



Antennal sensilla of two species of *Gymnetis* MacLeay, 1819 (Coleoptera: Scarabaeidae: Cetoniinae)

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ABSTRACT

The present study describes the morphology of the antennal sensilla of adults of *Gymnetis holosericea* (Voet, 1779) and *Gymnetis rufilateris* (Illiger, 1800). The adults of *Gymnetis* spp. were sexed and antenna of males and females were dissected. Both species have sensilla chaetica, trichodea, placodea (type I and II), coeloconica (type I and II), and ampullacea (or pore). Females of *G. holosericea* have a total of about 19,995 sensilla and males have about 23,273 sensilla, and females of *G. rufilateris* have about 16,633 sensilla and males have about 21,184 sensilla. Sensilla placodea are the predominant type of sensilla in males and females of *G. holosericea* and *G. rufilateris*.

Introduction

The flower chafer genus *Gymnetis* MacLeay, 1819 (Coleoptera, Scarabaeidae, Cetoniinae, Gymnetini) comprises 57 species distributed from USA to Argentina, of which 26 occurs in Brazil. The adults are usually found in plants during day light, where they feed on flowers, mature or rotting fruits, pollen grains, and sap flow (Ratcliffe, 2018; Ratcliffe and Nogueira, 2020). Some species have been associated with honeybee hives (*Apis mellifera*, Hymenoptera) and possibly with ant nets (Formicidae, Hymenoptera), but the association with Hymenoptera is possibly accidental (Tejada and Morón, 2015; Ratcliffe, 2018; Ratcliffe and Nogueira, 2020). The larvae of the genus feed on rich organic matter soil, roots, and some species were noted inside composter or associated with ant nests (Orozco and Pardo-Locarno, 2004; Rodrigues et al., 2016; Sánchez-Soto et al., 2017; Ratcliffe, 2018). The immatures of seven species have been described (Ibarra-Polesel et al., 2022).

Some reports were done about the diversity of the genus in Brazil. Rodrigues et al. (2013) listed *Gymnetis flava* (Weber, 1801) (named as *Gymnetis holosericea flava* (Weber, 1801), *Gymnetis hebraica* (Drapiez, 1820), *Gymnetis pantherina* (Burmeister, 1842)

(named as *Gymnetis pantherina meleagris* (Burmeister, 1842)), *Gymnetis rufilateris* (Illiger, 1800), *Gymnetis vandepolli* Bates, 1889 (named as *Gymnetis bajula vandepolli* Olivier, 1789) from Mato Grosso do Sul State. From Minas Gerais State, Gonçalves and Louzada (2005) registered *Gymnetis cupriventris* Janson, 1880 and *Gymnetis pantherina*, while Puker et al. (2014) noted *G. pantherina* and *G. undata* (Olivier, 1789). The occurrence of *G. undata* and *G. vandepolli* in Brazil is doubtful since the *Gymnetis* review (Ratcliffe, 2018) registered the former to Caribbean and Guyana regions, and the last to Colombia and Central America. Evangelista Neto et al. (2018) collected *G. hebraica*, *G. rufilateris*, *G. pantherina* (named as *G. rubrocincta* (Schürhoff, 1937)), *G. flavomarginata* Blanchard, 1837 in Brasília, but the last species registered must be checked because *G. flavomarginata* possibly does not occur in Brazil (Ratcliffe, 2018).

Adult scarabs usually detect other scarabs, food sources, or nesting places by the detection of pheromones (Leal and Mochizuki, 1993; Ochieng et al., 2002; Zarbin et al., 2007; Robbins et al., 2008, 2009), plant volatiles, and ambient odors (Renou et al., 1998; Hansson et al., 1999; Larsson et al., 2001). Scarabs use structures named sensilla to these chemical detections and those structures are mainly present in the beetle antennal clava or club (Meinecke, 1975; Bohacz et al., 2020). Sensilla are also abundant

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in the apex of maxillary and labial palpi, and palp sensilla are probably gustative and detect contact chemicals (Fombong et al., 2012).

Some studies show that the phytophagous scarab beetles, sometimes named as Pleurosticti (including Cetoniinae, Dynastinae, Melolonthinae, Rutelinae, and others minor subfamilies), have some types of sensilla in their antennal lamellae (those lamellae together form the antennal clava or club), as sensilla placodea, basiconica, coeloconica, ampullacea (pores), chaetica, and trichodea (Romero-López et al., 2004; Shao et al., 2019; Saldanha et al., 2020). The sensilla subtypes and its amount is variable regarding different species and the sensilla distribution often show variation between males and females (Tanaka et al., 2006; Romero-López et al., 2010; Costa et al., 2021).

The antennal sensilla of the genus *Gymnetis* is still undescribed until now and the present work aim to provide the first sensilla characterization of the genus based in the study of two species: *G. holosericea* and *G. rufilateris*. The morphological description provided here adds new characteristic to differentiate species and new information about to Scarabaeidae antennal structure.

Material and methods

The field activities were conducted at Fazenda Escola de São Luís (02° 35' 04.0" S, 44° 12' 33.3" W), Universidade Estadual do Maranhão, São Luís municipality, Maranhão State (MA), Brazil. This experimental area includes crops of *Euterpe oleracea* Mart. (Arecaceae, "açazeiro"), *Theobroma grandiflorum* (Willd. Ex Spreng.) K. Schum. (Malvaceae, "cupuaçuzeiro"), and *Musa* sp. (Musaceae, "bananeira"). Adults were collected using 12 traps baited with sugarcane juice as attractant. The trap was made with two liter PET bottle with lateral openings (method modified from Rodrigues et al., 2013). Traps were installed in the field from May 2021 to January 2022, and were inspected each two days. The attractant was replaced two times per week. Collected insects were preserved in 70% ethanol. The laboratory activities were conducted at Universidade Estadual do Mato Grosso do Sul, Cassilândia municipality, Mato Grosso do Sul State (MS).

Gymnetis species were identified according Ratcliffe (2018), and *G. holosericea* was also identified by Rafael Sousa at Museu de Zoologia da Universidade de São Paulo (MZSP), São Paulo municipality, São Paulo State (SP).

Antennal sensilla were studied by scanning of antennal clubs taken using an electron microscope in the Departamento de Física e Química, Universidade Estadual Paulista (UNESP), Campus of Ilha Solteira, SP (a method by Tanaka et al., 2006). Antennae of six males and six females of *G. holosericea* and four males and six females of *G. rufilateris* were dissected and maintained in 70% ethanol. Afterwards, appendages were successively washed in 80% and 90% ethanol for 15 minutes each, and then 100% ethanol for 20 minutes. The pieces were dried in a CO₂ critical point dryer (model Leica® CPD300). Subsequently, they were coated with gold using a Quorum® Q150TE turbo molecular pump. Images were obtained using a Zeiss® EVO LS15 scanning electron microscope (SEM). The images obtained in SEM were subjected to image enhancement filters available in the software Image-Pro Plus 6.0. The sensilla were quantified in the images obtained by SEM of the coverslips of six males and six females. The Student's t-test was used to compare the distribution of sensilla.

Sensilla terminology follows Keil (1999). The term sensilla trichodea is here used as a very comprehensive term, to quite variable hair-like sensilla (Snodgrass, 1935; Keil, 1999). And the term sensilla chaetica is here used as a restricted term to a hair-like sensilla that are distinguished from other hair-like sensilla by their distribution or form (usually as a stout or spine-like setae Snodgrass, 1935; Keil, 1999).

Results

The general structure of the antenna of *G. holosericea* and *G. rufilateris* is typical to Scarabaeoidea and comprises (basis to apex): a scape, a pedicel, funicle with 5 antennomeres, and the distal clava or club with three lamellate antennomeres (Fig. 1). The clava of both species have sensilla trichodea, chaetica, placodea, coeloconica, and ampullacea (pores). Sensilla trichodea are hair-like long setae distributed mainly on edges of lamellae (Fig. 4F). Sensilla chaetica are also hair-like, but are shorter than sensilla trichodea, and are mainly found grouped in the outer surface of the proximal lamella (Fig. 4F).

Sensilla placodea are distributed in inner side (the distal surface) of proximal lamella, inner and outer sides of medial lamella, inner side of distal lamella (the proximal surface), and in the posterior area of outer side of distal lamella (Figs. 4–7). Two types of sensilla placodea are found, type I and II (Figs. 2 and 3). The sensilla placodea type I are rounded plates surrounded by a peripheral ditch or furrow and

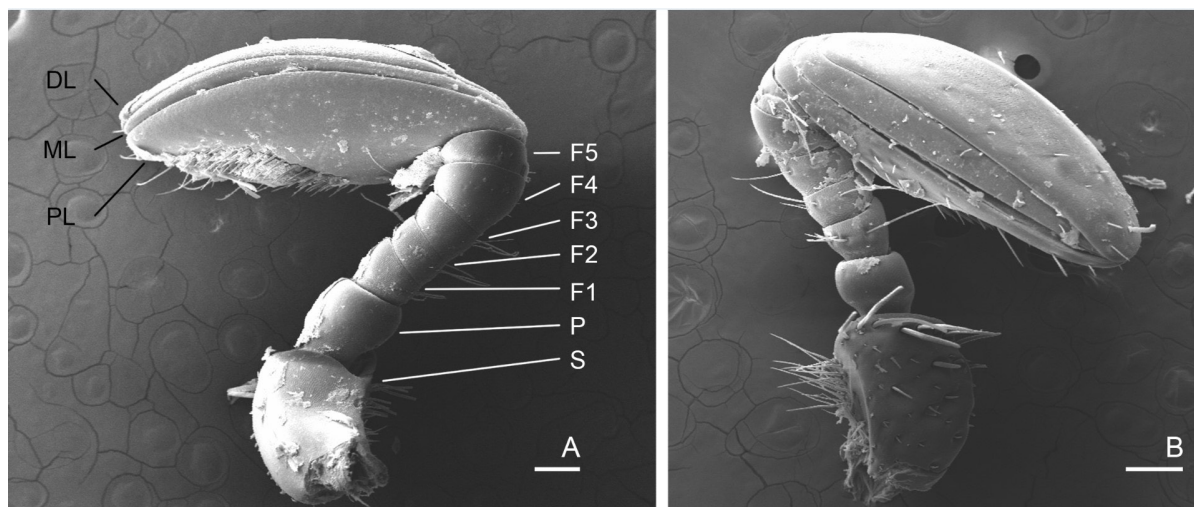


Figure 1 Female antenna of *Gymnetis holosericea* (A, inner view) and of *Gymnetis rufilateris* (B, outer view). Scape (S), pedicel (P), antennomeres of funicle (F1–F5), proximal lamella (PL), medial lamella (ML), distal lamella (DL). Scale = 200 µm.

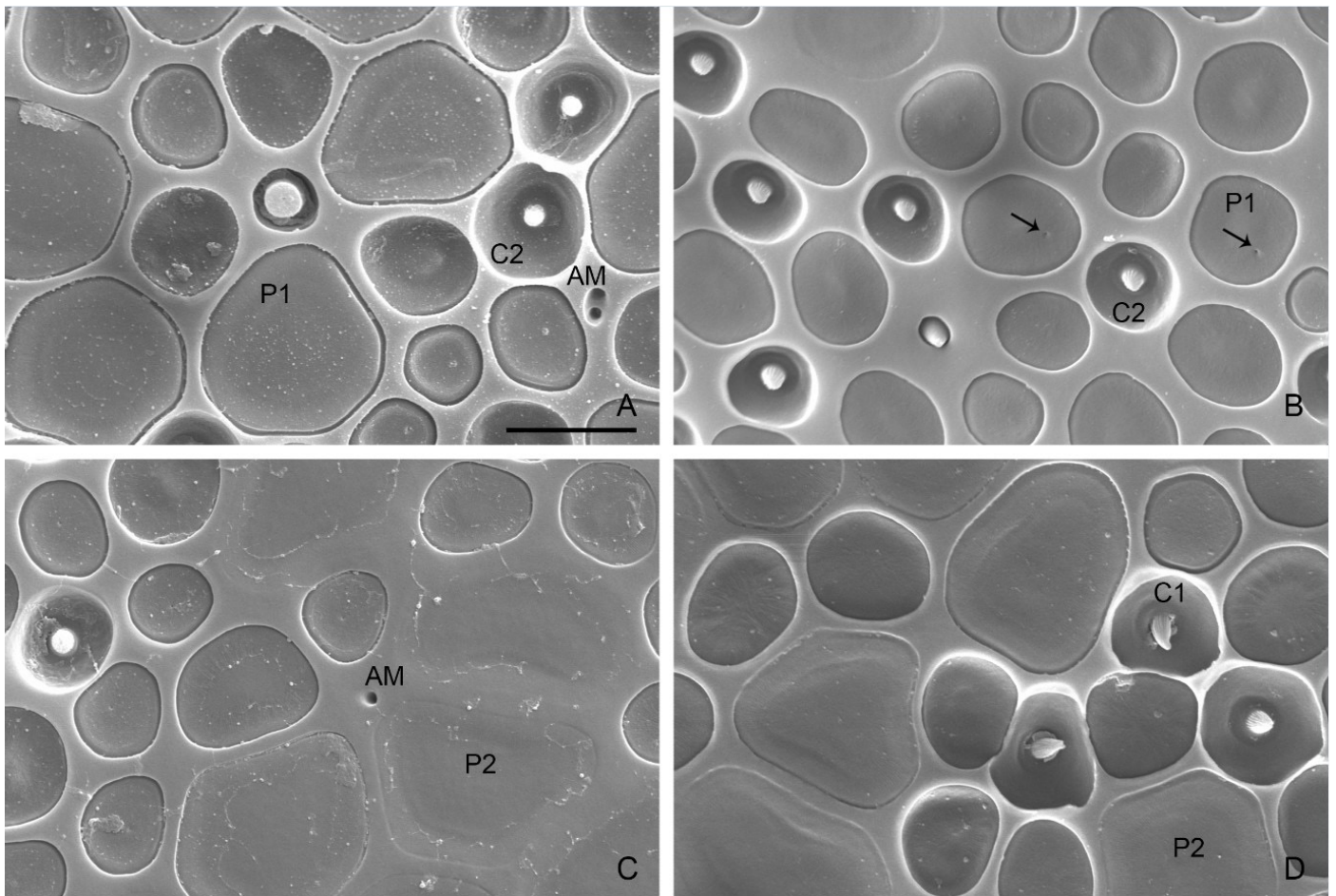


Figure 2 *Gymnetis holosericea*, antennal sensilla, inner side of proximal lamella of male (A), inner side of distal lamella of female (B), inner side of medial lamella of female, black arrow pointing to punctures present in some sensilla placodea type I (C), outer side of medial lamella of female (D). Sensilla ampullacea (AM), sensilla coeloconica type I (C1), sensilla coeloconica type II (C2), sensilla placodea type I (P1), sensilla placodea type II (P2). Scale = 10 μ m.

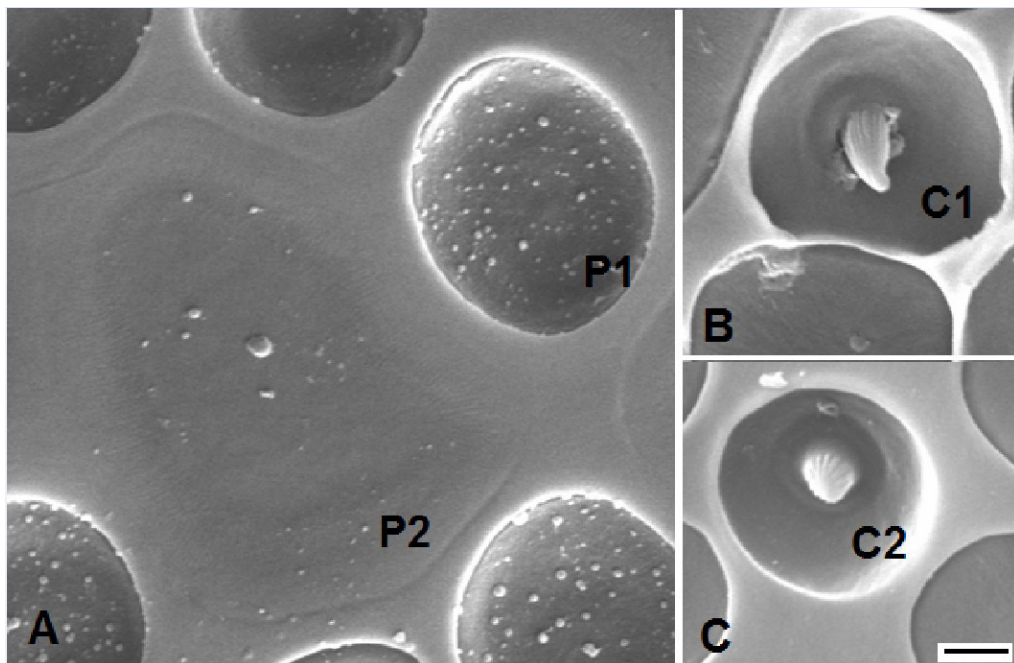


Figure 3 *Gymnetis holosericea*, antennal sensilla. A) Sensilla placodea type I (P1), sensilla placodea type II (P2). B) sensilla coeloconica type I (C1). C) sensilla coeloconica type II (C2). Scale = 2 μ m.

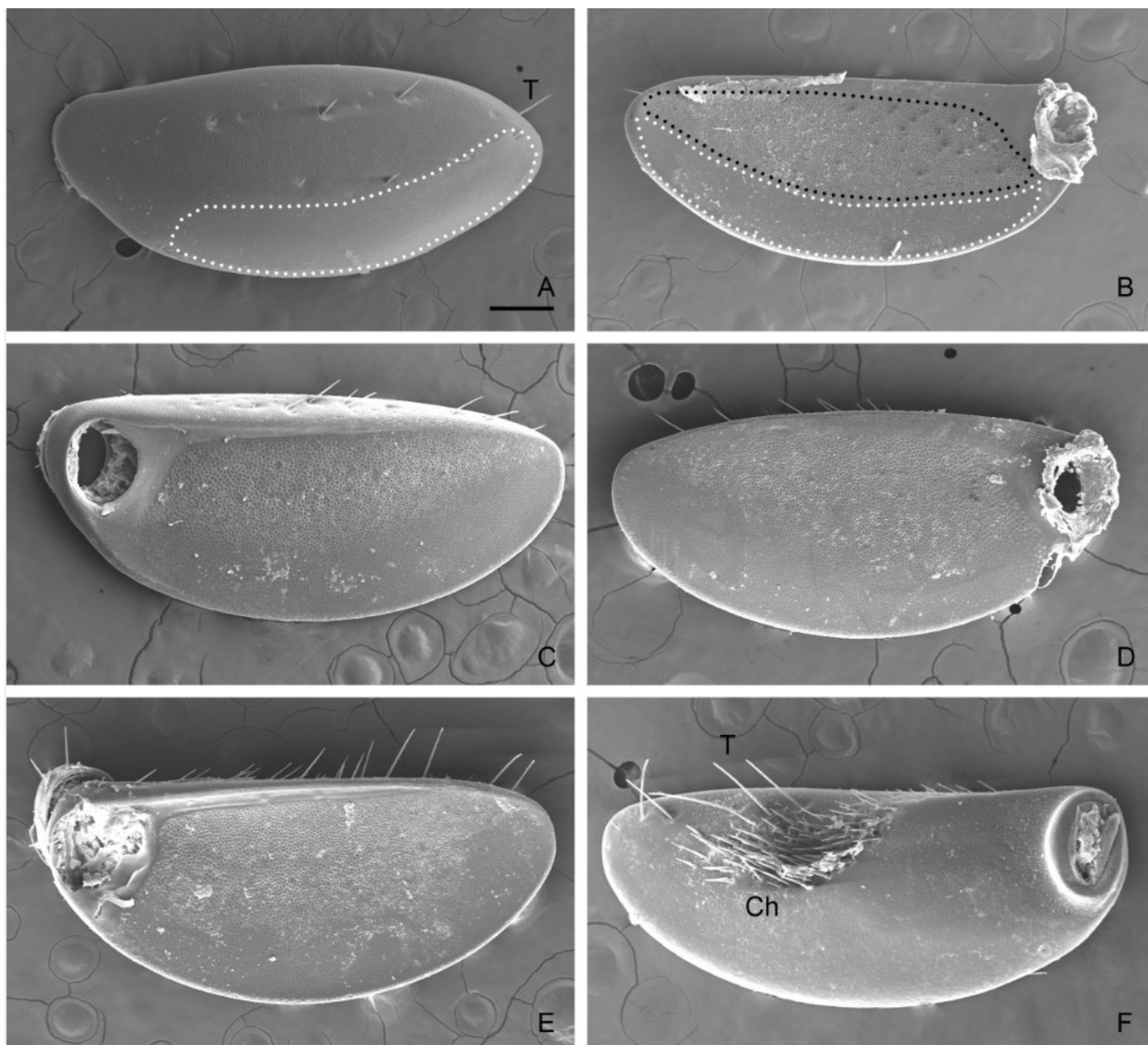


Figure 4 *Gymnetis holosericea*, female, antenna. Distal lamella, outer and inner sides (A, B), medial lamella, outer and inner sides (C, D), proximal lamella, outer and inner sides (E, F). White dotted line showing the posterior homogeneous area, black dotted line showing the anterior heterogeneous area. Sensilla chaetica (Ch), sensilla trichodea (T). Scale = 200 μ m.

have a diameter of $7.1 \pm 2.1 \mu\text{m}$ in *G. holosericea* and $5.8 \pm 2.1 \mu\text{m}$ in *G. rufilateris*. The sensilla placodea type II are rounded plates without peripheral furrow and have a diameter of $8.1 \pm 2.1 \mu\text{m}$ in *G. holosericea* and $10.7 \pm 2.1 \mu\text{m}$ in *G. rufilateris*. Few observed sensilla placodea type I show some minor punctures (Figs. 2B and 3).

The sensilla placodea type I are mainly arranged at medial and anterior area of inner side of proximal lamellae, inner and outer sides of medial lamella, inner side of distal lamella (Figs. 4B, 5B, 6B and 7B). Sensilla placodea type II are mainly arranged at posterior area of: inner side of proximal lamellae and inner and outer sides of medial and distal lamellae (Figs. 4A, 5A, 6A and 7A).

Sensilla coeloconica are small projections inside a pit, and are divided into type I, with sharp apex, and type II, with blunt apex (Figs. 2 and 3). These sensilla are distributed in anterior and medial areas of inner side

of proximal lamella, inner and outer sides of medial lamella, inner side of distal lamella. Both types can present some grooves in their surface or the grooves are indistinct and the surface seems smooth.

Sensilla ampullacea are visible as pores and they are scarcely distributed in all sides of lamellae (Figs 2A and C).

Gymnetis holosericea

Females ($n = 6$, Fig. 4) have 19,995 sensilla, of which 19,411 (97.08%) are sensilla placodea and 584 (2.92%) are sensilla coeloconica (Table 1). Males ($n = 6$, Fig. 5) have 23,273 sensilla, of which 22,217 (95.46%) are sensilla placodea and 1,056 (4.54%) are sensilla coeloconica (Table 1). The amounts of placodea and coeloconica sensilla between females and

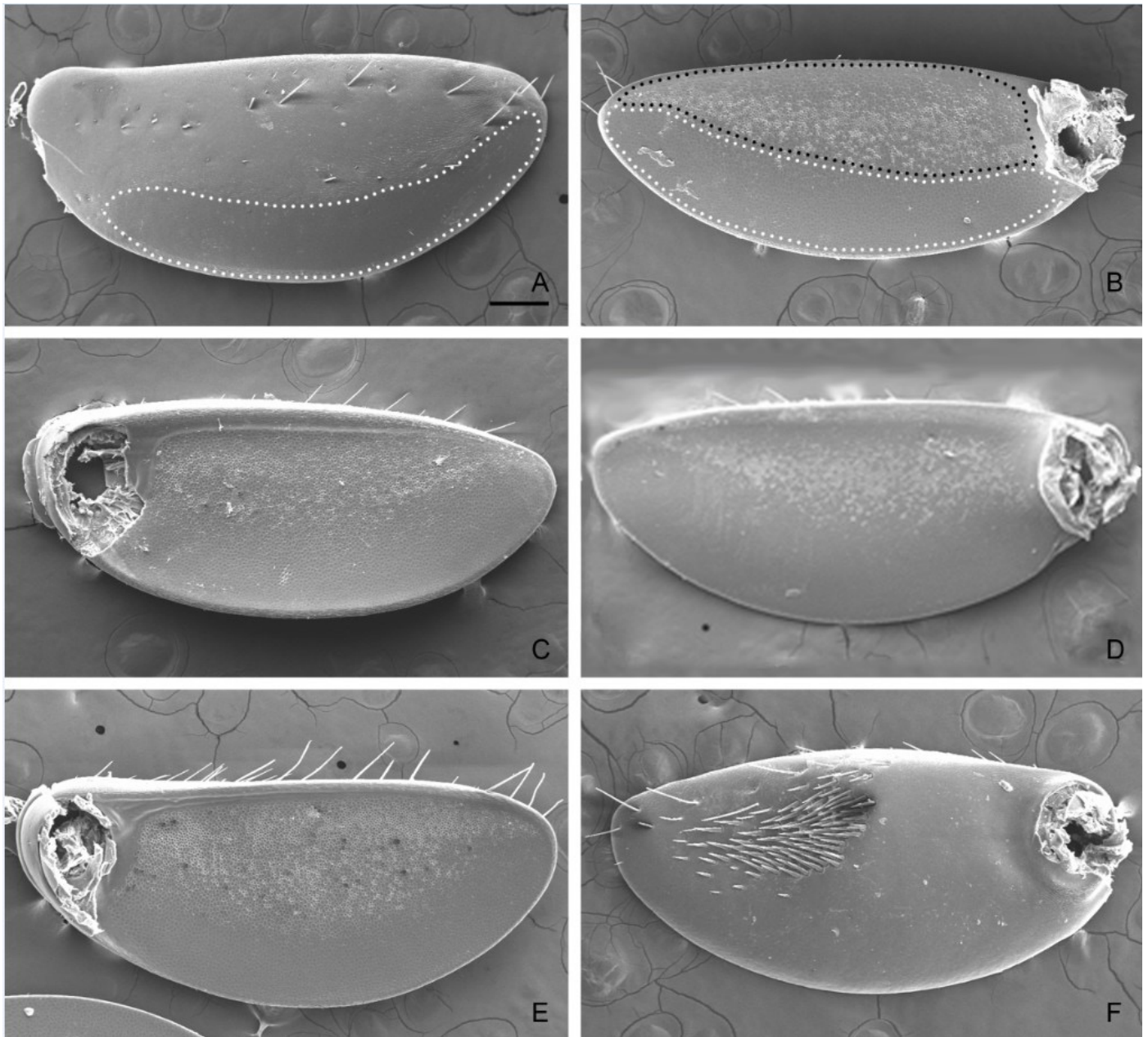


Figure 5 *Gymnetis holosericea*, male antenna. Distal lamella, outer and inner sides (A, B), medial lamella, outer and inner sides (C, D), proximal lamella, outer and inner sides (E, F). White dotted line showing the posterior homogeneous area, black dotted line showing the anterior heterogeneous area. Scale = 200 μ m.

Table 1

Number of sensilla placodea and sensilla coeloconica of *Gymnetis holosericea*.

sensilla	distal lamella		medial lamella		proximal lamella		Total (standard deviation)
	outer	inner	outer	inner	outer	inner	
female							
placodea	2477	3626	4201	4892	-	4215	19411±150.77
coeloconica	-	178	126	87	-	193	584 ±31.31
total	2477	3804	4327	4979	-	4408	19995±165.18
male							
placodea	2941	4323	5001	4030	-	5922	22217±173.97
coeloconica	-	378	206	125	-	347	1056±97.96
total	2941	4701	5207	4155	-	6269	23273 ±236.05

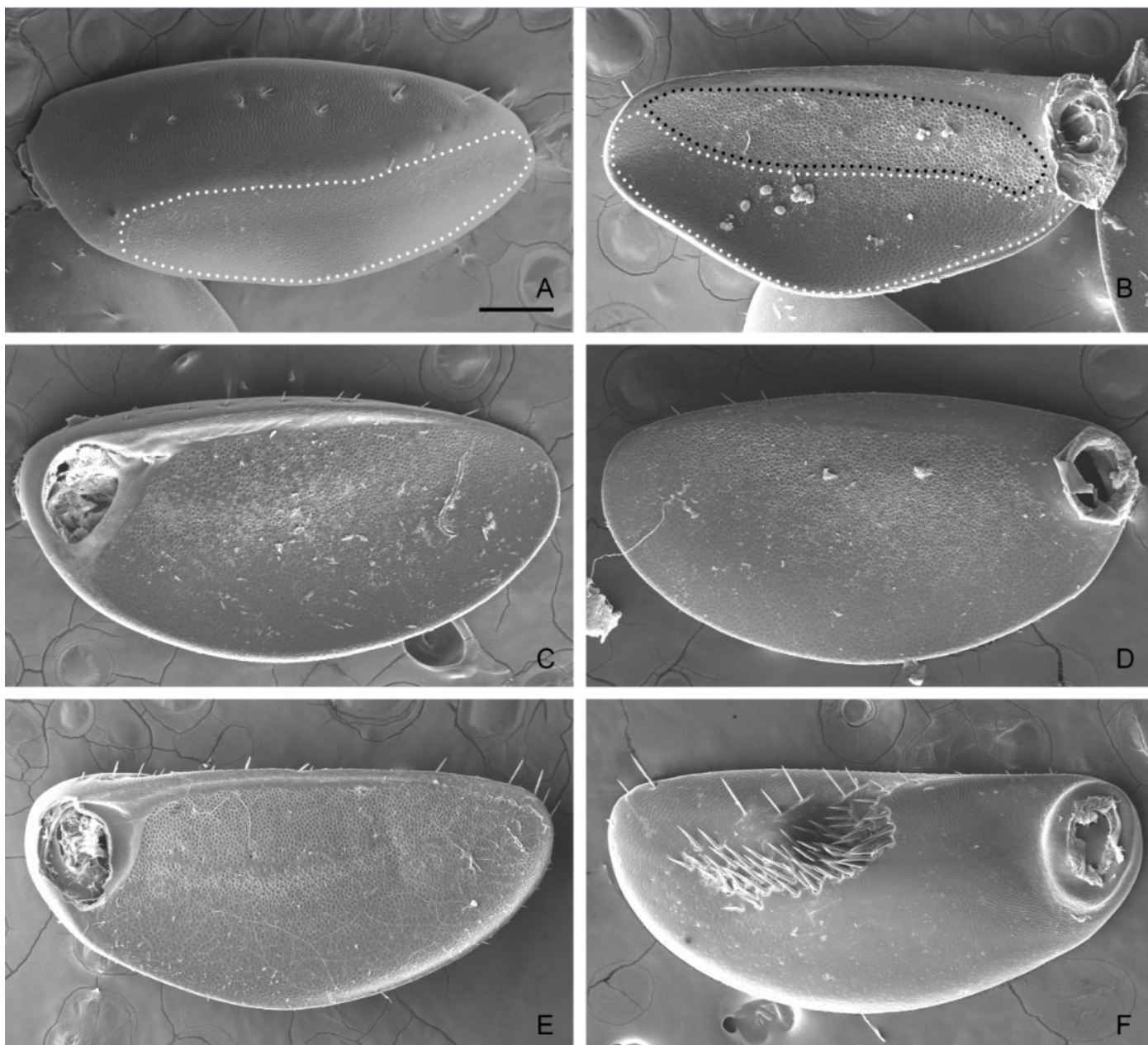


Figure 6 *Gymnetis rufilateris*, female antenna. Distal lamella, outer and inner sides (A, B), medial lamella, outer and inner sides (C, D), proximal lamella, outer and inner sides (E, F). White dotted line showing the posterior homogeneous area, black dotted line showing the anterior heterogeneous area. Scale = 200 μ m.

males of *G. holosericea* differ significantly from each other ($t=29.85$; $df=10$; $P<0.001$) and ($t=11.24$; $df=10$; $P<0.001$), respectively. For the total number of sensilla between females and males, significant differences were observed ($t=27.86$; $df=10$; $P<0.001$).

Gymnetis rufilateris

Females ($n = 6$, Fig. 6) have 16,633 sensilla, of which 16,026 (96.35%) are sensilla placodea and 607 (3.65%) are sensilla coeloconica (Table 2). Males ($n = 4$, Fig. 7) have 21,184 sensilla, of which 20,586 (97.18%) are sensilla placodea and 598 (2.82%) are sensilla coeloconica (Table 2). The amounts of placodea sensilla between females and males of *G. rufilateris* differ significantly from each other ($t=62.05$; $df=10$;

$P<0.001$), while the amounts of coeloconica sensilla do not differ from each other ($t=0.9$; $df=10$; $P<0.001$). For the total amount of sensilla, significant differences were observed between females and males ($t=61.22$; $df=10$; $P<0.001$).

When comparing the amounts of sensilla between the two species of *Gymnetis*, it was verified that the amounts of placodea sensilla between females of *G. holosericea* and *G. rufilateris* differ significantly from each other ($t=44.95$; $df=10$; $P<0.001$), among males, significant differences were also observed for the amount of placodea sensilla ($t=17.62$; $df=10$; $P<0.001$). The amounts of coeloconica sensilla between *G. holosericea* and *G. rufilateris* females do not differ from each other ($t=1.63$; $df=10$; $P<0.14$), however, they differ significantly between males ($t=11.22$; $df=10$; $P<0.001$). For the total amount of sensilla among females of

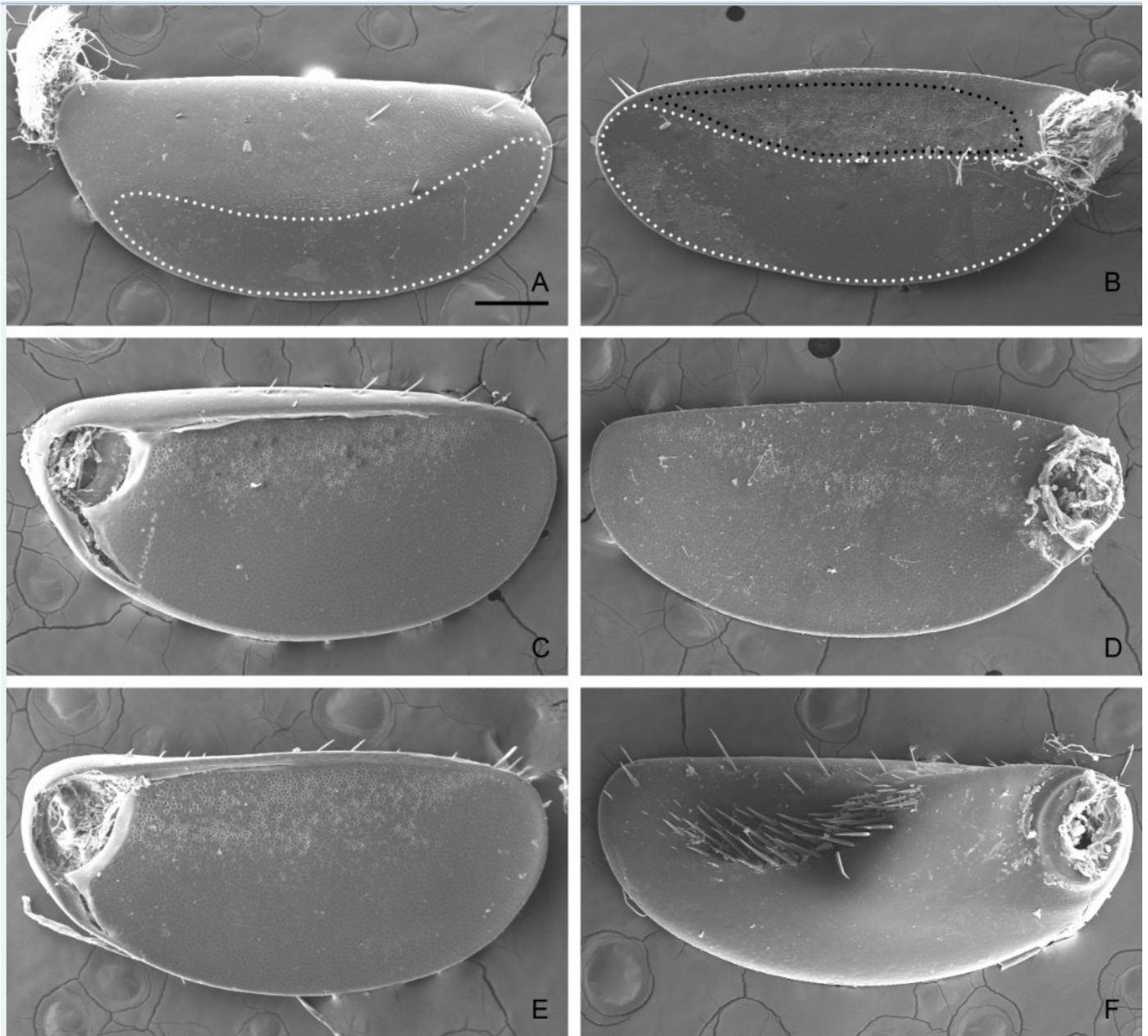


Figure 7 *Gymnetis rufilateris*, male antenna. Distal lamella, outer and inner sides (A, B), medial lamella, outer and inner sides (C, D), proximal lamella, outer and inner sides (E, F). White dotted line showing the posterior homogeneous area, black dotted line showing the anterior heterogeneous area. Scale = 200 μ m.

Table 2

Number of sensilla placodea and sensilla coeloconica of *Gymnetis rufilateris*.

sensilla	distal lamella		medial lamella		proximal lamella		Total (standard deviation)
	outer	inner	outer	inner	outer	inner	
female							
placodea	2275	2643	3399	3921	-	3788	16026 \pm 106.25
coeloconica	-	211	123	178	-	95	607 \pm 14.47
total	2275	2854	3522	4099	-	3883	16633 \pm 108.48
male							
placodea	2635	4041	3682	4085	-	6143	20586 \pm 145.29
coeloconica	-	141	102	98	-	257	598 \pm 19.55
total	2635	4182	3784	4183	-	6400	21184 \pm 146.21

G. holosericea and *G. rufilateris*, significant differences were observed ($t=41.71$; $df=10$; $P<0.001$), with significant differences also for the total amount of sensilla among males ($t=18.45$; $df=10$; $P<0.001$).

Discussion

With the development of the present study, the studies with the antennal sensilla of Cetoniinae were expanded to 20 species (Meinecke, 1975; Baker and Monroe, 2005; Zauli et al., 2016; Bohacz et al., 2020; Costa et al., 2021; present study): *Cetonia aurata* (Linnaeus, 1758), *Cotinis nitida* (Linnaeus, 1758), *Epicometis hirta* (Poda 1761), *Gnorimus nobilis* (Linnaeus, 1758), *Gymnetis holosericea*, *Gymnetis rufilateris*, *Hoplopyga albiventris* (Gory and Percheron, 1833), *Hoplopyga liturata* (Olivier, 1789), *Leucocelis elegans*, *Mausoleopsis amabilis*, *Osmoderma eremita* (Scopoli, 1763), *Oxythyrea funesta* (Poda 1761), *Pachnoda ephippiata* (Gerstaecker, 1867), *Pachnoda marginata* (Drury, 1773), *Pachnoda interrupta* (Olivier, 1789), *Potosia affinis* (Andersch, 1797), *Potosia cuprea* (Fabricius 1775), *Protaetia morio* (Fabricius, 1781), *Trichius fasciatus* (Linnaeus, 1758), and *Valgus hemipterus* (Linnaeus, 1758). Otherwise, the quantification of sensilla of males and females are known to (Costa et al., 2021; present study): *Hoplopyga albiventris*, *Hoplopyga liturata*, *Gymnetis holosericea*, and *Gymnetis rufilateris*.

Within the subfamily, *Valgus hemipterus* (Valgini) is separated from all other flower chafers, or even from other scarabaeoid beetle, by the presence of sensilla placode bearing invaginations like pores or points, those pores could be irregularly distributed or have a concentric distribution (Bohacz et al., 2020: sensilla 1E; Meinecke, 1975: E1, E2, E3). Some sensilla placodea type I of *Gymnetis* has some minor punctures and these punctures are quite distinctive from the invaginations above discussed to *Valgus*. The above differentiation of the puncture of *Gymnetis* and *Valgus* is given to show that both structures are possibly not homologous.

All Cetoniinae have the outer side of proximal lamella bearing a group of setae. These setae are designated here with sensilla chaetica, but other studies termed these setae as sensilla trichodea (as Bohacz et al., 2020). Despite the term used (see terminology in Material and Methods), the presence of this group of setae is a conspicuous character of Cetoniinae and part of Dynastinae (Bohacz et al., 2020; Costa et al., 2021), is inconspicuous to Rutelinae and part of Dynastinae (Cyclocephalini; Rodrigues et al., 2019; Saldanha et al., 2020; Nagamine et al., 2022), and is indistinct in Melolonthinae (Bohacz et al., 2020; Menis et al., 2021). In Rutelinae and Cyclocephalini, the outer side of proximal lamella has a sparse group of setae, and these setae are not evidently different from other setae present on lamellar edges (Rodrigues et al., 2019; Nagamine et al., 2022). To Melolonthinae, the outer side of proximal lamella has several setae that are not grouped (Menis et al., 2021). The presence of this grouped setae is here discussed to encourage future works investigate the evolution of this character, checking its homology, or even the possibility of usage of it as a diagnosis to family-group names.

Some here termed sensilla coeloconica type I and II have shallow striae on its surface (Bohacz et al., 2020: sensilla GSC). Sensilla coeloconica variations are sometimes difficult to clarify and intermediate types are common. Present study tried to classify these variations based on its apical shape, type I and II with blunt or acute tip, respectively. Despite the apex shape, the surface of sensilla coeloconica is smooth or grooved. The grooved sensilla coeloconica are common to all Scarabaeoidea and those with smooth surface is common to Scarabaeidae subfamilies: Cetoniinae, Rutelinae, Dynastinae, and part of Melolonthinae (Diplotaxini, Melolonthini) (Bohacz et al., 2020). To melolonthine species, Shao et al. (2019) proposed that the sensilla coeloconica are sensible to humidity

and temperature, and Romero-López et al. (2004) suggested that these sensilla detect plant volatiles.

Sensilla basiconica are here termed as finger-like sensilla with its length evidently longer than the depth of the surrounding pit (see Saldanha et al., 2020: fig. 7). Bohacz et al. (2020) interpreted these sensilla as a variation of sensilla coeloconica (see Bohacz et al., 2020: fig. 4A) and restricted the term sensilla basiconica to longer sensilla surrounded or not by a pit. The sensilla basiconica found on genera *Cyclocephala* (Dynastinae: Cyclocephalini; Saldanha et al., 2020; Nagamine et al., 2022) and *Anomala* (Rutelinae: Anomalini; Rodrigues et al., 2019) are not found in Cetoniinae genera of the tribe Gymnetini: *Cotinis* (described by Baker and Monroe, 2005), *Gymnetis* (by present study), and *Hoplopyga* (by Costa et al., 2021); and are also not found in other flower chafers as *Osmoderma* (Osmodermatini; by Zauli et al., 2016: sensilla basiconica sub-type 1 is here interpreted as a sensilla coeloconica variation) and *Pachnoda* (by Bengtsson et al., 2011).

The general structure of lamellar sensilla of *Gymnetis* is quite similar to other Gymnetini (*Cotinis*, *Hoplopyga*) and other cetoniine (*Osmoderma*, *Pachnoda*).

The inner side of proximal and distal lamella and both sides of medial lamella have two well defined area, a posterior homogeneous area mainly formed by sensilla placodea type II, and an anterior heterogeneous area with sensilla coeloconica (type I and II), and sensilla placodea (mostly type I). These areas are present in Cetoniinae, Dynastinae, Rutelinae, and Melolonthinae (Rodrigues et al., 2019; Bohacz et al., 2020; Costa et al., 2021; Menis et al., 2021; Nagamine et al., 2022). Larsson et al. (2001) noted that the sensilla of homogeneous area is responsible to the sexual pheromone detection, and those of heterogeneous area are responsible to other volatiles detection in the ruteline *Anomala cuprea* (Hope, 1839).

The number of sensilla are higher in males than in females of *Gymnetis*, and the sexual dimorphism is more evident in *G. rufilateris* than in *G. holosericea*. The intrageneric sexual variation of number of sensilla as similarly noted to *Hoplopyga* species, and the sexual dimorphism is more evident in *H. albiventris* than in *H. liturata* (Costa et al., 2021). Males of *G. rufilateris* have almost 24.4% more sensilla than females, and males of *G. holosericea* have about 14.1% more sensilla than females. And males of *H. albiventris* have about 34.5% more sensilla than females, males of *H. liturata* have almost 14.8% more sensilla than females.

Both *Gymnetis* and *Hoplopyga* have the anterior area of outer side of distal lamella bearing sensilla placodea type II homogeneously distributed (Costa et al., 2021).

The distribution and structure of the antennal sensilla are almost the same to *G. holosericea* and *G. rufilateris*, except the diameter of sensilla placodea that are slightly larger in *G. rufilateris* than in *G. holosericea* (see above). The main difference of antennal sensilla to both species is relative to the number of elements, especially regarding placodea sensilla present in posterior homogeneous area, and this area is relatively bigger in *G. rufilateris* than in *G. holosericea*.

Present study reveals that the distribution (into a well delimited anterior posterior areas), shape and size of antennal sensilla are variable between congeneric species. This variability may indicate different patterns of volatile detection and chemical communication. Despite the functional importance, sensilla interspecific variations are a rich data source for phylogenetic studies and also add new morphological descriptions to formerly described species.

Some differences and similarities regarding the structure and vestiture of the antennal lamella were discussed above. The significance of these comparative effort is to highlight the variation observed in the antennal sensilla of phytophagous scarab beetles, encourage future descriptive and comparative studies to found new differences and

similarities, and also support future cladistic studies by adding new primary homology hypothesis to be checked.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

RSS and EAA performed the collections in the field. RSS performed the photos in scanning electron microscopy. RSS, SRR and JF performed the analyzes and interpretations of the data. RSS, EAA, SRR and JF contributed to the critical review and addition of intellectual content. RSS, EAA, SRR and JF made the corrections and final writing.

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