Acta Zoologica
Published for The Royal Swedish Academy of Sciences
and The Royal Danish Academy of Sciences and Letters

Acta Zoologica (Stockholm) **95**: 73–83 (January 2014) doi: 10.1111/azo.12010

Ultrastructure and distribution of sensilla on the antennae of female fig wasp *Eupristina* sp. (Hymenoptera: Agaonidae)

Zongbo Li,[1,2] Pei Yang,[1] Yanqiong Peng[1] and Darong Yang[1]

[1]Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, 650223, China; [2]College of Forestry, Southwest Forestry University, Kunming, 650224, China

Keywords:
Eupristina sp., antennae, sensilla, scanning electron microscopy, transmission electron microscopy

Accepted for publication:
21 January 2010

Abstract

Li, Z., Yang, P., Peng, Y. and Yang, D. 2014. Ultrastructure and distribution of sensilla on the antennae of female fig wasp *Eupristina* sp. (Hymenoptera: Agaonidae). — *Acta Zoologica* (Stockholm) **95**: 73–83.

In the species-specific and obligate mutualism between the fig (Moraceae: *Ficus* spp.) and its pollinator (Hymenoptera: Agaonidae), the continuity of lifecycle of both partners completely depends on the female pollinator's ability to detect receptive figs. To better understand the chemical location mechanism, we examined the antennae and their sensilla of the female fig pollinator *Eupristina* sp. using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The antennae of female *Eupristina* sp. are geniculated, and in total, there were seven types of sensilla found on the antennae: two types of multiporous placoid sensilla (type 1 is sausage-like and type 2 is rounded), sensilla trichodea (ST), basiconic sensilla (BS), chaetica sensilla (ChS), coeloconic sensilla (CoS), and one specialized sensillum classified as sensillum obscurum (SO). We described external morphology, abundance, distribution, ultrastructure and discussed putative functions. We inferred from their ultrastructures as chemoreceptors that two types of multiporous placoid sensilla, BS and CoS, were innervated by sensory neurons. The aporous type ST, ChS, and SO were not innervated by dendrites which may function as mechanoreceptor/proprioceptor. These results were also discussed in relation to the interaction between *Eupristina* sp. and its host fig.

Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 88 Xuefu Road, Kunming 650223, China. E-mail: Yangdr@xtbg.ac.cn

Introduction

The Agaonidae (Hymenoptera: Chalcidoidea) is a family of highly specialized pollinator fig wasps (Bouček 1988). The biology of agaonids is intimately linked to *Ficus* (Moraceae) inflorescences (known as figs or syconia), which are typically pollinated only by their associated species of fig wasp. The interaction is generally highly host species-specific (Wiebes 1979), although some exceptional cases have been documented (Michaloud *et al.* 1996; Peng *et al.* 2008; Su *et al.* 2008). Pollinator fig wasps show extreme sexual dimorphism that reflects the different roles of the sexes (Weiblen 2002). Female wasps have functional wings and are responsible for colonizing new figs and pollinating them; male wasps have no wings and spend most or all of their lives in the cavity of their natal fig.

The lifecycles of both plants and wasps depend completely on the female wasp's ability to locate receptive figs (those ready to be pollinated) in a complex chemical and physical environment. Because of their typically synchronous crops, but with asynchrony between fig trees, and the short lifespan of female pollinators (1–3 days; Kjellberg *et al.* 1988; Dunn *et al.* 2008), they must quickly undertake long-range dispersal (Harrison 2003). Adult female pollinators are attracted over long distances by chemical cues released from receptive figs (Frank 1984; Van Noort *et al.* 1989; Ware *et al.* 1993; Hossaert-McKey *et al.* 1994; Ware and Compton 1994; Nason *et al.* 1996; Song *et al.* 2001; Grison-Pige *et al.* 2002a,b; Harrison 2003; Chen and Song 2009).

Generally, most chemoreceptors are situated on the antennae of insects (Keil 1999; Schneider 1969; Zacharuk 1985). To better understand the chemical location mechanism of pollinator fig wasps, it is necessary to identify and characterize their sensilla types and ultrastructure, to identify those involved in chemoreception. Furthermore, detailed information of their antennal sensilla will facilitate studies of sensilla electrophysiology and the behavioral ecology of the wasps. In the current paper, we present first the morphology, ultrastructure, abundance, and distribution of antennal sensilla in female *Eupristina* sp. and then discuss their putative function, based on ultrastructural features and previously published data.

Materials and Methods

Eupristina *sp.*

Eupristina sp. is an undescribed species of diminutive pollinating fig wasp. It is involved in an obligate mutualism with its host plant *Ficus curtipes*, breeding only inside the figs of this plant and being its sole pollinator. *F. curtipes* is a small monoecious South-East Asian hemi-epiphyte or tree, 5–10 m tall. It is found particularly on limestone substrata in mountains or near villages at altitudes of 500–1,500 m in southern China (Wu *et al.* 2003). Ripe figs are present on the tree all year round in Xishuangbanna, China (Zhang *et al.* 2008).

Wasp collection

Eupristina sp. females were obtained by collecting figs which were about to release adult wasps (D-stage figs) of *F. curtipes*. These figs are characterized by their relatively large size and spongy feel when squeezed, often coupled with a dark red to purplish red surface (Wu *et al.* 2003). The adult wasps emerged after 1 or 2 days. Newly emerged individuals were anesthetized by ethyl acetate, and the antennae were rapidly removed from the head and immersed into 3.5% glutaraldehyde containing 0.1 M phosphate-buffered solution (PBS) and 4% paraformaldehyde at a pH of 7.4 at 4 °C for 3 days.

Scanning electron microscopy (SEM)

Wasp samples were warmed up to room temperature and sonicated for 10 s in a 10% solution of detergent to remove impurities (Maher and Thiery 2004). The samples were then rinsed with PBS for 30 min and postfixed in 1% osmium tetroxide for 2 h. This postfixation process was followed by cleaning through PBS and then dehydration in a graduated series of ethanol concentrations (30%, 50%, 75%, 80%, 95%, and absolute ethanol) and isoamyl acetate (100%). Each step lasted for 10 min until they were finally dried in a critical point dryer. The specimens were then mounted on aluminum stubs and sputter-coated using a precision etching and coating system (PECS-682; Gatan Co. Ltd., Pleasanton, CA, USA) for 45 s and finally examined with a Philips XL-30 ESEM at 20–30 kV (Philips, the Netherlands). A total of six antennae were arranged with different views to obtain detailed information.

Transmission electron microscopy (TEM)

Methods were largely similar to those described above for SEM. The samples were moved from the 4 °C refrigerator, then fully rinsed by PBS five times for 30 min each, postfixed in 1% osmium tetroxide mixed with PBS at a PH of 7.4 at 4 °C for 2 h, and finally cleaned with distilled water. Dehydration was carried out once for 30 min in each of a graded ethanol series (as described above), then transferred into 100% epichlorohydrin for 10 min, and infiltrated with Epon 618 and acetone mixtures at ratio 1 : 1 (V: V) at room temperature for 2 days. Specimens were then directionally embedded in pure Epon resin under a stereomicroscope (model SZX12; Olympus, Tokyo, Japan) and polymerized at 60 °C for 2 days. Ultra-thin sections were cut with a diamond knife on a Leica-U ultramicrotome (Leica, Wetzlar, Germany) set at slice thickness of 50–70 nm, stained with uranyl acetate and 1% lead citrate for 10 min each, followed by air drying. Finally, the specimens were viewed with a JEM-1010 TEM (Hitachi, Minato-ku, Tokyo, Japan).

Analyses

Sensilla were classified and named according to morphological details as revealed by SEM and TEM. Nomenclature followed Zacharuk (1985), Isidoro *et al.* (1998), Keil (1999), Ochieng *et al.* (2000), and Bleeker *et al.* (2004). Antennal sensilla were identified, counted, and measured from photomicrographs on the computer screen. To obtain accurate images, the background color was removed from the photographs, and they were manipulated using Adobe Photoshop 7. A Kruskal–Wallis test (using SAS 8.0 software, SAS Institute Inc., Cary, NC, USA) was used to determine the differences in length and width of each flagellomere.

Results

Antennal map of female wasp

The antenna of female *Eupristina* sp. has ten segments that comprise a scape with radicula, pedicel, and flagellum (Figs 1A and 2A–E). The radicula (Rd) is distinct and barrel-like in section. As it is articulated with the broader antennal socket on the cranium, it may be considered as essential for turning the antennae around (Fig. 1B,C). It is a tiny segment measuring 18.49 ± 9.56 μm (mean ± SE, $N = 6$) in length. The triangular scape is the most heavily sclerotized segment with a rugged surface (Fig. 1A,C) and measures 134.71 ± 17.62 μm in maximum length. The pedicel (segment 2) is a rounded, lozenge-shaped segment (Fig 1A,D) measuring 73.46 ± 7.73 μm in length. It is followed by a highly modified third antennal segment. This segment is cylindrical, with a longer, sharp, hook-shaped branch (Figs 1A,D,E and 2C,E). Segment 3 is 36.66 ± 2.50 μm in length (excluding the hook-shaped branch). The rest of the flagellum (segments 4–10) is

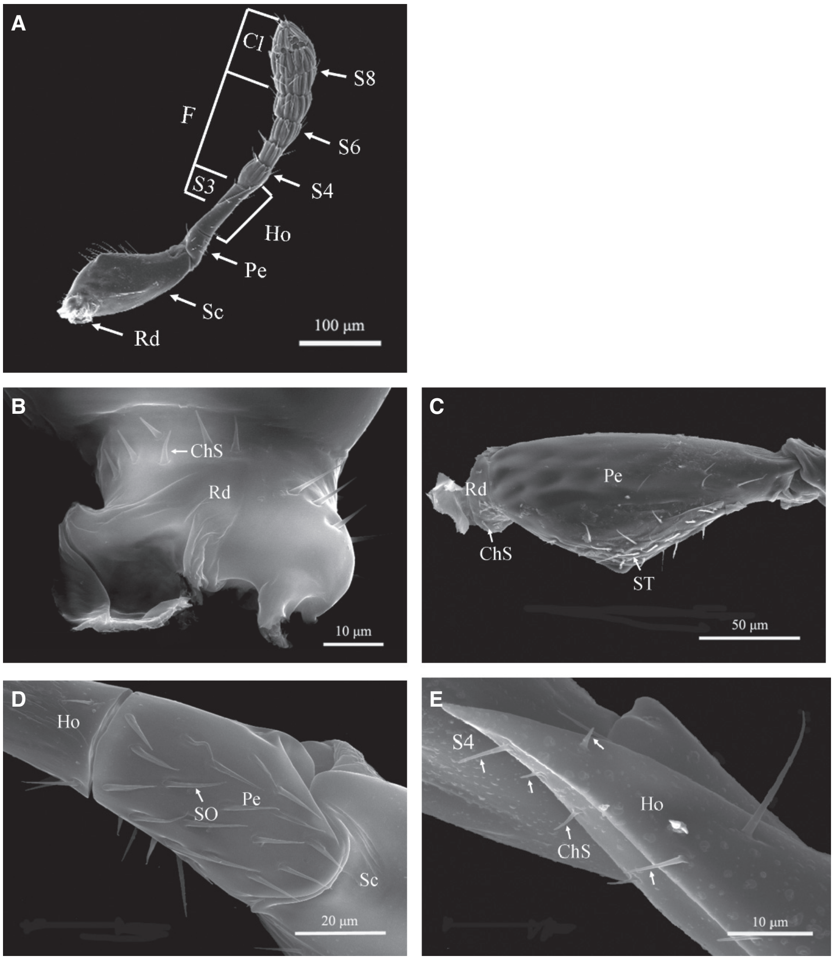

Fig. 1—Images of the antennae of *Eupristina* sp. female show general characters of the antennae and antennal segments. Rd, radicula; Sc, Scape; Pe, Pedicel; Ho, Hook-shaped branch; F, Flagellum; S3, 4, 6, 8, segments 3, 4, 6, 8, respectively; Cl, clava (club); ChS, chaetica sensilla; ST, sensilla trichodea; SO, sensillum obscurum. — **A**. Whole profile of the antennae. The arrows show different segments. — **B**. Inboard view of the radicula. — **C**. outboard view of the scape; — **D**. Dorsal view of the pedicel. — **E**. Morphological character of the hook-shaped branch. The arrows show chaetica sensilla of different sizes.

more elongate and broadens distally, with the last three segments forming a clava (club) (Figs 1A and 3A). The maximum length and width of each flagellar segment are given in Table 1. Segments 3–10 differ significantly in length ($\chi^2 = 17.86, P = 0.013$) and width ($\chi^2 = 18.61, P = 0.005$).

Antennal sensilla types and distribution

Based on size, shape, location, and cuticular character, seven main types of sensilla were recognized on the antennae of *Eupristina* sp. female. These include two types of multiporous placoid sensilla (type 1 is sausage-like and type 2 is rounded), sensilla trichodea (ST), basiconic sensilla (BS), chaetica sensilla (ChS), coeloconic sensilla (CoS), and one specialized sensillum classified as a sensillum obscurum (SO). The numbers and spatial variation in different sensilla types are shown in Table 2 and Fig 2, respectively.

Table 1 Mean length of the antennal segments 3–10 in female *Eupristina* sp. One antenna per wasp were removed and placed on the stub

Antennal segments	Length (μm)	Width (μm)
3	36.66 ± 2.50^{AB}	21.52 ± 0.19^{D}
4	40.95 ± 1.83^{A}	30.47 ± 1.65^{C}
5	31.90 ± 1.77^{B}	29.05 ± 2.57^{C}
6	36.92 ± 2.26^{AB}	37.48 ± 6.10^{C}
7	36.15 ± 1.46^{AB}	45.16 ± 1.52^{B}
8 (clava 1)	31.48 ± 5.15^{B}	46.64 ± 2.39^{B}
9 (clava 2)	36.62 ± 2.33^{AB}	54.44 ± 2.41^{A}
10 (clava 3)	41.88 ± 2.29^{A}	–
Total length	519.22 ± 54.5	

Values show mean ± SE ($N = 6$). In each column, values with the same letter are not significantly different at $P < 0.05$ level by Kruskal–Wallis test.
'–' Represents measurements were not obtained because of triangular shape.

Table 2 The numbers and distribution of different sensilla types on the antennae of female *Eupristina* sp.

Segment	ST	BS	MPS-E	MPS-R	ChS	CoS	SO
1	30 ± 2.2	–	–	–	27 ± 3.8	–	–
2	11 ± 2.1	–	–	–	9 ± 1.2	–	17 ± 1.9
3	3 ± 1.7	–	–	–	–	–	–
4	3 ± 1.3	1 ± 0.4	3 ± 1.2	–	–	–	–
5	4 ± 1.3	1 ± 0.5	3 ± 0.0	–	–	–	–
6	5 ± 0.5	1 ± 0.6	9 ± 1.2	–	–	–	–
7	5 ± 2.4	0.4 ± 0.5	11 ± 2.3	–	–	1 ± 0.0	–
8	6 ± 1.3	0.4 ± 0.5	8 ± 0.6	–	–	1 ± 0.0	–
9	6 ± 1.9	1 ± 0.0	11 ± 1.0	–	–	1 ± 0.0	–
10	4 ± 0.8	6 ± 0.5	6 ± 0.0	4 ± 0.0	–	1 ± 0.0	–
Total	77 ± 15.5	10.8 ± 4.0	61 ± 6.1	4 ± 0.0	36 ± 5.0	4 ± 0.0	17 ± 1.9

ST, sensilla trichodea; BS, basiconic sensilla; MPS-E, elongated multiporous placoid sensilla; MPS-R, rounded multiporous placoid sensilla; ChS, chaetica sensilla; CoS, coeloconic sensilla; SO, sensillum obscurum. The same as Table 3.
Values are estimated based on views of two-thirds of the surface area (mean ± SE, $N = 6$).

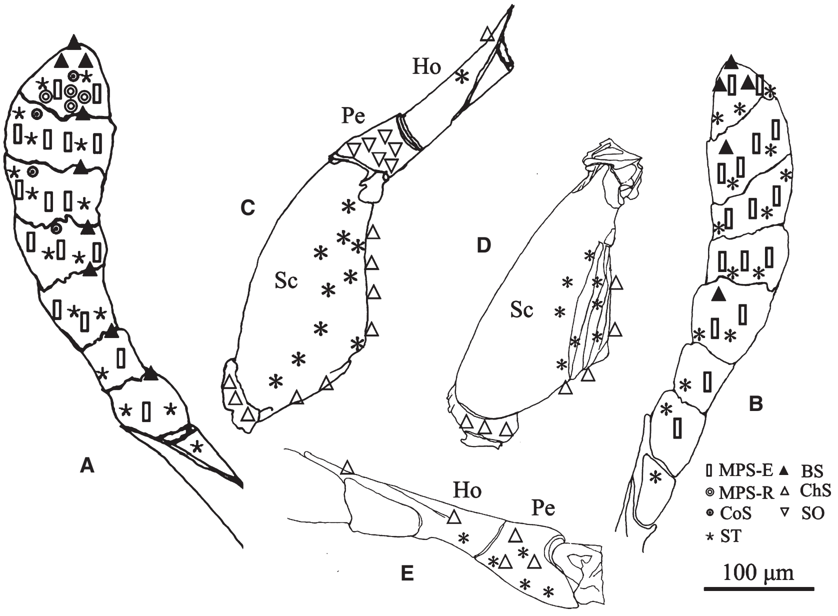

Fig 2—Diagrammatical representation of the distribution of sensilla on the antennae of *Eupristina* sp. female. Sc, scape; Pe, pedicel; Ho, hook-shaped branch; MPS-E, elongated multiporous placoid sensilla; MPS-R, rounded multiporous placoid sensilla; ST, sensilla trichodea; BS, basiconic sensilla; ChS, chaetica sensilla; CoS, coeloconic sensilla; SO, sensillum obscurum. — **A**. Ventral view of the flagellum; — **B**. Dorsal view of the flagellum. — **C**. Dorsal view of the scape and the pedicel; — **D**. Ventral view of the scape; — **E**. Ventral view of the pedicel and the hook-shaped branch.

Elongated multiporous placoid sensilla (MPS-E)

The MPS-E are the largest and most abundant sensilla on the segments 4–10. They have a ring-like distribution (Figs 1A, 2A,B and 3A) and are generally equidistant from one another. Each sensillum is an elongated and sausage-shaped structure elevated directly from the cuticle, slightly tapering slightly to a rounded tip at the distal apex, where they may separate from the antennal axis (Fig. 3A). The number of MPS-E gradually increases from the base to the distal end of the flagellum (Table 2; segment 10 is exceptional). The length and width of the MPS-E are 31.14 ± 4.77 and 5.17 ± 0.83 µm, respectively (mean ± SE, $N = 30$). The cuticles of the MPS-E contain numerous pores (Figs 3C and 4A). Estimated half way along their length, the multiple pores occur on the walls of the MPS at a density of $20 \pm 4.6/\mu m^2$ (mean ± SE, $N = 5$). The span between two pores is about 250 nm. In transverse

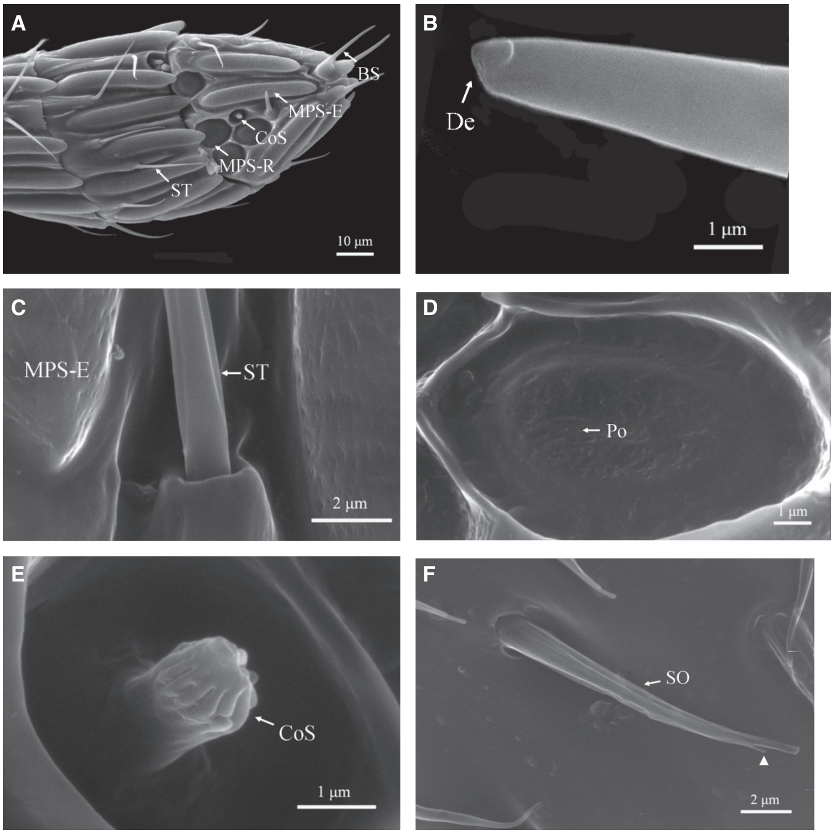

Fig 3—External morphology of different sensilla types recorded on the antennae of *Eupristina* sp. female. Po, pore; De, dent; ST, sensilla trichodea; BS, basiconic sensilla; MPS-E, elongated multiporous placoid sensilla; MPS-R, rounded multiporous placoid sensilla; ChS, chaetica sensilla; CoS, coeloconic sensilla; SO, sensillum obscurum. — **A**. Ventral view of 4 sensilla types recorded on the flagellar segments 7–8; — **B**. Terminal portion of a smooth-wall BS, showing small dents (arrow). — **C**. Cuticular character of ST and MPS-E recorded on the segment 9; — **D**. Cuticular character of MPS-R. — **E**. Cuticular character of CoS. — **F**. The external profile and cuticular character of SO. The white triangle shows a furcated branch.

section (Fig. 4A), the surface of these sensilla is convex and the area below it contains abundant dendrites that parallel the sensillum axis. Forty to 45 sensory neurons were counted. The septa (See in Fig. 4A) define one median and two lateral channels. When the dendrites enter into the median channel, they branch and turn toward the distal end of the sensillum, transversing its longitudinal axis. Each dendrite appears to terminate in a pore. The pore chamber/channel could be clearly discerned (Fig. 4A).

Rounded multiporous placoid sensilla (MPS-R)

The MPS-R are an unusual feature of the antennae of female *Eupristina* sp. only found on the proximal end of segment 10 (Figs 1A, 2A and 3A). Each sensillum possesses a rounded cuticular plates. These are set into a deeper cuticular depression of 8.76 ± 1.46 μm in diameter (mean ± SE, $N = 12$; Table 3), and radial pores are evident at the magnification (Fig. 3D). The estimated pore density is approximately $17 \pm 1.6/\mu m^2$ (mean ± SE, $N = 5$) in the middle section of the sensilla, where there are about two pores per 260 nm. With respect to appearance, TEM sections are similar to those of MPS-E (Fig. 4B), although the number of sensory neurons is fewer. Thirty to 35 neurons were counted under the septa. No pore chamber/channel could be discerned in connection with the pores (Fig. 4B).

Sensilla trichodea

Sensilla trichodea occur at three locations: the scape, the ventral surface of the pedicel, and the flagellum (Figs 1A,C, 2A–E and 3A). Their numbers are given in Table 2. Each sensillum is a slender and hair-like structure with deep longitudinal grooves that comes to a sharp apex (Fig. 3C). The sensillum cuticle is thick and aporous, as revealed by TEM. No dendrite branches were observed in the sensillum lymph (Fig. 4D). These sensilla are inserted into oval sockets, which are slightly elevated above the cuticle (Fig. 3A,C). They vary from 3.09 ± 1.01 to 32.78 ± 2.71 μm in length and from

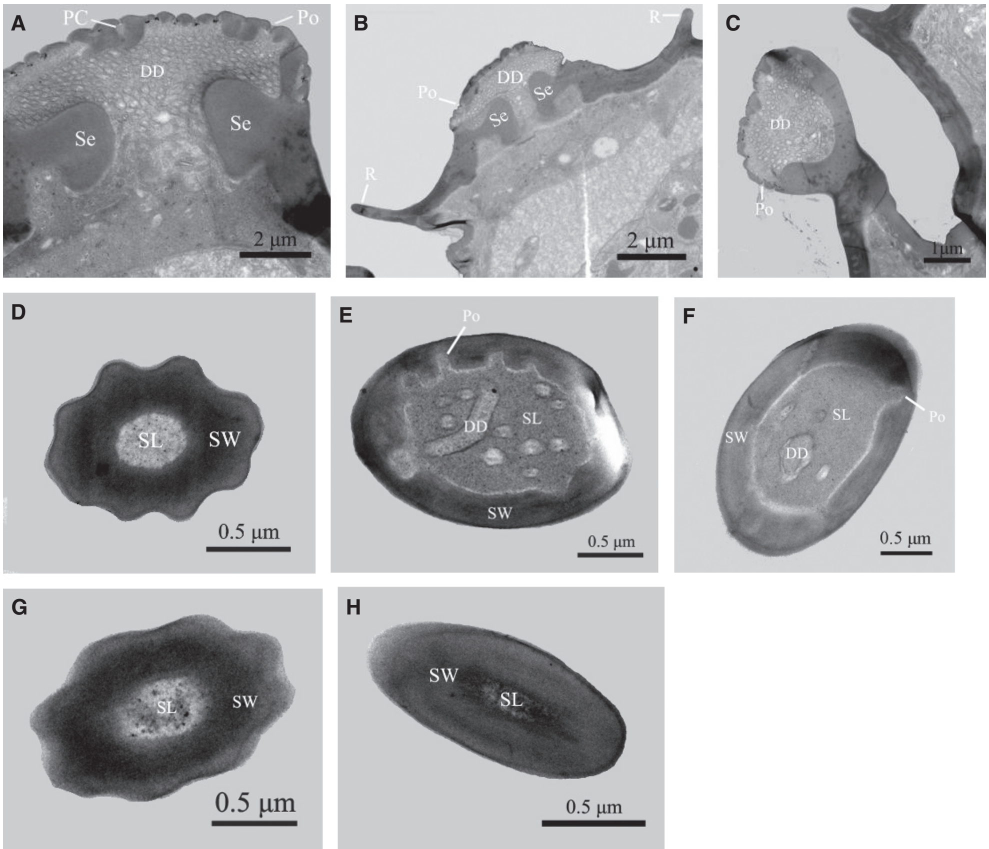

Fig. 4—Transmission electron sections of different sensilla types on the antennae of *Eupristina* sp. female. PC, pore chamber/channel; Po, pore; DD, dendrites; Se, septa; SL, sensillum lymph lumen; SW, sensillum wall. — **A**. Transverse section of elongated multiporous placoid sensilla; — **B**. Transverse section of rounded multiporous placoid sensilla; — **C**. Transverse section of coeloconic sensilla; — **D**. Transverse section of sensilla trichodea; — **E.** Transverse section of basiconic sensilla (BS) observed on the proximal portion; — **F**. transverse section of BS observed on the distal portion; — **G**. Transverse section of sensilla obscurum; — **H**. Transverse section of chaetica sensilla.

0.87 ± 0.10 to 1.74 ± 0.54 µm in basal width according to their location.

Basiconic sensilla

The BS are present on the antennal segments 4–10 and were encountered most often on the distal apex of segment 10 (Figs 2A,B and 3A). They have a smooth wall and project slightly more perpendicularly to the axis of the antenna than ST. Because of their length and orientation, the tips of BS are well above the level of the other sensilla. Generally, the great majority of BS are straight and tapering with a blunt apex (Fig. 3B), but some BS may appear slightly curved distally. This is especially true of the BS found on the dorsal field of segment 10. These sensilla have a thin cuticular wall surrounding the sensillum lymph lumen, with 5–11 dendrites of sensory nerve cells (Fig. 4E,F). They have a nonporous wall in their distal portion, but have multiporous walls on the proximal portion. The tip of this sensillum has also some circular dents (Fig. 3B), which may be considered as pores based on the distribution of the antennae of *Eupristina* sp. Each sensillum is inserted in a slightly elevated socket. They are 14.34 ± 5.26 µm in length and have a basal width of 1.58 ± 0.38 µm (mean ± SE, $N = 30$).

Chaetica sensilla

This type of sensilla is distinguished from ST and BS based on their external morphology, cuticular attachment, and distribution. ChS have tapered profiles with a smooth wall. Each is set into a broader cuticular depression (Fig. 1B) of 2.47 ±0.59 µm (mean ± SE, $N = 30$) in width. Each ChS measures 5.64 ± 1.41 µm in length and 1.07 ± 0.15 µm in width (Table 3). They are found on the radicula, scape, the ventral face of the pedicel, and the hook-shaped branch (Table 2; Fig 2C–E). The cuticle of this sensillum has a very thick and nonporous wall, which is not innervated by dendrites (Fig. 4H).

Coeloconic sensilla

The CoS are the shortest and the least numerous of the sensilla types found on the antennae of *Eupristina* sp. (Tables 2 and 3). They are scattered randomly on the distal–ventral face of segments 7–10, occurring in larger numbers around the MPS-R (Figs 2A and 3A). Each sensillum is composed of a circular depression (diameter: 4.77 ± 1.89 μm) with a mushroom-shaped protrusion from the central socket (Fig. 3E). The surface of the protrusion is perforated by numerous pores with multiple branch dendrites (Fig. 4C). They have a length and width of 2.98 ± 1.29 and 1.26 ± 0.13 μm (mean ± SE, *N* = 10), respectively.

Sensillum obscurum

This type of sensilla is highly distinctive, with a tapered shape, a bifurcated apex, and longitudinal grooves similar to the walls of ST (Figs 1D and 3F). Each SO measures 13.24 ± 1.46 μm in length and 1.39 ± 0.29 μm (mean ± SE, *N* = 30) in basal width (Table 3). These sensilla are closely attached to the cuticle at the shallow edge of an oval depression, which gradually separates from the antennal surface. TEM showed that the cuticle of SO is similar to ST, with a thick and non-porous wall surrounding the sensillum lymph lumen, where there are no dendrite branches (Fig. 4H). The SO are distributed in a patch on the dorsal field of the pedicel, pointing toward the head (Table 2), where they roughly align with the longitudinal antennal axis in several groups (Figs 1D and 2C). The number of SO is relatively stable (17 ± 1.9, mean ± SE, *N* = 2).

Discussion

In this study, we have characterized the female antennal sensilla of the pollinator fig wasp *Eupristina* sp. (Hymenoptera: Agaonidae). These sensilla were largely similar to those described for other hymenopteran parasitoids (Olson and Andow 1993; Isidoro *et al.* 1998; Pettersson *et al.* 2001; Bleeker *et al.* 2004; Gao *et al.* 2007; Onagbola and Fadamiro 2008), but with some exceptions, such as SO, which may be unique to fig wasps. One of the most characteristic features of fig wasp antennae, present in a large majority of species, is the third antennal segment with a larger hook-shaped branch (Fig. 1A). This is an adaptation for entering the fig ostiole (Kjellberg *et al.* 2005; Weiblen 2002).

Table 3 Sizes of different sensilla types on the antennae of female *Eupristina* sp.

Sensilla types	Length (μm)	Width (μm)
ST	17.81 ± 2.90	1.13 ± 0.24
BS	14.34 ± 5.26	1.58 ± 0.38
MPS-E	31.14 ± 4.77	5.17 ± 0.83
MPS-R	8.76 ± 1.46[a]	6.02 ± 0.81[b]
ChS	5.64 ± 1.41	1.07 ± 0.15
CoS	2.98 ± 1.29	1.26 ± 0.13
SO	13.24 ± 1.46	1.39 ± 0.29

Values are mean ± SE. Measurements obtained from at least 10 individual sensilla of the same type are dependent on the location and number of sensilla type.

For MPS-R, values with a letter indicate mean diameter; [a]The circular depression; [b]Inner plate.

Generally, the functional significance of antennal thickness may be related to an increase in sensilla numbers, which in turn should result in an increase in informational reliability and detectability (Vinson 1998). However, antennal thickness may be limited by the physical barrier of fig occluding bracts in the ostiole. Therefore, antennal design may be the result of coevolution between *Eupristina* sp. and *F. curtipes*.

Different morphological types of sensilla have different functions. Two morphological types of placoid sensilla were recorded on the flagellum of female *Eupristina* sp., and were obviously different to those described for other hymenoptera (Barlin and Vinson 1981; Olson and Andow 1993; Amornsak *et al.* 1998; Basibuyuk and Quicke 1999; Ochieng *et al.* 2000; Pettersson *et al.* 2001; Bleeker *et al.* 2004; Gao *et al.* 2007; Onagbola and Fadamiro 2008). Our study extends the findings of Barlin and Vinson (1981) which stated that only MPS-E are present in Chalcidoidea. The MPS-E are the most similar to those found in the closely related Chalcidoidea (Hymenoptera) (Barlin and Vinson 1981; Ware and Compton 1992), but externally, they bear some resemblance to the placoid sensilla found in the Ichneumonidae (Borden *et al.* 1978a; Basibuyuk and Quicke 1999; Li and Bai 2004), Braconidae (Ochieng *et al.* 2000; Gao *et al.* 2007), Aphidiidae (Borden *et al.* 1978b; Basibuyuk and Quicke 1999). Because of their multiporous surface and numerous dendrites, MPS-E have been regarded as olfactory receptors for plant volatiles (Steinbrecht 1997). Single-sensilla recording has demonstrated these putative functions (Ochieng *et al.* 2000). We believe that the MPS-E in *Eupristina* sp. are also for olfaction as reported by Steinbrecht (1997) and Ochieng *et al.* (2000), because their structures are similar. Based on the specialized lifestyle of *Eupristina* sp., the MPS-E may be odor specialists with the female-specific function of locating the receptive fig inflorescences (Barlin and Vinson 1981; Ware and Compton 1992). Zacharuk (1985) indicated that the body plan of insects may provide some functional advantages for insect sensory systems. For example, the smaller the body, the more rapid messages are delivered. Therefore, the diminutive pollinator *Eupristina* sp. may possess efficient receptivity and delivery. Fig wasps often have to disperse long distances and may have to detect host volatiles at very low concentrations, as the densities of receptive hosts are very low (Harrison 2003).

The MPS-R are broadly distributed on the antennae of Apoidea (Wcislo 1995; Basibuyuk and Quicke 1999), Vespidae (Krause 1960; Cited from Barlin and Vison 1981), Stephanidae, and Pelecinidae (Basibuyuk and Quicke 1999). Use of SEM and TEM in this study indicated the multiporous wall with 30–35 neurons innervated into the MPS-R typical of olfactory receptors. Olfactory recordings from individual MPS-R have been reported from the honeybee *Apis mellifera*

(Lacher and Schneider 1963; cited from Ochieng *et al.* 2000). Brockmann and Brückner (1993) found that MPS-R were able to receive chemical signals and sensory cells inside MPS-R interacted with each other in many ways (e.g., synergisms and inhibitions). It appears that there may not be separate classes of cells corresponding to different odorant spectra, as previous studies had suggested by Vareschi (1971; Cited from Brockmann and Brückner 1993). Such a structure may suggest its role in detecting long-range plant volatiles. However, the relatively fewer number and distribution found on the last flagellum of *Eupristina* sp. may indicate that MPS-R have bimodal functions (olfaction/gustation) in order to find receptive figs on a tree and in order to find the ostiole on a fig.

Recently, the pattern of multiporous placoid sensilla (MPS), including the density, distribution, morphology, and ultrastructure, has been used in discerning common ancestry and phylogenetic relationships of other Hymenoptera (Basibuyuk and Quicke 1999; Quicke *et al.* 1999). The morphology of the MPS-R in this study provides evidence for a relationship between the Agaonidae and other Chalcidoidea excluding the Mymarommatidae (Barlin and Vinson 1981; Gibson 1986). MPS-R are unusual in the Chalcidoidea. According to molecular phylogenies, the relationship between the Agaonidae and the Apoidea, the Vespidae, the Stephanidae, the Pelecinidae is distant. Why is MPS-R present on the antennae of the *Eupristina* sp.? The reason remains unclear. We assume that this characteristic could be phylogenetically informative within these species, but more work and research is needed.

Sensilla trichodea are the most abundant types observed on all segments of female *Eupristina* sp. (Table 2), as reported also for ST NP in Cotesia species (Bleeker *et al.* 2004), aporous type curved trichoidea in *Opius concolor* (Barbarossa *et al.* 1998), and fluted BS in *Cardiochiles nigriceps* (Norton and Vinson 1974), and ST in bracoid species (Ochieng *et al.* 2000; Gao *et al.* 2007). However, the number of ST in this study is fewer when comparing with other parasitoid wasps, and sparsely occurs on the antennae. We did not find any pore system and sensory neuron on the sensilla lymph of the ST dendrites of female *Eupristina* sp. under the TEM; thus, no olfactory function is attributed to this sensilla type. This conclusion is supported by previous studies suggesting that the function of sensilla can be deduced from the number of pores (Keil 1999; Bleeker *et al.* 2004). Therefore, ST of *Eupristina* sp. are most likely to be mechanoreceptor due to socket-like insertion into the antennal cuticle and their spatial distribution.

Basiconic sensilla described in the current study resemble sensilla basiconica B (Navasero and Elzen 1991), ST with wall pores (Pettersson *et al.* 2001; Bleeker *et al.* 2004), sensilla basiconica 2 (Ochieng *et al.* 2000; Gao *et al.* 2007), multiporous pitted ST C (Olson and Andow 1993), and multiporous type III sensilla trichoidea (Onagbola and Fadamiro 2008). In general, sensilla types with wall pores are presumed to function as olfactory receptors in insects (Steinbrecht 1997; Keil 1999; Bleeker *et al.* 2004). Some electrophysiological studies have also confirmed this proposal (Hansson *et al.* 1999). The BS have a thin wall with numerous pores and multiple sensory neurons suggest an olfactory function. On the last flagellar segment, the BS are likely to function as gustatory receptors. Behavioral observations suggest these sensilla may be involved in host examination and host discrimination because *Eupristina* sp. usually examines the host fig by brushing the exterior of the fig as well as drumming the surface with the antennal flagellum, which bears these sensilla.

Our study confirms that sensilla chaetica occur frequently on the radicula, the scape, and the pedicel, but not the flagellum (Navasero and Elzen 1991; Amornsak *et al.* 1998; Van Baaren *et al.* 1999; Ochieng *et al.* 2000; Gao *et al.* 2007). Amornsak *et al.* (1998) indicated that sensilla found on the scape and the pedicel were primarily mechanoreceptors. Based on the location and ultrastructure, the ChS in this study may also have mechanoreceptive functions for antennal movement and position (Ochieng *et al.* 2000). Fig pollinators need to enter into figs for reproduction. Therefore, some of the ChS in *Eupristina* sp., especially on the hook-shaped branch, are likely to possess functions related to fig entry (Weiblen 2002).

The CoS are easily distinguishable from other sensilla types by their conspicuous structural features and socket region (Altner *et al.* 1981). The mushroom-shaped sensilla are similar to CoS reported for *Pteromalus cerealellae* (Onagbola and Fadamiro 2008), to the 'pit organ' reported for honeybees (Wcislo 1995), and to coelocapitular sensillum reported for *Apis mellifera* (Yokohari *et al.* 1982). However, unlike on the bracoid species (Ochieng *et al.* 2000; Bleeker *et al.* 2004; Gao *et al.* 2007), the CoS on the flagellum of *Eupristina* sp. are located in larger sockets and are slightly elevated above the antennal surface. The CoS of *Eupristina* sp. are not differentiated into subtypes. In contrast, two morphologically distinct types of CoS were recorded for the Cotesia species (Bleeker *et al.* 2004; Roux *et al.* 2005). TEM investigation shows the mushroom-shaped protrusion contains numerous wall pores that are usually innervated by multiple dendrites. Thus, we believe that the CoS in this study have an olfactory function, as reported in previous studies (Keil 1999; Ochieng *et al.* 2000; Roux *et al.* 2005; Gao *et al.* 2007). However, this sensillum has been considered to have other or multiple functions (chemo-, thermo-, and hygroreceptor) in several nonparasitic species (Altner *et al.* 1983; Yokohari *et al.* 1982).

Up to now, the SO have not been found on the antennae of any insects, except for Agaonidae. Based on the location and arrangement, the SO occurring on the antennae of *Eupristina* sp. highly resemble those described for SO in *Liporrhopalum tentacularis* (T. Cockerill, unpublished data) and *Ceratosolen solmsi marchali* (Z.B. Li, P. Yang and D.R. Yang, unpublished data). However, unlike the latter, the SO are grooved hairs tapering to a thin forked apex. Yang *et al.* (2008) described sensilla furcatea, a furcated trichoid hair found on the antennae of *Coleophora obducta*, but this is likely to be distinct from SO due to distribution and taxonomic differences. Cockerill (unpublished data) suggested that SO may come from the evolution of ST. The similarity in morphology (Fig. 3C,F) and ultrastructure (Fig. 4F,G) substantiate this claim. The presentation of SO with a thick wall, aporous surface, oval flexible socket, and no dendrites is more likely to suggest a mechanofunction. Because of their location and spatial arrangement (Fig. 1D), SO are likely to play a vital role in aiding *Eupristina* sp. in getting through the ostiole bracts. The specific function in *Eupristina* sp., however, has yet to be illustrated by behavioral recording.

One of the main functions of female wasp antennae is to detect the volatile attractants released by figs. The wasp must have long-range olfactory sensitivity and short-range gustatory sensitivity in order to search for and find a receptive fig in which they can reproduce. The putative functions of these sensilla types found on the antennae of female *Eupristina* sp. suggest that the flagellum confers this long-range olfactory sense through the relatively sensitive and numerous MPS-E, and also possibly through MPS-R, BS, and CoS, should their function prove to be chemoreceptive. Short-range gustatory sensitivity is likely to be conferred by BS and possibly by MPS-R. The ST, ChS, and SO observed in this study are most likely to be associated with the specific prerequisite of that in order to reproduce *Eupristina* sp. negotiate the physical barrier of the ostiole bracts. These results substantiate earlier findings that suggested female fig wasps mainly depend on chemical cues to detect hosts, and also provide requisite background information for the further study on electrophysiology and behavior ecology of *Eupristina* sp.

Acknowledgements

This researcher was supported the Knowledge Innovation Program of the Chinese Academy of Sciences (NO. 30670358) and the Chinese Natural Science Foundation (NO. 30571507). We would like to thank Ms. Song Jingling (Kunming medical university, China) and Mr. Li Min (Kunming University of Science and Technology) for technological assistance with electron microscopy. We also thank Prof. Jean-Yves Rasplus (Centre de Biologie et de Gestion des Populations, France) for identifying wasps, Dr. Steve G. Compton (University of Leeds, UK) for permitting us to use their unpublished data and improving the quality of the manuscript, Dr. Rhett Harison (Xishuangbanna Tropical botanical Garden, China) for checking and improving the final manuscript, Prof. Thomas Keil (Max-Planck-Institut für Biochemie, German) for assisting with sensilla classification and Dr. Denis Thiery (Unité Mixte de Recherche en Santé Végétale, France) for imparting silver staining methods.

References

Altner, H., Routil, C. and Loftus, R. 1981. The structure of bimodal chemo-, thermo-, and hygroreceptive sensilla on the antenna of *Locusta migratoria*. – *Cell and Tissue Research* **215**: 289–308.

Altner, H., Schaller-Selzer, L., Stetter, H. and Wohlrab, L. 1983. Poreless sensilla with inflexible sockets. A comparative study of a fundamental type of insect sensilla probably comprising thermo- and hygroreceptors. – *Cell Tissue Research.* **234**: 279–307.

Amornsak, W., Cribb, B. and Gordh, G. 1998. External morphology of antennal sensilla of *Trichogramma australicum* girault (Hymenoptera: Trichogrammatidae). – *International Journal of Insect Morphology and Embryology* **27**: 67–82.

Barbarossa, I. T., Muroni, P., Dardani, M., Casula, P. and Angioy, A. M. 1998. New insight into the antennal chemosensory function of *Opius concolor* (Hymenoptera, Braconidae). – *Italian journal of zoology* **65**: 367–370.

Barlin, M. R. and Vinson, B. S. 1981. Multiporous plate sensilla in antennae of the chalcidoidea (Hymenoptera). – *International Journal of Insect Morphology and Embryology* **10**: 29–42.

Basibuyuk, H. H. and Quicke, D. L. J. 1999. Gross morphology of multiporous plate sensilla in the Hymenoptera (Insecta). – *Zoologica Scripta* **28**: 51–67.

Bleeker, M. A. K., Smid, H. M., Van Aelst, A. C., Van Loon, J. J. A. and Vet, L. E. M. 2004. Antennal sensilla of two parasitoid wasps: A comparative scanning electron microscopy study. – *Microscopy Research and Technique* **63**: 266–273.

Borden, J. H., Chong, L. and Rose, A. 1978a. Morphology of the elongated placoid sensillum on the antennae of *Itoplectis conquisitor*. – *Annals of the Entomological Society of America* **71**: 223–227.

Borden, J. H., Rose, A. and Chorney, R. J. 1978b. Morphology of the elongate sensillum placodeum on the antennae of *Aphidius smithi* (Hymenoptera: Aphidiidae). – *Canadian Journal of Zoology* **56**: 519–525.

Bouček, Z. 1988. Australasian Chalcidoidea (Hymenoptera): A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species. CAB International, Wallingford, UK.

Brockmann, A. and Brückner, D. 1993. Zur funktionellen organisation des antennalen und olfaktorischen systems der Aebeiterin der Honigbiene, *Apis mellifera* L. – *Apidologie* **24**: 219–233.

Chen, C. and Song, Q. 2009. Responses of the pollinating wasp *Ceratosolen solmsi* marchali to odor variation between two floral stages of *Ficus hispida*. – *Journal of Chemical Ecology* **131**: 46–57.

Dunn, D. W., Yu, D. W., Ridley, J. and Cook, J. M. 2008. Longevity, early emergence and body size in a pollinating fig wasp – Implications for stability in a fig-pollinator mutualism. – *Journal of Animal Ecology* **77**: 927–935.

Frank, S. A. 1984. The behavior and morphology of the fig wasps *Pegoscapus assuetus* and *P. jimerrezi*: Descriptions and suggested behavioral characters for phylogenetic studies. – *PSYCHE* **91**: 289–308.

Gao, Y., Luo, L. Z. and Hammond, A. 2007. Antennal morphology, structure and sensilla distribution in *Microplitis pallidipes* (Hymenoptera: Braconidae). – *Micron* **38**: 684–693.

Gibson, G. A. P. 1986. Evidence for monophyly and relationships of chalcidoidea, Mymaridae, and Mymarommatidae (Hymenoptera: terebrantes). – *Canadian Entomologist* **118**: 205–240.

Grison-Pigé, L., Bessière, J.-M. and Hossaert-McKey, M. 2002a. Specific attraction of fig-pollinating wasps: Role of volatile compounds released by tropical figs. – *Journal of Chemical Ecology* **28**: 283–295.

Grison-Pigé, L., Hossaert-McKey, M., Greeff, J. M. and Bessiere, J.-M. 2002b. Fig volatile compounds–a first comparative study. – *Phytochemistry* **61**: 61–71.

Hansson, B. L. S., Larsson, M. C. and Leal, W. S. 1999. Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. – *Physiological Entomology* **24**: 121–126.

Harrison, R. D. 2003. Fig wasp dispersal and the stability of a keystone plant resource in Borneo. – *Proceedings of the Royal Society B: Biological Sciences* **270**: S76–S79.

Hossaert-McKey, M., Gibernau, M. and Frey, J. 1994. Chemosensory attraction of fig wasps to substances produced by receptive figs. – *Entomologia Experimentalis et Applicata* **70**: 185–191.

Isidoro, N., Bartlet, E., Ziesmann, J. and Williams, I. H. 1998. Antennal contact chemosensilla in *Psylliodes chrysocephala* responding to cruciferous allelochemicals. – *Physiological Entomology* **23**: 131–138.

Keil, T. A. 1999. Morphology and development of the peripheral olfactory organs. In: Hansson, B. S. (Ed.): Insect Olfaction, pp. 5–48. Springer-Verlag, Berlin, Heidelberg.

Kjellberg, F., Doumesche, B. and Bronstein, J. L. 1988. Longevity of a fig wasp (*Blastophaga psenes*). – *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen (C)*, **91**: 117–122.

Kjellberg, F., Jousselin, E., Hossaert-McKey, M. and Rasplus, J. Y. 2005. Biology, ecology, and evolution of fig-pollinating wasps (Chalcidoidea, Agaonidae). In: Raman, A., Schaefer, C. W. and Withers, T. M. (Eds): Biology, Ecology and Evolution of Gall-Inducing Arthropods, Vol. 1 and 2, pp. 539–572. Science Publishers, Inc, Enfield, NH, USA, Plymouth, UK.

Krause, B. 1960. Elektronenmikroskopische Untersuchungen an den Plattensensillen des Insektenfühlers. – *Zoologische Beiträge* (*N.F.*) **6**: 161–205.

Lacher, V. and Schneider, D. 1963. Electrophysiologischer nachweis der riechfunktion von pornplatten (sensilla placodea) auf den antennen der drohne und arbeitsbiene (*Apis mellifera* L.). – *Zeitschrift fur Verengleichende Physiologie* **47**: 274–278.

Li, X. and Bai, S. F. 2004. Ultrastructural studies on the antennal sensilla of *Diadegma semiclausum* hellen (Hymenoptera: Ichneumonidae). – *Journal of Henan Agricultural University (China)* **38**: 45–48.

Maher, N. and Thiery, D. 2004. Distribution of chemo- and mechanoreceptors on the tarsi and ovipositor of female European grapevine moth, *Lobesia botrana*. – *Entomologia Experimentalis et Applicata* **110**: 135–143.

Michaloud, G., Carriere, S. and Kobbi, M. 1996. Exceptions to the one: One relationship between African fig trees and their fig wasp pollinators: Possible evolutionary scenarios. – *Journal of Biogeography* **23**: 513–520.

Nason, J. D., Herre, E. A. and Hamrick, J. L. 1996. Paternity analysis of the breeding structure of strangler fig populations: Evidence for substantial long-distance wasp dispersal. – *Journal of Biogeography* **23**: 501–512.

Navasero, R. C. and Elzen, G. W. 1991. Sensilla on the antennae, foretarsi and palpi of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). – *Proceedings of the Entomological Society of Washington* **93**: 737–747.

Norton, W. N. and Vinson, S. B. 1974. Antennal sensilla of three parasitic Hymenoptera. – *International Journal of Insect Morphology and Embryology* **3**: 305–316.

Ochieng, S. A., Park, K. C., Zhu, J. W. and Baker, T. C. 2000. Functional morphology of antennal chemoreceptors of the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). – *Arthropod Structure and Development* **29**: 231–240.

Olson, D. M. and Andow, D. A. 1993. Antennal sensilla of female *Trichogramma nubilae* (Ertle and Davis) (Hymenoptera: Trichogrammatidae) and comparisons with other parasitic hymenoptera. – *International Journal of Insect Morphology & Embryology* **22**: 507–520.

Onagbola, E. O. and Fadamiro, H. Y. 2008. Scanning electron microscopy studies of antennal sensilla of *Pteromalus cerealellae* (Hymenoptera: Pteromalidae). – *Micron* **39**: 526–535.

Peng, Y. Q., Duan, Z. B., Yang, D. R. and Rasplus, J. Y. 2008. Co-occurrence of two Eupristina species on *Ficus altissima* in Xishuangbanna, SW China. – *Symbiosis* **45**: 9–14.

Pettersson, E. M., Hallberg, E. and Birgersson, G. 2001. Evidence for the importance of odour-perception in the parasitoid *Rhopalicus tutela* (Walker) (Hym., Pteromalidae). – *Journal of Applied Entomology* **125**: 293–301.

Quicke, D. L. J., Basibuyuk, H. H. and Rasnitsyn, A. P. 1999. Morphological, palaeontological and molecular aspects of ichneumonoid phylogeny (Hymenoptera, Insecta). – *Zoologica Scripta* **28**: 175–202.

Roux, O., Van Baaren, J., Gers, C., Arvanitakis, L. and Legal, L. 2005. Antennal structure and oviposition behavior of the *Plutella xylostella* specialist Parasitoid: *Cotesia plutellae*. – *Microscopy Research and Technique* **68**: 36–44.

Schneider, D. 1969. Insect olfaction: deciphering system for chemical messages. – *Science* **163**: 1031–1037.

Song, Q. S., Yang, D. R., Zhang, G. M. and Yang, C. R. 2001. Volatiles from *Ficus hispida* and their attractiveness to fig wasps. – *Journal of Chemical Ecology* **27**: 1929–1942.

Steinbrecht, R. A. 1997. Pore structures in insect olfactory sensilla: A review of data and concepts. – *International Journal of Insect Morphology and Embryology* **26**: 229–245.

Su, Z. H., Iino, H., Nakamura, K., Serrato, A. and Oyama, K. 2008. Breakdown of the one-to-one rule in Mexican fig-wasp associations inferred by molecular phylogenetic analysis. – *Symbiosis* **45**: 73–81.

Van Baaren, J., Boivin, G., Le Lannic, J. and Nénon, J.-P. 1999. Comparison of antennal sensilla of *Anaphes victus* and *A. listronoti* (Hymenoptera, Mymaridae), egg parasitoids of Curculionidae. – *Zoomorphology* **119**: 1–8.

Van Noort, S., Ware, A. B. and Compton, S. G. 1989. Pollinator-specific volatile attractants released from the figs of *Ficus burtt-davyi*. – *South African Journal of Science* **85**: 323–324.

Vareschi, E. 1971. Duftunterscheidung bei der Honigbiene–Einzelzellableitungen trod Verhaltensreaktion. – *Zeitschrift für vergleichende Physiologie* **75**: 143–173.

Vinson, S. B. 1998. The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. – *Biological Control* **11**: 79–96.

Ware, A. B. and Compton, S. G. 1992. Repeated evolution of elongate multiporous plate sensilla in female fig wasps (Hymenoptera: Agaonidae: Agaoninae). – *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen(1990)* **95**: 275–292.

Ware, A. B. and Compton, S. G. 1994. Responses of fig wasps to host plant volatile cues. – *Journal of Chemical Ecology* **20**: 785–802.

Ware, A. B., Kaye, P. T., Compton, S. G. and Noort, S. 1993. Fig volatiles: Their role in attracting pollinators and maintaining pollinator specificity. – *Plant Systematics and Evolution* **186**: 147–156.

Wcislo, W. T. 1995. Sensilla numbers and antennal morphology of parasitic and non-parasitic bees (Hymenoptera: Apoidea). – *International Journal of Insect Morphology and Embryology* **24**: 63–81.

Weiblen, G. D. 2002. How to be fig wasp. – *Annual Review of Entomology* **47**: 299–330.

Wiebes, J. T. 1979. Co-evolution of figs and their insect pollinators. – *Annual Review of Ecology and Systematics* **10**: 1–12.

Wu, Z. Y., Zhou, Z. K. and Michael, G. G. 2003. Floral of China **5**: 37–71. Available at: www.efloras.org.

Yang, H., Yan, S. C. and Liu, D. 2008. Ultrastructural observations on antennal sensilla of *Coleophora obducta* (Meyrick) (Lepidoptera: Coleophoridae). – *Micron* **40**: 231–238.

Yokohari, F., Tominaga, Y. and Tateda, H. 1982. Antennal hygroreceptors of the honey bee, *Apis mellifera* L. – *Cell and Tissue Research* **226**: 63–73.

Zacharuk, R. Y. 1985. Antennae and sensilla. In: Kerkut, G. A. and Gilbert, L. J. (Eds): Comprehensive Insect Physiology Biochemistry and Physiology, Nervous System: Sensory, pp. 1–69. New York, Oxford, Pergamon.

Zhang, F. P., Peng, Y. Q., Guan, J. M. and Yang, D. R. 2008. A species of fig tree and three unrelated fig wasp pollinators. – *Evolutionary Ecology Research* **10**: 611–620.