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First Case of Gynandromorphism in the Orchid-Bee *Eulaema meriana* (Olivier) (Hymenoptera: Apidae)

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Abstract
The discovery of the first case of gynandromorphism in *Eulaema meriana* (Olivier) is described and illustrated. A specimen from Los Ríos, western Ecuador, discovered in the Instituto Nacional de Biodiversidad (MECN) collection, exhibits mostly male features on the left and female features on the right, except for the jugal comb of the hind wing and terminalia. This finding is compared to other reported cases in orchid bees.

Introduction

A significant important number of cases of phenotypic anomalies have been reported among bees. Specimens with intersex phenotypes are the majority of these cases. These anomalies consist of the manifestation of primary and secondary characteristics of both sexes in the same individual, a phenomenon called gynandromorphism (Michez et al., 2009).

The origin of gynandromorphy has been attributed to genetic anomalies. Though different hypotheses have been raised to explain it, the mechanisms that generate these abnormal individuals remain unclear. The phenomenon is presented in many groups within Hymenoptera (see Wcislo et al., 2004; Michez et al., 2009). The gynandromorphs have been categorized in three accepted patterns: transversal or antero-posterior, mixed or mosaic, and bilateral, of which mixed and transverse appear to be the most common (Dalla Torre & Friese, 1899; Wcislo et al., 2004; Hinojosa-Díaz et al., 2012).

Gynandromorphy has been reported in all families of Anthophila and all biogeographical regions. However, most cases have been documented from species of the Holarctic region (Hinojosa-Díaz et al., 2012). Fewer cases have been reported in the Neotropics, despite containing one of the most diverse bee faunas, with many endemic taxa.

Neotropical orchid-bees (tribe Euglossini) are conspicuous members of the corbiculate bees due to their long labiomaxillary complex, metallic coloration, and the male behavior of collecting fragrances from orchid flowers (hence their name). Some cases of gynandromorphism in orchid bees have been reported in the last decade. The first was in *Euglossa iopoecila* Dressler (Giangarelli & Sofia, 2011), and several cases have been reported since: *Euglossa tridentata* Moure

(Hinojosa-Díaz et al., 2012), *Eulaema atleticana* Nemésio (Silveira et al., 2012), *Euglossa pleosticta* Dressler (Camargo & Gonçalves, 2013), *Euglossa gorgonensis* Cheesman (González, 2014) and *Euglossa melanotricha* Moure (Suzuki et al., 2015).

This article describes the first recorded gynandromorph of the orchid-bee species *Eulaema meriana* (Olivier) coming from western Ecuador. This is the seventh case within the Euglossini and the first gynander bee from Ecuador.

Material and Methods

Dr. Marcio de Oliveira visited the entomological collection of the Instituto Nacional de Biodiversidad (Quito, Ecuador) in July 2019, intending to review and identify bee specimens. Along with the work, we found an individual of *El. meriana* exhibiting male and female-specific features on its body. We described the specimen according to morphological terminology used in Michener (2007); description of the genitalia follows Ospina-Torres (1998). Morphological measures and photographic records of characters were made using a stereomicroscope (Olympus SZ61R) adapted with a digital camera (The Imaging Source DFK23UX236) installed with the IC Measure v. 2.0.0.161 software. All measures were performed three times with IC Measure; the means of these measures are presented here and are given in millimeters (mm). Images were then focus stacked in Helicon Focus and edited in Adobe Photoshop CS5.

The genitalia of the specimen was dissected. Before dissection, the individual was placed in a sealed plastic container with water-soaked tissue paper for about 72 hours. Next, the terminalia were excised using an insect pin (size 0) and fine forceps to punch holes and pull apart the conjunctival membrane, separating the fifth and sixth metasomal terga and sterna. Excised terminalia were placed in a solution containing 10% potassium hydroxide (KOH) and water, boiled for about 5 minutes, and then put in acetic acid to neutralize the KOH. Finally, the genitals were transferred to a glass vial containing a small amount of glycerin for indefinite storage. The vial containing the genitals remains stored together with the specimen, pinned under it through the rubber cap. The gynander genitals were compared with those of a congeneric female, *Eulaema cingulata* (Fabricius), and the descriptions of male genitals were taken from the literature (Ospina-Torres, 1998).

Results

Gynandromorph description. The specimen is in good condition, except that most of the left antenna flagellomeres are missing. General measurements (mm): total body length 25.01, head width 7.64, upper interocular distance 3.07, lower interocular distance 4.43, intertegular distance 6.41, mesoscutellum length 3.06, right forewing length 21.06, left forewing length 21.39, right hind wing 14.01, left hind wing 13.99, maximum metasomal width 10.3, metasomal length 13.45. Overall coloration black in head, mesosoma, and legs, metasoma with integument of the first tergum with a green metallic luster, or blue to golden green. Marginal stripes of yellow hairs in the distal section of each tergum. Terga 4-7 covered with ferruginous hairs. Integument densely punctuate in head and legs. Long face, malar area clearly longer than the diameter of the third flagellomere; distance between the lower corner of the clypeus and the orbit greater than the diameter of the middle ocellus. Tongue elongate, nearly as long as the body. These general features match those of normal specimens of both sexes and the descriptions of the species (Moure, 2000; Oliveira, 2007).

Head. Bilaterally asymmetric, right side with mainly female features, left side with only male features, as follows (Fig 1a): right section of the labrum more developed with hairs twice as large as on the left side. Right mandible (3.98), noticeably larger and broader, with three well-developed teeth; external part of the mandible with long hairs uniformly distributed along its surface. Left mandible smaller (2.64) with no differentiable teeth and only a row of long hairs on the external margin, directed ventrally. Smooth and shiny spot on the right malar area (at the intersection of the clypeus, mandible, and compound eye) are more developed and with a patch of yellow hairs longer than those on the left side. The ventral part of the mandibular insertion of the left side with a conspicuous bifurcated lobe, stouter on the external side and broader but weaker on the internal side, with a medial concavity. Right side uniformly descending to the mandibular articulation, without lobes (Fig 1b). Right antenna with 12 segments with 10 flagellomeres, left antenna with the last flagellomeres missing. The dimensions of the head show little difference between right and left, the right side being slightly bulkier than the left one.

Mesosoma. The central thoracic axis presents slight differences in symmetry, the right side being more voluminous than the left. In addition, mesoscutelum with a small tuft is found, a character showed only by *Eulaema* females. The most notable differences are those of the legs. The right legs largely correspond to those of a normal female: foreleg without chemical gathering tufts (Fig 1c), without velvety area or tufts in mesotibia (Fig 1e), metatibia with a well-developed concave corbicula (Fig 1g).

The left legs were very similar to those of a normal male: foreleg with well-developed chemical gathering tufts on second through fourth protarsomeres (Fig 1d), an incipient velvety area, and tufts on the outer surface of mesotibia (Fig 1f). In the hind leg, a flattened metatibia with the appearance of a deformed corbicula can be observed. In addition, there is a slit with a very irregular shape in its lower portion, widened in its initial and final parts, while in the middle portion it becomes a thin line. In normal male specimens, the slit has a regular width throughout (Fig 1h).

Wings without notable difference in size. Both hind wings with a jugal comb (typical of male euglossine bees),

left wing with 20 blades, the right with only 15. Both wings differ in the number of hamuli, the left having 37, and the right 35. However, this is not a sex-related character. Based on our observations in specimens of both sexes and several species of *Eulaema*, the number of hamuli can vary in the wings of the same individual.

Metasoma. Abdomen with five visible terga and six sterna (tergite six is naturally lacking), which would be the number of visible terga for a normal female. It also appears to be bilaterally asymmetric, with slight differentiation in size between the right and left sides of the terga and sterna. The right side is slightly coarser than normal females, the left one being less bulky (Fig 1i). A well-developed stinger (7.15), as in normal females, emerges from the final segments (Fig 1j).

Terminalia. The genital parts, although slightly modified, match those of a female of the same species (Fig 2a).

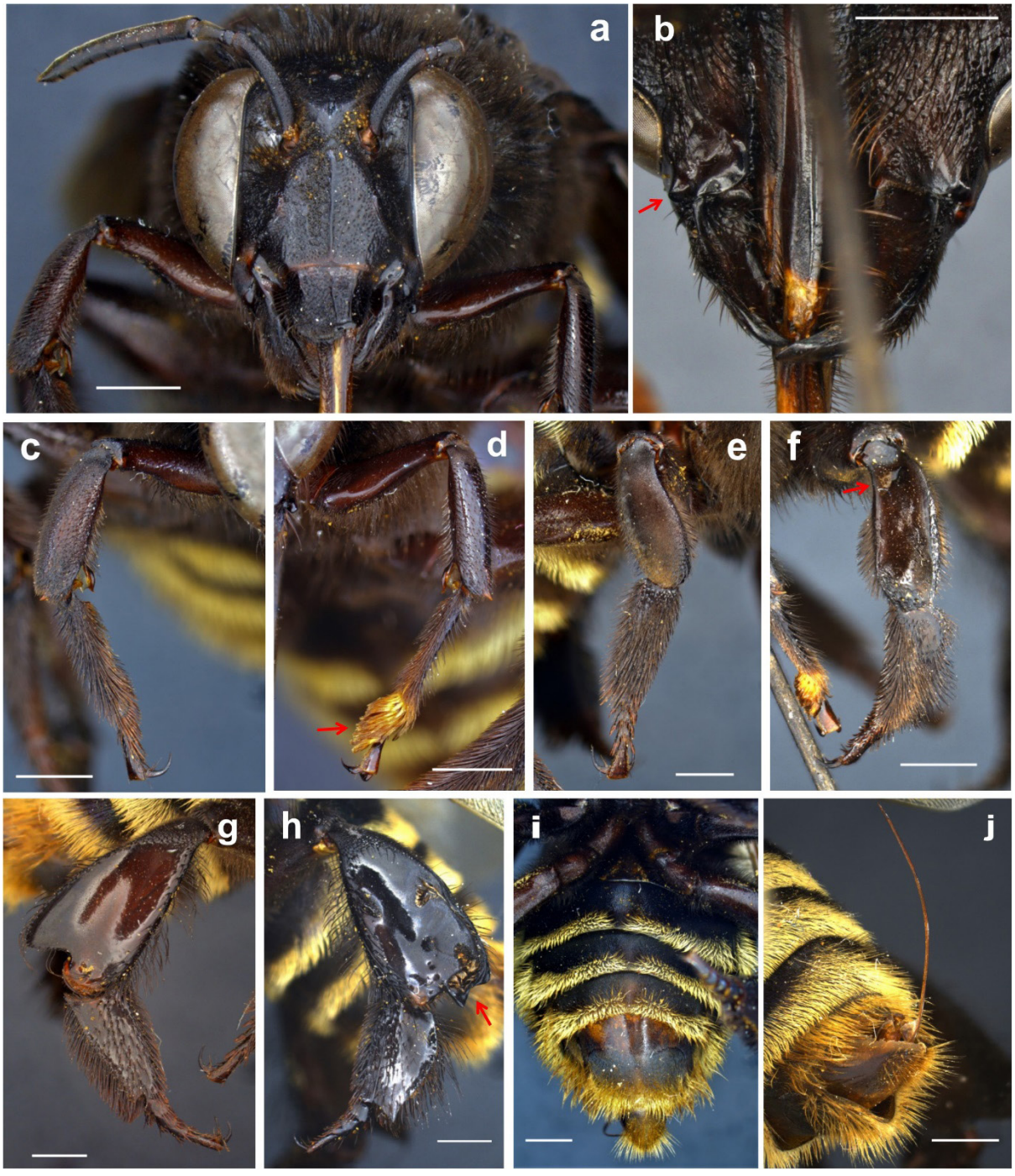

Fig 1. *Eulaema meriana* gynander: **a.** Front view of the head, **b.** Ventral part of the mandibular insertion (left side with a conspicuous bifurcated lobe), **c.** Right foreleg, **d.** Left foreleg, **e.** Right middle leg, **f.** Left middle leg, **g.** Right hind leg, **h.** Left hind leg (with malformation), **i.** Ventral view of the abdomen (bilaterally asymmetric), **j.** Final tergites of the abdomen with a well-developed sting. Scale bars = 2 mm.

A normal female has two rami of valvulae that converge to form the stinger; however, one of the rami of valvulae (the lower) is not connected to the sting in the gynander specimen and is ending freely in the genital capsule. The longer one emerged from the upper part of the capsule, appearing as a longer and thinner ramus. Sternite 6 similar to that of a normal female (Fig 2b).

Material examined. Ecuador, Los Ríos, Buena Fé, Patricia Pilar, Centro Científico Río Palenque, 140m, 00°34'55" S, 79°22'01" W; 12 Feb 1987; Calaway Dodson leg. (Collection method is not mentioned). Deposited in Instituto Nacional de Biodiversidad – INABIO [MECN]. Catalog number: MECN-EN-HYM-1626. M. L. Oliveira det.

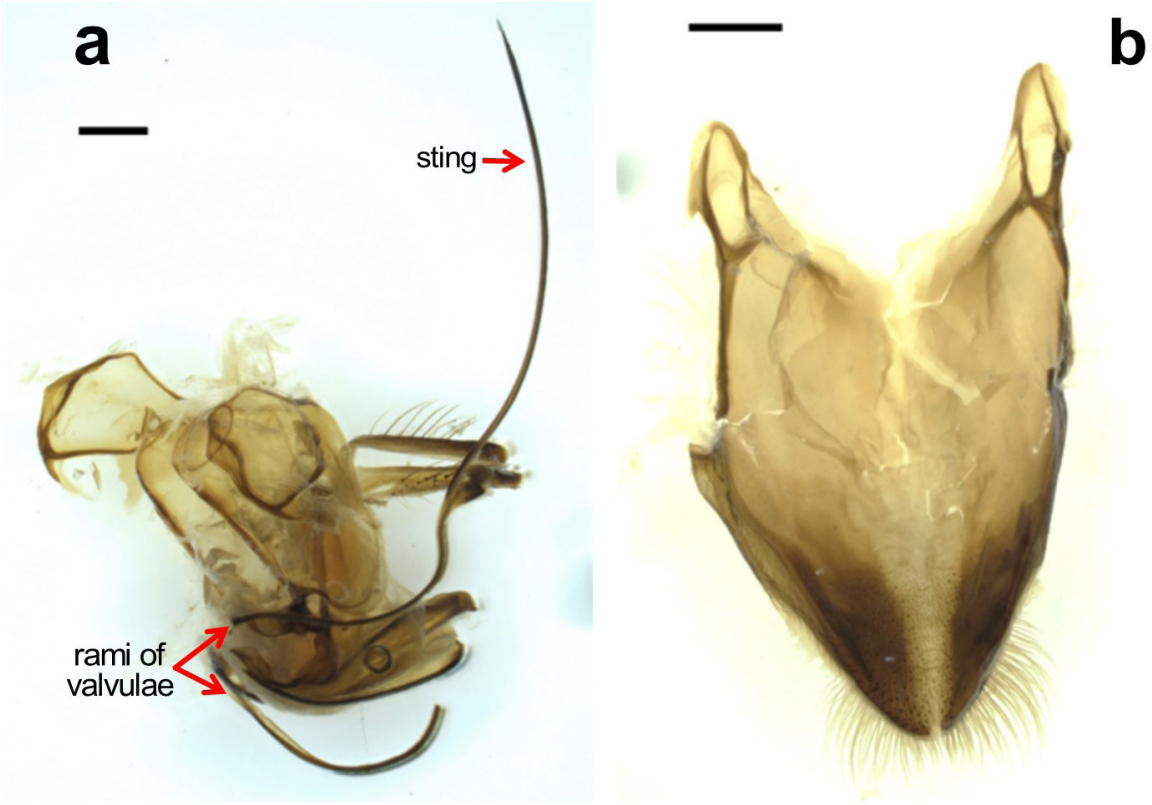

Fig 2. Terminalia of *El. meriana* gynander: **a.** Genitalia of gynander (in lateral view), sting emerges from the upper part of the genital capsule and not the lower one, **b.** Sternum 6 in dorsal view. Scale bars = 1 mm.

Discussion

Eulaema meriana (Olivier) is one of the most widely distributed orchid bee species in the Neotropical region, from Guatemala to Bolivia (Oliveira, 2007). Despite being a relatively common species, this is the first record of a specimen with morphological characteristics of both sexes and the seventh documented case in the tribe Euglossini.

In this specimen, most of the secondary sexual characters are distributed bilaterally, half male left and half female right. However, the hind wing jugal comb on both sides (male feature) and female terminalia would classify this case as mixed or mosaic gynandromorphy. The other known case within the genus, *Eulaema atleticana* Nemésio (Silveira et al., 2012), is also classified as mixed. Still, it differs from the case presented here because it does not present bilateral sexual secondary characteristics. On the contrary, each tagma has characteristics of one sex or another. Furthermore, the head and mesosoma have characteristics of male and the metasoma of female, while there is an alternation of characters on the legs.

The mixed or mosaic pattern of this case in *El. Meriana* corresponds with all reports of orchid-bee gynanders, where male and female structures were distributed in patches throughout the body. However, the distribution of characters was irregular among body sections in prior cases (Giangarelli & Sofia, 2011; Hinojosa-Díaz et al., 2012; Silveira et al., 2012; Camargo & Gonçalves, 2013; González, 2014; Suzuki et al., 2015) (see Table 1), establishing a pattern of phenotypic intersexual anomalies in Euglossini. This aligns with the trend of most known cases of gynandromorph bees worldwide that present this gynandromorph type (Wcislo et al., 2004; Michez et al., 2009; Hinojosa-Díaz et al., 2012).

Detection of gynandromorphism in orchid bees should be facilitated by the marked sexual dimorphism between males and females, a situation that does not occur in other groups of bees (Hinojosa-Díaz et al., 2012). Males have specialized morphological structures to collect fragrances for reproductive purposes, while females are morphologically homogeneous, to the point that many are not possible to identify species without an associated male (Roubik & Hanson, 2004; Rasmussen et al., 2015).

On the other hand, the methodology with which the specimen described here was collected has not been written on the original label. But we presume that it could be collected with attractants for a male orchid bee. Since our observations, most of the orchid bee specimens donated by C. Dodson to INABIO were collected with attractive fragrances such as eucalyptus oil, methyl salicylate, among others that only the males of this group collect for reproductive purposes.

Despite the notable morphological differences between the sexes and the anomalies that this specimen presents, it has taken more than 33 years since it was collected for a specialist to determine it as a case of gynandromorphism. This is due to the notable lack of knowledge of the Ecuadorian bee fauna. Although, in recent years, euglossines have been the relatively most studied group of this fauna, to the point that they are the only group that currently has a species checklist and several recent revisions (Botsch et al., 2017; Padrón et al., 2018; López, 2018; Yanouch et al., 2018; Suárez-Torres, 2019; Roubik, 2019).

The case published here follows this trend of increasing melittological research and a positive outlook for greater knowledge of this fantastic pollinator group in Ecuador and the Neotropical region.

Table 1. Comparison of characters among orchid bee gynanders recorded until today.

Species	Gynandromorph type	Deviant phenotypic characters	Reference
Euglossa iopoecila Dressler	Mixed	Head right half ♀, left half ♂; mesosoma: left half ♂, right half ♀, (mesoscutellum, ♀-like); metasoma ♀, except left half of the second sternum ♂	Giangarelli & Sofia, 2011
Euglossa tridentata Moure	Mixed	Head and mesosoma: right half ♀, left half ♂, but labrum with reversed sides, mesoscutellum ♀-like, legs mixed; metasoma: right half ♀, left half ♂ basally (distal segment lost)	Hinojosa-Díaz et al., 2012
Eulaema atleticana Nemésio	Mixed	head and mesosoma ♂, metasoma ♀, legs mixed	Silveira et al., 2012
Euglossa pleosticta Dressler	Mixed	Head right half ♂, left half ♀; legs mixed; mesosoma ♀; metasoma ♀	Camargo & Gonçalves, 2013
Euglossa melanotricha Moure	Mixed	Head, mesosoma, and metasoma ♀; right legs ♀, left legs ♂	Suzuki et al., 2015
Euglossa gorgonensis Cheesman	Mixed	Head and mesosoma ♂; legs mixed.	González, 2014
Eulaema meriana (Olivier)	Mixed	Head and mesosoma left half ♂; Right half ♀ (mesoscutellum ♀-like); genitalia ♀.	This work

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Author's Contribution:

MLO: conceptualization, writing-original draft, writing-review and editing, supervision.

APP: conceptualization, methodology, writing-original draft, writing-review and editing, supervision.

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