

Morphology and Distribution of Sensilla on the Antennae of *Hylamorpha elegans* Burmeister (Coleoptera: Scarabaeidae)

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Introduction

The scarab beetles belong to the insect superfamily Scarabaeoidea, with more than 31,000 species that have diversified during 200 million years of evolution. Most pest species are found within the family Scarabaeidae, recording about 2,300 genera and 25,000 species that are within 20 subfamilies and numerous tribes (Leal 1998, Jackson & Klein 2006). The scarabaeids are widespread and endemic beetles that can be found in all types of environments, wherein they can represent economically significant pests, both in the larval and adult stages, due to the damage that they inflict on roots and leaves, stems, flowers, and fruits of crops and trees (Leal 1998, Ochieng *et al* 2002, Quiroz *et al* 2007). One such beetle is the white grub, *Hylamorpha elegans* Burmeister, which is found in Central and Southern Chile and Southwestern Argentina (Ratcliffe & Ocampo 2002). The larvae of these beetles cause severe agricultural damage in Chile, where they feed on the roots of grasses and small grain cereals (Quiroz *et al* 2007, Millas & Carrillo 2011), and the adults occasionally cause severe defoliation on their secondary host, *Nothofagus* species, principally *Nothofagus*

Abstract

Considering that sensilla constitute important functional elements of sensory systems in insects, the aim of this study was to determine the type and distribution of sensilla in the antennae of *Hylamorpha elegans* Burmeister examined by scanning electron microscopy. *Hylamorpha elegans* antennae are lamellate and consist of the scape, pedicel, and flagellum. The antennal club of this beetle consists of three terminal plates: proximal, middle, and distal lamellae. Four types of sensilla were observed in the lamellae from both sexes: sensilla trichoidea, chaetica, coeloconica, and placodea. Antennal length was larger in males than in females, and significant sexual variation in the number of sensilla placodea and sensilla coeloconica was observed.

antarctica, *Nothofagus betuloides*, *Nothofagus dombeyi*, and *Nothofagus obliqua*, and young trees have been killed by huge numbers of *H. elegans* feeding on them (Ratcliffe & Ocampo 2002).

Biological and chemical controls have been unsuccessful in *H. elegans* control, and the use of semiochemicals such as pheromones and kairomones has been poorly described. To date, one study by Quiroz *et al* (2007) examined the chemical ecology in this species, indicating that 1,4-benzoquinone released by virgin females of *H. elegans* elicited an attraction response from males.

Olfaction is an important sensory modality for insects, used for locating mates, food, prey, and oviposition sites (Larsson *et al* 2001). The antennae contain a concentration of olfactory chemoreceptors (Klowden 2007), conferring the ability to discriminate a myriad of essential chemical signals from the irrelevant chemical compounds in the environment (Leal 2003). Considering these issues and the important role of chemical cues in the mating behavior of *H. elegans*, the purposes of this study were to describe the types and distribution of the antennal sensilla of *H. elegans* and to propose their putative function. The information generated here is

essential for future electrophysiological and behavioral studies of the antennal sensory system involved in chemical communication of *H. elegans*.

Material and Methods

Insect antenna

Imagoes of *H. elegans* were collected by using a light trap in the Regional Research Center INIA-Carillanca, La Araucanía, Chile. Sexual dimorphism in adults was described by Ratcliffe & Ocampo (2002), and these characteristics were used to determine the sex of individuals.

Light microscopy

The samples were prepared according to Romero-López & Morón (2013). Antennae from females and males of freshly killed beetles were soaked in 10% KOH at 80°C for 30 min, rinsed in distilled water at the same temperature for 30 min, and dehydrated in 70% and 100% ethanol for 30 and 60 min, respectively. The antennae were placed in xylene for 10 min for clearing, mounted in Canadian balsam, and observed under a light microscope (Motic, mod. BA310). Images of the antennae were obtained using the Motic Images Plus 2.0 software.

Scanning electron microscopy

The samples were prepared following Tanaka *et al* (2006) with modifications. The antennae of 20 males and 20 females were dehydrated in 30%, 50%, 70%, 90%, and 99% *v/v* ethanol for 3 min each. They were dried at the critical point of CO₂ and gold coated for 20 min in an Edwards S150 Sputter Coater critical point dryer (Edwards, UK). The specimens were viewed using a LEO 420 microscope (Carl Zeiss SMT, Oberkochen, Germany) at 5 kV.

Image and data analysis

The images obtained by scanning electron microscopy (SEM) were processed using ImageJ software v. 1.44p (National Institutes of Health, USA), and the data obtained from the length and width of lamellae and from the number of the different types of sensilla found were compared between sexes by means of Student's *t* test (Zar 1999) using StatsDirect v. 2.7.8.

Results

Hylamorpha elegans has a typical lamellate scarab antenna, subdivided into three usual parts: the basal segment, scape,

which is attached to the head; pedicel, which is attached to the scape; the flagellum composed of five flagellomeres; and three terminal plates. These are the distal, middle, and proximal lamellae (Fig 1). In males, proximal lamellae (pl), middle lamellae (ml), and distal lamellae (dl) were longer than in females. Furthermore, proximal and distal lamellae are wider in males than in females (Table 1, Fig 2).

Types of sensilla

Sensilla morphology used in this work is based upon terminology used by Keil (1999). Male and female antennae have four different types and some subtypes of sensilla: sensilla chaetica (sch), sensilla trichoidea (str), sensilla placodea (spc), and sensilla coeloconica (sco).

Sensilla chaetica

This sensillum type was found along the entire length of the antennae of both sexes, but mainly in the external side of the proximal lamella, where it is possible to observe sensilla having short bristles, each with a broad base and a pointed apex. These are designated as sensilla chaetica type I (sch 1) (Fig 3a) and sensilla chaetica type II (sch 2) (Fig 3b) and they are setae of different lengths with pointed tips and small cuticular outgrowths (spine-like), more abundant in the apical region. Sensilla type II are also abundant in the scape. Both sensilla types could be considered as mechanoreceptors, and their basal diameters are similar in both sexes ($p > 0.05$). However, only sch 2 were significantly longer in male than in female antennae (104 ± 7.9 versus 90 ± 6.2 μm) ($t = 3.24$, $df = 9$, $p = 0.0102$).

Sensilla trichoidea

The sensilla trichoidea were localized in the peripheral edges of all lamellae. These sensilla are setae with slightly curved blunt tips. They are about 20 μm in length (Fig 3c).

Sensilla placodea

In both sexes, sensilla placodea are the most abundant morphological type and are more abundant in males than in females (Table 2). They consist of thin oval cuticular plates and diameter ranged from 7.1 to 15.0 μm , with no difference in size between sexes ($t = 1.22$, $df = 77$, $p = 0.2249$, and $t = 0.074$, $df = 58$, $p = 0.9409$). Two different types were found in the smooth surface of the lamella (Fig 3d), whereas in the heterogeneous surface of the lamella, the sensilla with pores and plates bulged outwards into bizarre folds are the dominant type (Fig 3e–g).

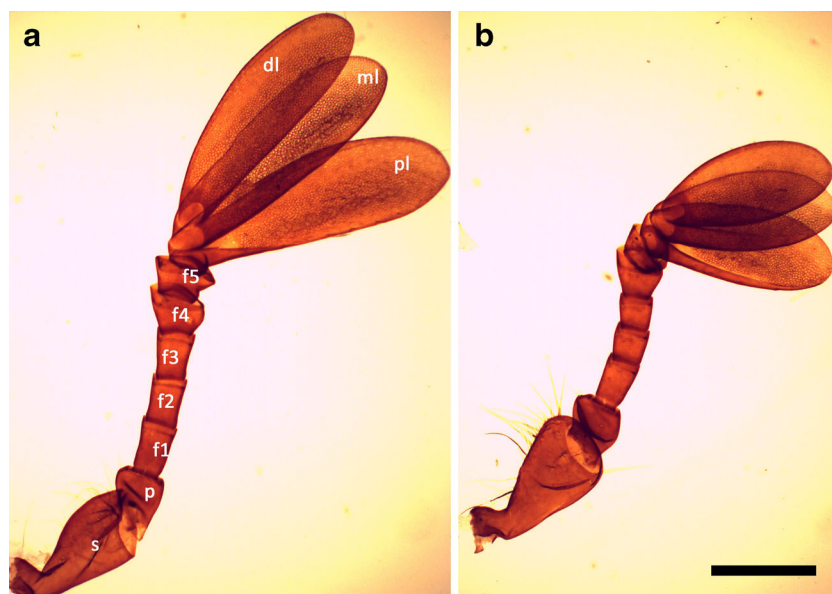


Fig 1 Light microscope image of the entire left antenna of males (**a**) and females (**b**) of *Hylamorpha elegans*, showing the scape (*s*), pedicel (*p*), flagellomeres (*f1* to *f5*), and distal (*dl*), middle (*ml*), and proximal (*pl*) lamellae. Scale bar=500 μ m.

Sensilla coeloconica

Sensilla coeloconica are short ($\sim 2.5 \mu\text{m}$) and stand in a pit. Three types of these sensilla are present in the heterogeneous surface of all lamellae (sco I, sco II, and sco III). Sco I and sco II have a wall consisting of a pyramid-shaped palisade of cuticular fingers, meeting each other at the tip, whereas sco III has finger-like structures on the distal portion (Fig 3h–j). Sensilla coeloconica were significantly more abundant in males (125.3 ± 22.5) than in females (86.3 ± 19.5) in the middle lamella ($t=2.619$, $df=6$, $p=0.0397$).

Discussion

In common with other scarab beetles, *H. elegans* presents sexual differences in the length of the antennae. Males have longer antennae than females, principally lamellar club segments. This species has four different types of sensilla,

Table 1 Comparison of length and width at the base of the different lamellae found on the antennae of male and female *Hylamorpha elegans*.

Lamellae	Length (μm)		Width (μm)	
	Female	Male	Female	Male
Proximal (pl)	1,046.5 \pm 18b	1,486.1 \pm 72a	372.7 \pm 15b	420.4 \pm 27a
Middle (ml)	1,033.3 \pm 22b	1,458.5 \pm 28a	390.3 \pm 14a	395.2 \pm 21a
Distal (dl)	882.0 \pm 66b	1,307.6 \pm 56a	310.8 \pm 45a	375.7 \pm 21a

Values are mean \pm standard deviation; $n=10$. Different letters within a sex and lamella type indicate a significant difference between sexes (Student's *t* test, $p \leq 0.05$).

but no sex-specific sensillum type has been found. Putative olfactory and thermo- and hygro-sensitive sensilla were present on the inner surfaces of proximal and distal lamellae and on both sides of the middle lamella, showing one heterogeneous surface consisting of different morphological types of sensilla. Additionally, one smooth surface was found, consisting only of sensilla placodea, whereas in external surfaces, the proximal and distal lamellae predominate: sensilla trichoidea and chaetica that have putative mechanosensory functions.

Mechanical stimuli, such as touch, air currents, sound, and gravity, serve different purposes. According to Keil (1997), sound receptors are involved in intraspecific communication and avoidance of predators or parasites, whereas touch receptors not only report direct mechanical contact, but are also involved in regulation of body position. In scarabaeids, specifically melolonthids, this function is associated with sensilla trichoidea and sensilla chaetica (Ochieng et al 2002, Romero-López & Morón 2013). The abundance of sensilla chaetica in the scape of *H. elegans* suggests that it has a role in the proprioception of the antenna position and movement.

Similar to other scarab beetles, *H. elegans* adults emerge from the ground in the spring–summer season when the evenings are relatively warm. Hygro- and thermoreceptive sensilla are expected to be important in determining the environmental conditions. In this regard, according to Keil (1999), sensilla coeloconica or sensilla styloconica respond to changes in humidity and temperature. Here, those functions could be carried out by sco III in a bimodal way, as described by Tichy (1979) for *Carausius morosus* (Br. Stabheuschrecke).

Information regarding the chemical communication in Rutelinae and Melolonthinae, mainly in the identification of

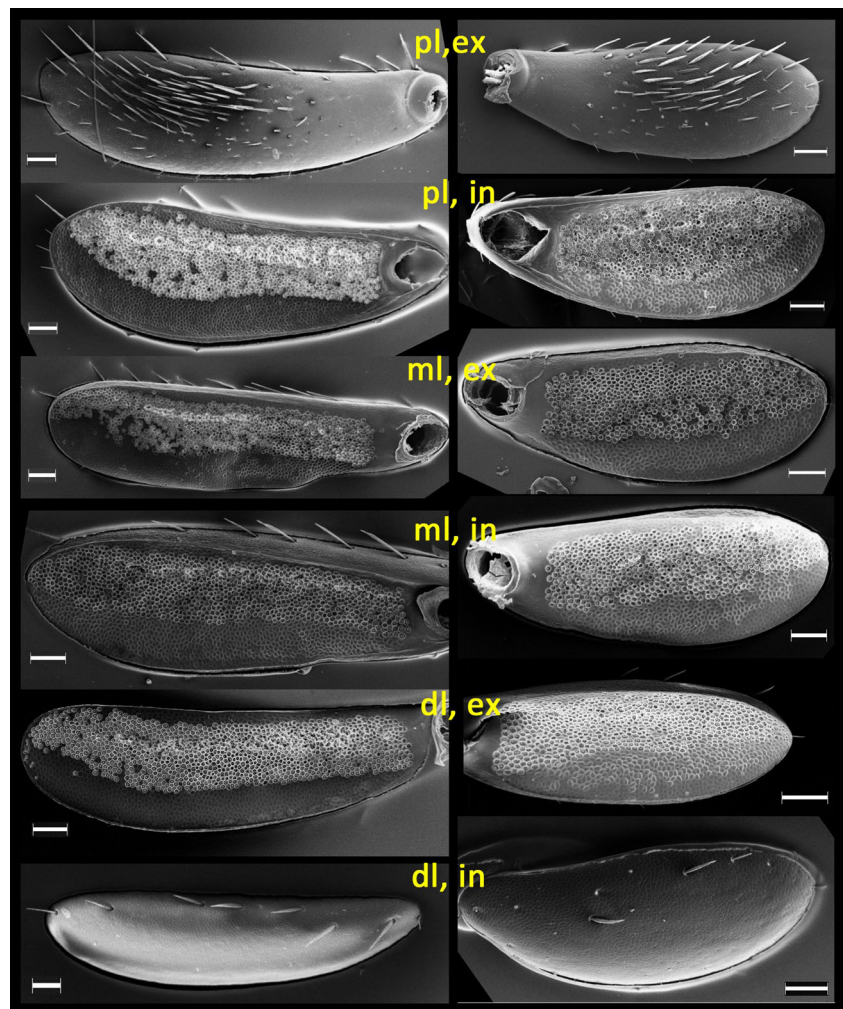


Fig 2 Scanning electron micrographs of the surface of the three lamellae in the antennae of male (*left*) and female (*right*) *Hylamorpha elegans* showing the internal (*in*) and external (*ex*) surfaces of the proximal (*pl*), middle (*ml*), and distal (*dl*) lamellae. Scale bars=100 μm .

sex pheromones and the role of plant volatiles in sexual behavior, has been described (Landolt 1997, Leal 1998, Kim & Leal 1999, Ruther *et al* 2000, Tóth *et al* 2003). Furthermore, the marked sexual dimorphism in antennal size and shape is often observed in species that use sex pheromones, as described in melolonthid species (Romero-López *et al* 2010). In this regard, Quiroz *et al* (2007) studied the male behavioral responses of *H. elegans* to odors released from virgin conspecific females and identified a putative sex pheromone. Moreover, whereas females remain feeding on host leaves, the flight and mating encounters were observed on the foliage, suggesting a role of plant volatiles in enhancing response towards the sex pheromone released by females of *H. elegans*.

Generally, sensilla basiconica, sensilla coeloconica, and sensilla placodea are considered the main sensilla involved in chemoreception (Romero-López *et al* 2010). In this study, the abundance of sensilla placodea was significantly larger in males than in females. This pattern is similar to that reported in other rutelines (Agren 1985,

Kim & Leal 2000) and melolonthines (Allsopp 1990, Romero-López *et al* 2004, Tanaka *et al* 2006, Romero-López *et al* 2010), suggesting that males of ruteline beetles have a larger potential for detecting their conspecific pheromone, reflecting their mate-searching behavior (Kim & Leal 2000). The function associated with the sensillum placodeum is reported by Hansson *et al* (1999) for the Japanese scarab beetle *Phyllopertha diversa* (Waterhouse), indicating that, through electrophysiological studies, all sensilla containing pheromone-specific olfactory receptor neurons (ORNs) were found in the smooth surface which houses sensilla placodea. Moreover, Leal & Mochizuki (1993) inserted a single-cell recording electrode in sensillum placodeum of *Anomala cuprea* (Hope), which reacted to the respective pheromone. Similarly, electrophysiological studies indicated that sensilla placodea of *Popillia japonica* house ORNs specific for both optical isomers of its sex pheromone (Kim & Leal 2000). On the other hand, Ochieng *et al* (2002) reported two types of sensilla coeloconica, type I and II, similar to those described in this study, with

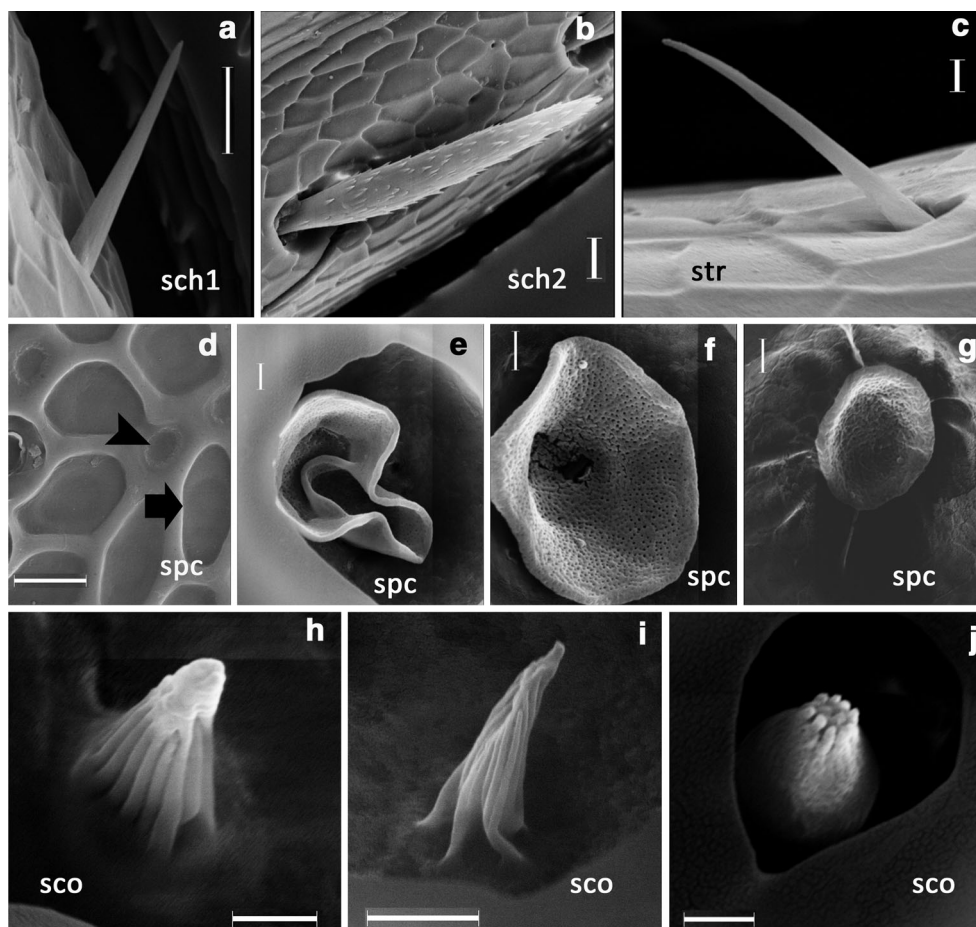


Fig 3 Scanning electron micrographs of the sensilla types present in the antennae of male and female *Hylamorpha elegans*. Sensillum chaeticum type I (*sch 1*) (a), sensillum chaeticum type II (*sch 2*) (b), sensillum trichoideum (c), and sensilla placodea (*spc*) that were present mainly on smooth surfaces. The *arrowhead* shows the groove sensillum, and the *arrow* shows the smooth sensillum (d). Sensilla placodea (*spc*) present in heterogeneous surface (e–g), sensillum coeloconicum (*sco*) type I (h), sensillum coeloconicum (*sco*) type II (i), and sensillum coeloconicum (*sco*) type III (j). Scale bars a=b=d=10 μ m; c=i=2 μ m; e=f=g=h=j=1 μ m.

olfactory functions in *Phyllophaga anxia* LeConte antennae. Kim & Leal (2000) and Romero-López et al (2004) suggest that sensilla coeloconica could be involved in the reception of plant volatiles.

The lack of a unified system of nomenclature for sensilla coeloconica makes it challenging to compare our results. The sensillum coeloconicum has been described under various synonyms: smooth basiconica sensilla (Norton & Vinson 1974), multiporous grooved sensilla (Barlin & Vinson 1981), sensillum basiconicum type II, or grooved peg (Jourdan et al 1995, Hu et al 2009). It has also been associated with multiple functions. In this sense, future

observations using transmission electron microscopy of the cross sections of the sensilla are therefore necessary to determine if these setae are capable of detecting chemical stimuli, as well as to assign a function to each sensilla described here.

Finally, the results obtained in this study suggest that the antennae of *H. elegans* are able to detect the sex pheromone and host volatiles. The fact that males of *H. elegans* have more chemosensory sensilla than females is likely to be related to the male's ability to find and recognize a conspecific mate on the foliage of the host plant. Considering that olfaction in scarab beetles, specifically sex pheromone perception, is a very important event (Leal 1998, Leal et al 1998), and the sensilla constitute the most important structural and functional elements of the sensory system (Romero-López et al 2004), the knowledge on antennal sensory organs of *H. elegans* together with the identification of a pheromone-binding protein present in males and females will be the basis for further electrophysiological, immunological, and behavioral studies to explore the chemical ecology of this species.

Table 2 Comparison of the number of sensilla placodea in the different lamellae of the antenna of *Hylamorpha elegans*.

Lamellae	Female	Male
Proximal (pl)	520.0±17b	1,224.5±29a
Middle (ml)	668.7±51b	1,046.0±131a
Distal (dl)	659.5±96b	1,152.5±235a

Values are mean±standard deviation; n=10. Different letters within a sex and lamella type indicate a significant difference between sexes (Student's *t* test, $p \leq 0.05$).

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