morphological characters more alike the extant minute bees (Melo, 2020).

The ancestral body size of stingless bees has implications for the interpretation of the evolution of biological traits in the tribe. Stingless bees exhibit a diversity of brood cell organization strategies, including the most common construction of combs, as well as the building of irregular combs and cell clusters (Michener, 1961). Most minute stingless do not exhibit the characteristic brood cell combs found in most genera, instead building clusters of cells (Michener, 2001). Similar clusters can be found in orchid bees and in species of *Bombus (Mendacibombus)*, the first lineage to split among extant Bombini (Cameron et al., 2007; Williams et al., 2016). Consequently, the expected groundplan of corbiculate bee nest architecture is the construction of cell clusters, a character also hypothesized as present in the ancestor of Meliponini (Michener, 1961, 1964). Our results (Figs. 2, 5) support this interpretation because all early diverging lineages of stingless bees retained the ancestral habit of building cell clusters (e.g., *Austroplebeia, Tetragonula, Trigonisca*). In this scenario, comb building evolved independently in different lineages in both hemispheres (Roubik, 2006; Grüter, 2020, and references therein for a summary of the cell organization). From these, reversions can be hypothesized, such as in the clade *Duckeola* + *Frieseomelitta* + *Trichotrigona*, and in some species of *Plebeia* (Grüter, 2020). However, a formal reconstruction of the evolutionary history of this trait is yet lacking and may reveal interesting patterns in the future.

In the Afrotropical clade, we confirmed the hypothesis of Michener (1990) for the placement of *Cleptotrigona* as sister to *Liotrigona*. These genera share a similar structure of the male genitalia, which exhibits a curling of the anterior portion (Michener, 1990). Bees of the genus *Cleptotrigona*, however, are obligately cleptobiotic, plundering nests of *Hypotrigona* and perhaps of *Liotrigona* (Michener, 1990). Cleptobiotic stingless bees construct nests but rely on resources from other bee nests to provision their own. The species of *Cleptotrigona* resemble those of the Neotropical *Lestrimelitta*, due to morphological adaptations for a cleptobiotic life. This behavior is obligate in species of both genera, although species in other bee genera can behave as robbers occasionally (Nogueira-Neto, 1970; Grüter et al., 2016). In the past, this has led to the grouping of species of *Cleptotrigona* and *Lestrimelitta* in a single genus (Friese, 1912; Schwarz, 1948). Here, we maintain Wille's (1979) interpretation that this morphological similarity is due to evolutionary convergence linked to the independent development of cleptobiosis, since both genera are distantly related (Fig. 2).

At least three independent lineages from Africa evolved to explore cavities in the ground, representing the genera *Plebeiella*, *Plebeina*, and *Meliplebeia* (Smith, 1954; Portugal-Araujo, 1963). It can be hypothesized that the general habit of stingless bees is the opportunistic use of preexisting cavities above the ground, especially in hollow trunks. This hypothesis is supported by the rather derived position of groups with subterranean or aerial exposed nests in the present analysis. The repeated evolution of ground-nesting can be related to exploring habitats with more open vegetation, which are abundant in the Afrotropical region. The same pattern can be identified in the Neotropical region, where the ground-nesting *Mourella*, *Schwarziana*, *Melipona (Melikerria) quinquefasciata* Lepeletier, 1836, and species of the *Paratrigona lineata* (Lepeletier, 1832) group are related to open habitats south to the Amazon rainforest (Camargo and Wittmann, 1989; Camargo and Moure, 1994; Camargo et al., 2023). In the Indo-Australasian region, nests in the ground are only confirmed in *Tetragonilla* (Li et al., 2021), even though they are probably in association with root systems and subterranean termites.

4.4. Western Hemisphere stingless bees

Within the Neotropical clade, *Camargoia*, *Meliwillea*, and *Paratrigonoides* were recovered in positions similar to those based on morphological data (e.g., Camargo, 1996; Camargo and Roubik, 2005; Camargo and Pedro, 2003b). The two included species of *Camargoia* form a clade sister to *Ptilotrigona*, with this larger clade placed as sister to *Tetragona*. These relationships had already been suggested by Camargo (1996) based on the presence of setae on the propodeum in both genera, as opposed to *Tetragona* (see also Camargo and Pedro, 2003b). The monotypic *Meliwillea* was recovered as the sister group of *Scaptotrigona*, agreeing with the results of Costa et al. (2003). Both genera share several morphological traits, such as the shape of the metabasitarsus, and the presence of long and sinuous setae on metasomal sterna (Roubik et al., 1997). Finally, the also monotypic *Paratrigonoides*, a genus endemic to northeastern Colombia, is recovered as sister to *Aparatrigona* + *Paratrigona*. The three genera are characterized by the opaque integument and elongate tongue (Camargo and Roubik, 2005). On the other hand, the recently described genus of Neotropical Meliponini based on *Plebeia nigrifacies* (Friese, 1900), *Plectoplebeia* (Melo, 2016; see also Wille, 1960) was recovered among species of the genus *Plebeia*, suggesting that the unique features of that species actually are derived traits that do not justify its separation from other *Plebeia*.

Results for perhaps one of the most neglected stingless bees in the Neotropics, the minute bees included in the genera *Celetrigona*, *Dolichotrigona*, *Leurotrigona*, and *Trigonisca*, indicate a need for systematic revisions. Rasmussen and Cameron (2010) first showed that *Dolichotrigona* is nested within *Trigonisca*, rendering the latter paraphyletic. Currently, *Dolichotrigona* includes ten species (reviewed by Camargo and Pedro, 2005), while *Trigonisca* includes 28 species, with many yet to be described (Albuquerque and Camargo, 2007; Camargo et al., 2023). Given that our results indicate a paraphyletic *Trigonisca* split into at least four independent lineages, future efforts should treat species of *Dolichotrigona* under *Trigonisca*, to avoid the need to propose many genus-level names for the latter. It is worth noting that *Trigonisca bidentata* Albuquerque and Camargo, 2007 was previously recovered as sister to all other species + *Dolichotrigona* (Rasmussen and Cameron, 2010) and may belong to a fifth lineage not represented herein. In our analyses, the species *Leurotrigona gracilis* Pedro and Camargo, 2009 and *L. muelleri* (Friese, 1900) formed a monophyletic group. Previously, Rasmussen and Cameron (2010) had retrieved a paraphyletic *Leurotrigona*, even though with relatively low support values for the composing clades. This discrepancy cannot be assessed here because our analyses did not include *Leurotrigona pusilla* Moure and Camargo, 1988, which proved to be a key part of this puzzle in previous inferences.

Melipona is the largest genus of stingless bees, with more than 70 described species, being the only genus for which subgenera have been adopted herein (Grüter, 2020; Camargo et al., 2023). Although the sampling of the genus was relatively small in relation to its known diversity, the inclusion of respective type species of all five subgenera allows important considerations about their phylogenetic relationships. *Melipona (Meliponiella) illustris* Schwarz, 1932 was recovered as sister to all other species, validating the use of *Melipona (Meliponiella)* as a separate subgenus (Melo, 2021). The adoption of this subgenus is also supported by the results of Rasmussen and Cameron (2010), which recovered *M.bradleyi* Schwarz, 1932 as sister to other *Melipona*, and of Ramirez et al. (2010), which recovered *M. micheneri* Schwarz, 1951 as related to *M. illustris*. *Melipona (Eomelipona)* Moure, 1992 was grouped with *Melipona (Michmelia)*, having *Melipona (Melikerria)* as their sister subgenus. In its turn, *Melipona (Melipona)* came out as the sister group of the clade formed by these three other subgenera. This is the first time this grouping is recovered, resolving relationships within this contentious clade (see Ramirez et al., 2010).

The clade including *Aparatrigona, Nogueirapis, Parapartamona, Paratrigona*, *Paratrigonoides* and *Partamona* form a group characterized by diverse nesting habits. As far as is known, all stingless bees belonging to these genera have nests that are not built in preexisting and unused cavities in wood (Camargo and Moure, 1994; Camargo and Pedro, 2003a; Roubik, 2006), deviating from the general plan of stingless bees. (although the nest of *Paratrigonoides mayri* Camargo and Roubik, 2005 is unknown). The use of substrates other than cavities in wood was likely present in the common ancestor of the clade. This entire lineage is also remarkable for including various groups with obligate association with nests of termites and ants (Camargo and Moure, 1994; Camargo and Pedro, 2003a; Grüter, 2020). In the Eastern Hemisphere stingless bees, obligate association with nests of other social insects is only fully documented for *Sundatrigona* (Sakagami et al., 1989), with *Tetragonilla* being another potential case yet to be investigated (Li et al., 2021). In the Neotropics, this biology can also be found in species of *Scaura* and *Trigona* (Schwarz, 1938; Rasmussen and Camargo, 2008), which seems to constitute independent events given their distant relationships to *Paratrigona*, *Partamona*, and closely related genera (Figs. 2, 5). Finally, *Partamona* is one of the four groups to have evolved the capacity to build completely exposed nests, with other cases being *Dactylurina*, *Tetragonisca weyrauchi* (Schwarz, 1943), and in a single lineage of *Trigona* (Roubik, 2006; Rasmussen and Camargo, 2008; Grüter, 2020).

Our results confirm the paraphyly of the genus *Plebeia* as currently defined (Camargo et al., 2023; Engel et al., 2023). This taxon includes small bees with shiny black integument and yellowish maculations and represents one of the most diverse genera in the Neotropical region (Michener, 2007; Camargo et al., 2023). *Plebeia franki* (Friese, 1900) and *P. minima* (Gribodo, 1893) formed a clade recovered as sister to another clade composed by *Friesella schrottkyi* (Friese, 1900), the genus *Lestrimelitta* and other *Plebeia* species. The latter group includes species related to the type species of the genus, *P. mosquito* (Smith, 1863). While the monotypic *Friesella* includes bees relatively similar to *Plebeia* in behavior and size, *Lestrimelitta* includes obligatory cleptobiotic species (see above). Recently, the genus *Asperplebeia* Engel, 2021 was proposed to include *Plebeia moureana* Ayala, 1999 and *P. tica* (Wille, 1969), with the latter designated as the type species for the new genus (Engel et al., 2021). Additionally, a new classification was proposed for *Plebeia*, with the description of *Plebeia (Nanoplebeia)* Engel, 2021 to include a few minute species of the genus, having *P. minima* as its type species (Engel et al., 2021). This proposal is not congruent with the results of available molecular analyses and is not supported herein. Based on the available information, either *Nanoplebeia* or *Asperplebeia* should be used for *P. tica* and related species, including *P. minima* and *P. franki* (H. A. Werneck et al., *unpublished results*). Despite recent efforts, *Plebeia* includes groups with elusive taxonomy and several undescribed species, requiring proper taxonomic revisions.

Another expected result confirmed by our inferences is the non-monophyly of *Scaura*. The genus, as defined by most authors (e.g., Schwarz, 1948; Camargo et al., 2023), is paraphyletic in relation to *Schwarzula*. The most striking character shared by species in both genera is the swollen hind basitarsus, which helps in their unusual pollen-collecting behavior (Laroca and Lauer, 1973). In all analyses, *Scaura longula* (Lepeletier, 1836) was recovered as sister to *S. tenuis* (Ducke, 1916), while *S. argyrea* (Cockerell, 1912) grouped with species of *Schwarzula*. Although some initiatives have been made to treat species of *Schwarzula* under *Scaura* (Grüter, 2020; Engel et al., 2021), an alternative approach would be to erect a new genus to reflect the behavioral uniqueness of the groups. All analyses recovered *Trichotrigona* as a lineage within the genus *Frieseomelitta*, rendering the latter paraphyletic. A putative behavioral synapomorphy of the two genera + *Duckeola* is the lack of regular brood cell combs, especially in *Frieseomelitta* + *Trichotrigona*, which have cells organized in typical clusters, while *Duckeola* builds irregular combs (Camargo and Pedro, 2007; Grüter, 2020). *Trichotrigona* species are remarkable for their cleptobiotic behavior, inferred from the reduced corbicula in the hind legs and lack of other structures for handling pollen, besides the absence of pots for food storage within their nests (Camargo and Moure, 1983; Camargo and Pedro, 2007; Pedro and Cordeiro, 2015). Their nests have been rarely found, but, in some occasions, were observed in association with nests of *Frieseomelitta*, which are their presumed hosts (Camargo and Moure, 1983; Camargo and Pedro, 2007). Among stingless bees, obligatory cleptobiotic behavior is also found in the Neotropical genus *Lestrimelitta* and the African genus *Cleptotrigona*. While workers of these genera obtain food through aggressive raids over nests of other species, it is hypothesized that workers of *Trichotrigona* act individually on nests of their hosts (Camargo and Pedro, 2007). This kind of solitary-foraging cleptobiosis could be explained by the absence of foraging recruitment in the common ancestor of *Trichotrigona* and *Frieseomelitta*, since there is evidence that species in the latter genus do not have any kind of recruitment strategy for specific locations (Jarau et al., 2003; Melo, 2020).

The most remarkable findings regarding Neotropical taxa are related to the position of genera belonging to the *Frieseomelitta*, *Nannotrigona*, *Plebeia*, and *Trigona* clades (Fig. 5). Phylogenetic affinities among those major clades have shown to be contentious in previous analyses, suggesting the difficulty of resolving these relationships. The clade *Scaura* + *Schwarzula* was recovered as the sister group of a clade including *Duckeola*, *Frieseomelitta*, *Tetragonisca*, and *Trichotrigona* in all analyses. This entire clade was recovered in a position closer to the *Trigona* clade in most analyses, including GGI (node 2 in Fig. 5), while Rasmussen and Cameron (2010) recovered it as closer to the *Nannotrigona* and *Plebeia* clades. It is worth noting that the branches leading to the clades mentioned are some of the shortest in the entire tree, indicating a rapid divergence that could be difficult to reconstruct by most phylogenetic methods. Additionally, the most favored hypothesis we advocate (Fig. 5) is more congruent with morphological evidence since genera such as *Frieseomelitta* and *Tetragonisca* resemble groups related to *Trigona* rather than those related to *Plebeia.* This overall similarity is due in part to the morphology of the hind tibia, which bears a narrow keirotrichiate zone on its posterior surface, in addition to the presence of numerous plumose setae on its dorsal margin, characters of wide relevance for the identification of Meliponini genera (Schwarz, 1948; Michener, 2007). The grouping of *Oxytrigona* with *Scaptotrigona* recovered by Rasmussen and Cameron (2010) was not replicated by concatenated (Fig. 2) and ASTRAL-hybrid analyses either. The GGI results do, however, unequivocally favor the grouping of both genera (Fig. 5). Species of *Oxytrigona* and *Scaptotrigona* share with *Meliwillea* the wide frons, long malar space, and presence of a transverse declivity on the base of the mandible, among other characteristics interpreted as derived within the clade. A similar case can be visualized in the position of the genus *Cephalotrigona* Schwarz, 1940, recovered as sister to *Trigona* by Rasmussen and Cameron (2010), but herein placed either in a clade with *Camargoia*, *Ptilotrigona*, and *Tetragona* (Fig. 2) or as sister group of *Geotrigona* (Figs. 3, 5). The latter hypothesis was favored by GGI, being morphologically supported by the lack or reduction of the mesotibial spur in both genera. Regarding relationships among species of *Trigona*, our results are mostly congruent with previous investigations focused on the genus (Rasmussen and Camargo, 2008; Marconi et al., 2023), with the only exception being the position of *Trigona dallatorreana* Friese, 1900 and *Trigona nigerrima* Cresson, 1878. We also included *Trigona dimidiata* Smith, 1854, a species belonging to a group not represented in previous analyses. The subgeneric classification of *Trigona* proposed by Engel et al. (2021) was not supported, with three of their proposed subgenera being recovered as paraphyletic on concatenated analyses with the full dataset (Fig. 2).

5. Conclusion

This work helps solving longstanding gaps in our knowledge about the evolutionary history and systematics of stingless bees. A backbone of the phylogenetic relationships was provided, in part, thanks to the scientific collections allied to our capacity to generate molecular sequences from old specimens using high-throughput sequencing methods. In addition, we explored the potential of a large genomic dataset to yield well-supported hypotheses for elusive phylogenetic problems using up-to-date phylogenetic tools. Nevertheless, many important questions remain and should be addressed in the next years taking advantage of the fruitful literature regarding stingless bee management and behavior, besides their relatively rich fossil record. Regarding the systematics of Meliponini, the presence of several short branches within genera and groups of genera suggests the need for increased taxon sampling to resolve their fine-scale relationships. The generated dataset and resulting hypotheses can be used as bases for investigations regarding further questions in stingless bee systematics and evolution.

CRediT authorship contribution statement

Anderson Lepeco: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Michael G. Branstetter:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Gabriel A.R. Melo:** Writing – review & editing, Supervision, Project administration, Investigation, Data curation, Conceptualization. **Felipe V. Freitas:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation. **Kerrigan B. Tobin:** Writing – review & editing, Investigation. **Jenny Gan:** Writing – review & editing, Investigation. **Jeremy Jensen:** Writing – review & editing, Investigation. **Eduardo A.B. Almeida:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are thankful to the curators and collection technicians who facilitated the access to specimens used for DNA extraction: James M. Carpenter, Christine LeBeau, Corey Smith (AMNH), Terry Griswold (BBSL), and José Amilcar Tavares Filho (RPSP). AL and EABA are particularly grateful to J. M. Carpenter for making possible a long-term visit of the junior author of this research to the AMNH. We are also grateful to Matheus Mourão Carvalho and Diego A. Guevara for collecting and helping identify some specimens, and to Vinicius H. Franceschini-Santos for his help with the GGI analyses. This research would not have been possible without the invaluable contributions of numerous collectors who made the AMNH, BBSL, DZUP, and RPSP collections representative enough—for that we are especially thankful to G. E. Bohart, J. M. F. Camargo, T. Dikow, T. Griswold, S. Mateus, M. Mazucato, C. Menezes, C. D. Michener, M. Middleton, F. G. L. Oliveira, L. Packer, F. D. Parker, S. R. M. Pedro, C. Rasmussen, D. W. Roubik, B. F. Santos, J. A. Tavares, P. Vit, and A.Wille. We are thankful to people who kindly provided us with beautiful photographs of stingless bees: Adriana Tiba and Julio Pupim, Alain Pauly, Felipe B. Fraga (whose photographs were financed by the Espírito Santo Research Foundation - Fapes project T.O. 959/2022), Fiora Li, Graham Stott, Jürg Sommerhalder, Mark Chao, Nicolas Vereecken, Omar Martín, and Subramanian Sevgan. We also thank one anonymous reviewer and the editor-in-chief, Dr. G.Orti, for comments that greatly improved the manuscript. This work was financed by the São Paulo Research Foundation (FAPESP grants 2018/09666-5, 2019/09215-6, 2021/12082-8, and 2023/00647-6), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001, the U.S. Department of Agriculture, Agricultural Research Service (Project# 2080-21000-019-000-D), and the U.S. National Science Foundation (DEB# 2127744).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2024.108219.

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