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Facultad de Ciencias
Departamento de Biología
Sede Bogotá

UNIVERSIDAD
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ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE

WING PHENOTYPIC DIVERSITY IN STINGLESS BEES GENERA (APIDAE: MELIPONINI) FROM ECUADOR AMAZONIA

Diversidad fenotípica alar en géneros de abejas sin aguijón (Apidae: Meliponini) de la Amazonía Ecuatoriana

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Received: 13th February 2023. **Revised:** 13th March 2023. **Accepted:** 16th June 2023.
Associate editor: Héctor Jaime Gasca Álvarez

Citation/ citar este artículo como: Bonilla-F, K., Liria Salazar, J., Rasmussen C. (2024). Wing Phenotypic Diversity in Stingless Bees Genera (Apidae: Meliponini) from Ecuador Amazonia. *Acta Biol Colomb*, *29*(1), 112-118. https://doi.org/10.15446/abc.v29n1.106788

ABSTRACT

Native stingless bees, Meliponini, are the only social bees that produce honey in addition to honeybees. These bees have been managed in meliponiculture and for crop pollination. In Ecuador, meliponiculture studies are scarce and limited by the lack of species recognition. Traditionally the taxonomic recognition of the species is based on morphological characteristics. In Hymenoptera, the wing shape has proven to be a valuable tool in combination with traditional characters. Due to this, the wing shape was evaluated for discriminating morphospecies in Ecuador's Amazonian meliponaries. A total of 127 specimens grouped into six genera were analyzed: *Melipona (Melikerria)* sp., *Melipona (Michmelia)* sp1., *Melipona (Michmelia)* sp2., *Tetragonisca* sp., *Paratrigona* sp., *Nannotrigona* sp., *Scaura* sp., and *Plebeia* sp. The most significant differences are located at the vein intersection: cubital and cubital 1 (Cu + Cu1), posterior margin of the marginal cell (r and Rs), and basal and posterior marginal cell (M and Rs).

Keywords: Meliponiculture, *Plebeia*, Procrustes, *Scaura*, wing vein.

RESUMEN

Las abejas nativas sin aguijón, Meliponini, junto con las melíferas son las únicas abejas sociales que producen miel. Las especies de esta Tribu han sido manejadas en meliponicultura y para la polinización de cultivos. En Ecuador, los estudios de meliponicultura son escasos y limitados por la falta de reconocimiento de especies. Tradicionalmente el reconocimiento taxonómico se basa en características morfológicas; en Hymenoptera, la forma del ala ha demostrado ser una herramienta valiosa en combinación con los caracteres tradicionales. Debido a esto, se evaluó la forma del ala para morfoespecies en meliponarios amazónicos de Ecuador. Se analizaron 127 ejemplares agrupados en seis géneros: *Melipona (Melikerria)* sp., *Melipona (Michmelia)* sp1., *Melipona (Michmelia)* sp2., *Tetragonisca* sp., *Paratrigona* sp., *Nannotrigona* sp., *Scaura* sp., y *Plebeia* sp. Los cambios más significativos se localizan en la intersección de las venas: cubital y cubital 1 (Cu + Cu1), margen posterior de la celda marginal (r y Rs), y celda marginal basal y posterior (M y Rs).

Palabras clave: Meliponicultura, *Plebeia*, Procrustes, *Scaura*, venación alar.

INTRODUCTION

The native stingless bees, Meliponini, are part of the group of bees named corbiculate bees (Apidae: Apinae) (Ayala, 1999; Arnold et al., 2018). Within these, a modified, reduced, and non-functional stinger characterizes the workers (Nates-Parra and Rosso, 2013). The global diversity estimate is 526 native stingless bee species grouped in 61 genera (Rasmussen and Gonzales, 2013). However, the actual richness appears to be greater than 800 spp (Rasmussen and Delgado, 2019; Burgett et al., 2020). In Central and South America are reported about 400 species (75 % of the total species described worldwide) within 32 genera, distributed from northern Mexico to Argentina (Barquero-Elizondo et al., 2019; Assefa et al., 2021).

Modern native stingless bee management in Ecuador dates since the 1980s (Jácome and Guarderas, 2016). Studies published between 2006 and 2009 are related to meliponiculture and management of *Melipona indescisa* Cockerell 1920 and *M. mimetica* Cockerell 1914 (Mejia and Jiménez, 2006; Rivas, 2009). Later, between 2014 and 2017 were reported the use for honey production of the following species: *Cephalotrigina capitata* Smith, 1854, *Geotrigona fumipennis* Camargo and Moure 1996, *Melipona eburnean* Friese 1900, *M. indecisa*, *M. mimetica*, *M. rufiventris* Friese 1900, *Nannotrigona* sp., *Oxytrigona mellicolor* Packard, 1869, *Paratrigona* aff. *eutaeniata*, *Paratrigona eutaeniata* Camargo y Moure 1994, *Scaptotrigona ederi* Engel, 2022, *Scaptotrigona postica* Latreille 1807, *Scaptotrigona* sp. cf. *postica*, *Scaptotrigona* sp., *Tetragonisca* sp., *Trigona fulviventris*, and *Trigona* "matera" nom. nud, recorded for the Eastern and Southern part of Ecuador (Ramirez et al., 2014; Vit et al., 2015). In the Amazonian Ecuador, Oriental region, *Geotrigona leucogastra* Cockerell, 1914, *Melipona grandis* Guérin-Méneville 1844 and *Scaptotrigona ederi*, have been reported used by Kichwa communities (Vit et al., 2017). Despite these fragmentary reports, in Ecuador, knowledge and management of Native stingless bees are still incipient (Jácome and Guarderas, 2016). There is a lack of basic faunistic knowledge on meliponiculture, as well as collection, processing, and analysis of managed species (Ramirez et al., 2014; Assefa et al., 2021).

Traditional taxonomic identification methods include the use of dichotomous keys and morphological knowledge of the group to be identified, where phenotypic characteristics of bees are used (Combey et al., 2013; Francoy et al., 2009). Nevertheless, in megadiverse insect taxa such as Hymenoptera, particularly in Meliponini, it is difficult, first establish the actual number of species and then to identify them due to as there appear new species every so often and the presence of cryptic species (Nates, 2001a; Michener, 2007). However, several studies suggested that phenotypic quantification by characterizing Hymenoptera wings could be a helpful tool for recognizing different taxonomic levels as a complement to traditional taxonomy, where morphology is mainly used (Francoy et al., 2008; dos Santos et al., 2009a; dos Santos et al., 2019b). Specifically, stingless bee wings can be used as part of species identification even though the forewing venation is reduced (Ayala, 1999; Álvarez, 2015). In this context, geometric morphometry can be defined as a quantitative study of shape after removing the effects of scale, translation, and rotation; could be used for this purpose (Benítez and Püschel, 2014). Geometric morphometry uses multivariable statistical techniques to analyze the coordinates of landmarks based on anatomical homologous points that provide a quantifiable result of the differences in shape (Owen, 2012; Benítez and Püschel, 2014). With this tool, it is possible to discriminate and separate taxonomic groups through the shape, describing the differences using landmark displacements. Several studies have already shown the application of geometric morphometry as an efficient method of differentiation between closely related bee taxa (Combey et al., 2013; Vijayakumar and Jayaraj, 2013; Rattanawannee et al., 2015; Nogueira et al., 2019; dos Santos et al., 2009a; dos Santos et al., 2019b).

Based on these reports, wing shape characterization using geometric morphometry is here tested as an additional tool for describing the taxonomic diversity of native stingless bees from meliponaries in the Napo province of Ecuador.

MATERIALS AND METHODS

Native stingless bee sampling. Bee collections were made from three localities in the Napo province, two in the Tena canton: the community of Aguapungo close to the city of Tena (site 1: 0° 58' 10.3" S, and 77° 48' 16.2" W; site 2: 0° 58' 14.6" S, and 77° 48' 27.8" W); and one in Archidona canton: city of Archidona (site 3: 0° 54' 11.1" S, and 77° 48' 09.7" W). Ministerio del Ambiente, Agua y Transición Ecológica del Ecuador, an environmental regulation entity, approved the scientific collection according to authorization #1340/2021. At least 15 individuals of each morphospecies were taken directly from a single nest of meliponaries per group, for *Tetragonisca* sp. 15 individuals were taken, due to the due to availability and accessibility, and 16 for the rest morphospecies. Specimens were placed in vials with 70 % alcohol (Márquez, 2005). and later pin-mounted for identification using dichotomous keys (Ayala, 1999; Michener, 2007; Álvarez, 2015; Rasmussen and Delgado, 2019). From each specimen, the right fore wing was removed, and from the bottom, the microscope slide was mounted with Euparal medium, and photographed (Fig. 1), at the same scale (dos Santos et al., 2009; Nogueira et al., 2019). From each slide 11 anatomical landmarks (Lm), were digitized (Fig. 2) with TpsDig2 v2.11 (following Rohlf, 2018). The 11 anatomical landmarks were located on intersection points of venation, Lm type I (following Prado-Silva et al., 2016).

Geometric morphometrics analysis. The Geomorph package (Adams and Otárola-Castillo, 2013) in the R v4.1.0 program

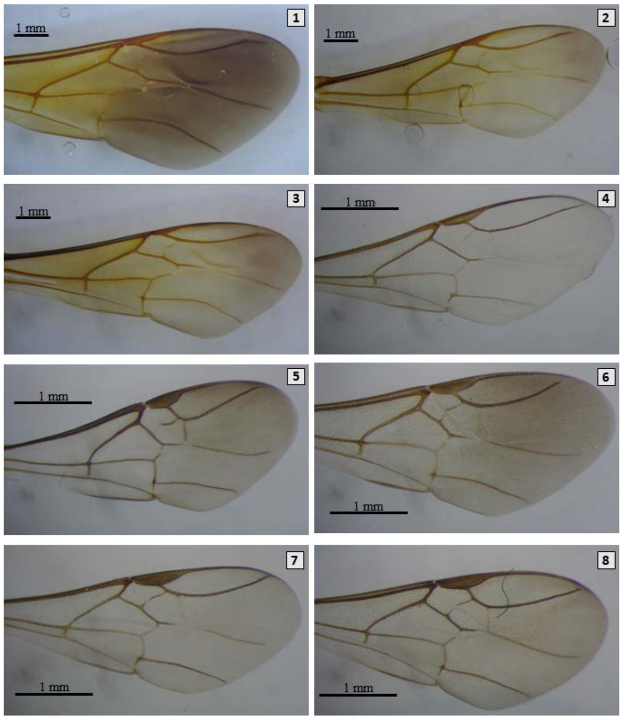

Figure 1. Right fore wing of eight morphospecies collected in Napo meliponaries: 1, *Melipona grandis*. 2, *Melipona eburnea*. 3, *Melipona illota*. Smaller morphospecies 4, *Tetragonisca angustula*. 5, *Paratrigona* sp. 6, *Nannotrigona melanocera*. 7, *Scaura* sp. 8, *Plebeia* sp.

was used to perform Principal Component Analysis (PCA), and the centroid size (CS) analysis, differences between them were evaluated statistically using the Kruskal-Wallis. MorphoJ 2.0 (Klingenberg, 2011) was used for a Canonical Variable Analysis (CVA) in order to statistically evaluate a posteriori re-classification of the previously identified stingless bees genera and morphospecies (García-Olivares et al., 2015; Nogueira et al., 2019); all Lm wing shape differences were represented through deformation grids.

RESULTS

The final native stingless bees wing dataset comprises 127 specimens grouped into eight morphospecies in six genera: *Melipona* (3 morphospecies), *Tetragonisca* (1 mspp), *Paratrigona* (1 mspp), *Nannotrigona* (1 mspp), *Scaura* (1 mspp), and *Plebeia* (1 mspp). The CS showed significant differences (Kruskal-Wallis test, x2=113.15; $p < 0.001$), between the large morphospecies (Fig. 3): *Melipona grandis* (5.014±0.005 mm), *Melipona eburnea* (4.150±0.011 mm) and *Melipona illota* Cockerell, 1919 (3.816±0.020 mm), and the smaller morphospecies: *Nannotrigona melanocera* (Schwarz, 1938) (2.002±0.023 mm), *Plebeia* sp (1.967±0,042 mm), *Tetragonisca angustula* Latreille, 1811 (1.950±0,127 mm), *Scaura* sp. (1.840±0.113 mm) and *Paratrigona* sp. (1.770±0.026 mm) (Table1).

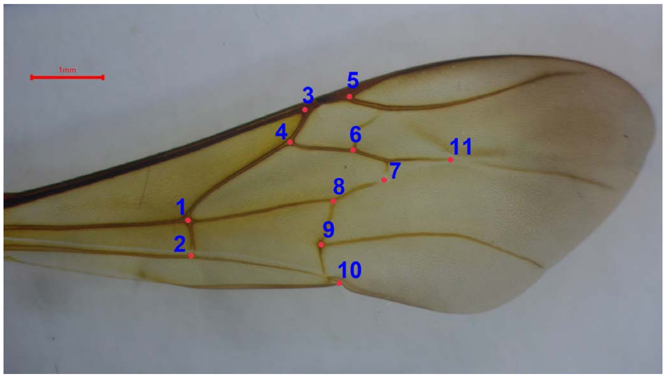

Figure 2. Right fore wing of Melliponini showing the landmarks (1-11) configuration analyzed.

Figure 4, shows the PCA shape for the main variation axes, with six differentiated groups and two partially overlapping morphospecies (both in the *M. (Michmelia)* subgenus). The CVA (Fig. 5) showed significant differences between morphospecies; a priori group morphospecies assignments based on traditional morphology was correctly (100 %) assigned to a posteriori group based on MANOVA average Mahalanobis distances (Wilk's $\lambda = 0.000$; $p < 0.0001$).

At generic level, all taxa represented in the PCA showed shape variation concentrated between wing cells: Marginal, 1st submarginal, and 2nd submarginal, in contrast with Lm 1 and 2 that do not show differences among genera. Particularly, the three *Melipona* morphospecies were characterized by a diagonal displacement of Lm 9 and variation between Lm 3, 4, and 5; where Lm 3 and 4 are displaced diagonally, while Lm 5 is displaced to the left. In *Tetragonisca* sp., there are variations of Lm 3, 6, and 11; Lm 3 and 6 have a left displacement, while Lm 11 has a left displacement. Then, *Paratrigona* sp. showed a marked variation in Lm 3, 4, and 5 indicating a diagonal displacement to the right, while *Nannotrigona* sp., *Plebeia* sp., and *Scaura* sp. present displacements of Lm 9 diagonally to the right. Finally, *Scaura* sp. showed a diagonal displacement to the left in Lm 3 and 4.

Table 1. Stingless bees morphospecies collected in each site; the a priori identification was based on traditional characters, and used as groups for a posteriori reclassification based on wing geometric morphometrics.

Genus	Specie	Local name	Site 1	Site 2	Site 3
Melipona	*grandis*	Miski	X		X
Melipona	*eburnea*	Miski	X		X
Melipona	*illota*	Miski	X		
Tetragonisca	*angustula*	Angelita	X	X	X
Paratrigona			X		X

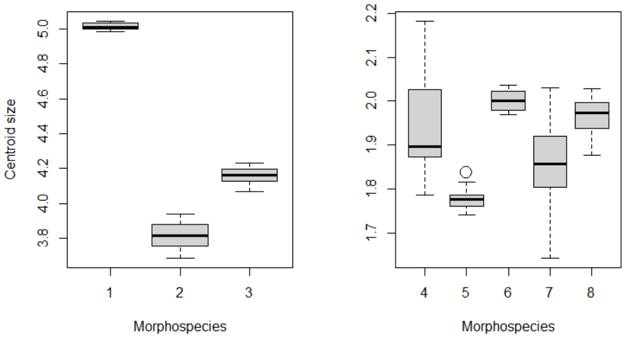

Figure 3. Centroid size boxplot of eight morphospecies collected in Napo meliponaries. Large morphospecies: 1, *Melipona grandis*. 2, *Melipona eburnea*. 3, *Melipona illota*. Smaller morphospecies 4, *Tetragonisca angustula*. 5, *Paratrigona* sp. 6, *Nannotrigona melanocera*. 7, *Scaura* sp. 8, *Plebeia* sp

Genus	Specie	Local name	Site 1	Site 2	Site 3
Nannotrigona	*melanocera*		X		X
Scaura					X
Plebeia				X	

DISCUSSION

The central size differences could be associated with the capacity of each phenotype under different genetic and environmental characteristics, which respond to micro and macroevolutionary causes (Benítez and Püschel, 2014; Jaramillo-Ocampo, 2014). Wing variation may be closely related to flight distance, with the largest bees able to fly distances between 1,500 and 2,500 meters from their nests, while the smallest restricted between 600 to 950 m (Roubik and Aluja, 1983; Nogueira et al., 2019).

Nunes et al. (2013), state that wing shape is more affected by evolutionary factors than size; the shape is not related to altitude and geographic variations. Additionally, Benítez and Püschel (2014), reported a close relationship between size and shape, pointing out that the first PCA axes can be associated with shape variation due to size. Our results showed that the first PCA axes explain more than 70 % of the variance, suggesting a possible allometric effect in some of the morphospecies studied. Allometry in Hymenoptera was verified by Belyaev and Farisenkov (2019), showing that body size increased according to the contraction in the forewing contour. Danforth (1989), stated that the wings of small hymenopterans tend to be broad and spatulate, while the hymenopteran's wings tend to be apically tapered.

Particularly, the partial overleaping between the wing shape of the two morphospecies of *Melipona (Michmelia)*, could be explained due to the evolutionary closeness - and the inadequateness of geometric morphometrics analysis to separate closely related species; Phylogenetic studies suggest *Michmelia* is a clearly monophyletic group (Rasmussen and Cameron, 2010). On the other hand, the shape similarities of *Plebeia* and *Scaura* could be related to the fact that these taxa are hypothesized as a monophyletic clade (Rasmussen and Cameron, 2010). However, this result could be a kind of flawed, due to the geometric morphometry in this work having been used to separate single representatives from each genus, therefore - for a good comparison of the partial overlapping species - care should be taken when applying this method between closely related species.

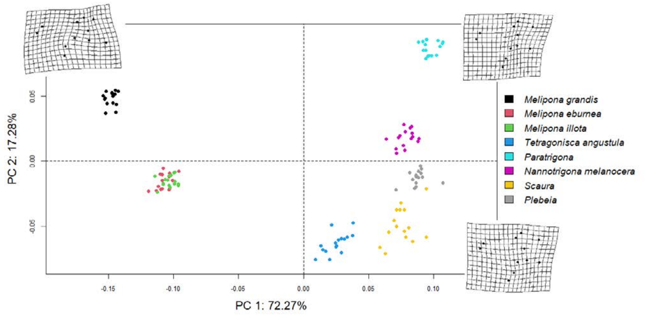

Figure 4. Principal Component Analysis diagram of two principal components (within percentage explained variance contribution) for 127 specimens and eight native stingless bees morphospecies recollected in meliponaries of Napo. The deformation grid represents the extreme variation axes

The deformation grids allowed us to visualize the differences in the wing conformation according to a consensus configuration (Fig. 4). The most relevant characters for morphospecies discrimination were Lm9 (veins Cu - cubitus and Cu1- cubitus1 intersection), Lm3 (veins R- radius and Rs - radial sector intersection), Lm4 (veins M - medius and Rs - radial sector intersection), and Lm5 (stigma and Rs - radial sector intersection). The function of most veins in Hymenoptera is unknown, the only veins with known functionality are those anchored to the base, which are important for the wing stability during flight (Francoy et al., 2009). Some authors have hypothesized that the reduction in Meliponini wing venation could be related to an increase in wing flexibility, while others suggested the relationship with the distribution of sensory receptors. We believe that the reduction is because the ancestral stingless bees were tiny, and therefore lost most of the venation during this dwarfism, until the group again gained size, but did not regain the lost venation. As part of these results, six landmarks are necessary for differentiation of genus level, in this case, they are Lm3, Lm4, Lm9, and Lm5 which were mentioned already, Lm6, and, Lm1, in order of relevance (Fig. 2). All of them present an important shape variation in the wing coordinates.

Several authors have demonstrated a commercial interest in the stingless bee genera reported in this work: *Melipona* sp. (Ramírez et al., 2014; Vit et al., 2015, 2017), *Tetragonisca*

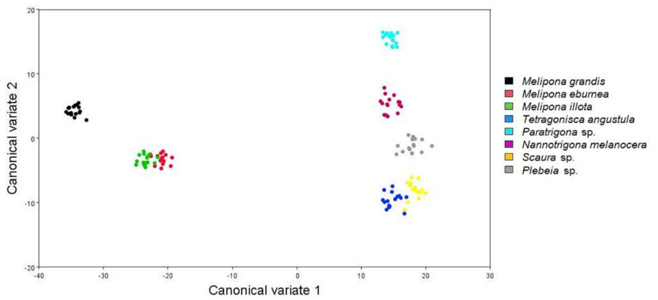

Figure 5. Canonical Variates Analysis diagram for 127 specimens and eight stingless bee morphospecies recollected in meliponaries of Napo.

sp. (Ramírez et al., 2014; Vit et al., 2016), *Paratrigona* sp. (Ramírez et al., 2014; Vit et al., 2015), and *Nannotrigona* sp. (Ramírez et al., 2014). However, the genera *Scaura* and *Plebeia* have not been previously reported for meliponiculture in Ecuador. According to Nates (2001a, 2001b) and Francoy (2011), the commonly used Meliponini genera are *Melipona* and *Tetragonisca*, due to the quantity and quality of honey produced and the wide distribution range of these (Francoy et al., 2011; Nates-Parra and Rosso, 2013). While the use of species like *Nannotrigona* and *Scaura* is limited, due to the low honey production. Additionally, nests of one species of *Scaura* are always associated with termites. Those genera producing low quantities of honey could be managed for conservation, recreation activity, or pollination (Nates-Parra and Rosso, 2013).

Our results showed that geometry morphometrics allows for the discrimination of morphospecies in Napo meliponiaries. In particular, we found that by only six measurements, it is possible to classify the colonies according to genus and sometimes species. The use of wing descriptors in Meliponini reveals the importance of this structure for taxonomic proposes (Rattanawannee et al., 2015; Nogueira et al., 2019). This method is a quantitative technique that allows for obtaining reliable and comparative results traditionally obtained by morphology (Vijayakumar and Jayaraj, 2013; Jaramillo-Ocampo, 2014). Several authors concluded that geometry morphometrics applied to the Hymenoptera wing contributes usefully to the discrimination and classification of genera and even some species of Meliponini (Francoy et al., 2009; García-Olivares et al., 2015). Finally, we suggest that quantitative phenotypic description of additional anatomical partitions (e.g., wings, thorax, legs) could be combined with molecular techniques to identify an easily recognizable classification in Meliponini.

AUTHORS PARTICIPATION

The first author was responsible for the written content and performing statistical analyses. The second author provided written assistance, additional support, and contributed to editorial improvements. Meanwhile, the third author oversaw identifying the bee species and made some revisions to the text.

ACKNOWLEDGMENT

We thank to the meliponicultures: Uli Zebisch, Brian Ruano and Jaime Marti of Napo for allowed the bee collection.

CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest associated with the publication of this manuscript. They have no financial, personal, or professional interests that might impact the research or this content.

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