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Sexual Dimorphism in Antennae of Mexican Species of *Phyllophaga* (Coleoptera: Scarabaeoidea: Melolonthidae)

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Additional information is available at the end of the chapter

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1. Introduction

Sexual dimorphism in body, antennae, lamellae and chemosensilla types of some Mexican species of *Phyllophaga* are recorded. In *Phyllophaga obsoleta* and *Phyllophaga ravidata* the male's body is slightly larger than the female's, and its antennae and lamellae are longer than the females. Meanwhile, in *Phyllophaga opaca* the body and its antennae and lamellae of the males are very similar in size than the females. The external morphology of sensilla on the antennae has been described using scanning electron microscopy. The antennal club of these beetles consists of three terminal plates: proximal, middle, and distal lamellae. In all these species, the main sensilla types were identified on the internal and external surfaces of lamellae from both sexes: placodea (PLAS), auricilica (AUS), basiconica (BAS), coeloconica (COS), trichodea (TRS), and chaetica (CHS). The first four types have been considered as chemosensilla and the last two as mechanoreceptor sensilla. For *P. opaca*, fifteen types of chemosensilla were found: four types of PLAS (I, II, III and VIII), four types of AUS (I, II, III and IV), five types of BAS (I, II, III, IV and V), and two types of COS (I and III). This is very similar to that observed in *P. ravidata* and therefore it is suggested that these data can relate to the taxonomy of the genus *Phyllophaga*, since both species belong to the same subgenus. To give continuity to this type of comparative studies with other Mexican species of Melolonthidae to complement the information on the phylogeny of the group, is necessary in addition to their chemical communication, information about their sexual dimorphism focused on antennal micro-morphology, genital structure, and reproductive behavior.

2. Sexual dimorphism in Coleoptera

Outstanding structural differences between males and females of many species of beetles have been described by naturalist and scientists during the last 200 years. Most part of such

differences is located on the head and prothorax of males, such as in the form and size of the mandibles, length of antennal or palpi segments, horn-like structures on the frons or on the pronotal surface. Other differences are found in the size of whole body, development of elytra and hind wings, as well as in the length of fore and hind legs, or in the shape of pygidial plate. Less frequent is the strong difference in color or body vestiture. Flightless females are commonly found in species of many families, but larval-like females are the rule into the species of Phengodidae [1,2,3,4]. Sexual dimorphism is more common in the species of the suborder Polyphaga than in the suborder Adephaga, and appears to be a recent feature, because few fossil specimens of diverse families dated from Pliocene and Pleistocene shown dimorphic structures [5,6,7].

3. Sexual dimorphism in Coleoptera Scarabaeoidea

“A most remarkable distinction between the sexes of many beetles is presented by the great horns which rise from the head, thorax and clypeus of the males... These horns, in the great family of Lamellicorns, resemble those of various quadrupeds, such as stags, rhinoceroses, etc. and are wonderful both from their size and diversified shapes. ... The females generally exhibit rudiments of the horns in the form of small knobs or ridges; but some are destitute of even a rudiment.... The extraordinary size of the horns, and their widely different structure in closely-allied forms, indicate that they have been formed for some important purpose; but their excessive variability in the males of the same species leads to the inference that this purpose cannot be of a definite nature. ... The most obvious conjecture is that they are used by the males for fighting together; but they never had been observed to fight...” [8].

Members of the superfamily Scarabaeoidea (Lamellicornia and Pectinicornia) exhibit ones of the most striking dimorphic structures of the beetle species. Cephalic horn-like projections are present in the males of species from the subfamilies Dynastinae, Rutelinae, Cetoniinae, Scarabaeinae, and Geotrupinae. Much enlarged mandibles are common in the males of the subfamilies Lucaninae, Dorcinae, Lampriminae, Chiasognathinae and Odontolabinae. Males of Melolonthinae, Rutelinae and Pleocomidae frequently show the segments of the antennal club enlarged and expanded. Pronotal horn-like or tooth-like projections are developed in the males of the subfamilies Dynastinae, Cetoniinae, Scarabaeinae, Geotrupinae and Orphninae. Much enlarged fore legs (femur, tibia, tarsus) are characteristic in males of the subfamily Euchirinae, meanwhile males of the genus *Pachylomera* (Scarabaeinae) have fore femora much swell. Enlarged hind legs (femur and tibia) are observed in males of some genera of Rutelinae and Scarabaeinae. Females apterous or with short hind wings have been reported in nearly all the subfamilies of Scarabaeoidea, but the female of *Pachypus candidae* Petagna (Pachypodinae) lacks also the elytra. Except for horn-like structures, usually, males have smaller and slender bodies than females, but females of the family Pleocomidae are much larger and stouter than males [9,10].

3.1. Dimorphism and behavior in Melolonthidae (chafers, rhinoceros beetles)

Following the classification of [11] updated by [12], the family Melolonthidae (“melolonthids”) is formed by the world wide members of Melolonthinae (sensu lato),

Rutelinae and Dynastinae. Some species of these subfamilies are famous by its striking sexual differences. First example, each antenna of the male of *Polyphylla petiti* Guérin (Melolonthinae) is formed by seven enlarged, flattened antennomeres that are five times longer than the remainder three basal antennomeres, meanwhile each antenna of the female is formed by five briefly elongated antennomeres that are as long as the remainder five antennomeres. It is evident that the much expanded olfactory surface of the male is developed in correspondence with the perception of sex compounds produced by the female [13].

Second example, metasternum of the male of *Chrysina macropus* Francillon (Rutelinae) strongly produced, hind coxa is wide, hind femur is swollen and provided with a strong spine, and the tibia is enlarged and curved with setae on the inner border (Figures 1 A-B), in the female the metasternum is flattened, hind coxa and femur are much narrower than in male and hind tibia is short and nearly straight, without setae on the inner border. Looks like the male embraces female with his large hind legs during coupling, but really these legs are not used in this form. It is much possible that are useful during combat with other co-specific males as forceps formed between femur and tibia, that may produce much force derived from the increased musculature inside the metathorax, coxa and femur; such forceps also may be used as defense against big predators [Morón unpublished data].

Third example, horn-like projections on the head and pronotum of *Dynastes hercules* L. (Dynastinae) may be as long as body length or even longer than this. Both projections may act as long forceps, applying force derived from cervical muscles to head projection. Such force is sufficient to cut or broken the elytra of other male of "Hercules beetle", but is useful to embrace it a turnout of the tree branches where they inhabit, after fighting during some minutes. But this conclusion needed many years to mature, because much speculation surrounded the observation of dead specimens in collections, and studies of live beetles in nature or in captivity were scarce and incomplete during near a century. After a long discussion on the dimorphic structures in horned beetles, with arguments and data of other authors, including Darwin, [14] concluded that "The horns of a beetle, the size of which is increasing gradually as generations succeed one another, will as a result become more and more disproportionate in size, regardless of the fact that they may be quite useless, and the absence of the restraining and modeling influence of natural selection will be a contributory cause of the acquisition of fantastic forms."

A large number of observations on captive Hercules beetles in Venezuela support the following comments: "The sequence of the beetle encounter is unvarying. The two meet head on, and the projecting horns touch and click, spread wide and close, the whole object of the opening phase being to get a grip outside the opponent's horns. When the four horns are closed together, there is a deadlock. All force is now given to pinching, with the apparent desire to crush and injure some part of head or thorax. Again and again, both opponents back away, freeing their weapons, and then rush in for a fresh grip. Once this hold is attained and a firm grip secured, the beetle rears up and up to an unbelievably vertical stance. At the zenith of this pose it rests upon the tip of the abdomen and the tarsi of



Figure 1. *Chrysina macropus* Francillon. (A) Male, ventral view, showing the hypertrophied hind legs. (B) Female, ventral view. Scale lines 5 mm. Photos M. A. Morón.

the hind legs, the remaining four legs outstretched in mid air, and the opponent held sideways, kicking impotently... after 2-8 seconds the victim is slammed down or is carried away ... before to banging to earth will take place” [15]. Other academics discussed on the essential components of such phenomenon or on details of the above described behavior, as [16] who comments: “The display of species showing extreme intrasexual selection function both to attract females to intimidate other males. Precopulatory displays are short or absent. The male Hercules beetle, for example, evidently engages in none whatever. Occasionally he picks a female up and carries her aimlessly about for a short while, but the significance of the behavior is unknown. During both transportation and copulation the female remains outwardly passive”. With elegance and precision, [17] objectively wrote: “The only function for beetle horns which has been confirmed by detailed observations is that of weapons for use in intraspecific fights. Many horn designs remain to be studied... More data are needed to answer outstanding questions about the significance of multiple horn designs and the selective pressures favoring fighting in some species. Several factors may have predisposed beetles to evolve horns more readily than other insects”.

3.2. Study of dimorphism in melolonthid beetles in Mexico

The males of nearly all the species of Melolonthidae usually present a number of small structural differences more or less directly associated with the search of females and coupling behavior. Most of these modifications are located in the legs, as tarsal pads, specially curved tarsal claws, tibial spurs, rows of tarsal setae, etc. Other modifications are

developed on the last sternites and pygidial plate [18]. Many of these are frequently applied as taxonomic characters, but its function remains generalized. Few studies on the allometric growth of hypertelic structures in species with strong sexual dimorphism had been published in Mexico [19,20].

Scarce research had been carried on the microstructures, mainly on those related with sensorial functions, as the diverse types of sensilla placed on the lamellar surfaces of antennomeres such as was detailed by [21]. These structures were briefly studied in some Mexican species by [18,22,23,24]. The structure, distribution and function of the antennal sensilla reveal much interest, because they represent the main way to understand the pre-reproductive behavior, and the evolution of the chemical communication in this group of insects.

4. Studies of sexual dimorphism in antennae of Mexican species of *Phyllophaga*

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 [25]. Chemicals released from melolonthid females are captured in the sensilla located on both sides of male antennal lamellae [21,18,26]. The genus *Phyllophaga* is formed by more than 800 species, but only in *Phyllophaga anxia* LeConte [27] and in the antennae of Mexican species *Phyllophaga obsoleta* Blanchard [23] and *Phyllophaga ravidata* Blanchard [24] have the different types of sensilla been studied. In the following sections data from *P. obsoleta* and *P. ravidata* are remembered and compared with the data of another species of *Phyllophaga* distributed in Mexico.

4.1. Methodology for the study of antennae of Mexican melolonthids

4.1.1. Measurement of body, antennae and lamellae dimensions

After taxonomical identification using the keys proposed by Morón [18], females and males of *P. obsoleta*, *P. ravidata* and *Phyllophaga opaca* Moser were randomly chosen for length measurement with the Image Tool 3.0 software program [28]. Length was measured in each specimen from the clypeus to the pygidium. The head of each previously measured specimen was separated from the body and preserved in 70% ethanol. The antennae of females and males of each species were separated and measurements of total length, width, and area of each lamella, 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 (internal or external surfaces). The lamella located farther away from the head was denominated distal lamella (DL), while the nearest was called proximal lamella (PL) and the one between these two, middle lamella (ML).

4.1.2. Specimen preparation for light microscopy studies

Antennae from females and males of each species 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.

4.1.3. Specimen preparation for scanning electron microscopy studies

Lamellae from additional specimens were prepared following the methods proposed by [29] and were examined at 25kV under a scanning electron microscope (JEOL Mod. JSM-5600LV).

4.1.4. Statistics

Data on body, antennae and lamellae dimension for *P. opaca*, *P. ravidata* and *P. obsoleta*, and adult males and females were analyzed with Student's t or Mann-Whitney Rank Sum tests (SigmaStat 3.1; 3.5). Unless otherwise stated, all values reported are mean \pm standard error.

5. Sexual dimorphism in antennae of *Phyllophaga obsoleta*

This is one of the most noxious *Phyllophaga* species in México [30]. *Phyllophaga obsoleta* female's body length is larger than males, but the lamellar club is significantly longer and wider in males than females (Figures 2 A-B, Table 1). In males, PL and ML were longer and had a larger area than in females (Table 1). Also, DL in males were longer, had a larger area and greater width than in females (Table 1).

Classification and terminology of sensilla types used here are based principally on [31] and in part on [21,32,27]. Six types of sensilla in both surfaces of antennal lamellae of both sexes of *P. obsoleta* were identified: sensilla placodea (PLAS), sensilla auricilica (AUS), sensilla basiconica (BAS), sensilla coeloconica (COS), sensilla trichodea (TRS) and sensilla chaetica (CHS). In Figure 3, these different sensilla types are showed. PLAS are thin-walled plates or are low dome shaped and BAS are cone-shaped. AUS are characterized by a "rabbit-ear" shape. COS are rarely found as aggregations of long rods located inside cuticle cavities. COS are observed only on internal surfaces of all lamellae from both sexes. TRS have a long hair-like structure that occurs along the peripheral edges while CHS present a short-bristle- or spine-like structure; they occur along the peripheral edges and some are in the lamellar center. According with above cited authors, TRS and CHS types are most likely mechano-receptors, but PLAS, AUS, BAS, and COS are considered chemo-receptor types.



Figure 2. Habitus of species of *Phyllophaga*. *Phyllophaga obsoleta*, (A) male; (B) female. *Phyllophaga ravida*, (C) male; (D) female. *Phyllophaga opaca*, (E) male; (F) female. Scale lines 5 mm. Photos M. A. Morón.

MEASURE (mm)	POBS			PRAV			POP		
	F	M	p	F*	M*	p	F**	M**	p
Body	18.18 ± 0.3	17.19 ± 0.2	<0.05	22.13 ± 0.3	21.17 ± 0.2	<0.05	22.12 ± 0.2	21.16 ± 0.5	0
Entire antennae	3.30 ± 0.08	3.90 ± 0.04	<0.001	3.37 ± 0.02	4.07 ± 0.05	<0.001	2.88 ± 0.03	3.08 ± 0.05	<0.001
Antennal flagellum	2.15 ± 0.08	2.20 ± 0.04	0	0.99 ± 0.01	1.06 ± 0.01	<0.05	1.80 ± 0.03	1.98 ± 0.04	<0.001
Antennal club	1.15 ± 0.02	1.70 ± 0.02	<0.001	1.15 ± 0.01	1.71 ± 0.02	<0.001	1.09 ± 0.01	1.10 ± 0.01	0
PL length	1.07 ± 0.02	1.66 ± 0.04	<0.001	1.19 ± 0.01	1.71 ± 0.04	<0.001	0.92 ± 0.02	0.95 ± 0.02	<0.05
PL area (mm ²)	0.28 ± 0.01	0.51 ± 0.02	<0.001	0.38 ± 0.01	0.63 ± 0.02	<0.001	0.25 ± 0.01	0.26 ± 0.01	0
PL width	0.35 ± 0.01	0.38 ± 0.01	0	0.41 ± 0.01	0.46 ± 0.01	<0.05	0.35 ± 0.01	0.36 ± 0.01	0
ML length	1.10 ± 0.02	1.60 ± 0.03	<0.001	1.13 ± 0.01	1.67 ± 0.03	<0.001 ⁽¹⁾	0.81 ± 0.01	0.90 ± 0.02	<0.001
ML area (mm ²)	0.29 ± 0.03	0.48 ± 0.04	<0.001	0.36 ± 0.01	0.61 ± 0.02	<0.001	0.19 ± 0.01	0.22 ± 0.01	<0.05
ML width	0.36 ± 0.01	0.37 ± 0.01	0	0.41 ± 0.01	0.47 ± 0.01	<0.05	0.30 ± 0.01	0.32 ± 0.01	<0.05
DL length	1.04 ± 0.03	1.58 ± 0.04	<0.001	1.04 ± 0.01	1.60 ± 0.03	<0.001	0.69 ± 0.02	0.82 ± 0.01	<0.001
DL area (mm ²)	0.23 ± 0.01	0.44 ± 0.01	<0.001	0.31 ± 0.01	0.54 ± 0.01	<0.001	0.13 ± 0.02	0.17 ± 0.01	<0.001
DL width	0.31 ± 0.01	0.37 ± 0.01	<0.001	0.38 ± 0.01	0.42 ± 0.01	<0.001	0.24 ± 0.01	0.27 ± 0.01	<0.05

Values are mean ± standard error of the mean; *Student t*-test; 0= not significant; ⁽¹⁾= Mann-Whitney Rank Sum test
 POBS: *Phyllophaga obsoleta*, n= 12; PRAV: *Phyllophaga ravida*, n= 14; POP: *Phyllophaga opaca*, n= 13; F= females; M= males; * n=20; **= n=6; PL = proximal lamella; ML = middle lamella; DL = distal lamella

Table 1. Comparison of the body and antennae dimensions, length, width and area of the lamellae of females and males of *Phyllophaga obsoleta*, *Phyllophaga ravida* and *Phyllophaga opaca*.

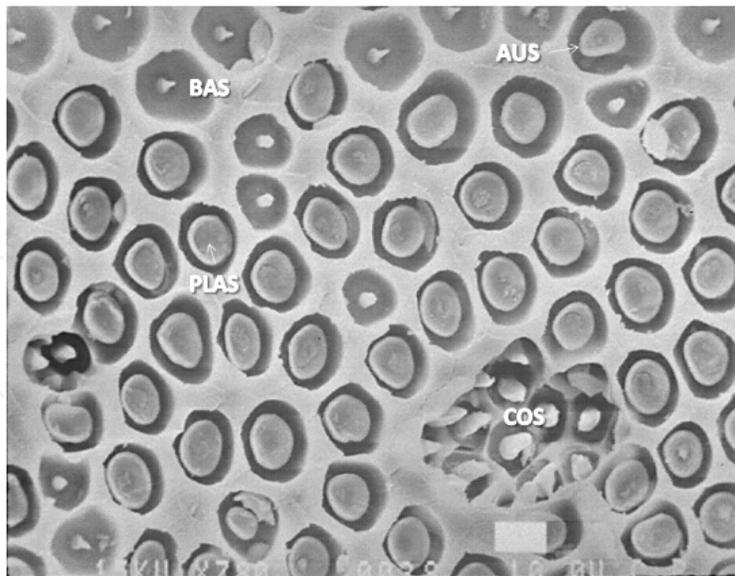


Figure 3. Scanning electron-micrograph of the internal surface (IN) of male *Phyllophaga obsoleta* middle lamella (ML). The IN is almost entirely covered by four sensillar types. In the periphery of the lamella, TRS and CHS are observed. B) TRS (long hair or setae shape). C) CHS (spine shape). D) PLAS (thin-walled plate or low dome), BAS (cone- or rod-shape) and AUS (‘‘rabbit-ear-like’’) on the IN of the ML. E) Another view of PLAS, BAS and AUS. F) PLAS, BAS, AUS and COS (long rod aggregations located inside cuticle cavities). G) Detail of PLAS, BAS and COS. Micrographies by J. Valdez.

6. Sexual dimorphism in antennae of *Phyllophaga ravidata*

Phyllophaga ravidata is included in the “*dentex*” complex of the “*ravidata*” species group, subgenus *Phyllophaga* (*sensu stricto*), and is one of the main white grub species of agricultural and economic importance in Mexico [30]. Several sex-related differences in antennae are observed. Although the body of adult *P. ravidata* females is larger than that of males, the entire male antenna is considerably longer (Figures 2 C-D, Table 1). In males, DL is longer, wider, and cover a larger area than in females. Furthermore, the male ML is longer, with a larger area and greater width. Finally, male PL is longer, with a larger area and greater width (Table 1). Several types of sensilla were observed on *P. ravidata* antennae using light- and scanning electron-microscopy: PLAS, AUS, BAS, COS, TRS and CHS (Table 2). TRS have a long spine- or hair-like structure and CHS present a short-spine, being shorter than TRS (Table 2). For *P. ravidata*, sixteen chemo-sensilla types were found: three types of PLAS, four of AUS, five of BAS, and four of COS.

Morphological characteristics of each chemo-sensilla type are described in the Table 2.

Sensilla types	Morphological characteristics
PLAS I	large spherical plates
PLAS II	spherical or elliptical and thin-walled plates
PLAS III	small and elliptical thin-walled plates or low dome-shaped plates
AUS I	elliptical and thin walled-plates with “rabbit-ear” shape
AUS II	small “rabbit-ear” shaped structures, elliptical and low dome-shaped
AUS III	“human-ear” shaped structures
AUS IV	small “rabbit-ear with neck” or “raisin with neck” shaped structures
BAS I	large peg- or cone-shaped
BAS II	short-spine shaped
BAS III	short peg- or cone shaped
BAS IV	serrated-cone shaped
BAS V	long-rod shaped
COS I	aggregations of 2 to 16 long peg- or cone-shaped structures (BAS I)
COS II	aggregations of 2 to 3 serrated cone-shaped structures (BAS IV)
COS III	long, rod-shaped aggregations (BAS V)
COS IV	aggregations of a long cone (BAS I) and a spherical plate (PLAS I)
TRS	long hair-like structure
CHS	short-bristle- or spine-like structure

PLAS= sensilla placodea; AUS= sensilla aurilica; BAS= sensilla basiconica; COS= sensilla coeloconica; TRS= sensilla trichodea; CHS= sensilla chaetica.

PLAS, AUS, BAS and COS are present on internal surface of lamellae while TRS and CHS are structures that occurs along the peripheral edges of each lamella and some are in the lamellar center.

Table 2. Morphological description of each of the types of antennal sensilla observed in the lamellae of males or females of *Phyllophaga ravidata*.

For both sexes, PLAS type I were observed randomly distributed on internal surface (IN) of all lamellae mainly in the center, except on peripheral edges of lamellae. PLAS type II were observed randomly distributed on IN and at the peripheral edges of all lamellae in both male and females. PLAS type III were located on IN of all lamellae, principally on pit, basal, and peripheral edges in both sexes. AUS type I were observed randomly distributed on IN and peripheral edges of all lamellae for both sexes. AUS type II was observed only in male lamellae, distributed on both sides (except on the external surface of the DL). AUS type III was located only on male lamellae, randomly distributed on IN and on peripheral edges (except on the IN of DL). AUS type IV were restricted to the center of PL in males. BAS type I were observed randomly distributed mostly on IN of all club lamellae of both sexes (except on peripheral edges and on the IN of ML). BAS type II are present for both sexes, they are situated on IN and peripheral edges of all lamellae. For both sexes, BAS type III were observed randomly distributed principally on IN of all club lamellae, except on peripheral edges. BAS type IV were found only in females at the center of the IN of ML and PL. BAS type V were found only in males, mainly at the center of the IN of PL. For both sexes, COS type I are restricted principally to the center, appearing only at the IN of all club lamellae. COS type II are located in both sexes, on the floor of cuticle cavities. COS type II are restricted only to females, mostly at the center and only on the IN of all club lamellae, except on DL. COS type III are located inside cavities in the antennal cuticle. COS III are restricted principally to the center, appearing only on the IN of all club lamellae in both sexes, except on male PL. COS type IV are found only in males and are restricted to the center and IN of PL. They are located inside depressions or cavities in the antennal cuticle.

7. Sexual dimorphism in antennae of *Phyllophaga opaca*

Phyllophaga opaca is distributed in the states of Michoacan and Sinaloa, Mexico [30]. Some general characteristics of its flight and eating habits [33], the nature of its chemical sexual communication, and the structures involved in the production of its sex pheromone [25] are known, although there are no data regarding of its sexual dimorphism in body and antennae. Although the body of adult *P. opaca* males is very similar in size than the females, the entire male antenna is significantly longer (<0.001) (Figures 2 E-F, Table 1). In males, DL and ML were longer and had a larger area than in females (Table 1). Also, PL in males were longer than in females (Table 1).

PLAS, AUS, BAS, COS, TRS and CHS were observed on *P. opaca* antennae (females and males) using light- and scanning electron-microscopy (Figures 4 and 5). TRS have a long spine- or hair-like structure and CHS present a short-spine, being shorter than TRS (Table 2, Figure 4). For *P. opaca*, fifteen chemo-sensilla types were found: four types of PLAS, four of AUS, five of BAS, and two of COS.

For both sexes, PLAS type I were observed randomly distributed on IN of all lamellae mainly in the center, except on peripheral edges of lamellae; they are rounded and elongated plates (Figures 4C-D, Table 2). PLAS type II were observed in both males and females, randomly distributed on IN of all lamellae mainly in the center; they are spherical

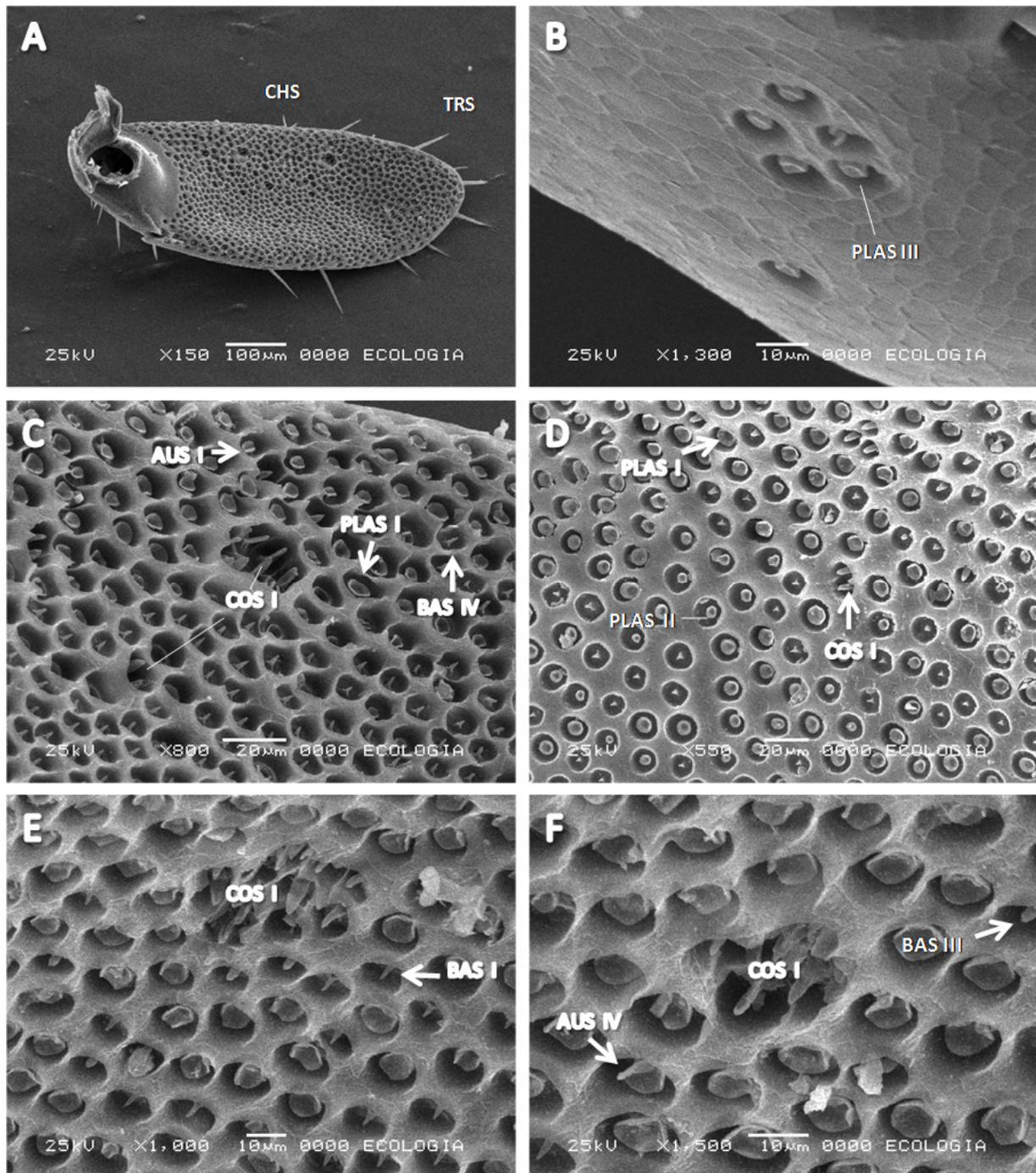


Figure 4. Sensilla on the antennae of female *Phyllophaga opaca*. A) Internal surface (IN) of distal lamella (DL). In the periphery of the lamella, trichodea (TRS) and chaetica sensillum (CHS) are observed. B) Detail of placodea sensilla (PLAS) type III on external (EX) of proximal lamella (PL). C) Coeloconica sensilla (COS) type I, auricillica sensilla (AUS) type I, basiconica sensilla (BAS) type IV and PLAS I on IN of DL. D) Detail of COS I, PLAS I y PLAS II on IN of medium lamella (ML). E) COS I and BAS I on IN of PL. F) Detail of COS I, AUS IV and BAS III on IN of PL. Micrographies by T. Laez.

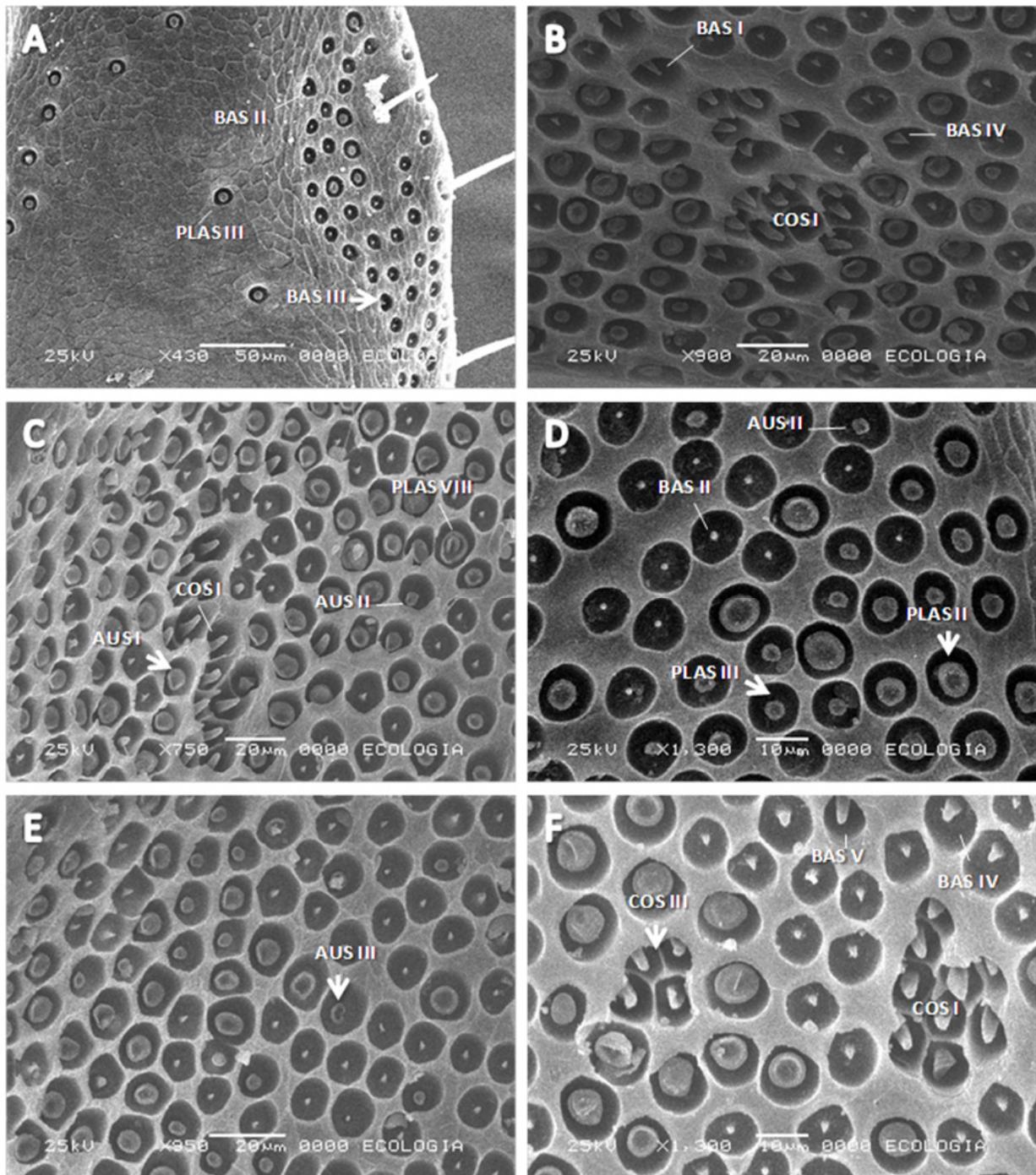


Figure 5. Sensilla on the antennae of male *Phyllophaga opaca*. A) External surface (EX) of proximal lamella (PL). Detail of placodea sensilla (PLAS) type III, basiconica sensilla (BAS) type II and III. B) Coeloconica sensilla (COS) type I, BAS I and BAS IV on internal surface (IN) of distal lamella (DL). C) Detail of auriclica sensilla (AUS) type I and II, COS I and PLAS VIII on IN of PL. D) PLAS II, PLAS III, AUS II and BAS II on IN of medium lamella (ML). E) Detail of AUS III on IN of PL. F) COS I, COS III, BAS IV and BAS V on IN of PL. Micrographies by T. Laez.

or elliptical and thin-walled plates (Figures 4D-5D, Table 2). PLAS type III are located on both sides of all lamellae, principally on pit and peripheral edges in both sexes; they are small, elliptical, thin-walled plates or low dome-shaped plates (Figures 4B-5A, Table 2). PLAS type VIII are chemoreceptors not reported previously; they are spherical or elliptical and thin-walled plates with “human-ear” like structure in the center. PLAS VIII were observed only in males lamellae, randomly distributed on IN of DL (Figure 5C).

AUS type I were observed randomly distributed on IN of all lamellae for both sexes; they are characterized by a “rabbit-ear” shape or elliptical and thin-walled plates (Figures 4C-5C, Table 2). AUS type II were observed only in male lamellae, distributed on IN of ML and DL; they are “rabbit-ear” shaped structures, elliptical and low dome-shaped (Figures 5C-D, Table 2). AUS type III were located only on male lamellae, randomly distributed on IN of lamella center (except on the IN of DL); they are characterized by a “human-ear” shape (Figures 5E, Table 2). AUS type IV were restricted to the center of PL in both sexes; their shape is characterized as “rabbit-ear with neck” or “raisin with neck” structures (Figure 4F, Table 2).

BAS type I were observed randomly distributed mostly on IN of all club lamellae of both sexes (except on peripheral edges and on the external surface of ML); they are large peg- or cone-shaped (Figures 4E-5B, Table 2). BAS type II are short-spine shaped; for both sexes, they are situated on both sides of all lamellae (Figure 5D, Table 2). BAS type III were observed for both sexes distributed on both sides of all lamellae; they are short-cone shaped (Figures 4F-5A, Table 2). BAS type IV were found randomly distributed on IN of all club lamellae of both sexes of PL and DL; they are serrated-cone shaped (Figures 4C-5B-5F, Table 2). BAS type V were found only in males, mainly at the center of the IN of PL; they are long-rod shaped (Figure 5F, Table 2).

For both sexes, COS type I are restricted principally to the center, appearing only at the IN 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 (Figures 4 and 5, Table 2). COS type II were found as aggregations of two to three serrated cone-shaped structures (BAS IV) located on the floor of cuticle cavities. These cavities vary in size between 11.58 and 17.54 μm on the largest axis. COS type III are restricted principally to the center, appearing only on IN of male PL (Figure 5F, Table 2).

8. Analysis between species - sexual dimorphism

As described in this chapter, in the melolonthids there is a marked sexual dimorphism at different levels. In the case of the members of the genus *Phyllophaga*, mainly for Mexican species have been studied some morphological traits that allow differentiate between females and males. For antennae, at least three species studied show sexual dimorphism in body size, length of the antennae and lamellae, and the presence/absence of certain types of sensilla. Sexual dimorphism in antennae of these species is evident when considering antennal/body length ratio. For *P. obsoleta* and *P. ravida* males, the length of the antennal club represent almost one fourth of the body length (Table 1). This is evident in the antennae

of several melolonthid species, in which males have longer antennae than females [18,10,34,35]. 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 [23]. Because *P. obsoleta*, *P. ravidata* and *P. opaca* males have longer antennae, longer and wider lamellae, and greater antennal area, males can be regarded as the receptors in their sexual chemical communication [25]. Previous studies with these species have 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 [36,25].

In *P. opaca*, the size and proportions of antennae of male is nearly similar as in female, so may be hypothesised that olfactory sensilla works in different form or that sexual attractant is distinct. Functional olfactory antennal surface (club lamellar area) in male of *P. opaca* is two times smaller than in the antennae of *P. ravidata*, and 1.5 times smaller than in the antennae of *P. obsoleta* (Table 1).

The morphological study for *P. opaca* receptors is the second for melolonthids with several different types of antennal sensilla (after the *P. ravidata* record). In similar studies, antennal

Sensilla types	POBS	PRAV	POP
PLAS I	X	X	X
PLAS II	X	X	X
PLAS III	X	X	X
AUS I	X	X	X
AUS II	X	X	X
AUS III		X	X
AUS IV		X	X
BAS I	X	X	X
BAS II	X	X	X
BAS III	X	X	X
BAS IV		X	
BAS V		X	
COS I	X	X	X
COS II		X	
COS III		X	X
COS IV		X	
TRS	X	X	X
CHS	X	X	X

PLAS= sensilla placodea; AUS= sensilla auricilica; BAS= sensilla basiconica; COS= sensilla coeloconica; TRS= sensilla trichodea; CHS= sensilla chaetica.

PLAS, AUS, BAS and COS are present on internal surface of lamellae while TRS and CHS are structures that occurs along the peripheral edges of each lamella and some are in the lamellar center.

POBS: *Phyllophaga obsoleta*

PRAV: *Phyllophaga ravidata*

POP: *Phyllophaga opaca*

Table 3. Comparison of the different sensilla types of the lamellae of females and males of *Phyllophaga obsoleta*, *Phyllophaga ravidata* and *Phyllophaga opaca*.

lamellae of other species have a maximum of six sensilla types [27,23,35]. For Coleoptera in general, only thirteen types of sensilla have been reported in three different Carabidae species [37]. Previous studies for others species use terminology and classification based largely on [21] and [31]; these papers report the main sensilla types as PLAS, BAS, and COS [34,38], including the AUS [27,23]. Part of the results obtained in *P. opaca* clearly show a distinct sexual dimorphism principally with PLAS VIII and COS III, observed only in males. The data obtained at the time allow viewing two main perspectives to continue with studies of this type: data useful in taxonomy and data that allow relating the morphology of the sensilla with the sexual behaviour of the Mexican species of *Phyllophaga*. On the one hand, we found evidence that the types of antennal receptors could be support some subgroups into the studied genus. For example, the main types of chemo-sensilla were presented in both *P. ravidata* and *P. opaca* studied: PLAS I, PLAS II, PLAS III, AUS I, AUS II, AUS III, AUS IV, BAS I, BAS II, BAS III, COS I and COS III (Table 3), which belong to the subgenus *Phyllophaga*.

Phyllophaga obsoleta, *P. ravidata* and *P. opaca* belongs to three of the groups of Mexican species proposed by [18,30]. During forthcoming years we expect to study representative species of most of the remaining 38 groups, their sexual dimorphism, phocused on antennal micro-morphology, genital structure, production of chemical attractants and reproductive behavior.

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