

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Sexual Dimorphism in Antennal Receptors of *Phyllophaga ravida* Blanchard (Coleoptera: Scarabaeoidea: Melolonthidae)ANGEL ROMERO-LÓPEZ¹, MIGUEL MORÓN¹, JORGE VALDEZ²¹Red de Biodiversidad y Sistemática. Instituto de Ecología, 91000 Xalapa, Veracruz, Apartado Postal 63, México; aaromelo@gmail.com; miguel.moron@inecol.edu.mx²Instituto de Fitosanidad, Colegio de Posgraduados, Campus Montecillo, 56230 Estado de México, México; jvaldez@colpos.mx

Edited by Roberto A Zucchi – ESALQ/USP

Neotropical Entomology 39(6):957-966 (2010)

ABSTRACT - The external morphology of sensilla on the antennae of males and females of *Phyllophaga ravida* Blanchard is described using scanning electron microscopy. Sexual dimorphism in body and antennal dimensions and in antennal receptor types was found. The female's body is slightly larger than the male's, although male antennal lamellae are longer than in females. Sixteen types of sensilla were identified on the proximal and distal surfaces of lamellae from both sexes, most of them in males: three types of placodea sensilla, four types of auricilica sensilla, five types of basiconica sensilla, and four types of coeloconica sensilla. Also, two types of mechanoreceptor sensilla were present on the lamellae periphery. Furthermore, males had larger placodea, auricilica and some types of basiconica sensilla.

KEY WORDS: Antenna, morphology, sensilla, sexual behavior

Phyllophaga ravida Blanchard is included in the *dentex* complex of the *ravida* species group, subgenus *Phyllophaga* (*sensu stricto*), and is one of the white grub species of agricultural and economic importance in Mexico (Morón 2003). The systematics and biology of this species are known (Ramírez-Salinas *et al* 2000, Aragón *et al* 2005, Morón 2006), but little research has addressed its ecology and behavior. Reports on behavior show that *P. ravida* adults present a sexual activity similar to that of other *Phyllophaga* species, with a sex attractant involved (Morón 1986, Romero-López *et al* 2007). Sexual chemical communication in some Melolonthidae involves the production and release of specific chemicals by the emitter and the detection and olfactory processing of these signals leading to appropriate behavioral responses in the receiver (Leal 1998, Kim & Leal 2000, Oliveira *et al* 2003, Romero-López *et al* 2005). Chemicals released from melolonthid females are captured in the sensilla located on both sides of male antennal lamellae (Meinecke 1975, Morón 1986, Leal 1998). The genus *Phyllophaga* is formed by more than 800 species, but only *Phyllophaga anxia* LeConte (Ochieng *et al* 2002) and *Phyllophaga obsoleta* Blanchard (Romero-López *et al* 2004) have the different types of sensilla been studied, although differences in receptors were not morphologically observable. The main purpose of this paper is to describe sexual dimorphism in body, antennal, and lamellar dimensions as well as in the sensilla distribution in the antenna of this beetle, as a first step to better understand the dynamics of sexual chemical communication in this species.

Material and Methods

Collection of specimens. *Phyllophaga ravida* specimens used in this study were collected during May and July of 2008 with light traps in golf fields located at Puebla, Mexico and preserved in 70% ethanol.

Measurement of body dimensions. After taxonomical identification using the keys proposed by Morón (1986, 2006), 12 females and 12 males were randomly chosen for length measurement with the Image Tool 3.0 software program (Wilcox *et al* 2002). Length was measured in each specimen from the clypeus to the pygidium.

Measurement of antennae and lamellae. The head of each previously measured specimen was separated from the body and preserved in 70% ethanol. The antennae of 12 females and 12 males were separated and measurements of total length, width, and area of each lamellae, as well as sensilla dimensions, were obtained with the Image Tool 3.0 software program. Afterwards, the three lamellae forming each antennal club were separated, labeled, and grouped according to sex and lamellar side (distal or proximal surfaces). The lamella located farther away from the head was denominated distal lamella, while the nearest was called proximal lamella and the one between these two, middle lamella.

Specimen preparation for light microscopy studies. Antennae from an additional 10 females and 10 males were

kept in 10% KOH at 80°C for 60 min, rinsed in distilled water, and placed in 70% ethanol in order to separate the lamellae, which were then dehydrated in 80%, 90%, and absolute ethanol. Lamellae were placed in xylene during 10 min for clearing, mounted in Canadian balsam and observed under a light microscope (Iroscope, Mod. MG-11J). Images from these slides and from non-cleared lamellae were obtained with a photo-microscope III (Carl Zeiss) and a Tessovar microscope (Carl Zeiss), both including a PaxCam 3 digital camera.

Specimen preparation for scanning electron microscopy studies. Lamellae from additional specimens were prepared following the methods proposed by Bozzola & Russell (1998) and were examined at 25kV under a scanning electron microscope (JEOL Mod. JSM-5600LV).

Measurements of sensilla. Images of the three lamellae of two *P. ravida* females and males were from scanning electron microscopy. The different types of sensilla (except the mechanoreceptors) were measured with Image Tool 3.0. The circular- or spherical-shaped sensilla were measured from the diameter of the largest axis; sensilla with other shapes were measured from maximum length and width (pegs, cones, rods, spines, etc.). Aggregations of rods or cones located inside cuticular cavities were measured from the diameter of the largest axis cavity, and rods or cones were recorded.

Statistics. Data on body, antennae, lamellae, and sensilla dimension for *P. ravida* adult males and females were analyzed with Student's *t* or Mann-Whitney Rank Sum tests (SigmaStat 3.1). Unless otherwise stated, all values reported are mean \pm standard error.

Results

Body, antennae, and lamellae dimensions. Several sex-related differences in antennae were observed. Although the body of adult *P. ravida* females is larger than that of males ($t = -2.42$; $df = 38$; $P < 0.05$), the entire male antenna is considerably longer ($t = -11.67$; $df = 34$; $P < 0.001$), with a significantly longer antennal club ($t = -11.67$; $df = 34$; $P < 0.001$) (Table 1). Other noticeable differences are the superior

Table 1 Comparison of the body and antennae dimensions of females and males of *Phyllophaga ravida*.

| Measure length (mm) | n | Female | Male | P |
|---------------------|----|-------------------|-------------------|-------------|
| Body | 20 | 22.13 \pm 0.30 | 21.17 \pm 0.25 | $P < 0.05$ |
| Scape | 16 | 0.957 \pm 0.016 | 0.973 \pm 0.027 | ns |
| Pedicel | 16 | 0.265 \pm 0.004 | 0.313 \pm 0.006 | $P < 0.001$ |
| Flagellum | 16 | 0.988 \pm 0.011 | 1.064 \pm 0.018 | $P < 0.05$ |
| Antennal club | 16 | 1.154 \pm 0.016 | 1.717 \pm 0.024 | $P < 0.001$ |
| Entire antennae | 16 | 3.371 \pm 0.028 | 4.073 \pm 0.05 | $P < 0.001$ |

Values are mean \pm standard error of the mean; Student *t*-test; ns not significant.

length of the male pedicel ($t = -6.24$; $df = 34$; $P < 0.001$), flagellum ($t = -3.40$; $df = 34$; $P < 0.05$), and lamellar club ($t = -19.27$; $df = 34$; $P < 0.001$).

Male antennal length represents approximately 19% of total body length, while female antennal length is no more than 15% of body length (Table 1). In males, distal lamellae are longer ($t = -14.53$; $df = 28$; $P < 0.001$), wider ($t = -4.432$; $df = 28$; $P < 0.001$), and cover a larger area ($t = -10.69$; $df = 28$; $P < 0.001$) than in females (Table 2). Furthermore, the male middle lamellae are longer (Mann-Whitney test; $df = 28$; $P < 0.001$), with a larger area ($t = -10.58$; $df = 26$; $P < 0.001$) and greater width ($t = -4.41$; $df = 26$; $P < 0.001$) (Table 2). Finally, male proximal lamella are longer ($t = -11.39$; $df = 26$; $P < 0.001$), with a larger area ($t = -8.31$; $df = 26$; $P < 0.001$) and greater width ($t = -3.30$; $df = 26$; $P < 0.05$) (Table 2).

Sensilla dimensions. Several sensilla types were observed on *P. ravida* antennae using light- and scanning electron-microscopy (Figs 1, 2). Classification and terminology of sensilla types used here are based principally on Schneider (1964) and in part on Meinecke (1975), Zacharuk (1980), and Ochieng *et al* (2002). Basic sensilla trichodea (TRS), chaetica (CHS), placodea (PLAS), auricilica (AUS), basiconica (BAS), and coeloconica (COS) were identified. TRS have a long hair-like structure that occurs along the peripheral edges (Fig 1a). CHS present a short-bristle- or spine-like structure; they occur along the peripheral edges and some are in the lamellar center (Fig 1a). Both types are most likely mechanoreceptors. For the

Table 2 Comparison of the length, area, and width of the lamellae of females and males of *Phyllophaga ravida*.

| Measure (mm) | Female | Male | P |
|--------------------------|-------------------|-------------------|--------------------------|
| Proximal lamellae length | 1.199 \pm 0.013 | 1.717 \pm 0.043 | $P < 0.001$ |
| Proximal lamellae area | 0.384 \pm 0.008 | 0.632 \pm 0.028 | $P < 0.001$ |
| Proximal lamellae width | 0.414 \pm 0.008 | 0.459 \pm 0.011 | $P < 0.05$ |
| Middle lamellae length | 1.137 \pm 0.009 | 1.665 \pm 0.037 | $P < 0.001$ ¹ |
| Middle lamellae area | 0.365 \pm 0.007 | 0.616 \pm 0.022 | $P < 0.001$ |
| Middle lamellae width | 0.416 \pm 0.007 | 0.467 \pm 0.009 | $P < 0.05$ |
| Distal lamellae length | 1.043 \pm 0.018 | 1.605 \pm 0.034 | $P < 0.001$ |
| Distal lamellae area | 0.311 \pm 0.009 | 0.541 \pm 0.019 | $P < 0.001$ |
| Distal lamellae width | 0.381 \pm 0.005 | 0.423 \pm 0.008 | $P < 0.001$ |

Values are mean \pm standard error of the mean; $n = 14$; Student *t*-test.

¹Mann-Whitney Rank Sum test

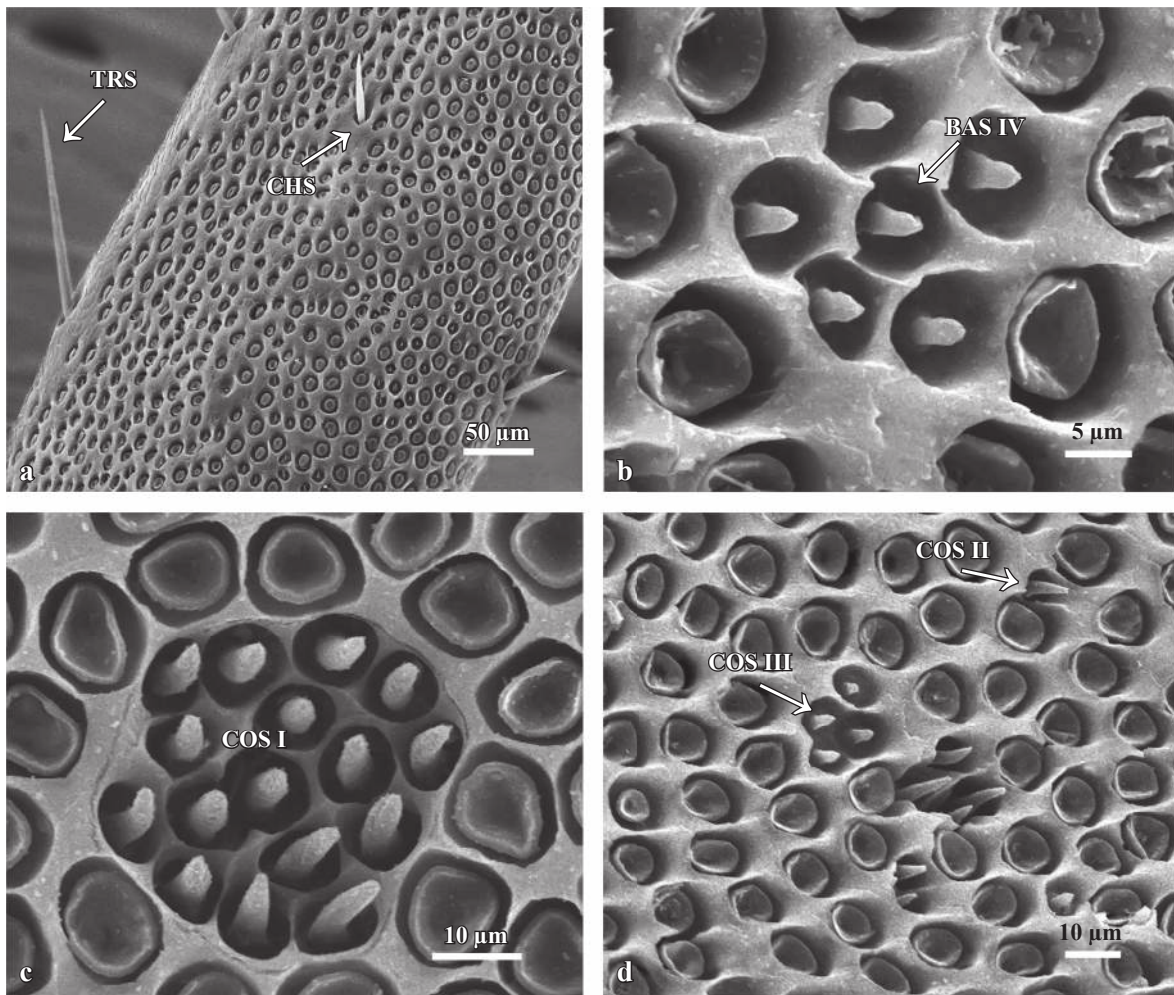


Fig 1 Sensilla on the antennae of female *Phyllophaga ravida*. a) Distal surface of distal lamella. Trichodea (TRS) and chaetica sensillum (CHS) are seen in the periphery of the lamella and CHS is in the center; b) Detail of basiconica (BAS) sensilla type IV on proximal surface of proximal lamella; c) Coeloconica (COS) sensilla type I on proximal surface of proximal lamella; d) Detail of coeloconica (COS) sensilla type II and III on proximal surface of medium lamella.

purposes of the present study, we focused primarily on PLAS, AUS, BAS, and COS, which are considered chemoreceptor types. In this case, sixteen chemosensilla types were found: three types of PLAS, four of AUS, five of BAS, and four of COS.

In both sexes, PLAS type I are randomly distributed on both sides of all lamellae mainly in the center, except on peripheral edges of lamellae; they are rounded and elongated plates that vary in size between 6.88 μm and 9.22 μm in diameter (Fig 2a, Table 3). PLAS type II are randomly distributed on both sides and at the peripheral edges of all lamellae in both male and females; they are spherical or elliptical and thin-walled plates that vary in diameter between 5.65 μm and 7.69 μm (Fig 2a, Table 3). PLAS type III are located on both sides of all lamellae, principally on pit, basal, and peripheral edges in both sexes; they are small, elliptical, thin-walled plates or low dome-shaped plates that vary in size between 3.04 and 3.87 μm on the largest axis (Fig 2a, Table 3).

AUS type I are randomly distributed on both sides and

peripheral edges of all lamellae for both sexes; they are characterized by a “rabbit-ear” shape or elliptical and thin-walled plates, that varied in size between 5.92 and 7.07 μm on the largest axis (Fig 2b, Table 4). AUS type II are present only in male lamellae, distributed on both sides (except on the distal surface of the distal lamellae); they are “rabbit-ear” shaped structures, elliptical and low dome-shaped, and smaller than AUS I (size between 3.27 and 5.17 μm on the largest axis) (Fig 2b, Table 4). AUS type III are located only on male lamellae, randomly distributed on both sides and on peripheral edges (except on the proximal surface of distal lamellae); they are characterized by a “human-ear” shape that varies in size between 5.77 μm and 7.02 μm on the largest axis (Fig 2b, Table 4). AUS type IV are restricted to the center of proximal lamellae in males; their shape is characterized as “rabbit-ear with neck” or “raisin with neck” structures that vary in size between 3.92 μm and 5.57 μm on the largest axis (Fig 2c, Table 4).

BAS type I are randomly distributed mostly on both sides of all club lamellae of both sexes (except on peripheral

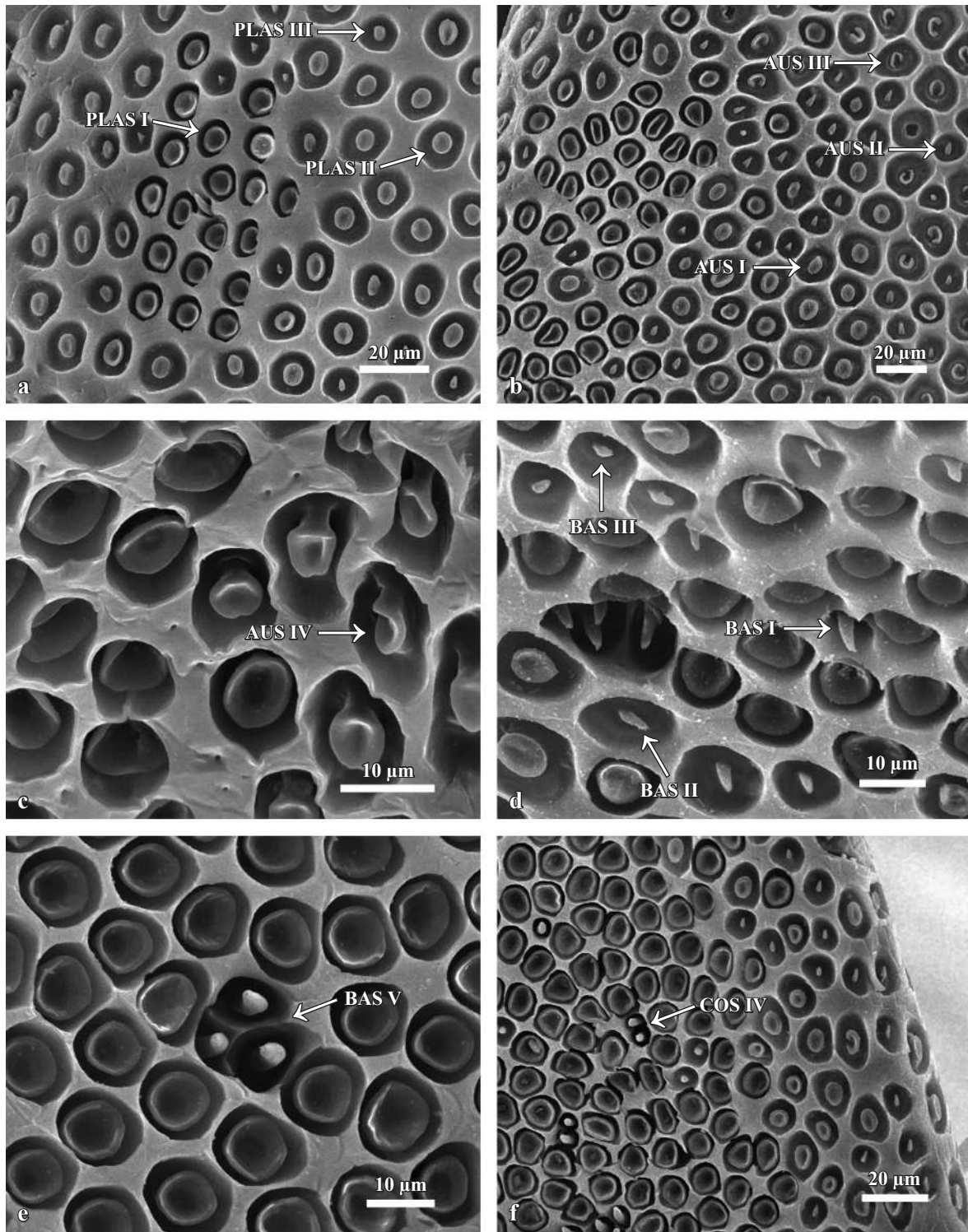


Fig 2 Sensilla on the antennae of male *Phyllophaga ravid*a. a) Proximal surface of proximal lamella. Detail of placodea (PLAS) sensilla type I, II and III; b) Auricilica (AUS) sensilla type I, II and III on proximal surface of medium lamella; c) Detail of auricilica (AUS) sensilla type IV on proximal surface of proximal lamella; d) Basiconica (BAS) sensilla type I, II and III on proximal surface of proximal lamella; e) Detail of basiconica (BAS) sensilla type V on proximal surface of proximal lamella; f) Coeloconica (COS) sensilla type IV on proximal surface of proximal lamella.

Table 3 Measurements of sensilla type placodea on lamellae of both sexes of *Phyllophaga ravida*.

| Sensilla | Lamellae | Surface | Sensilla diameter (μm) ¹ | |
|----------|----------|---------|--|--------------------------------------|
| | | | Female | Male |
| PLAS I | DL | P | 7.97 \pm 0.21 | 8.20 \pm 0.12 |
| | | D | 6.88 \pm 0.15 | 7.96 \pm 0.01 ^{0.001} |
| | ML | P | 7.33 \pm 0.11 | 9.21 \pm 0.16 ^{0.001 (2)} |
| | | D | 7.25 \pm 0.08 | 8.60 \pm 0.21 ^{0.001 (2)} |
| | PL | P | 8.68 \pm 0.23 | 9.09 \pm 0.17 |
| | | D | 7.74 \pm 0.08 | 7.99 \pm 0.14 |
| PLAS II | DL | P | 6.99 \pm 0.21 | 7.69 \pm 0.11 ^{0.05 (2)} |
| | | D | 5.65 \pm 0.14 | 6.89 \pm 0.11 ^{0.001} |
| | ML | P | 7.05 \pm 0.12 | 7.48 \pm 0.12 ^{0.05} |
| | | D | 6.89 \pm 0.07 | 7.67 \pm 0.11 ^{0.001} |
| | PL | P | 6.93 \pm 0.11 | 7.31 \pm 0.09 ^{0.05} |
| | | D | 7.37 \pm 0.09 | 7.05 \pm 0.09 ^{0.05} |
| PLAS III | DL | P | 3.76 \pm 0.06 ^{0.001} | 3.04 \pm 0.08 |
| | | D | 3.31 \pm 0.09 | 3.64 \pm 0.08 ^{0.05} |
| | ML | P | 3.69 \pm 0.54 | 3.80 \pm 0.06 |
| | | D | 3.57 \pm 0.07 | 3.78 \pm 0.05 ^{0.05 (2)} |
| | PL | P | 3.75 \pm 0.06 | 3.87 \pm 0.06 |
| | | D | 3.81 \pm 0.05 | 3.77 \pm 0.05 |

PLAS = placodea sensilla; DL = distal lamellae; ML = medium lamellae; PL = proximal lamellae; P = proximal surface; D = distal surface

Values are mean \pm standard error of the mean; n = 20; Student *t*-test; ¹major axis; ²Mann-Whitney Rank Sum test; ^{0.05} = P < 0.05; ^{0.001} = P < 0.001.

edges and on the distal surface of middle lamellae); they are large peg- or cone-shaped and vary in length between 5.05 μm and 7.53 μm (Fig 2d, Table 5). BAS type II vary in length (3.11-3.98 μm) and are short-spine shaped; for both sexes, they are situated on both sides and peripheral edges of all lamellae (Fig 2d, Table 5). For both sexes, BAS type III were observed randomly distributed principally on both sides of all club lamellae, except on peripheral edges; they are short-cone shaped and vary in length between 1.47 μm and 1.83 μm (Fig 2d, Table 5). BAS type IV are present only in females at the center of the inner surface of medium and proximal lamellae; they are serrated-cone shaped and vary in length between 3.52 μm and 5.25 μm (Fig 1b, Table 5). BAS type V occur only in males, mainly at the center of the proximal surface of proximal lamellae; they are long-rod shaped and vary in length between 6.59 μm and 7.50 μm (Fig 2e, Table 5).

For both sexes, COS type I are restricted principally to the center, appearing only at the proximal surface of all club lamellae. They are found as aggregations of 2 to 16 long peg- or cone-shaped structures (BAS I) located inside depressions or cavities in the antennal cuticle, which varies in size between 8.91 and 38.83 μm on the largest axis (Fig 1c, Table 6). COS type II are found as aggregations of two

to three serrated cone-shaped structures (BAS IV) located on the floor of cuticular cavities. These cavities vary in size between 11.58 and 17.54 μm on the largest axis. COS type II are restricted only to females, mostly at the center and only on the proximal surface of all club lamellae, except on distal lamellae (Fig 1d, Table 6). COS type III are found as rare aggregations of a long cone (BAS I) and a spherical plate (PLAS I) located inside cavities in the antennal cuticle. These cavities vary in size between 9.54 μm and 24.13 μm on the largest axis. COS III are restricted principally to the center, appearing only on the proximal surface of all club lamellae in both sexes, except on male proximal lamellae (Fig 1d, Table 6). COS type IV are found only in males and are restricted to the center and proximal surface of proximal lamellae. They are found as long, rod-shaped aggregations (BAS V) located inside depressions or cavities in the antennal cuticle, which varies in size between 9.28 μm and 17.34 μm on the largest axis (Fig 2f, Table 6).

Discussion

Antennal specializations for detecting pheromones are usually visible at a first glance, as marked sexual dimorphism

Table 4 Measurements of sensilla type auriculica on lamellae of both sexes of *Phyllophaga ravid*a.

| Sensilla | Lamellae | Surface | Sensilla diameter (μm) ¹ | | |
|-----------------|----------|---------|--|-------------------------------------|-------------------------|
| | | | Female | Male | |
| AUR I n = 20 | DL | P | 6.72 \pm 0.17 | 7.02 \pm 0.11 | |
| | | D | 5.92 \pm 0.12 | 6.63 \pm 0.23 ^{0.05 (2)} | |
| | ML | P | 6.48 \pm 0.15 | 6.95 \pm 0.14 ^{0.05} | |
| | | D | 6.07 \pm 0.14 | 7.07 \pm 0.13 ^{0.001} | |
| | PL | P | 6.70 \pm 0.14 | 6.51 \pm 0.15 | |
| | | D | 6.83 \pm 0.11 | 6.91 \pm 0.16 | |
| | AUR II | DL | P | - | 3.50 \pm 0.12 (n = 7) |
| | | | D | - | 5.17 \pm 0.18 (n = 7) |
| ML | | P | - | 4.25 \pm 0.10 (n = 14) | |
| | | D | - | 4.04 \pm 0.08 (n = 20) | |
| PL | | P | - | 3.27 \pm 0.09 (n = 8) | |
| | | D | - | 3.66 \pm 0.13 (n = 12) | |
| AUR III | DL | P | - | - | |
| | | D | - | 6.97 \pm 0.19 (n = 11) | |
| | ML | P | - | 6.57 \pm 0.16 (n = 15) | |
| | | D | - | 7.02 \pm 0.11 (n = 20) | |
| | PL | P | - | 6.10 \pm 0.13 (n = 20) | |
| | | D | - | 5.77 \pm 0.11 (n = 20) | |
| AUR IV | DL | P | - | - | |
| | | D | - | - | |
| | ML | P | - | - | |
| | | D | - | - | |
| | PL | P | - | 4.85 \pm 0.14 (n = 13) | |
| | | D | - | - | |

AUR = auriculica sensilla; DL = distal lamellae; ML = medium lamellae; PL = proximal lamellae; P = proximal surface; D = distal surface

Values are mean \pm standard error of the mean; Student *t*-test; ¹Major axis; ²Mann-Whitney Rank Sum test; ^{0.05} = $P < 0.05$; ^{0.001} = $P < 0.001$.

of antennal size and shape is often observed in species that use sex pheromones (Steinbrecht 1987). This is evident in the antennae of several melolontid species, in which males have longer antennae than females (Morón 1986, Kim & Leal 2000, Tanaka *et al* 2006). As in *P. obsoleta* (Romero-López *et al* 2004), the antenna is sexually dimorphic in *P. ravid*a: males have longer pedicel, flagellum, lamellar club segments, and the entire antenna. Furthermore, sexual differences in antennal length are mostly evident in the lamellar club, which is the most important sensorial zone for pheromone and allelochemical perception for these insects (Romero-López *et al* 2004). Because *P. ravid*a males have longer antennae, longer and wider lamellae, and greater antennal area, males can be regarded as the receptors in the sexual chemical communication in this species. Previous studies with *P. obsoleta* provided morphological and biological evidence

that females display a calling behavior during which they expose the protractile genital chamber from the abdominal tip and release chemical compounds that are attractive to males (Romero-López *et al* 2005, 2009).

In several melolontid species, this sexual dimorphism is not evident at the sensilla level, since males and females have identical sensilla types. For example, both sexes of *P. anxia* and *P. obsoleta* have five sensilla types (Ochieng *et al* 2002, Romero-López *et al* 2004). In other genera, there are cases in which a particular sex has two or three identical PLAS types, as in *Phyllopherta horticola* L. (Agren 1985), *Adoryphorus couloni* Burmeister (McQuillan & Semmens 1990), *Anomala cuprea* Hope (Leal & Mochizuki 1993), *Phyllopherta diversa* Waterhouse (Hansson *et al* 1999), and *Dasylepida ishigakiensis* Nijjima & Kinoshita (Tanaka *et al* 2006). Results obtained for *P. ravid*a are similar, although

Table 5 Measurements (μm) of sensilla type basiconica on lamellae of both sexes of *Phyllophaga ravida*.

| Se | La | Su | Length | | Width | |
|---------------------|----|----|--------------------------|--------------------------------------|-----------------|--------------------------------------|
| | | | Female | Male | Female | Male |
| BAS I | DL | P | 5.22 \pm 0.24 (n = 12) | 5.11 \pm 0.28 (n = 7) | 1.80 \pm 0.09 | 1.98 \pm 0.05 |
| | | D | 5.19 \pm 0.19 (n = 20) | 5.05 \pm 0.16 (n = 20) | 1.89 \pm 0.07 | 1.74 \pm 0.09 |
| | ML | P | 6.22 \pm 0.24 (n = 20) | 6.53 \pm 0.27 (n = 6) | 1.56 \pm 0.67 | 1.85 \pm 0.04 ^{0.05 (1)} |
| | | D | - | - | - | - |
| | PL | P | 7.39 \pm 0.28 (n = 20) | 7.09 \pm 0.19 (n = 14) | 1.91 \pm 0.05 | 2.41 \pm 0.08 ^{0.001 (1)} |
| | | D | 7.53 \pm 0.60 (n = 5) | 7.28 \pm 0.47 (n = 4) | 1.71 \pm 0.16 | 2.08 \pm 0.06 |
| BAS II (n = 20) | DL | P | 3.46 \pm 0.13 | 3.62 \pm 0.06 | 1.35 \pm 0.07 | 1.55 \pm 0.04 ^{0.05} |
| | | D | 3.11 \pm 0.15) | 3.75 \pm 0.07 ^{0.001 (1)} | 1.31 \pm 0.04 | 1.49 \pm 0.06 ^{0.05} |
| | ML | P | 3.74 \pm 0.05) | 3.94 \pm 0.05 ^{0.05} | 1.62 \pm 0.10 | 1.79 \pm 0.05 ^{0.05 (1)} |
| | | D | 3.76 \pm 0.07 | 3.85 \pm 0.05 | 1.49 \pm 0.06 | 1.75 \pm 0.05 ^{0.05 (1)} |
| | PL | P | 3.86 \pm 0.05 | 3.61 \pm 0.08 ^{0.05} | 1.78 \pm 0.05 | 1.68 \pm 0.04 |
| | | D | 3.98 \pm 0.06 | 3.65 \pm 0.06 ^{0.001} | 1.81 \pm 0.03 | 1.76 \pm 0.02 |
| BAS III (n = 20) | DL | P | 1.55 \pm 0.03 | 1.49 \pm 0.04 | 1.55 \pm 0.07 | 1.41 \pm 0.04 |
| | | D | 1.52 \pm 0.03 | 1.59 \pm 0.06 | 1.42 \pm 0.04 | 1.48 \pm 0.05 |
| | ML | P | 1.60 \pm 0.10 | 1.83 \pm 0.04 ^{0.05} | 1.58 \pm 0.06 | 1.74 \pm 0.03 ^{0.001 (1)} |
| | | D | 1.47 \pm 0.10 | 1.63 \pm 0.06 | 1.38 \pm 0.04 | 1.58 \pm 0.05 ^{0.001} |
| | PL | P | 1.54 \pm 0.04 | 1.51 \pm 0.05 | 1.46 \pm 0.04 | 1.47 \pm 0.05 |
| | | D | 1.50 \pm 0.04 | 1.67 \pm 0.05 ^{0.05} | 1.47 \pm 0.03 | 1.60 \pm 0.05 ^{0.05} |
| BAS IV | DL | P | - | - | - | - |
| | | D | - | - | - | - |
| | ML | P | 3.86 \pm 0.10 (n = 10) | - | 2.27 \pm 0.03 | - |
| | | D | - | - | - | - |
| | PL | P | 4.50 \pm 0.09 (n = 20) | - | 1.99 \pm 0.02 | - |
| | | D | - | - | - | - |
| BAS V | DL | P | - | - | - | - |
| | | D | - | - | - | - |
| | ML | P | - | - | - | - |
| | | D | - | - | - | - |
| | PL | P | - | 7.0 \pm 0.09 (n = 9) | - | 2.0 \pm 0.03 |
| | | D | - | - | - | - |

Se = sensilla; La = lamellae; Su = surface; BAS = basiconic sensilla; DL = distal lamellae; ML = medium lamellae; PL = proximal lamellae; P = proximal surface; D = distal surface

Values are mean \pm standard error of the mean; Student *t*-test; ¹ = Mann-Whitney Rank Sum test; ^{0.05} = $P < 0.05$; ^{0.001} = $P < 0.001$.

some differences among sexes were observed. In fact, only males show AUS type II, III, and IV, BAS type V and COS type IV, while only females show BAS type IV and COS type II. This sexual dimorphism at sensillar type level is the first record for a melolontid and is less common for other beetles. There is a similar report for *Trogossita japonica* Reitter, a carnivorous beetle, in which only females show a

sensilla-type stylochonica and some sensilla-type basiconica (Rani & Nakamuta 2001).

This morphological study of *P. ravida* receptors is the first for melolontids with several different types of antennal sensilla. In similar studies, antennal lamellae of other species have a maximum of six sensilla types (Ochieng *et al* 2002, Romero-López *et al* 2004, Tanaka *et al* 2006). For Coleoptera

Table 6 Measurements of sensilla type coeloconica on lamellae of both sexes of *Phyllophaga ravidata*.

| Sensilla | Lamellae | Surface | Diameter sensilla measure (μm) ¹ | | | |
|----------|----------|---------|--|---------------------------|--------|------|
| | | | Cuticular cavity | | BAS/CC | |
| | | | Female | Male | Female | Male |
| COS I | DL | P | 16.70 \pm 0.95 (n = 10) | 15.62 \pm 2.63 (n = 3) | 3.60 | 2.67 |
| | | D | - | - | - | - |
| | ML | P | 16.56 \pm 2.21 (n = 7) | 12.21 \pm 1.60 (n = 3) | 3.30 | 2.67 |
| | | D | - | - | - | - |
| | PL | P | 18.40 \pm 3.20 (n = 13) | 16.89 \pm 1.05 (n = 10) | 4.60 | 2.90 |
| | | D | - | - | - | - |
| COS II | DL | P | - | - | - | - |
| | | D | - | - | - | - |
| | ML | P | 14.30 \pm 0.53 (n = 2) | - | 3.0 | - |
| | | D | - | - | - | - |
| | PL | P | 12.33 \pm 0.75 (n = 2) | - | 2.0 | - |
| | | D | - | - | - | - |
| COS III | DL | P | 15.67 \pm 1.87 (n = 2) | 18.20 \pm 3.16 (n = 3) | 2.0 | 2.0 |
| | | D | - | - | - | - |
| | ML | P | 16.26 \pm 1.09 (n = 2) | 11.05 \pm 0.10 (n = 2) | 2.0 | 2.0 |
| | | D | - | - | - | - |
| | PL | P | 9.99 \pm 0.45 (n = 2) | - | 2.0 | - |
| | | D | - | - | - | - |
| COS IV | DL | P | - | - | - | - |
| | | D | - | - | - | - |
| | ML | P | - | - | - | - |
| | | D | - | - | - | - |
| | PL | P | - | 12.73 \pm 0.88 (n = 9) | - | 2.33 |
| | | D | - | - | - | - |

COS = coeloconic sensillae; DL = distal lamellae; ML = medium lamellae; PL = proximal lamellae; P = proximal surface; D = distal surface; CC = cuticular cavity; BAS/CC = basiconic sensillae number (mean)

Values are mean \pm standard error of the mean; Student *t*-test; ¹Major axis.

in general, only thirteen types of sensilla are reported in three different Carabidae species (Ploomi *et al* 2003). Previous records for others species use terminology and classification based largely on Meinecke (1975) and Schneider (1964); these papers report the main sensilla types as placodea, basiconica, and coeloconica (Leal & Mochizuki 1993, Kim & Leal 2000, Baker & Monroe 2005), including the auricular type (Ochieng *et al* 2002, Romero-López *et al* 2004). Some also use different nomenclatures: sensilla types I to VI for one species (Tanaka *et al* 2006) and placoid, basiconical, or trichoid sensilla types A, F, G, H, J, K, L for several species of *Hoplia*, *Isonychus*, *Pelidnota*, *Callirhinus*, *Cotinis*, *Euphoria*, *Tomarus*, and *Aegidium* (Carrillo-Ruiz & Morón 2008).

According to several authors (Schneider 1964, Leal & Mochizuki 1993, Larsson *et al* 1999, Ochieng *et al* 2002,

Romero-López *et al* 2004), TRS and CHS of *P. ravidata* are mechanoreceptors, whereas the other basic types (BAS, COS, and PLAS) are chemoreceptors. Generally, PLAS, BAS and COS are considered the main sensilla involved in chemoreception. PLAS are the most common chemosensilla on male lamellae of melolontids and were reported to be involved in the chemical perception of sex pheromones (Leal & Mochizuki 1993, Romero-López *et al* 2004). Although AUS are traditionally classified as a special type of PLAS (Hansson *et al* 1999, Tanaka *et al* 2006), they have been recently considered a separate type of sensillum and their participation in the perception of sex attractants is suggested (Ochieng *et al* 2002, Romero-López *et al* 2004).

Part of the results obtained in *P. ravidata* clearly shows a sexual dimorphism in AUS, with three or four types being

observed only in males. Almost all lamellae in males of *P. ravida* show three PLAS types and AUS type I, which all have a larger diameter in males than in females (Table 4). This coincides with other reports for melolontids, as *Cotinis nitida* L. and *D. ishigakiensis*, in which PLAS types are larger in males than in females (Baker & Monroe 2005, Tanaka *et al* 2006). For BAS, the size differences are less marked (type II and III principally), and COS in both sexes are of similar dimensions (Tables 5, 6). This represents an evident sexual dimorphism relating to sensilla morphology in *P. ravida*, mainly in PLAS and AUS.

In conclusion, the presence of a large array of different-shaped receptors on the antennal lamellae of *P. ravida* shows a marked sexual dimorphism and suggests a well-developed capability for chemical recognition in this species. The size of lamellae and sensory organs appears consistent with the species' sexual chemical communication, since males have the structures (PLAS and AUS) with the greatest contact surface to receive the sex pheromone released by females. Nevertheless, these data should be complemented with transmission electronic microscopy and traditional electrophysiological assays to elucidate the specific role of PLAS and AUS types. Likewise, the criteria for sensilla classification in melolontids must be unified in order to further elucidate the sexual chemical communication and phylogeny of the group.

Acknowledgments

A. Aragón (BUAP) helped with collection of specimens, T. Laez (INECOL) with scanning electron microscope images and G. Angeles (INECOL) with antennae preparation. A.A.R.L. is grateful to INECOL for financial support during his postdoctoral stay.

References

- Agren L (1985) Architecture of a lamellicorn flagellum (*Phyllopertha horticola*, Scarabaeidae, Coleoptera, Insecta). *J Morphol* 186: 85-94.
- Aragón-García A, Morón M A, López-Olguín J F, Cervantes-Peredo L M (2005) Ciclo de vida y conducta de adultos de cinco especies de *Phyllophaga* Harris, 1827 (Coleoptera: Melolonthidae: Melolonthinae). *Acta Zool Mex* (n s) 21: 87-99.
- Baker G T, Monroe W A (2005) Sensilla on the adult and larval antennae of *Cotinis nitida* (Coleoptera: Scarabaeidae). *Microsc Microanal* 11: 170-171.
- Bourdais D, Vernon P, Krespi I, Lannic J L, Baaren J V (2006) Antennal structure of male and female *Aphidius rhopalosiphi*: description and morphological alterations after cold storage or heat exposure. *Microsc Res Tech* 69: 1005-1013.
- Bozzola J J, Russell L D (1998) *Electron microscopy*. 2nd ed, London, Jones & Bartlett Publishers Inc., 670p.
- Carrillo-Ruiz H, Morón M A (2006) Study of the phylogenetic relationships of the hoplids (Coleoptera: Scarabaeoidea). *Proc Entomol Soc Wash* 108: 619-638.
- Hansson B S, Larsson M C, Leal W S (1999) Green leaf volatile-detecting olfactory receptor neurons display very high sensitivity and specificity in a scarab beetle. *Physiol Entomol* 24: 21-126.
- Kim J Y, Leal W S (2000) Ultrastructure of pheromone-detecting sensillum placodeum of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). *Arthropod Struct Dev* 29: 121-128.
- Larsson M C, Leal W S, Hansson B S (1999) Olfactory receptor neurons specific to chiral sex pheromone components in male and female *Anomala cuprea* beetles (Coleoptera: Scarabaeidae). *J Comp Physiol A* 184: 353-359.
- Leal W S (1998) Chemical ecology of phytophagous scarab beetles. *Annu Rev Entomol* 43: 39-61.
- Leal W S, Mochizuki F (1993) Sex pheromone reception in the scarab beetle *Anomala cuprea*. *Naturwissenschaften* 80: 278-281.
- McQuillan P B, Semmens T D (1990) Morphology of antenna and mouthparts of adult *Adoryphorus couloni* (Burmeister) (Coleoptera: Scarabaeidae: Dynastinae). *J Aust Entomol Soc* 29: 75-79.
- Meinecke C C (1975) Riechsensillen un Systematik der Lamellicornia (Insecta, Coleoptera). *Zoomorphologie* 82: 1-42.
- Morón M A (1986) El género *Phyllophaga* en México (Insecta: Coleoptera). Morfología, distribución y sistemática supraespecífica. Publ No 20, Instituto de Ecología, México, 342p.
- Morón M A (2003) Las especies de *Phyllophaga* (*s.str.*) del grupo *rugipennis* (Coleoptera: Melolonthidae), p.19-34. In Onore G, Reyes-Castillo P, Zunino M (eds) *Escarabeidos de Latinoamérica: estado del conocimiento*. Monografías Tercer Milenio, vol. 3, SEA, Zaragoza, 86p.
- Morón M A (2006) Revisión de las especies de *Phyllophaga* (*Phytalus*) grupos *obsoleta* y *pallida* (Coleoptera: Melolonthidae: Melolonthinae). *Folia Entomol Mex* 45: 1-104.
- Ochieng S A, Robbins P S, Roelofs W L, Baker T C (2002) Sex pheromone reception in the scarab beetle *Phyllophaga anxia* (Col: Scarabaeidae). *Ann Entomol Soc Am* 95: 97-102.
- Oliveira L J, García M A (2003) Flight, feeding and reproductive behavior of *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae) adults. *Pesq Agropec Bras* 38: 179-186.
- Ploomi A, Merivee E, Rahi M, Bresciani J, Ravn H P, Luik A, Sammelselg V (2003) Antennal sensilla in ground beetles (Coleoptera, Carabidae). *Agron Res* 1: 221-228.
- Ramírez-Salinas C, Morón M A, Castro-Ramírez A (2000) Descripción de los estados inmaduros de seis especies de *Phyllophaga* (Coleoptera: Melolonthidae: Melolonthinae) de la región Altos de Chiapas, México. *Folia Entomol Mex* 109: 73-106.
- Rani P U, Nakamuta K (2001) Morphology of antennal sensilla, distribution and sexual dimorphism in *Trogossita japonica* (Coleoptera: Trogossitidae). *Ann Entomol Soc Am* 94: 917-927.

- Romero-López A A, Aragón A, Arzuffi R (2007) Estudio comparativo del comportamiento sexual de cuatro especies de *Phyllophaga* (Coleoptera: Melolonthidae), p.275-281. In Estrada E G, Equihua A, Luna C, Rosas-Acevedo J L (eds) Entomología mexicana. Vol. 6, Publicación especial de la Sociedad Mexicana de Entomología, México, 728p.
- Romero-López A A, Arzuffi R, Morón M A (2005) Feromonas y atrayentes sexuales de coleópteros Melolonthidae de importancia agrícola. Folia Entomol Mex 44: 233-245.
- Romero-López A A, Arzuffi R, Valdez J, Morón M A (2009) Morfología y protrusión-retracción de la cámara genital femenina de *Phyllophaga obsoleta* (Coleoptera: Melolonthidae). Acta Zool Mex (n s) 25: 315-321.
- Romero-López A A, Arzuffi R, Valdez J, Morón M A, Castrejón V, Villalobos F J (2004) Sensory organs in the antennae of *Phyllophaga obsoleta* (Coleoptera: Melolonthidae). Ann Entomol Soc Am 97: 1306-1312.
- Schneider D (1964) Insect antennae. Annu Rev Entomol 9: 103-122.
- Steinbrecht R A (1987) Functional morphology of pheromone-sensitive sensilla, p.353-384. In Prestwich G D, Blomquist G J (eds) Pheromone biochemistry. Orlando, Academic Press, 565p.
- Sukontason K, Sukontason K L, Vogtsberger R C, Boonchu N, Chaiwong T, Piangjai S, Disney H (2005) Ultrastructure of coeloconic sensilla on postpedicel and maxillary palp of *Megaselia scalaris* (Diptera: Phoridae). Ann Entomol Soc Am 98: 113-118.
- Tanaka S, Yukuhiro F, Wakamura S (2006) Sexual dimorphism in body dimensions and antennal sensilla in the white grub beetle, *Dasylepida ishigakiensis* (Coleoptera: Scarabaeidae). Appl Entomol Zool 41: 455-461.
- Wilcox D, Dove S, McDavid W, Greer D B (2002) UTHSCSA Image Tool for Windows version 3.0. The University of Texas Health Science Center in San Antonio, USA.
- Zacharuk R Y (1980) Ultrastructure and function of insect chemosensilla. Annu Rev Entomol 25: 27-47.

Received 17/XI/09. Accepted 31/V/10.
