



RESEARCH ARTICLE - BEES

The Colour and the Shape: Morphological Variation on a Facultatively Eusocial Bee *Augochlora (Augochlora) amphitrite* (Schrottky)

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Abstract

Augochlorine bees exhibit a large array of foraging and social behaviors, nest substrates and architecture. The huge diversity of behaviors is frequently linked with morphological traits. All levels of variation should be analyzed in order to provide a broader view of evolution. *Augochlora (Augochlora) amphitrite* Schrottky occurs from northern of Argentina to southeastern of Brazil. The species nests in decaying wood and is facultatively eusocial. Color variation and head polymorphism were already mentioned in the literature and the main goal of the present paper is to evaluate the morphological variation of the species. For this purpose, we examined 720 specimens and carried out qualitative and quantitative analyses with traditional morphometrics. Other 25 *Augochlora* species were studied and we propose a revised diagnosis for *A. amphitrite*. A remarkable color variation is described, there are three morphs: green, dark blue, and black. There are no geographical patterns linked with the color variation. We propose that *Odontochlora lethe* Schrottky and *Odontochlora styx* Schrottky are junior synonyms of *Odontochlora amphitrite* Schrottky. Those names refer to black male and female occurring within *A. amphitrite* distribution. There is a continuous variation on size and shape of head. Again, we do not find any relation of morphology with distribution. Besides gena swelling, the adductor ridge of mandible is strongly developed on macrocephalic females. Due variations showed, *A. amphitrite* is a bee candidate to be a model for studies to link morphology, function and behavior.

Introduction

The tribe Augochlorini Beebe is a monophyletic bee lineage with 663 species distributed in 35 genera (Gonçalves, 2016), known for the brilliant green coloration present in most of its representatives (Engel, 2000). The tribe is restricted to the Western Hemisphere, being very abundant in tropical South America (Engel, 2000; Michener, 2007). Augochlorine bees exhibit a large array of foraging and social behaviors, nest substrates and architecture (Eickwort & Sakagami, 1979; Engel, 2000; Schwarz et al., 2007). Species may be solitary to primitively eusocial (Danforth & Eickwort, 1997; Michener, 2007; Gonçalves, 2016), nest on soil or decaying wood (Eickwort & Sakagami, 1979) or even be cleptoparasites (Engel, 2013). Some behavioral aspects, as nesting habits and division of labor, are frequently linked with morphological

traits (Eickwort, 1969). For example, substrate usage for nesting can be inferred by morphology, since females have developed ridges and teeth on mandible to excavate wood (Eickwort, 1969). As another example, allometry on head growth for some species may be indicative of social interactions (Sakagami & Moure, 1965). Head polymorphism is known for *Augochlora* Smith, *Corynurella* Eickwort, *Megalopta* Smith and *Rhinocorynura* Schrottky (Sakagami & Moure, 1965; Eickwort, 1969; Gonçalves, 2010; Gonçalves & Melo, 2012). Females whose heads are enlarged, especially due to the swelling of vertex and gena have been denominated as macrocephalic (Sakagami & Moure, 1965; Santos & Silveira, 2009; Gonçalves & Melo, 2012).

Augochlora is the second most speciose genus of Augochlorini with approximately 114 species (Moure, 2012). The genus is a monophyletic lineage belonging to the



Augochlora clade (Eickwort, 1969; Engel, 2000; Gonçalves, 2016; Meira & Gonçalves, 2018). The two extant subgenera use different nesting substrates. *Augochlora sensu stricto* nest in decaying wood (Eickwort 1969; Wcislo et al., 2003; Dalmazzo & Roig-Alsina, 2012, 2015) and was formerly considered solitary based on the study of Stockhammer (1966), however recent studies indicate facultative primitively eusocial behavior for some species (Wcislo et al., 2003; Dalmazzo & Roig-Alsina, 2012, 2015). *Augochlora (Oxystoglossella)* species nest in the soil (Eickwort, 1969), being the studied species considered primitively eusocial with some caste differentiation (Eickwort & Eickwort, 1972). Macrocephalic females are known for *Augochlora (Oxystoglossella) iphigenia* Holmberg (Sakagami & Moure, 1965; Dalmazzo & Roig-Alsina, 2011) and for *Augochlora (Oxystoglossella) empusa* Engel, Hinojosa-Díaz and Bennett (Engel et al., 2012). The *Augochlora* species from southern Argentina and Uruguay were revised by Dalmazzo and Roig-Alsina (2011), beside this, no taxonomic revisions for the entire genus were taken so far.

Augochlora (Augochlora) amphitrite Schrottky occurs from northern Argentina to southeastern Brazil (Moure, 2012). This resilient species is found in natural, rural and urban areas (Gonçalves & Melo, 2005; Gonçalves et al., 2014; Taura & Laroca, 2001). The few studies with the species include the description of a mostly bilateral gynandromorphy by Alvarez et al. (2014) and diet analysis and pollen host selection by Dalmazzo and Vossler (2015a; 2015b). The species was formerly described as *Odontochlora amphitrite* Schrottky, whose type specimen is probably lost (Moure, 2012). The same occurs with other type specimens of Curt Schrottky (Rasmussen et al., 2009), which may include names that correspond to morphological variations. Despite that Dalmazzo and Roig-Alsina (2011) were able to identify the species based on the original description and the distribution pattern. *A. amphitrite* possesses the typical spine on the first sternum and a longitudinal furrow on its scutellum (Schrottky, 1909; Dalmazzo & Roig-Alsina, 2011), a combination of features found in a small group of *Augochlora sensu stricto*. The specimens are usually metallic green with blue and violet reflections (Dalmazzo & Roig-Alsina, 2011).

According to Michener (2007), primitively eusocial behavior includes division of labor between foundresses and workers, which differs more distinctly in physiology and behavior than in morphology. *A. amphitrite* can also be considered facultative because single and multiple female nests have been found (Dalmazzo & Roig-Alsina, 2012). The authors also recognized that morphological variation is related to social behavior, since some females never observed outside the nests were distinctly larger than foragers. In addition, they had allometrically enlarged heads and developed ovaries. On the other hand, the forager females had worn mandibles and wings, and undeveloped ovaries. Still, intermediate states of wearing and ovary development were also observed in

foragers and suggested a continuous variation. Other studied *Augochlora sensu stricto* species probably present facultative primitively eusocial behavior, but morphological variation seems to be restricted to size (Wcislo et al., 2003; Dalmazzo & Roig-Alsina 2015).

The main goal of the present paper is to evaluate the morphological variation of *A. amphitrite*. We described and studied the color variation and its putative relationship with distribution. Macrocephalism is investigated with morphometrics to search for castes and for a relation to geographic distribution.

Material and methods

We examined material from ‘Coleção Entomológica Pe. Jesus Santiago Moure, Universidade Federal do Paraná’ (DZUP). The *Augochlora* collection with about 10,000 specimens was sorted to pull out the specimens of *A. amphitrite*. The species identification followed the diagnosis and redescription of Dalmazzo and Roig-Alsina (2011), accompanied by search for additional characters. Size and coloration were considered *a priori* as variation and not used to diagnose the species. Structure terminology follows Eickwort (1969) and Michener (2007) except where we refer to the basal area of propodeum as the metapostnotum. We use the abbreviations T1, T2 etc., to denote the metasomal terga; S1, S2 etc., to denote metasomal sterna. Sculpturing terminology follows Harris (1979).

In the course of identification, we studied 25 South American morphospecies from the DZUP collection to refine the delineation of *A. amphitrite*. A sum of seven males from different regions and also four males from other species had their genitalia prepared to provide additional characters. Males were softened in wet chamber from two to three days. Genitalia were removed with entomological pins and clarified in 10% KOH for 24 hours. The dissected structures were finally preserved in glycerol. We studied 720 specimens of *A. amphitrite*, 358 of them being measured and included in the morphometric analyses. The map for the distribution records was made with QGIS 2.18.15 ‘Las Palmas’.

Photographs of specimens were taken with a Nikon D700 and 107 mm Sigma Macro lens using Helicon Remote for controlling image capture. The same configuration was used for all images. All photographs were taken in manual mode, 1/160, 5.6, ISO 100, ev 0.3. A white sheet was used as background and the illumination followed the system of Kawada and Buffington (2016). Image stacking was made with Helicon Focus (version 6.0.18) – render method based on Method C (Pyramid). For color determination we followed the method of Aguiar (2005). We use the color picker tool (sample average option) on GIMP 2.8.16 (©The GIMP Team). The color sample was made on an area of 100x100 pixels, always on the same specimen portion. The corresponding RGB color code was recorded.

Measurements were made with Leica Stemi DV4 and a micrometric rule. The following measurements were taken: intertegular distance (IT), taken at middle portion of tegulae; ocular distance, measured at eye notch (OD); vertex length, in frontal view (VL); eye width, in lateral view (EW), taken from eye notch in an orthogonal line to the eye posterior margin; gena width, in lateral view (GW), in a continuation to the measurement of eye width; and mandible width (MW), taken at distal part of the adductor ridge. Principal components analysis was carried out for the entire dataset and separately for subsets containing only specimens from the Curitiba and Palotina regions (Paraná, Brazil). First PCA axis was considered as representing the overall body size. Grouping tests (LDA and Manova) and linear regression were carried out. For this analysis we used as attributes the color and distribution. Data was log transformed prior to the analysis. All analyses were carried out with R (R Core Team, 2017). The R packages *ade4* (Dray & Dufour, 2007), *ggfortify* (Horikoshi & Tang, 2016), *ggplot* (Wickham, 2010), *MASS* (Venables & Ripley, 2002) and *vegan* (Oksanen et al., 2018) were used for processing analysis and plot graphs.

Results

Taxonomy

Augochlora (*Augochlora*) *amphitrite* (Schrottky, 1909)
(Figs 1-5)

Odontochlora amphitrite Schrottky, 1909: 142 (females from Argentina, Buenos Aires and La Plata, types whereabouts unknown).

Odontochlora styx Schrottky, 1909: 143 (female from Paraguay, Alto Paraná, Puerto Bertoni, type whereabouts unknown). **New synonym.**

Odontochlora thebe Schrottky, 1909: 143–144 (male from Argentina, Buenos Aires, type whereabouts unknown). Decision for synonymy: Dalmazzo & Roig Alsina (2011).

Odontochlora lethe Schrottky, 1909: 144 (male from Paraguay, Alto Paraná, Puerto Bertoni, type whereabouts unknown). **New synonym.**

Females of *A. amphitrite* can be distinguished from most other *Augochlora sensu stricto* by the scutellum with a median longitudinal furrow which is strongly depressed posteriorly and by the S1 with a spiniform median projection. They can be further separated from similar species by the combination of the following features: clypeus mostly polished, microreticulate only on basal corners; supraclypeal area sparsely punctate and microreticulate; facial carina short, becoming a line above eye notch; distal portion of mandibular adductor ridge produced; metapostnotum with longitudinal, weakly sinuose, carinae; T1 punctate-puncticulate, with some scattered coarser punctures; T2 punctate-puncticulate, with few coarser punctures; and T3 puncticulate.

Males can be distinguished from other species of *Augochlora sensu stricto* by the combination of the following features: clypeus mostly polished, microreticulate only on basal corners; supraclypeal area densely punctate and microreticulate, facial carina very short; scutellum with a median longitudinal furrow and with polished sublateral spots; mesepisternum areolate-rugulose anteriorly to mesepisternal furrow and punctate posteriorly to the furrow; metapostnotum

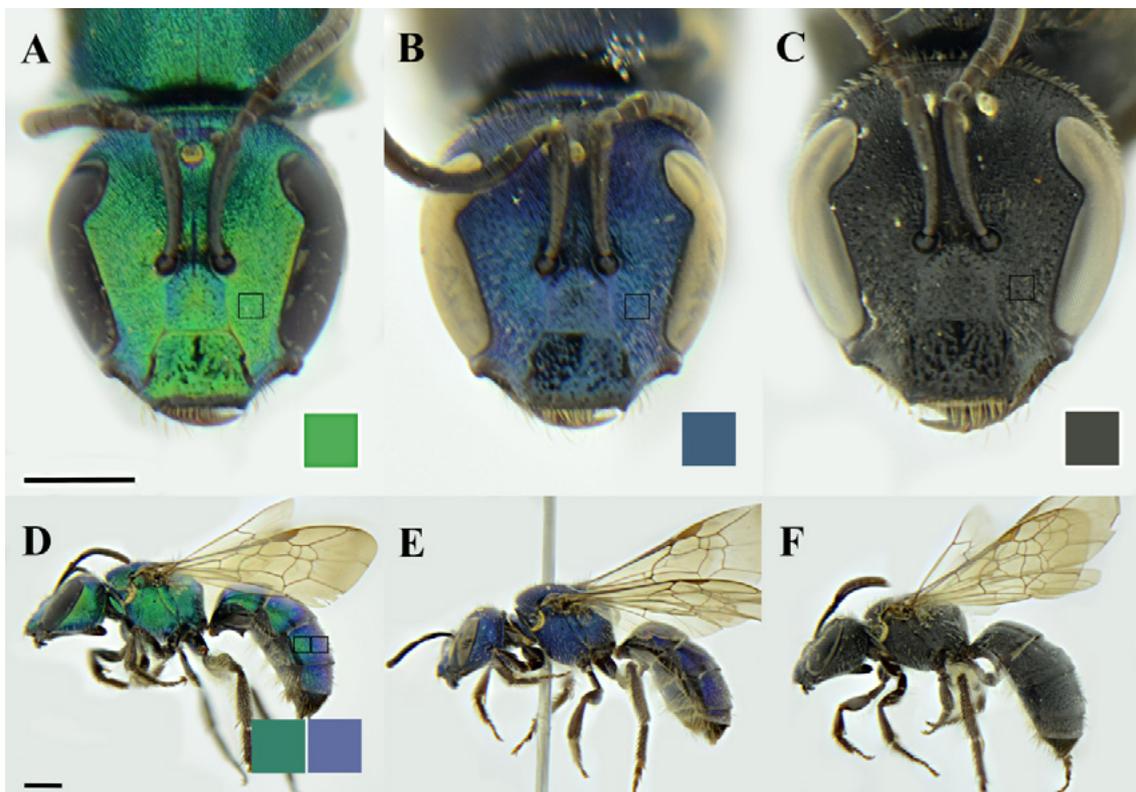


Fig 1. Color variation of *Augochlora amphitrite*. A. B. C. D. E. F. (bars correspond to 1mm).

with longitudinal, weakly sinuose, carinae; tergal discs mostly punctate, with impunctate marginal zones; S1 slightly tuberculate; and genitalia wider than longer.

Color variation

We found striking color variation on *A. amphitrite* (Fig 1), with most examined material ($n = 530$; 69.86 %) being predominantly metallic green (RGB 79:174:87, Fig 1A). The green morph is found along the entire species distribution (Fig 2). Some predominantly green specimens also exhibit a bluish iridescence (RGB 95:109:161, Fig 1D), most frequently observed on their head and metasoma, these specimens can be found elsewhere. Entirely dark blue (RGB 63:95:125, Fig 1B, E) specimens ($n = 119$; 16.53%) are known from north of Argentina and western Paraná, Brazil. The third morph, entirely black ($n = 98$; 13.61%) was found from the same collection series (RGB 71:74:66, Fig 1C, F) and was also sampled in other localities, predominantly in Paraná State (Fig 2).

The close similarity in structure, microsculpturing, pubescence and male genitalia supports the hypothesis that the color morphs belong to a single species. The size and shape variation described below was found in all three-color morphs. Finally, the sympatric distribution of these morphs reinforces this hypothesis, and we therefore relied on it to propose the taxonomic synonymies presented above.

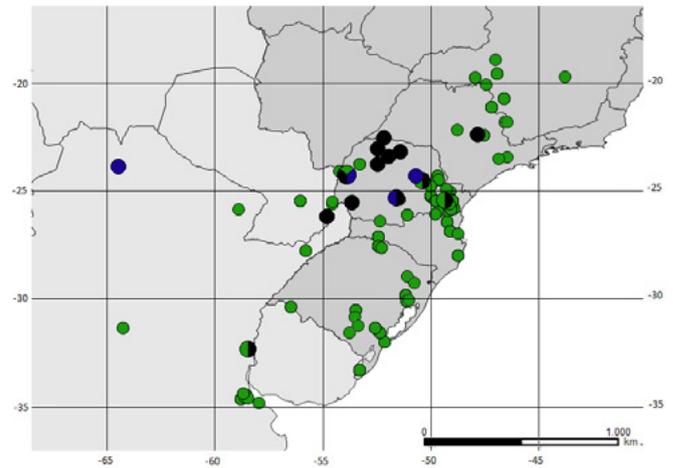


Fig 2. Distribution records for *Augochlora amphitrite*. Color morphs are indicated by circle colors.

Schrottky (1909) described *O. styx* and *O. lethe*, black female and male, respectively forms from Puerto Bertoni (Paraguay). Their type locality is very close to known occurrences of the black color morph of *A. amphitrite* and the original descriptions agree closely with *A. amphitrite*. Indeed, only color was used to separate *O. styx* from other *Augochlora* and *O. lethe* from *A. amphitrite* in Schrottky's (1910) key. Unfortunately, the whereabouts of the involved

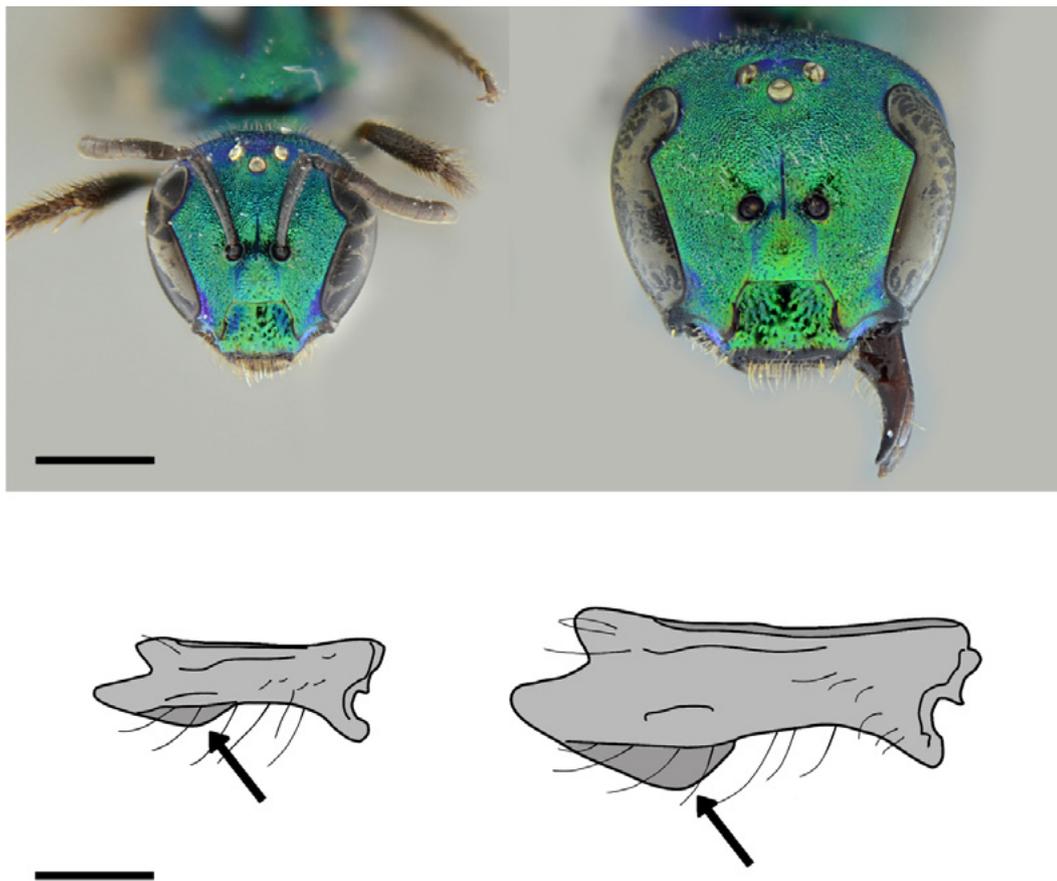


Fig 3. Cephalic polymorphism in *Augochlora amphitrite*. A) Small female, B) Macrocephalic female, C) Left mandible of a small female, D) Left mandible of a macrocephalic female. Arrows indicate the mandibular adductor ridge. A and B, and C and D, respectively, at same scale (bars correspond to 1mm and 0.5mm respectively).

type specimens is unknown, but in the course of an ongoing genus revision we examined several species from Argentina, Paraguay and Brazil and no entirely black morph deserving species status was found.

Morphometric variation

Females of *A. amphitrite* exhibit different body sizes and head shapes (Fig 3). However, according to PCA the variation is continuous and enlarged head females did not form a separated group (Fig 4). Those females are mainly distributed on the left upper corner of the PCA graph. There is no association of morphometric variation with body color and distribution. As expected, the variables vectors show the

same direction with PC1 axis showing a positive relation with to body size, however some structures are also variable on shape. The vector of gena length was strongly related to PC1 (Fig 5A). The mandible width and vertex length were more associate with PC2 axis than other variables. However, they showed different trends, with mandible expansion of the adductor ridge associated to macrocephalic females, while the vertex increased with body size but not necessarily with any other morphological measurement females. Mandible attributes are related to body size but the macrocephalic females are more dispersed from the regression line (Fig 5B, upper right). According to these results the distal portion of the mandibular adductor ridge (Fig 3 C-D) came out as an additional important feature of the macrocephalic females.

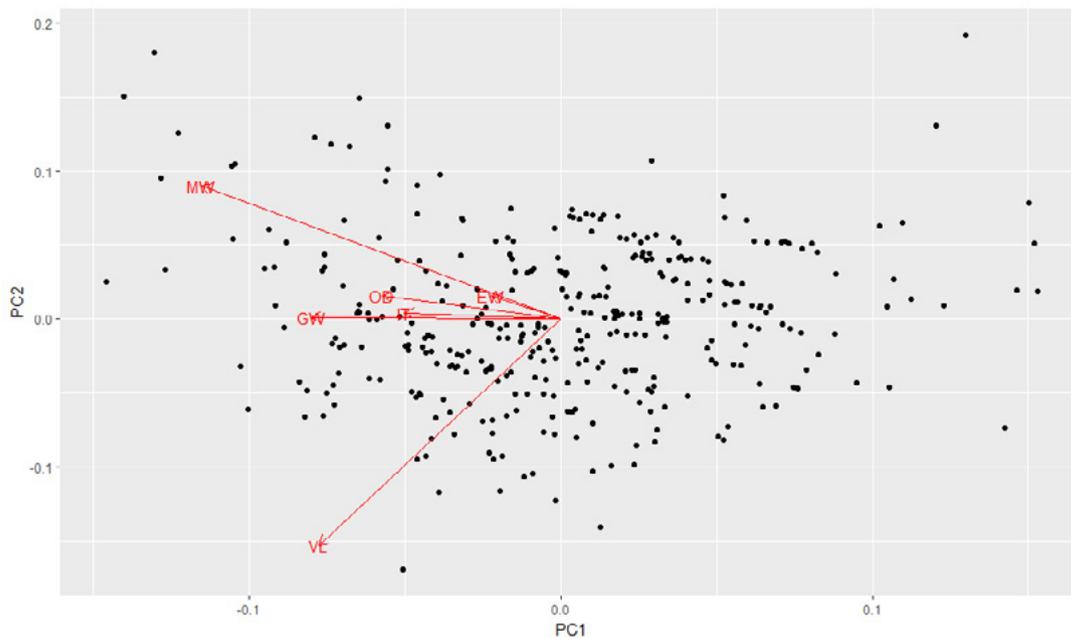


Fig 4. Principal component analysis of *Augochlora amphitrite*. Vectors: intertegular distance (IT); ocular distance (OD); vertex length (VL); eye width (EW); gena width (GW); and mandible width (MW).

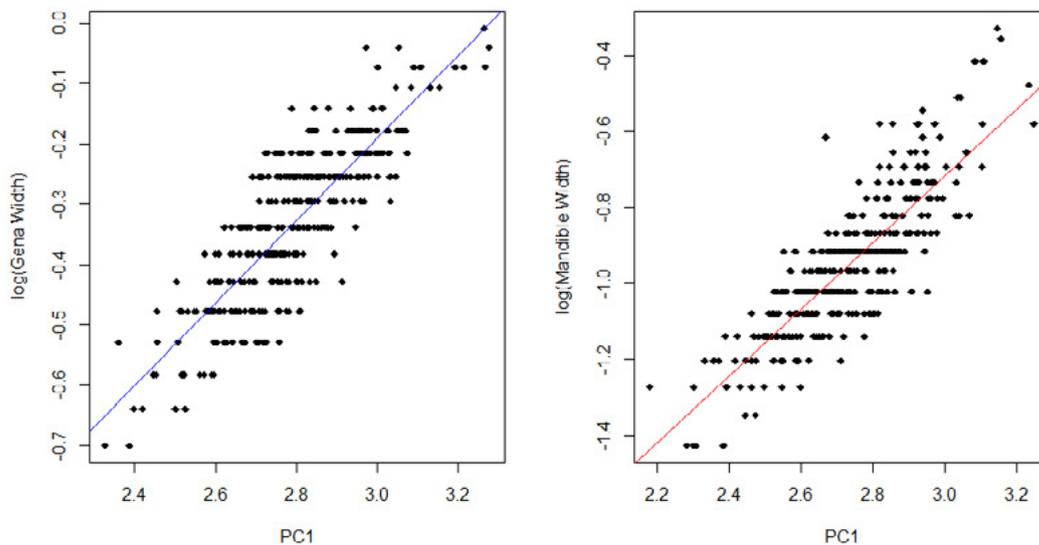


Fig 5. Plot of PC1 gena and mandible width of *Augochlora amphitrite*. Regression line indicated in color.

Discussion

Color variation

Although most *A. amphitrite* specimens have a predominantly metallic green integument, the blue and black morphs are not rare, corresponding together to 30% of the sample. It is noteworthy that one third of the analyzed specimens were taken from Palotina, where black and dark blue specimens are remarkably common. Dalmazzo and Roig-Alsina (2011) already mentioned entirely blue specimens from central Argentina. Additionally, we found the dark blue morph in three series from the Paraná state, Brazil (Guarapuava, Palotina and Telêmaco Borba municipalities) and a single specimen from northern Argentina (Jujuy Department). Black specimens were found from several localities in Brazil and also from Argentina. The black color morph should occur in Paraguay and it is plausible that the two names proposed by Schrottky (1909) correspond to black morphs of *A. amphitrite*. The three-color morphs are not distinguished by any diagnostic feature, the male genital capsules are similar and the morphometric analyses do not separate the color morphs. Lastly, a preservation artifact is discarded because the three morphs were sampled from a single locality (Palotina) and preserved under the same conditions by the senior author.

Color difference was documented for several bee groups as intraspecific variation. As an example, Carolan et al. (2012) studied three species of *Bombus* Latreille delimited with molecular data, but they found that coloration of the body setae was not useful to recognize the involved species, with a given color pattern being the same for different species. As another example, Ferrari and Melo (2014) studied some species of *Euglossa* Latreille with different color morphs and allopatric distributions. The authors had investigated the relationships of the specimens with molecular data and found that blue and green morphs corresponded to the same species (see also Penha et al., 2015; Frantini-Silva et al., 2017).

Intraspecific color variation has already been documented for halictines, the most common variation involving the metasomal color of Halictini Thomson. The color morphs of *Halictus lutescens* Friese and *Lasioglossum apristum* Vachal are related to variation in body size (Sakagami & Okazawa, 1985; Miyanaga et al., 1999). On the other hand, color morphs of *Lasioglossum politum* Schenck are linked with geographical distribution (Murao & Tadauchi, 2011). However, in Augochlorini color variation is not necessarily related to distribution or behavior. Previous cases have been reported by Gonçalves (2017) for *Paroxystoglossa mourella* Gonçalves and by Smith-Pardo (2010) for *Neocorynura pseudobaccha* Cockerell and in both of them no relation with distribution and behavior was found. The blackish bees are unusual within Halictinae, the previously known cases of black specimens on Augochlorini coming from *Ariphanartha palpalis* Moure, *Megalopta*, *Neocorynura* Schrottky and *Pseudaugochlora* Michener.

Dark brown and black species can be found in most Augochlorini lineages but no phylogenetic optimization was carried out to map the evolution of color within this bee clade. The black morph of *A. amphitrite* probably represents a derived condition. Most of the examined species of *Augochlora* are metallic green or blue, with few exceptions as the black and cupreous *Augochlora perimelas* Cockerell. The other species of the *Augochlora* clade are metallic green, with some darkened specimens of *Augochlorella* Sandhouse (Coelho, 2004).

The visible green and blue colors result from light interaction with the integument microstructure (Berthier, 2007; Seago et al., 2009). On the other hand, the black color results from a melanization process, being an example, the complex regulation of melanin given by Wittkopp and Beldade (2009). The final insect color depends on a complex interaction of pigments and structural organization (Ghiradella, 2009). The biochemical and genetic determination of the integument color can be even more complex and are an open field of investigation for bees.

Morphometric variation

A. amphitrite exhibits a remarkable variation in body size and head shape (Fig 3). The variation is continuous and does not correspond to true dimorphism among females, with a distinct macrocephalic morph as found in other groups such as *Rhinocorynura* (Gonçalves & Melo, 2012). Nevertheless, we use herein the term macrocephalic for the large females with enlarged gena and developed distal part of the mandibular adductor ridge. Polymorphism in social species is usually associated with latitudinal and altitudinal gradients and also with seasonality (Davison & Field, 2016) but we did not find any pattern for *A. amphitrite*.

Division of labor involving females of *A. amphitrite* from the same nest was documented by Dalmazzo and Roig-Alsina (2012). The authors found that macrocephalic females stay inside the multi-female nests and probably act as dominant. This link of behavior, function and form has been widely documented for Halictinae (Richards & Packer, 1996; Soucy, 2002; Richards et al., 2003) but no previous case involving *Augochlora* sensu stricto was published. Dalmazzo and Roig-Alsina (2015) found that in *Augochlora phoemonoe* Schrottky there is no apparent structural differentiation between dominant and subordinate individuals, but body size and physiology differ between them. In *Augochlora istmii* Schwarz there is only size variation among nest mates (Weislo et al., 2003). More studies can reveal different degrees of morphological and behavior variation for species of *Augochlora*.

The swollen gena was already discussed as an important feature to identify macrocephalic forms in eusocial species of Augochlorini (Sakagami & Moure 1965; Santos & Silveira, 2009). There is no study on head musculature for the tribe, but in ants the major forms with enlarged heads have developed muscles, including those for moving the mandibles

(Lillico-Ouachour et al., 2018). The mandible is also modified in *A. amphitrite* (Fig 3 C-D) with enlargement of the distal part of the adductor ridge in macrocephalic females. In addition to its role in nest construction, the mandible is also used in intranidal disputes, with important consequences on the dominance exerted by foundresses (Pabalan et al., 2000; Packer et al., 2003). Females with enlarged heads in *A. amphitrite* probably have stronger musculature for mandible control, a hypothesis to be tested in further studies.

Other lineages of Augochlorini have also intraspecific variation in head morphology. In *Megalopta*, another group with wood nesting facultative eusocial species, the gena show different degrees of swelling and a genal projection of variable length is present (Santos & Silveira, 2009). In this bee tribe, the most remarkable cases of cephalic polymorphism come from the *Rhinocorynura* group (Gonçalves & Melo, 2012). Significant variation in size among females is observed within all species of *Rhinocorynura* and in some species of its sister group (Gonçalves, 2010). In the clade formed by *Rhinocorynura inflaticeps* Ducke + *Rhinocorynura vernoniae* Schrottky, the variation is also mostly continuous, but the females differ qualitatively: the larger ones have distinct lateral prongs in their clypeus. There is clearly a continuous variation among the larger females in the size of the lateral clypeal projection, but in the small females, the lateral projections are completely lacking.

Concluding remarks

We presented a revised diagnosis for *A. amphitrite* to broader comparison with additional 21 species not studied by Dalmazzo and Roig-Alsina (2011). The species revealed remarkable cases of color and shape polymorphisms. The color variation includes discrete morphs with unknown causes. The morphometric variation is probably related to nest interaction based on previous observations and do not follow any clear geographical pattern. Based on the evidences from this study and from Dalmazzo and Roig-Alsina (2012) *A. amphitrite* is considered facultative primitively eusocial and is a candidate to be a model for studies to link form, function and behavior within bees.

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Authors' Contribution

RB GONÇALVES conceived the study. A LEPECO performed the measurements, statistical analyses and specimen imaging. A. LEPECO and RB GONÇALVES contributed equally to the writing of the manuscript.

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