



Host plants and antennal sensilla of *Anomala testaceipennis* Blanchard (Coleoptera: Scarabaeidae: Rutelinae)

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Abstract. Adults of *Anomala testaceipennis* feed on leaves or flowers of plants, while the larvae feed on the roots of cultivated plants. This study was conducted at the Universidade Estadual de Mato Grosso do Sul, Cassilândia, MS, Brazil from September 2017 to December 2021 in a Brazilian Cerrado biome. Adults of *A. testaceipennis* were collected associated with host plants and taken to the laboratory for studies. Some phytophagous scarab beetles found host plants through detection of plant volatiles. The detection of those odorants is intermediated by antennal sensilla. The main goals of the present study are to describe the antennal sensilla of *A. testaceipennis* and check the host plants used as food resource. This specie was found feeding on flowers of: *Anadenanthera colubrina* var. *cebil* (Fabaceae), *Azadirachta indica* (Meliaceae), *Buchenavia* sp. (Combretaceae), *Cordia glabrata* (Boraginaceae), *Inga edulis* (Fabaceae), *Moquilea tomentosa* (Chrysobalanaceae), *Paubrasilia echinata* (Fabaceae), *Tabernaemontana catharinensis* (Apocynaceae), *Tapirira guianensis* (Anacardiaceae), and *Xylophragma pratense* (Bignoniaceae). To the sensilla study, antennae were dissected and images of the sensilla were obtained using a scanning electron microscope. Antennae of *A. testaceipennis* have sensilla chaetica, trichodea, placodea (type I, II, and III), coeloconica (type I and II), basiconica (type I), and ampullacea (or pore). Males have a total of about 6,243 sensilla of which 5,868 (93.99%) are sensilla placodea, 370 (5.93%) are sensilla coeloconica, and 5 (0.08%) are sensilla basiconica. Females have a total of about 5,119 sensilla of which 4,820 (94.16%) are sensilla placodea, 270 (5.27%) are sensilla coeloconica, and 29 (0.57%) are sensilla basiconica.

Keywords: Chemical communication; Neotropical; Pleurosticti; Phytophagous; Ultrastructure.

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Scarab beetles (Coleoptera: Scarabaeidae) are a speciose and diverse taxon, and some species are phytophagous and are potential pests to crops, and according to MORÓN (2001) 19,280 species are registered in the subfamilies Melolonthinae, Rutelinae, Dyastinae and Cetoniinae. In Brazil, reports were made about crop damage caused by species of scarab beetles (e.g., *Phyllophaga cuyabana* (Moser) (Melolonthinae) (OLIVEIRA & GARCIA 2003), *Phyllophaga capillata* (Blanchard) (OLIVEIRA *et al.* 2007), *Liogenys suturalis* Blanchard (Melolonthinae) (SANTOS & ÁVILA 2009), *Diloboderus abderus* Sturm (Dynastinae) (SILVA & BOSS 2002; SILVA & COSTA 2002), *Liogenys fusca* Blanchard and *Anomala testaceipennis* Blanchard (Rutelinae) (RODRIGUES *et al.* 2011).

The genus *Anomala* (Rutelinae, Anomalini) includes more than 1,000 described species, of which about 300 species are registered in Brazil (JAMESON *et al.* 2003; RAMÍREZ-PONCE & MORÓN 2009). Some works regarding the biology of the genus were published, like the biology of *Anomala testaceipennis* described by RODRIGUES *et al.* (2008). The mating behavior of *Anomala orientalis* (Waterhouse) and *Anomala albopilosa sakishimana* Nomura were described by FACUNDO *et al.* (1999) and ARAKAKI *et al.* (2004) respectively. The pheromone glandular system of *A. albopilosa albopilosa* (Hope) by TADA & LEAL (1997). The sexual pheromones of *Anomala cuprea* (Hope) were identified by LEAL (1991) and the pheromone detection was showed by LEAL & MOCHIZUKI (1993) and the detection of plant volatiles was characterized by LARSSON *et al.* (2001).

To Brazil, the specie *A. testaceipennis* was reported damaging crops of soybean (*Glycine max* L. Merrill, Fabaceae), corn (*Zea mays* L., Poaceae), wheat (*Triticum aestivum* L., Poaceae), and oats (*Avena strigosa* Schreb., Poaceae) (ÁVILA & SANTOS 2009). The larvae of this species consume roots of cultivated plants or pastures (ÁVILA & SANTOS 2009; RODRIGUES *et al.* 2011). Adults were found consuming flowers of "oiti" (*Moquilea tomentosa* Benth., Chrysobalanaceae), and louro-preto (*Cordia glabrata* A.DC., Boraginaceae) (RODRIGUES *et al.* 2014). The adults swarm mainly from September to December, and the life cycle of the species least about 140 days (RODRIGUES *et al.* 2008). The species was also recorded as potential pest to *Rosa* sp. (Rosaceae; BALLOU 1935).

Anomala testaceipennis find host plants to nutrition and also as matting sites. These beetles evidently use chemical communication, intermediated by antennal structures named sensilla (RODRIGUES *et al.* 2014).

The main goal of present study is describing the antennal sensilla and update the host plant list to the species.

MATERIAL AND METHODS

The studies were performed at the Campus Cassilândia of the Universidade Estadual de Mato Grosso do Sul (UEMS), Cassilândia municipality, Mato Grosso do Sul State (MS), Brazil, from September 2017 to December 2021. The study area is fragment of Brazilian Cerrado biome, and flowering plants were observed from 18:00 to 22:00h, a period identified by RODRIGUES *et al.* (2014) as the swarm period to *A. testaceipennis*. When present, adults were collected (five specimens per plant). Plant exsiccatae were made to the identification of the plant species, and deposited at herbarium of Universidade Federal da Grande Dourados, Dourados municipality, Mato Grosso do Sul State, Brazil.

Collected adults of *A. testaceipennis* (Figure 1) were preserved at ethanol 70° GL and housed at Campus Cassilândia of UEMS. The identification of the specimens was made by comparison and used specimens housed at UEMS collection. The lamellae of the antennal club of ten males (n=10) and ten females (n=10) were dissected using a Motic stereoscopic microscope and stored in 20 mL glass flasks containing 70% alcohol. The lamellae were maintained in 80% alcohol for 10 minutes, 90% alcohol for 15 minutes, and 100% alcohol for 20 minutes for cleaning (TANAKA *et al.* 2006; ROMERO-LÓPEZ *et al.* 2013).



Figure 1. Adult of *Anomala testaceipennis* Blanchard.

The lamellae were dried to a critical point using a Leica® CPD300 dryer at the Universidade Estadual Paulista (UNESP), Ilha Solteira campus, São Paulo State (SP), Brazil. Subsequently, they were coated with gold using a Quorum® Q150T E turbo molecular pump. Images were obtained using a Zeiss® EVO LS15 scanning electron microscope (SEM), following the methodology adapted from ROMERO-LÓPEZ *et al.* (2013). Sensilla terminology follows MEINECKE (1975).

RESULTS

Host plants. Present study found adults of *A. testaceipennis* feeding on flowers of: *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul ("Angico vermelho", Fabaceae), *Azadirachta*

indica A. Juss ("Nim", Meliaceae), *Buchenavia* sp. ("Boca boca", Combretaceae), *Cordia glabrata* (Mart.) A. DC. ("Louro de mato grosso", Boraginaceae), *Inga edulis*, Mart. ("Ingá do cerrado", Fabaceae), *Moquilea tomentosa* Benth. ("Oiti", Chrysobalanaceae), *Paubrasilia echinata* (Lam.) Gagnon, H.C.Lima & G.P.Lewis ("Pau-brasil", Fabaceae), *Tabernaemontana catharinensis* A. DC. ("Leiteiro-de-folha-fina", Apocynaceae), *Tapirira guianensis* Aubl. ("Peito de pomba", Anacardiaceae), and *Xylophragma pratense* (Bureau & K. Schum.) Sprague ("Cipó rosa", Bignoniaceae).

Additionally, some specimens housed at Museu de Zoologia da Universidade de São Paulo (MZSP), São Paulo State (SP) were collected at Louveiro, São Paulo State damaging apple tree crops (*Malus* sp., Rosaceae) and vines (*Vitis* sp., Vitaceae).

Some observed flowering plants as: *Campomanesia pubescens* (Mart. ex DC.) O. Berg ("Gabirola", Myrtaceae), *Thaumatococcus lundii* (Warm.) Sakur., Calazans & Mayo (Araceae), *Cissus* sp. (Vitaceae), and *Pouteria caimito* (Ruiz & Pav.) Radlk. ("Abiu", Sapotaceae) were not visited by *A. testaceipennis*.

Adults of *A. testaceipennis* land on flowers and start to eat flower parts. At this structure in the plant the couples were formed and started the copulation. Those beetle activities were observed on flowers at three to 12 meters high.

Antennal clava sensilla. The antenna of adults of *A. testaceipennis* are divided in (proximal to distal) scape, pedicel, funicle (F1, F2, F3, F4), and a three lamellate clava (PL, ML, DL) (Figure 2A, B). The lamellae have sensilla trichodea, sensilla chaetica, sensilla placodea, sensilla coeloconica, sensilla basiconica, and some pores (sensilla ampullacea). The sensilla trichodea are found as long setae present on outer surface of proximal and distal lamellae, and on peripheral area of medial lamella (Figure 3A- C, E). The sensilla chaetica are similar to the sensilla trichodea, but are shorter and slightly widened, and are mainly grouped on the distal half of outer surface of proximal lamella (Figure 3A).

The sensilla coeloconica and sensilla basiconica are found on inner surface of proximal and distal lamella and on inner and outer surface of medial lamella (Figure 3D). Sensilla coeloconica could be of type I (MEINECKE 1975: L1), when it is pointed, and type II (MEINECKE 1975: L2), when it has a blunt tip (Figure 2D). The sensilla basiconica seem like a short seta enclosed by a ditch. It is mainly found grouped inside some foveae (Figure 3D).

The predominant type of sensilla is sensilla placodea, and they are identified as type I, II and III (Figure 3D). Sensilla placodea of the type I (MEINECKE 1975: G1) have a peripheral ditch, a shallowly reticulated surface and mean diameter of 10,53 μm (9,3-13,3) (Figure 3); sensilla of the type II (MEINECKE 1975: H1) have a peripheral ditch, a plane surface and mean diameter of 6,56 μm (5,3-9,3) (Figure 3D); and sensilla of the type III (MEINECKE 1975: H3) do not have peripheral ditch, have an almost plane surface and mean diameter 14,96 μm (9,3-20) (Figure 3D). The sensilla placodea are found mainly on inner surface of proximal and distal lamella, and inner and outer surface of medial lamella (Figure 3B, C, E). Sensilla placodea type III also occurs on posterior third of outer surface of distal lamella (Figure 4A, B). Some sensilla placodea type I as found grouped inside some foveae (Figure 4D). Pores or sensilla ampullacea are small holes found on the surface of the lamella (Figure 4D).

Males have more sensilla than females (Table 1): with on average 6,243 sensilla in lamella, of which 5,868 (93.99%) are sensilla placodea, 370 (5.93%) are sensilla coeloconica, and 5 (0.08%) are sensilla basiconica. Females, on the other

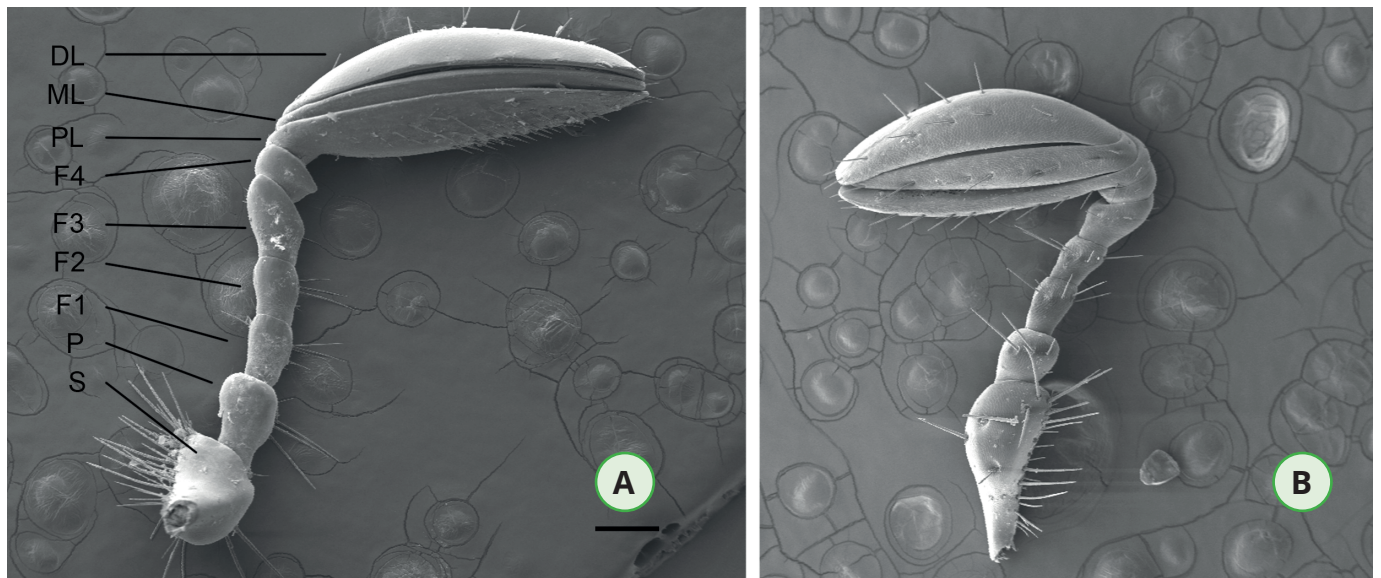


Figure 2. Antenna of *Anomala testaceipennis* Blanchard. A) male. B) female. DL = distal lamella, F1-4 = antennomeres of funicle, ML = medial lamella, P, pedicel, PL, proximal lamella, S, scape. Scale = 100 µm.

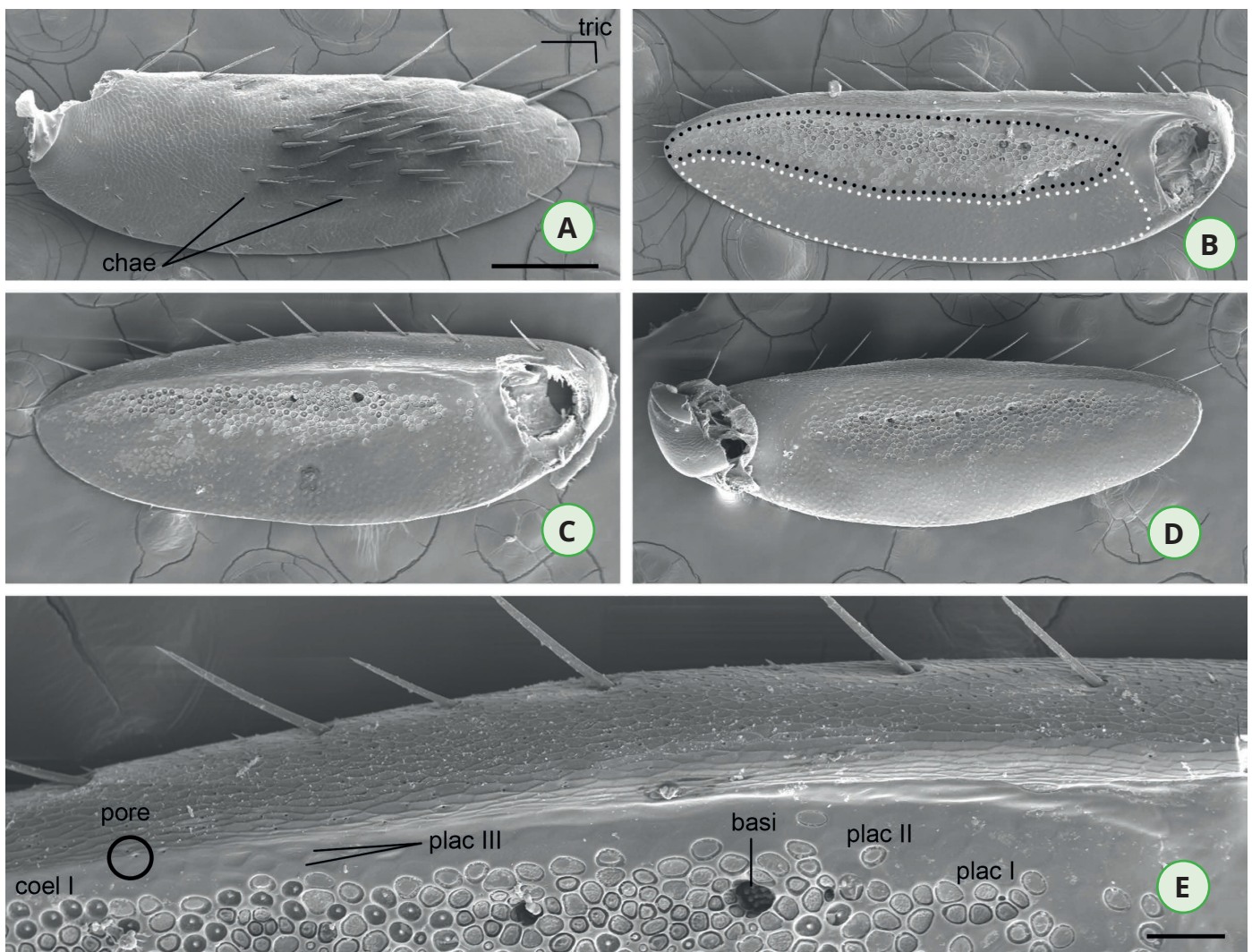


Figure 3. Antennal lamella of female of *Anomala testaceipennis* Blanchard. A) proximal lamella, outer surface. B) proximal lamella, inner surface. C) medial lamella, outer surface. D) medial lamella, outer surface, detail. E) distal lamella, inner surface. Anterior area (black dotted line) with sensilla placodea type I and II, sensilla coeloconica type I and II, and sensilla basiconica. Posterior area (white dotted line) with sensilla placodea type III homogeneously distributed; Basi = sensilla basiconica (grouped into a fovea), Chae = sensilla chaetica, Coel I = sensilla coeloconica type I, Plac I = sensilla placodea type I, Plac II = sensilla placodea type II, Plac III = sensilla placodea type III, Pore = sensilla ampullaceum, Tric = sensilla trichodea. Scale of A, B, C, E = 200 µm; scale of D = 40 µm.

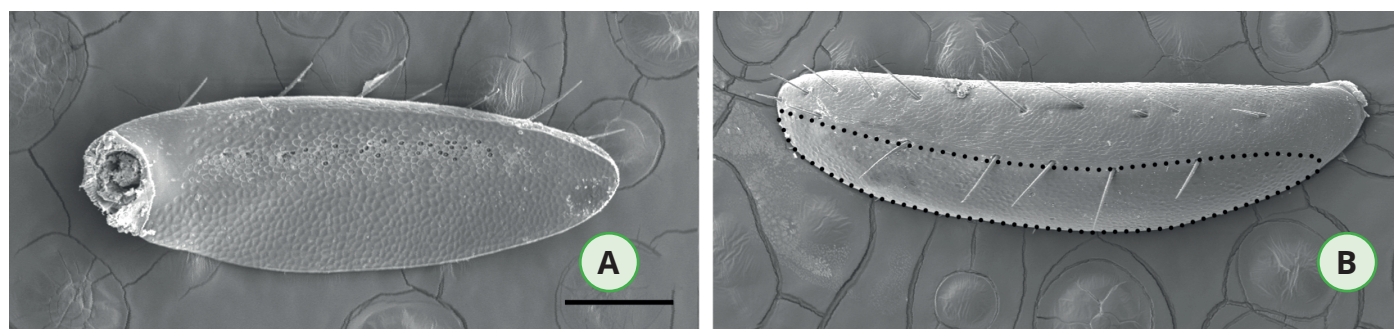


Figure 4. Antennal lamella of male of *Anomala testaceipennis* Blanchard. A) medial lamella, inner surface. B) distal lamella, outer surface (black dotted line showing posterior area with sensilla placodea type III). Scale = 200 μ m.

Table 1. Number of antennal sensilla of *Anomala testaceipennis* Blanchard.

Sensilla	Proximal lamella		Medial lamella		Distal lamella		Total
	Inner surface	Outer surface	Inner surface	Outer surface	Inner surface	Outer surface	
Female							
Placodea	1,193	0	1,135	941	1,075	476	4,820
Coeloconica	74	0	82	46	65	3	270
Basiconica	8	0	10	11	0	0	29
Total	1,275	0	1,227	998	1,140	479	5,119
Male							
Placodea	1,218	0	1,390	1,050	1,629	581	5,868
Coeloconica	90	0	67	52	157	4	370
Basiconica	2	0	0	0	3	0	5
Total	1,310	0	1,457	1,102	1,789	585	6,243

hand, have on average 5,119 sensilla in lamellae, of which 4,820 (94.16%) are sensilla placodea, 270 (5.27%) are sensilla coeloconica, and 29 (0.57%) are sensilla basiconica.

DISCUSSION

Host plants. *Anomala testaceipennis* is a generalist florivorous associated to several Eudicots plants, as Fabids (e.g., Anacardiaceae, Chrysobalanaceae, Fabaceae), Malvids (e.g., Combretaceae, Meliaceae), Lamiids (e.g., Apocynaceae, Bignoniaceae, Boraginaceae), and maybe others Rosids (e.g., Rosaceae, Vitaceae) (ÁVILA & SANTOS 2009; BALLOU 1935; RODRIGUES et al. 2014; and present study). Even feeding on several different plant, the species do not consume other native plants from Brazilian cerrado, as *Campomanesia pubescens* (Mart. ex DC.) (Myrtaceae), *Thaumatococcus lundii* (Warm.) Sakur., Calazans & Mayo (Araceae), *Cissus* sp. (Vitaceae) and *Pouteria caimito* (Ruiz & Pav.) (Sapotaceae).

RODRIGUES et al. (2014) listed *Moquilea tomentosa* (Chrysobalanaceae) and *Cordia glabrata* (Boraginaceae) as host plants for *A. testaceipennis*. Ten new host plants were observed in present study: *Anadenanthera colubrina* var. *cebil* (Fabaceae), *Azadirachta indica* (Meliaceae), *Buchenavia* sp. (Combretaceae), *Inga edulis* (Fabaceae), *Malus* sp. (Rosaceae), *Paubrasilia echinata* (Fabaceae), *Tabernaemontana catharinensis* (Apocynaceae), *Tapirira guianensis* (Anacardiaceae), *Vitis* sp. (Vitaceae), *Xylophragma pratense* (Bignoniaceae).

Antennal sensilla. The same types of sensilla such trichodea, chaetica, placodea, coeloconica, basiconica and pores were found in *A. testaceipennis*, *A. inconstans* Burmeister (RODRIGUES et al. 2019), and *Anomala cuprea* (LEAL & MOCHIZUKI 1993).

The general distribution of the sensilla show is quite similar to that found in others phytophagous scarab of Cetoniinae (e.g., COSTA et al. 2020), Dynastinae (e.g., SALDANHA et al. 2020; NAGAMINE et al. 2022) and Rutelinae (e.g., RODRIGUES et al. 2019). Those beetles usually have: 1) a group of sensilla chaetica on the outer surface of proximal lamella, 2) the inner surface

of proximal and distal lamella and the inner and outer surface of medial lamella have a heterogeneous area and a homogeneous anterior area, the heterogeneous area includes mainly sensilla placodea type I and II, sensilla coeloconica and sensilla basiconica, and some foveae, and the homogeneous area include only sensilla placodea type III.

The predominant sensilla of *A. testaceipennis* is sensilla placodea. LEAL & MOCHIZUKI (1993) demonstrated that sensilla placodea of *A. cuprea* are responsible to the sexual pheromone detection. Otherwise, to the Melolonthinae beetle *Holotrichia oblita* (Faldermann) the sensilla placodea seem to be related to plant volatiles detection (SUN et al. 2014). LARSSON et al. (2001) found evidence of sensilla placodea in *A. cuprea* detecting sexual pheromones and plant odorants. Furthermore, sensilla coeloconica also are abundant in antennae of *Anomala*. That kind of sensilla were associated to the plant volatiles detection (KIM & LEAL 2000; ROMERO-LÓPEZ et al. 2004).

Rutelinae and Dynastinae have a quite similar sensilla organization, and the antennal sensilla of Rutelinae show a distinctive characteristic regarding the antennae of Dynastinae. The sensilla placodea type I have a shallow reticulated surface, and those of the dynastine beetles have a deep reticulation. This assumption was also observed by BOHACZ et al. (2020) and the authors used this difference to separate these kinds of sensilla placodea as sensilla type F (sensilla placodea type I with deep reticulation) and as sensilla type G (sensilla placodea type I with shallow reticulation).

Comparing the amounts of sensilla in species of *Anomala*, females of *A. inconstans* have more sensilla than females of *A. testaceipennis*, otherwise males of *A. testaceipennis* have more sensilla than males of *A. inconstans* (Tables 1 and 2). Males of *A. testaceipennis* have evidently more antennal sensilla than females, but the opposite is observed in *A. inconstans*. Sexual pheromone or plant volatiles may have different importance in the aggregative behavior from both species, regarding the above-mentioned difference. Further studies on *Anomala* antennal structural and functional morphology are needed to clarify these differences.

Table 2. Number of antennal sensilla of *Anomala inconstans* Burmeister (modified from RODRIGUES *et al.* 2019).

Sensilla	Proximal lamella		Medial lamella		Distal lamella		Total
	Inner surface	Outer surface	Inner surface	Outer surface	Inner surface	Outer surface	
Female	1,804	0	1,696	998	1,983	1,303	7,784
Male	1,289	0	1,102	1,255	1,203	892	5,741

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