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ARTICLE

Descriptions of new gynandromorphs of *Xylocopa augusti* Lepeletier (Hymenoptera: Apidae: Xylocopini) from Argentina

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Abstract. Gynandromorphs are individuals that display both male and female features throughout the body and are rarely found in nature. We document and describe two new gynandromorphs of the large carpenter bee *Xylocopa* (*Neoxylocopa*) *augusti* reared from a trap-nest in La Plata, Buenos Aires, Argentina. In addition, based on a literature review, we assessed the frequency of the different types of gynandromorphs, and the body part affected, among large carpenter bees. Both gynandromorphs were assigned to the mixed category, the most common category reported in the literature (22 of 25 specimens). The remaining three specimens exhibit a bilateral pattern in all tagmata. The presence of both sexes' secondary sexual characteristics occurred more frequently on the mesosoma than on the head or metasoma. Trap nests used in bee hotels promote the conservation and study of wild bees and might facilitate the discovery of rare cases of gynandromorphs that would remain unknown otherwise under normal nesting conditions in the wild.

Keywords. Anthophila; Carpenter bees; *Neoxylocopa;* Gynandromorphism.

INTRODUCTION

Numerous morphological aberrations have been described in bees, but the most distinctive is gynandromorphism, when individuals display secondary characters of both sexes throughout the body (Michener, 1943; Wcislo *et al.,* 2004; Engel, 2007; Hopwood, 2007). To date, gynandromorphs have been found in more than 143 species and 37 genera belonging to all families of bees except Stenotritidae (Reviewed in Wcislo *et al.,* 2004; Michez *et al.,* 2009; Hinojosa-Díaz *et al.,* 2012; Fateryga *et al.,* 2011; Alvarez *et al.,* 2019; Barrett, 2021). Gynandromorphs may go undetected by researchers in bee groups that exhibit little sexual dimorphism in terms of pilosity or coloration. However, for taxa with strong sexual dimorphism, as in the case of large carpenter bees of the genus *Xylocopa* Latreille subgenus *Neoxylocopa* Michener, where females are black and males are yellow, gynandromorphs are detected easily in the field or among specimens in insect collections. Indeed, half of gynandromorphs of *Xylocopa* are in that neotropical subgenus (9 of 18 species) (Lucia & Gonzalez, 2013; Lucia *et al.,* 2015; Villamizar, 2020).

Here, we document two gynandromorphs of *Xylocopa* (*Neoxylocopa*) *augusti* Lepeletier de Saint-Fargeau, which were detected during on-going studies of the biology of this species using trap-nests in Argentina. The first gynandromorph of *X. augusti* was recorded by Lucia *et al.* (2015), who described a mixed specimen from Pehuen-Có, Buenos Aires, Argentina. In addition, based on a literature review, we also explored the occurrence of gynandromorphism in *Xylocopa* and the distribution of different sexual characteristics among tagmata.

MATERIAL AND METHODS

The gynandromorph described here was reared from a trap nest of *X. augusti* built inside an internode of cane (*Arundo donax* L., Poaceae) and collected on 12-XII-2018 in the Unidad Vivero

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Forestal, Facultad de Ciencias Agrarias y Forestales of the Universidad Nacional de La Plata (34°54′39″S, 57°55′37″W, 18 m.a.s.l.), La Plata, Buenos Aires province, Argentina. To document brood development, we transported the nest to the laboratory, opened it lengthwise and secured both halves with tape (Fig. 1a), which allowed us to monitor it on daily basis. We studied and measured bee external morphological features using a Nikon SMZ 745T stereomicroscope with an ocular micrometer and took images with a Canon T6 digital camera attached to a stereomicroscope using a 60 mm Oshiro Macro lens. Multiple photographs taken at different depths were then merged using the Combine ZMT software package. Morphological terminology generally follows that of Hurd & Moure (1963) and Michener (2007). We measured total body length from the head to the apex of metasoma in lateral view, forewing length from the apex of the costal sclerite to the wing apex, mesosoma width between the outer borders of the tegulae, and metasoma width across the second tergum using a stereomicroscope with an ocular micrometer. In the descriptions, we used T and F for metasomal terga and flagellar segments. To facilitate comparisons, we followed the classification of gynandromorphs by Dalla Torre & Friese (1899) as follows: bilateral (divided in left-right), antero-posterior, transverse (dorsal-ventral), and mixed (mosaics or different combinations).

To assess the occurrence of gynandromorphism in *Xylocopa* and the distribution of the female and male features among tagmata, we reviewed the literature (supplementary material; table 1) and classified each gynandromorph in bilateral (symmetrical distribution of male and female features) or mixed (mosaic of male and female features) due to its features and their body part affected. To evaluate differences between types of gynandromorphs and among body parts, we used the Chi-Square goodness of fit test at a 5% significance level. Tests were analyzed using the statistical software R version 4.0.5 (R Core Team, 2021).

RESULTS

Xylocopa (*Neoxylocopa*) *augusti* Lepeletier de Saint Fargeau, 1841

Gynandromorph 1 (Figs. 1b-f)

Description: Body length 22.1 mm, head length 5.1 mm, head width 7.1 mm, mesosoma width 9.5 mm, metasoma width 10.7 mm. The specimen displays female and male traits distributed irregularly along the body (mixed). **Head:** (Fig. 1b) with a mixture of female and male characteristics. The left half of the supraorbital area, vertex, frons, paraocular area, supraclypeal area and clypeus with yellow integument and pubescence as in the male. The right half with integument and pubescence dark brown to black, female-like, except male-like yellow integumental maculations as follows: frons, lower side of clypeus and left upper and lateral paraocular area. Genal areas female-like with integument and pubescence dark brown to black, except medially and basally with a yellow spot and yellow setae. Labrum with integument and setae as in the male, except for a small apical portion on its right side with integument and features as in the female. Mandibles female-like, except right mandible with a small yellow spot basally, similar to that of the male. Upper interorbital distance 3.9 mm; lower interorbital distance 4.2 mm; interalveolar distance 1.1 mm. Antennae with 11 flagellomeres as in the male, integument with a combination of coloration of both sexes, dorsally predominantly yellowish and ventrally dark brown (length of scape, pedicel, and F1: left 2.72 mm, 0.28 mm, and 0.97 mm, respectively; right side, 2.35 mm, 0.25 mm and 0.85 mm, respectively). **Mesosoma:** (Fig. 1c-d) female-like; right foreleg female-like, left foreleg with mixed features of both sexes, structurally female-like; profemur with three yellow stripes on ventral surface; protibia with yellow stripes in internal, ventral, and dorsal surfaces. Left procoxa and trochanter with yellow setae mixed with black. Middle and hind legs female-like. Wing dark brown with violet highlights. **Metasoma:** predominantly female-like (Fig. 1c-d), with six exposed terga and sterna. Terga with integument and pubescence as female. Sterna with mixed features of both sexes, T1-2 female-like, T3-6 with some spots tawny to black with yellow setae mixed with black setae. Genitalia bilaterally asymmetric, right half composed of hemi-male genitalia showing part of the gonobase, a penis valve and gonostylus; left half female-like with part of a sting (Fig. 1e-f).

Nesting and biological data: The nest from which the gynandromorph emerged (Fig. 1a) had five brood cells (cell 1 is the innermost cell, farthest from the nest entrance); the foundress female was killed by the conopid fly *Physochepala wulpi* Camras (Diptera: Conopidae). When the nest was found, cells 1-3 contained pupae in an advanced stage of development, cell 4 had an intact pollen mass (no larva developed), and cell 5 had a post-feeding larva that pupated on 17-XII-2018. Normal females emerged from cell 1 and 2 on 28-XII-2018 and 30-XII-2018 respectively, the gynandromorph emerged from cell 3 on 31-XII-2018, and a male emerged from cell 5 on 01-I-2019.

Material examined: One gynandromorph of *Xylocopa* (*Neoxylocopa*) *augusti;* Argentina, La Plata, Buenos Aires, collected on 31-XII-2018, Col. M. Lucia; deposited in the collection of División Entomología del Museo de La Plata, Argentina (MLP).

Gynandromorph 2 (Fig. 2)

We observed and photographed this second gynandromorph (Fig. 2) on 09-XI-2018 from a trap nest located within the same group of trap nests of the same bee hotel where we found the first gynandromorph. We did not collect it because disappeared on the second day of its detection in the field. However, we observed that this

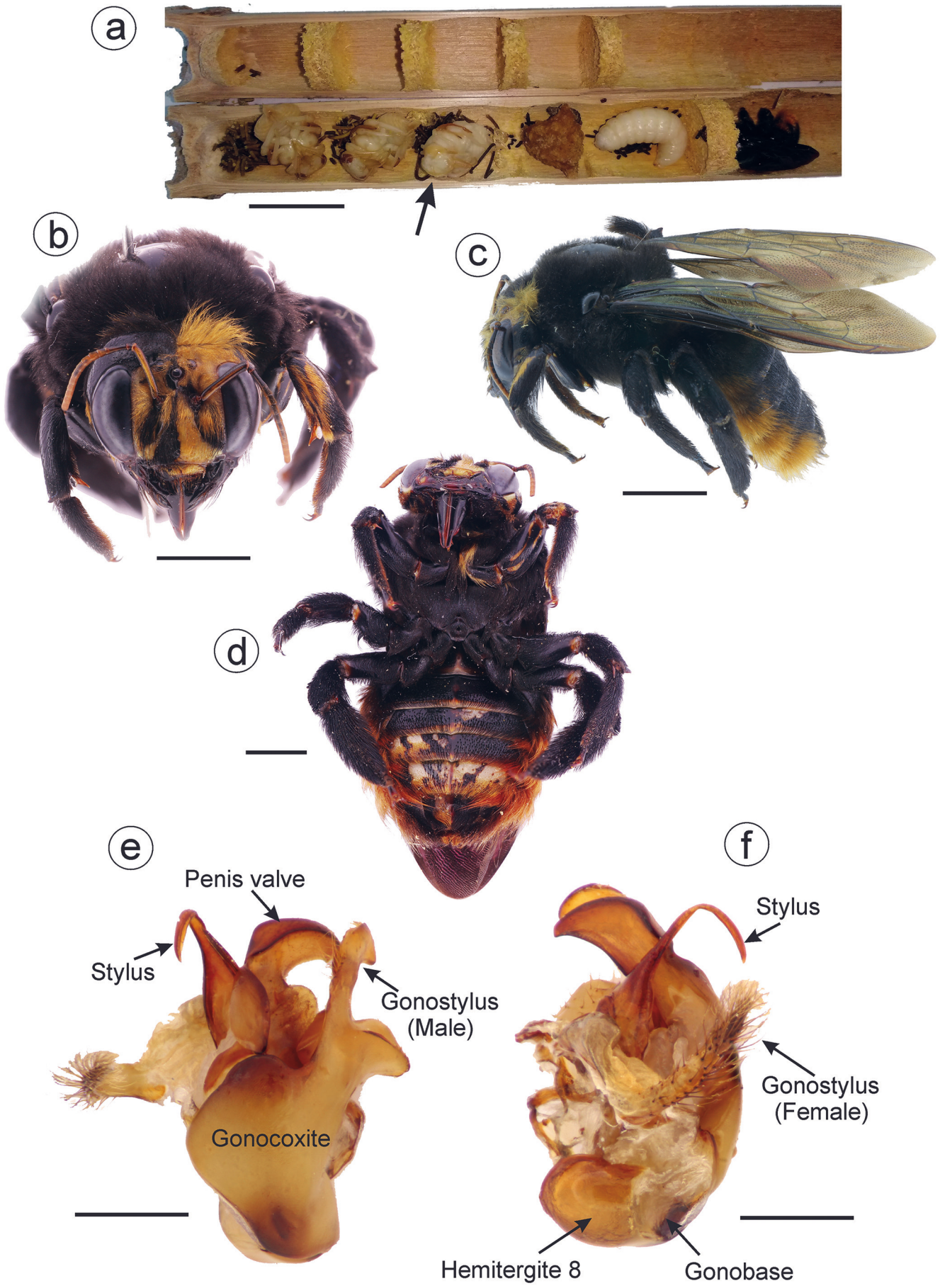

Figure 1. Gynandromorph of *Xylocopa* (*Neoxylocopa*) *augusti* Lepeletier de Saint Fargeau: (A) Sagittal section of a trap nest showing different developmental stages and pupa of Gynandromorph 1, which is indicated by the arrow in the third cell. (B) Head in frontal view. (C) Habitus in lateral view. (D) Habitus in ventral view (E) Genitalia in ventral view; (F) Genitalia in lateral view. Scale bars: (A) 2 cm; (B) 2 mm; (C-D) 1 cm; (E-F) 1 mm.

gynandromorph had the general aspect of a female except for its head (mesosoma and metasoma female-like). Based on a photograph (Fig. 2), we could characterize its head. **Head:** left eye, left half of the supraorbital area, vertex, frons, paraocular area, supraclypeal area, clypeus, and labrum with integument and pubescence as in the male, right side of the head female-like.

Frequency of gynandromorph types in *Xylocopa*

Both gynandromorphs described here are in the mixed category, the most common type recorded in *Xylocopa*. Among the 25 gynandromorphs reported for *Xylocopa* (Villamizar, 2020; supplementary matreial; table 1), the overall proportion of mixed to bilateral types is 7 to 1. No specimen shows the transverse or anterior-posterior types, according to Michez *et al.* (2009). Mixed phenotypes are recorded in at least a tagma of 22 specimens recorded to date. The remaining three specimens exhibit a bilateral pattern in at least a tagma. Although the mixed pattern occurs in at least a tagma (head 45.5%, mesosoma 68.2% and metasoma 47.6%), it was significantly more common on the mesosoma ($\chi^2 = 6.36$, df = 1, $p < 0.01$; Fig. 3) than in other tagmata. The head and metasoma showed a similar tendency to display a mixed pattern (head $\chi^2 = 0.22$, df = 1, $p = 0.63$; metasoma $\chi^2 = 1.67$, df = 1, $p = 0.19$). The bilateral condition exhibited different proportions among tagmata, as well as in the occurrence of female and male features on each side of the body.

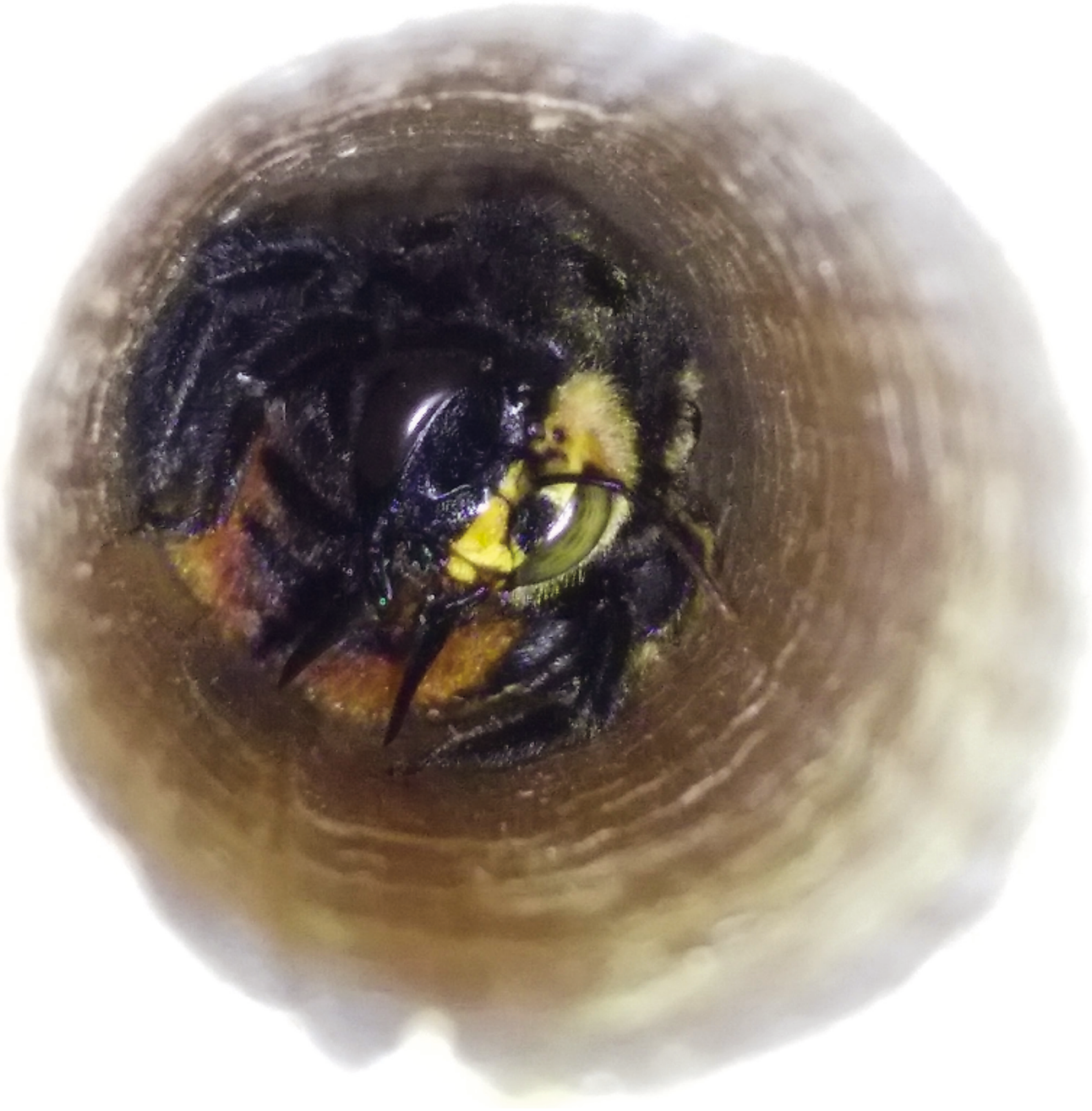

Figure 2. Gynandromorph 2 in frontal view inside the trap nest. This individual was not captured.

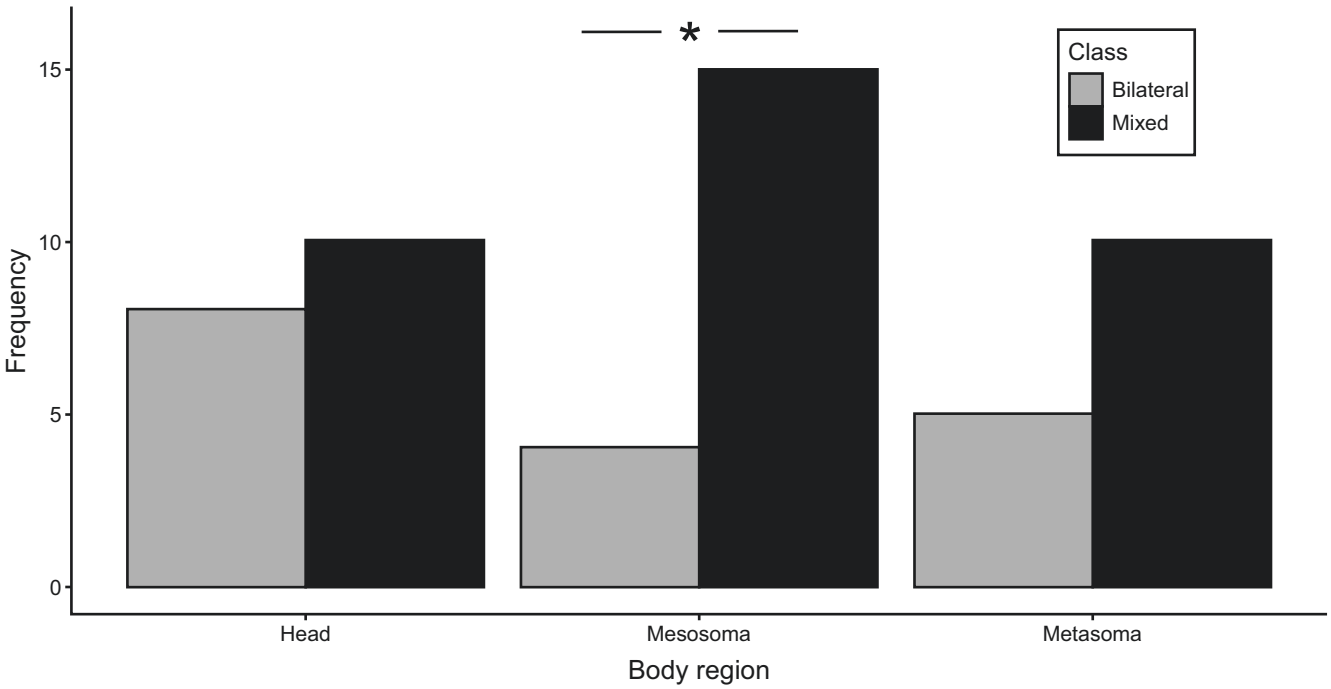

Figure 3. Frequency of gynandromorph types among tagmata in *Xylocopa.*

Table 1. Gynandromorph records of *Xylocopa augusti* Lepeletier. To explore gynandromorph records of *Xylocopa* Latreille, 1802, see Villamizar (2020) and Supplementary material.

Species	Category	Phenotype	Locality	Repository	Reference
Xylocopa (*Neoxylocopa*) *augusti* Lepeletier de Saint-Fargeau, 1841	Mixed	Mixture of ♂ and ♀ features in all tagmata	ARGENTINA, Buenos Aires, Pehuen-Có	MLP	Lucia *et al.* (2015)
	Mixed	**Gynandromorph 1:** Mixture of ♂ and ♀ features only in the head region. **Head:** left male and right female, Labrum with integument and setae as in the male, except for a small apical portion on its right side with integument and features as in the female. Mandibles female-like, except right mandible with a small yellow spot basally, similar to that of the male. **Mesosoma:** ♀ = like, **Metasoma:** predominantly ♀ = like.	ARGENTINA, Buenos Aires, La Plata	MLP	This paper
		Gynandromorph 2: General aspect of a female except for its face. Left eye, left half of the supraorbital area, vertex, frons, paraocular area, supraclypeal area, clypeus, and labrum with integument and pubescence as in the male.			

DISCUSSION

The number of gynandromorphs documented in *Xylocopa* has increased in recent years. Including the new specimens described here, there are 25 cases of gynandromorphism in *Xylocopa* belonging to 18 species. Half of them have been found in the subgenus *Neoxylocopa* Michener. The gynandromorphs described in this paper have a mixture of female and male features in at least a tagma, and thus they can be assigned to the mixed category. This is, by far, the most common pattern among *Xylocopa,* accounting for 88% (22/25) of gynandromorphs (Villamizar, 2020; Table 1; Supplementary material). We found that the presence of both sexes' secondary sexual characteristics occurred more frequently on the mesosoma. The underlying mechanisms that explain such a pattern are unknown. However, we believe that the presence of large appendages (legs) in this body region can make it easier for scientists to observe and describe gynandromorphy, at least superficially and without dissection, compared to head appendages or genitalic structures. Trap nests and bee hotels are extraordinary tools to promote the conservation and study of wild bees while engaging the public (McNally *et al.,* 2018). In addition, these tools might facilitate the discovery of rare cases of gynandromorphs, as demonstrated here, that would remain unknown otherwise under normal nesting conditions in the wild.

AUTHORS' CONTRIBUTIONS: VA, ML: Methodology; **VA, ML, VG:** Conceptualization, Writing – review & editing; **PJR:** Software, Data curation, Formal analysis. All authors actively participated in the discussion of the results, they reviewed and approved the final version of the paper.

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