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Comparison of the antennal sensilla of females of four fig-wasps associated with *Ficus auriculata*

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Host-parasitoid community, *Ficus auriculata*, Foundress, Antennal sensilla

Abstract

A comparison was performed of the antennal sensilla of females of four chalcid wasp species *Ceratosolen emarginatus* Mayr, 1906, *Sycophaga* sp., *Philotrypesis longicaudata* Mayr, 1906, and *Sycoscapter roxburghi* Joseph, 1957, which are specific and obligatory associated with *Ficus auriculata* (Lour, 1790). The four species exhibit different oviposition strategies in the fig ovules where their offspring hatch and develop. Antennal sensilla morphology was evaluated using scanning electron microscopy. Females of the four species present 11 morphologically similar types of sensilla: trichoid sensilla, sensilla obscura, chaetica sensilla 1 and 2, which all have mechanosensory functions; uniporous basiconic sensilla, which are presumably contact chemosensilla; basiconic capitate peg sensilla, coeloconic sensilla 1, multiporous basiconic and placoid sensilla, which may be regarded as olfactory sensilla, and coeloconic sensilla 2 and 3, which are presumed to be proprioceptors or pressure receptors. The four species have significant differences in the abundance and arrangement of trichoid sensilla and chaetica sensilla 1 on the flagellum. The coeloconic sensilla and sensilla obscura only occur on the antennae of *C. emarginatus* that enter figs. The chemosensilla which are presumably involved in host discrimination, i.e., basiconic sensilla, multiporous placoid sensilla and basiconic capitate peg sensilla, are similar in shape and configuration, although they present some differences in abundance. These findings provide practical information on the adaptations of fig wasps and the relationship between multisensory antennae and functions in fig wasp behaviour.

1. Introduction

Fig wasps (Hymenoptera: Chalcidoidea) are chalcid wasps that oviposit almost exclusively in fig ovules. Many of these wasp species develop on a single *Ficus* species (Cook and Rasplus, 2003; Weiblen, 2002). They locate their host using stage-specific chemical cues emitted by the figs of their host species (Hossaert-Mckey et al., 2010; Proffit et al., 2007, 2008; Wang et al., 2013). In insects, the antenna is an organ capable of detecting odours. It is a multisensory detector carrying various receptors that sense a series of signals, including smell, but also taste, temperature, sound, gravity, wind speed, and even the geomagnetic field (de Oliveira et al., 2009; Isidoro et al., 1996; Meyhöfer et al., 1997; Ochieng et al., 2000; Romani et al., 2010; Staudacher et al., 2005; van Baaren et al., 2007; Zacharuk, 1985). The extent to which variation in the structure of the antennae as well as the presence and distribution of different types of sensory receptors among wasp species can be explained by differences in behaviour and oviposition strategies are poorly known (but see Polidori and Nieves-Aldrey, 2014). For fig wasps, the few studies of the sensilla on the antenna have almost exclusively focused on pollinators. These wasps enter figs to oviposit and the host figs are specialized to attract them (Li et al., 2009, 2014; Ware and Compton, 1992). Little is known about the sensory organs on

the antennae of non-pollinating fig wasps (NFWs), which oviposit from outside the fig without providing any known benefits to the mutualism (Cook and Rasplus, 2003; Meng et al., 2016).

In this study we contrast the antennal ultrastructure of four fig-wasp species associated with one host species, *Ficus auriculata*: a pollinator (*Ceratosolen emarginatus* Mayr, 1906), belonging to the Agaonidae, and three NFW species, one belonging to the Sycophaginae (*Sycophaga* sp.) and two belonging to the Sycoryctinae (*Philotrypesis longicaudata* Mayr, 1906, *Sycoscapter roxburghi* Joseph, 1957). The aim of the study was to investigate similarities among species but also differences that may be associated with how the antennae contact the fig surface and with the fig-entering behaviour of the pollinating wasps. Our working hypothesis is that several traits of the first three segments of pollinator antennae are associated with the fig-entering behaviour, while the distal segments of the antennae would show many more similarities with NFW antennae. A second hypothesis is that wasps that contact the fig surface with the whole length of the flagellomere will have different sensilla from wasps that only tap the surface of the fig with the distal segment of the antennae. A third hypothesis is that since pollinators only live a few hours as adults while NFW adults are longer lived, they may present a more specialized, less diversified, detection system.

We compared here the morphology, number and distribution of the different types of sensilla on the antennae of four fig wasps associated with the host *F. auriculata* using SEM imagery. We discuss the likely roles of different antennal sensilla according to their morphology and interpret their presence according to global functioning of the antennae in connection with fig wasp behaviour.

2. Materials and methods

2.1. The study species

Ficus auriculata Lour., 1790, is a widespread species ranging from Pakistan to Thailand and Indochina through south China (Berg and Corner, 2005). The tree is pollinated by the species complex of *C. emarginatus* and it is host to 4 non-pollinating fig wasps: *Sycophaga* sp1., *Sycophaga* sp2., *P. longicaudata* and *S. roxburghi* (Peng et al., 2005; also see figweb (<http://www.figweb.org>)). These wasps exhibit strong sexual dimorphism: the females are winged, as in most chalcid wasps, and effect host plant choice while the males are wingless and usually do not leave the cavity of their natal fig. *Sycophaga* sp2. was present at low frequencies, while the other species were more abundant and may occur together in a fig (Peng et al., 2005; personal observation). *Ficus auriculata* produces large figs, and the different wasp species oviposit sequentially at different fig development stages (Fig. 2). Therefore, the different wasp species are probably exposed to different stage-specific chemicals or mixes of chemicals. Further, the pollinating wasp *C. emarginatus*, enters the fig to oviposit, losing part of the flagellomere in the process while the other species oviposit from outside the fig.

2.2. Oviposition and behavioural events of fig wasps

Ficus auriculata is a gynodioecious fig species that produces a globose-shaped inflorescence called the syconium or fig. The development of the fig is separated into 5 developmental stages (A–E) (Berg and Corner, 2005). The four most abundant wasp species associated with *F. auriculata* figs oviposit at different stages of fig development (Peng et al., 2005; Personal observation; Fig. 1). *Sycophaga* sp. is an as yet undescribed galler that oviposits early in fig development before female flower receptivity, from outside the fig into ovules in male and seed figs, using its long ovipositor (Peng et al., 2005). Females of this species are notably larger than the other fig wasps associated with *F. auriculata*.

Ceratosolen emarginatus penetrates figs when female flowers are receptive. In female figs it only pollinates while in male figs it oviposits into fertilised flowers, transforming them into galls. The larvae develops feeding on endosperm. *Philotrypesis longicaudata* and *S. roxburghi* oviposit later in the development of male figs, after female receptivity but before male anthesis. *Philotrypesis longicaudata* is a kleptoparasite that feeds on the galls containing developing *Sycophaga* sp. This species oviposits about 20 days after fig receptivity. *Sycosapter roxburghi* is the last NPFW wasps to oviposit. It lays its eggs into the developing figs about 30 days after fig visitation by pollinators. *Philotrypesis longicaudata* and *S. roxburghi* have an overlapping oviposition window of 1–2 days (Fig. 1). Subsequently, fig wasps and seeds develop in the male and seed figs, respectively. Adult mated females leave male figs at male anthesis. The seed figs ripen synchronously, attracting fig-eating frugivores. During a development cycle, figs usually undergo a series of changes in size, colour, odour and wall thickness (Fig. 2).

The pollinating wasps exhibit a characteristic behavioural sequence leading to entry into the fig (Fig. 2A–D). Upon landing on a fig, *C. emarginatus* foundresses hold their antennae upward and begin walking over the fig surface (Fig. 2A1). Then they lower the antennae and touch the surface of the fig with the tip of the final flagellomere by curving the antennae downward at the distal end (Fig. 2A2, 2A3). If the fig is clearly not receptive, they lift again their antennae and rapidly fly away. If the fig is receptive, the wasp continuously taps the surface of the fig with its antennae while walking around on the fig. Then the wasp begins to sweep the surface of the fig with the ventral part of the flagellum. While performing these actions, the wasp gradually approaches the ostiole located at the apex of the fig and shows an increased sweeping frequency. Once the ostiole is reached, the wasp lifts the ostiolar bracts using the tooth located on the third antennal segment at the base of the flagellomere (Fig. 2A4; Fig. 3A; Fig. 4D). This tooth is typical of fig pollinating wasps and facilitates entry into the fig cavity. If the fig is not at the right stage, the wasps do not perform the sweeping behaviour and instead fly away to find an appropriate-stage fig in the same fig tree. The oviposition behaviour of the three NPFWs can be separated into two groups. *Sycophaga* sp. introduces its ovipositor into the fig by positioning itself close to the fig surface, holding its ovipositor mainly parallel to the fig surface (Fig. 2B). The extremity of the ovipositor is inserted into the fig wall and the wasp moves backwards

progressively inserting the whole length of the ovipositor in the process. While ovipositing, the wasp hold its antennae straight in front of it. If an ant comes near, the wasp flattens on the fig surface and is generally not detected by the ant (for a description of this behaviour in the related genus *Idarnes*, see Jandér, 2015). Therefore, the whole length of the flagellum may contact the fig surface. On the other hand, *P. longicaudata* and *S. roxburghi* position the ovipositor shaft orthogonally to the fig surface, and progressively insert their ovipositor while maintaining the position of the shaft of the ovipositor (Fig. 2C and D). Hence the head of the wasps does not come close to the fig surface and only the tip of the antennae is used to contact the fig surface.

2.3. Antennae preparation for SEM

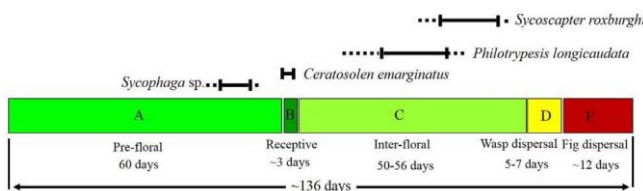


Fig. 1. Oviposition sequence of four fig wasp species associated with *F. auriculata* figs along the different development stages (A–E) of figs. The dashed lines show the first and the last arrival of wasps landing on the fig surface. The solid lines show the range of oviposition time.

Figs from which wasps were soon to emerge were placed in a nylon bag so that females could be collected as soon as they emerged through the exit hole chewed by male pollinators. SEM samples were prepared for observation using two techniques. First, foundresses were fixed in 2% glutaraldehyde and the number and distribution of sensilla on the antennae of each species were examined. Second, typical characteristics of the sensillar surface, including the cuticular grooves, pores, and basal sockets, were examined in detail. The female wasps were cleaned, stored in 3.5% glutaraldehyde, and then dehydrated in a graded series of alcohol as well as pure isoamyl acetate (Li et al., 2009, 2014). After dehydration, the samples were critical-point dried with a Quorum K850 drier (Quorum Technologies, Deben, UK), gold-coated in a Quorum Q150R sputter device (Quorum Technologies, Deben, UK), and observed with a Zeiss EVO-LS10 (Zeiss, Oberkochen, Germany) at 10 kV. The antennae were viewed from the ventral, dorsal, lateral and top sides. The antennae of more than 12 female wasps were examined for each species.

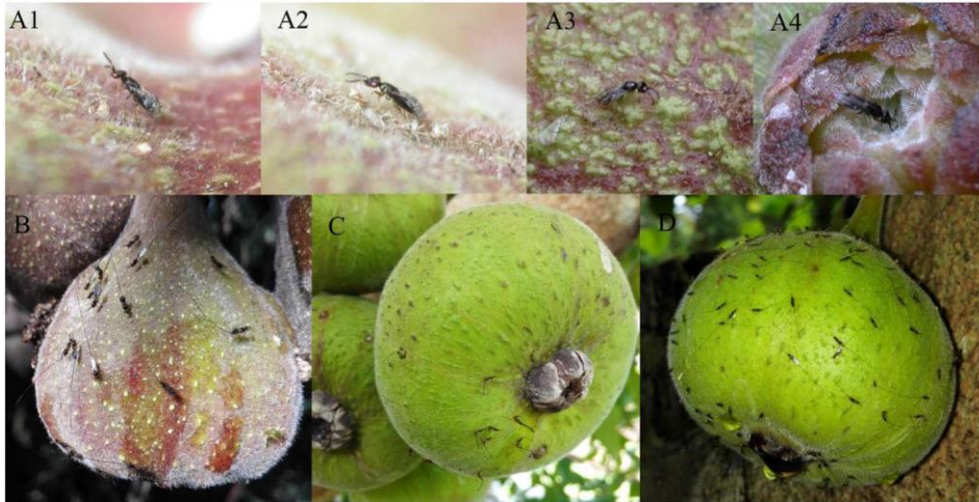


Fig. 2. Females of the four wasp species landing or ovipositing from outside the figs of *F. auriculata*. (A) Fixed sequence of behavioural events in the pollinator *C. emarginatus*, including holding the antennae upward (A1), lowering the antennae (A2), tapping the fig surface with the tip of the final flagellomere by curving the antenna downwards (A3), and lifting the ostiolar bracts with the modified spine-like expansion of the third antennal segment (A4). The bottom pictures show aggregative oviposition by the three nonpollinators, *Sycophaga* sp. (B), *P. longicaudata* (C), *S. roxburghi* (D).

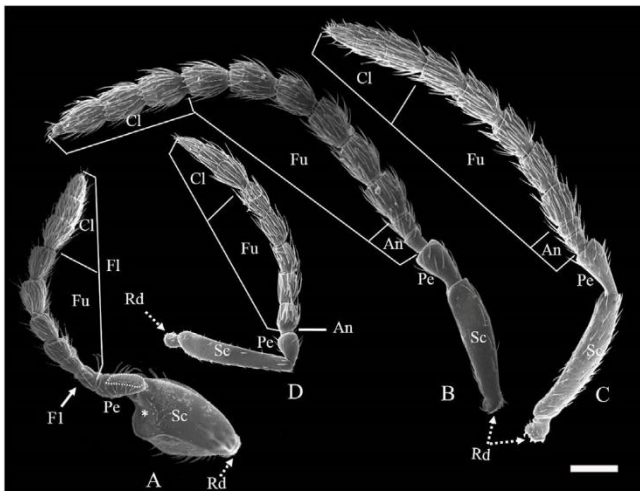


Fig. 3. Female antennae of four fig wasps associated with *F. auriculata*. The figure shows SEM graphs of excised geniculate antennae of females of the four fig wasp species *C. emarginatus* (A), *Sycophaga* sp. (B), *P. longicaudata* (C), and *Sycospater roxburghi* (D) and depicts the Radicula (Rd), Scape (Sc), Pedicel (Pe), and Flagellum (Fl), which is subdivided into the Anellus (An), Funicle (Fu) and Clava (Cl). Important morphological differences among the four species are indicated with a solid arrow (flagellomere one (F1) base with a spine-like expansion), a dashed loop (sensilla obscura on the dorsal side of the pedicel), an asterisk (deep socket), and a dashed arrow (radicula). Scale bar = 100 μ m.

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2.4. Sensilla number and analysis

The sensilla on the different sides of the antennae of each species were identified, recorded, and measured from the printed SEM pictures. For the irregular antennal segments, such as the scape on the antennae of *C. emarginatus*, sensilla numbers were directly counted on the ventral and dorsal sides. When counting sensilla on the cylindrical segments, such as the flagellomere (except for the terminal segment, which is conical and examined from the top side, see Fig. 4B), the total numbers were calculated from 2/3 sides of the total viewing surface area (Li et al., 2009; Ware and Compton, 1992). Each sensillary type was measured in at least 20 individuals from each species. To test for significance of variation among species, Kruskal–Wallis tests were used to analyse the distribution and density of multiporous placoid sensilla, which are a synapomorphy of chalcid wasps and were the most conspicuous type of sensillum and occurred on the flagella of females of the four wasp species.

3. Results

3.1. Antennae

The female antennae of the four species studied here are geniculate (angular in presentation) and consist of three functional segments: the scape with radicula (the area that is located proximally on the scape, is limited distally by a constriction and bears the basal knob proximally), the pedicel (the antennal segment that is connected proximally to the scape and distally to the flagellum) and the flagellum (Fig. 3 A–D). The mean length of the different segments are shown in Table 1. The radicula (Rd) is separated from the scape by a constriction, particularly in the two Sycoecine species, *P. longicaudata* and *S. roxburghi* (Fig. 3 C, D). It is relatively short for chalcid wasps (Heraty et al., 2013; Onagbola and Fadamiro, 2008). It has a particular type of sensillum (Figs. 3C and 5G). The radicula fits into the antennal socket, where it connects with the head through a ball-and-socket joint that allows movement in all directions. The scape (Sc) in *C. emarginatus* is irregular in shape and has two deeper grooves on the ventral side (Fig. 3A) that may serve to hold the basal part of the flagellum when the pollinators pass through the bracts. On the other hand, in NPFWs, the scape is cylindrical and is approximately 5-times longer than wide (Fig. 3 B–D). The pedicel (Pe) in the four species is a short, pear-shaped segment that connects to the scape by a hinge-joint articulation, thus restricting the movement of the antennae to one plane. The elongate flagellum consists of eight, eleven, eleven and nine distinct flagellomeres in *C. emarginatus*, *Sycophaga* sp., *P. longicaudata* and *S. roxburghi*, respectively; thus, the entire antenna is composed of varying segment numbers. We did not determine which segments were fused in the context of the basic organisation of chalcid wasp antennae, so we did not number the segments of the flagellum. The flagellum is subdivided into a basal ring-like sub-segment, the anellus (An), the median funicle (Fu) and the distal club-shaped clava (Cl) (Fig. 3). In this study, flagellomere one is only described in detail for *C. emarginatus* because its base is irregular and produces a pointed spine-like expansion,

traditionally called a tooth, which is clearly visible on the antenna (Figs. 3A and 4D). The surfaces of the antennae of the four species present a similar aspect, although the type, number and distribution of the antennal sensilla on the four species differ. These morphologically different sensilla have been given various names in the literature. We will use the terms trichoid sensilla, chaetica sensilla 1 and 2, uniporous and multiporous basiconic sensilla, multiporous placoid sensilla, basiconic capitate peg sensilla, sensilla obscura and coeloconic sensilla 1 to 3. These eleven different types of sensilla were never observed to occur together in a single species (Table 2).

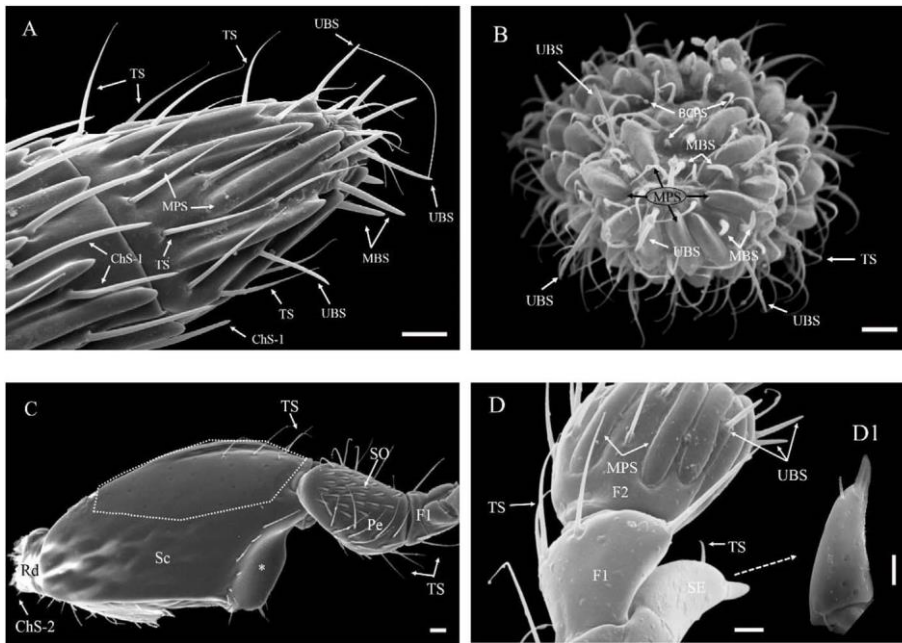


Fig. 4. Different types of antennal sensilla recorded on four fig wasp species associated with *F. auriculata*. (A) Micrograph of the tip flagellomere of *P. longicaudata* showing the trichoid sensilla (TS), chaetica sensilla type 1 (ChS-1), uniporous (UBS) and multiporous basiconic sensilla (MBS), and multiporous placoid sensilla (MPS) from the dorsal view. The UBS tips are well above the other sensilla (the arc). (B) Distribution of different sensilla, including basiconic capitate peg sensilla (BCPS), occurring on the tip of the clava of *C. emarginatus*. (C) Basal segment of antennae of *C. emarginatus* shows the specific sensory organs, including the sensilla obscura (SO) and chaetica sensilla type 2 (ChS-2). The dashed loop shows the mixed distribution of coeloconic sensilla 2 and 3. (D) Spine-like expansion detached from the base of first flagellomere (F1); the dashed arrow shows the enlarged image of the spinelike expansion with coeloconic sensilla 2 and 3 (pits on the surface, D1). Scale bars = 10 μ m

3.2. Antennal sensilla and their locations

3.2.1. Trichoid sensilla (TS)

Trichoid sensilla are present on all segments where they are abundant except for the flagellum of *S. roxburghi*, where they are few (Table 2; Fig. 6). These sensilla are inserted at a sharp angle, inclined in the direction of the axis of the antennae, and their tips are usually curved and point toward the distal end of the antennae (Fig. 4A–D; Fig. 5A and B). In the four species, TS vary in length between 4.13 μm and 49.54 μm depending on their location on the antennae. They present 8–11 longitudinal grooves reaching up to the tip. The base is inserted in a small asymmetric socket with a slightly raised ridge, and we did not detect any pore (Fig. 5 E, E1). Trichoid sensilla were most abundant in *C. emarginatus* and *Sycophaga* sp., slightly less so in *P. longicaudata* and were distinctly fewer in *S. roxburghi* (Table 2).

3.2.2. Chaetica sensilla (ChS)

We classified these sensilla into two subtypes: Chaetica sensilla type 1 (ChS-1) and type 2 (ChS-2). These subtypes present differences in morphology, size, location and cuticular attachment (Fig. 5D, F). ChS-1 is elongate and was only observed on the flagellum of *P. longicaudata* and *S. roxburghi* (Table 2; Fig. 6E–G), where they are intermixed with trichoid sensilla and partly compensate for the lower number of TS in these two species when compared to *C. emarginatus* and *Sycophaga* sp. (Fig. 5A). These sensilla are straight or medially bent bristles that arise directly from the cuticle. Their diameter decreases progressively finishing in a pointed apex. In these two species, the length of the ChS-1

Table 1

varies noticeably in different locations on the antennae, ranging from 0.89 μm to 8.35 μm . ChS-1 are the most numerous sensilla on *S. roxburghi* antennae, representing 40% of the total number of sensilla, while they are less abundant on *P. longicaudata* antennae (Table 2). ChS-2 is only observed on the base of the radicle and at the scape–pedicel joint in the antennae of all species (Table 2; Fig 4C; Fig. 6 A, C, D, G, H). There are about 20 per antenna. This type of sensillum is constituted by a short, tapered bristle with a bulbous base that projects perpendicularly to the antennal axis from a wide flexible socket (Fig. 5 F). ChS-2 has a constant length of 4.01 ± 0.24 μm and a basal diameter of 1.11 ± 0.26 μm . Both types of ChS are smooth and do not have apparent pores (Fig. 5 D, F).

3.2.3. Basiconic sensilla (BS)

Two types of basiconic sensilla were observed. Uniporous basiconic sensilla (UBS) are erect, and they were only present at the distal end of the terminal flagellomere of the antennae in all species. Because of their length (limited variation among species; 24.75 ± 5.93 μm in length, 2.33 ± 0.39 μm in basal diameter) and orientation, the tips of these sensilla project further than the other sensilla TS/ChS-1 (Fig. 4A and B; Fig. 6A, B, F). UBS are inserted in flexible basal sockets, present a longitudinal groove on their shafts, and their tips are blunt, suggesting the presence of a single apical pore (Fig. 5A). Multiporous basiconic sensilla (MBS) are short, upright, straight or medially slightly bent bristles distributed on the lateral-ventral side of the

flagellomere, on the funicle and clava of all species (Fig. 4A and B; Fig. 6A, B, E, F). They are however much more abundant in *C. emarginatus* and in *Sycophaga* sp. than in the two other wasp species. These MBS present limited variation and are $21.41 \pm 4.09 \mu\text{m}$ long and their basal diameter measures

$2.02 \pm 0.25 \mu\text{m}$. The shaft arises from a slight depression in the cuticle

Mean length of the female antennal segments of the four wasps associated with *F. auriculata*.

Antennal segments	<i>C. emarginatus</i> ^a	<i>Sycophaga</i> sp.	<i>P. longicaudata</i>	<i>S. roxburghi</i>
Radicula	17.60 ± 1.67	60.29 ± 14.26	47.72 ± 2.61	40.69 ± 7.74
Scape	232.07 ± 14.35	253.32 ± 4.49	275.29 ± 11.28	270.28 ± 3.97
Pedicel	101.76 ± 5.68	114.88 ± 1.75	100.60 ± 4.23	75.53 ± 0.97
Flagellum	596.40 ± 20.50	989.97 ± 48.00	681.79 ± 23.51	496.20 ± 24.85
Total	930.23 ± 31.47	1418.46 ± 55.59	1105.39 ± 37.34	882.70 ± 27.01

^a Flagellar length does not include the tooth-like expansion because it detaches from the antennal axis. Values show mean length ($\mu\text{m} \pm \text{S.E}$, $n = 12$).

Table 2

Abundance and distribution of different types of female antennal sensilla.

Sensilla	<i>C. emarginatus</i>	<i>Sycophaga</i> sp.	<i>P. longicaudata</i>	<i>S. roxburghi</i>
TS	346 ± 37	311 ± 16	226 ± 15	69 ± 9
UBS	6 ± 1	6 ± 1	6 ± 1	6 ± 1
MBS	24 ± 2	25 ± 7	6 ± 1	4 ± 1
MPS	69 ± 7	121 ± 5	81 ± 2	52 ± 7
BCPS	10 ± 1	12 ± 1	8 ± 1	8 ± 1
ChS-1	–	–	63 ± 3	102 ± 10
ChS-2	24 ± 2	22 ± 1	22 ± 2	18 ± 2
SO	27 ± 1	–	–	–
CoS-1	1	–	–	–
CoS-2	24 ± 2	–	–	–
CoS-3	34 ± 4	–	–	–
Total	564 ± 14	497 ± 23	410 ± 18	256 ± 11

Values are the mean \pm S.E of different types of antennal sensilla on each antennae per species ($n = 12$). The letter abbreviations state the type of sensilla described in text.

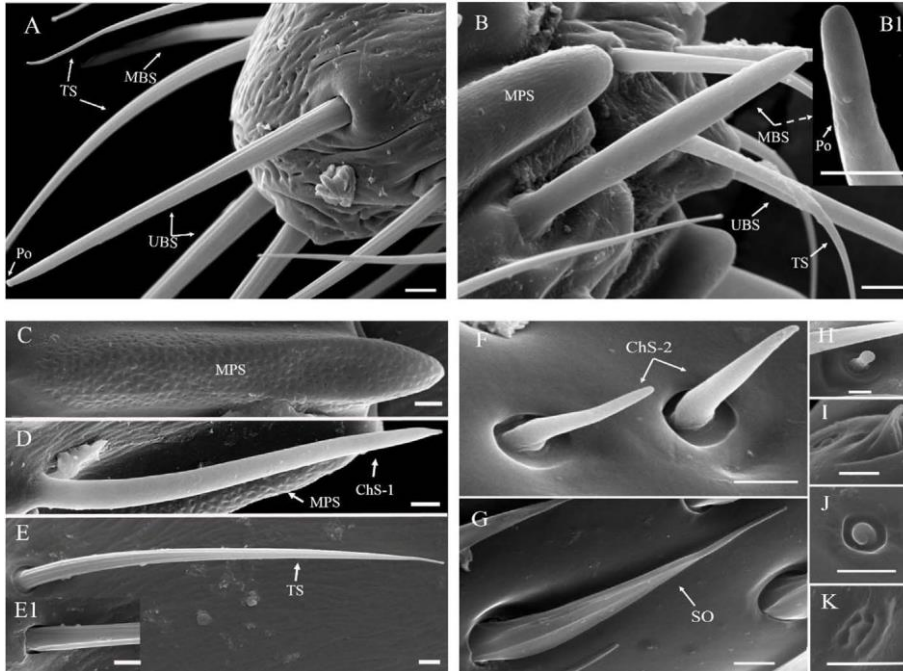


Fig. 5. Fine structure of different female antennal sensilla of the four fig wasp species associated with *F. auriculata*. (A) Uniporous basiconic sensilla (UBS) with a blunt tip (Po) occurred on the terminal part of the clava of the antennae of *Sycophaga* sp. Other sensilla are also labelled in the figures. (B) Multiporous basiconic sensilla (MBS) with many surface pores (B1) on the terminal claval segment of *P. longicaudata*. (C) Multiporous placoid sensilla (MPS) on *C. emarginatus*. (D) Chaetica sensilla type 1 (ChS-1) on *P. longicaudata*. (E) Trichoid sensilla (TS) with longitudinal grooves insert into a flexible socket (E1) on *Sycophaga* sp. (F) Chaetica sensilla type 2 (ChS-2) on *Sycophaga* sp. (G) Sensilla obscura (SO) with longitudinal grooves inserts into an oval socket. (H) Basiconic capitate peg sensilla (BCPS) on *P. longicaudata*. (I) Coeloconic sensilla type 1 (CoS-1). (J) Coeloconic sensilla type 2 (CoS-2). (K) Coeloconic sensilla type 3 (CoS-3). Scale bar = 2 μm

and decreases gradually toward the terminal apex (Figs. 4A and 5B). The MBS wall is relatively smooth and presents apparent pores on high resolution images (Fig. 5B1).

3.2.4. Multiporous placoid sensilla (MPS)

Multiporous placoid sensilla are numerous and conspicuous sensory structures on the antennae of all species (Fig. 3 A–D; Fig. 6 A, B, E, F). This type of sensillum rises directly from the antennal cuticle to form a sausage-shaped plate, and they cover each funicular and claval flagellomere, in a ring-like arrangement (Fig. 4 A–B, D). Multiporous placoid sensilla lie parallel to the antennal axis, present a pointed end and are always located between rows of TS/ChS-1 (Fig. 4A, D). The MPS plate is smooth and presents two pores per 400 nm at median length (Fig. 5C). The pore density of MPS surface per 1 μm^2 is

27.86 ± 3.44 in *C. emarginatus*, 27.75 ± 5.94 in *Sycophaga* sp., 26.00 ± 4.92 in *P. longicaudata*, and 26.13 ± 6.10 in *S. roxburghi* (n = 8), without significant difference among the four species ($\chi^2 = 0.579$, P = 0.901).

3.2.5. Basiconic capitate peg sensilla (BCPS)

Basiconic capitate peg sensilla are found on the lateral-ventral side of the apical end of each flagellomere on the funicle and clava on the antennae of all species (Fig 4B; Fig. 6E and F). BCPS are nipple-like structures with an expanded terminal part and a short stalk that sits within a distinct cuticular depression. In the four species, the BCPS shapes are slightly variable and present major differences in the length of the stalk and the diameter of the nipple. There are usually 1, sometimes 2, and very occasionally 4 BCPS per flagellomere (Fig. 4B). Their nipples contain 11 finger-like projections jointing at the tip of the stalk. The grooves between projections are relatively deep and may indicate the existence of pores (Fig. 5H).

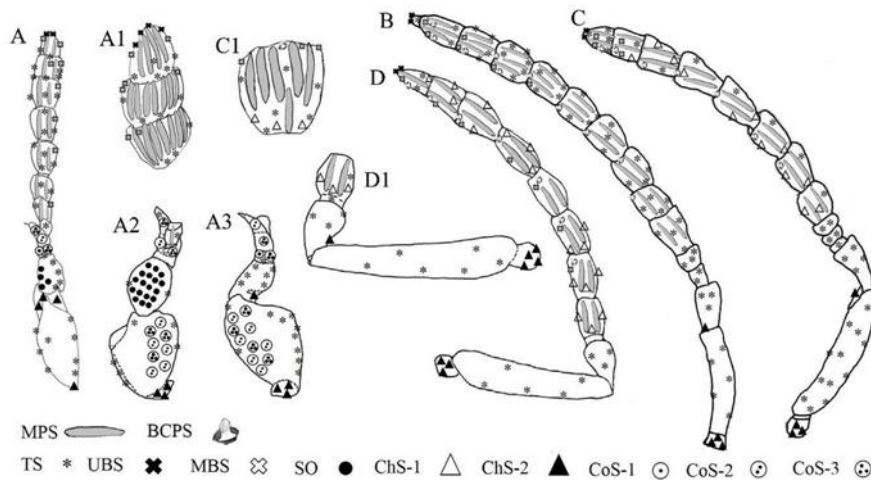


Fig. 6. Schematic diagrams of the distribution of 11 types of sensilla on the antennae of the four fig wasp species associated with *F. auriculata*. (A) Dorsal-ventral side of the whole antennae of *C. emarginatus*. (A1) Dorsal side of the clava on *C. emarginatus*. (A2) Dorsal/ventral (A3) side of the scape and pedicel, as well as one flagellomere shape on *C. emarginatus*. (B–D) Lateral-ventral side of the whole antennae of *Sycophaga* sp. (B), *P. longicaudata* (C) and *S. roxburghi*. (D), respectively. (C1) Ventral side of the second claval flagellomere on *P. longicaudata*. (D1) Enlarged drawing of the scape and pedicel on *S. roxburghi*

3.2.6. Coeloconic sensilla (CoS)

Coeloconic sensilla are recessed in a pit and only distributed on segments one and three of the antennae of the pollinator *C. emarginatus* (Table 2; Fig. 6 A, C, D). Based on the peg exterior appearance, location and cuticular attachment, three different types of CoS were identified (Fig. 5I–K). Type 1 CoS (CoS-1) is ear-shaped, rare (only 1 per antennae), and always close to the crossed groove on the tooth-like expansion. This sensillum sits in a shallow oval socket with a central opening, and its cuticle has 4–5 deep longitudinal grooves reaching the terminal tip (Fig.

5I). Type 2 CoS (CoS-2) is a spiral, bud-shaped protrusion that covers the tooth-like expansion and the top dorsal side of the scape, and it mixes with type 1 (CoS-1) and 3 CoS (CoS-3). The shaft is inserted into a deep, circular socket and has a diameter of 0.78 μm that tapers gradually (Fig. 5J). CoS-3 is a small-coned protrusion that is obviously lower than the depth of the wide circular socket (Fig. 5K). CoS-3 always mixes with CoS-2 but never with CoS-1. Both CoS-2 and CoS-3 have smooth walls without pores (Fig. 5J–K).

3.2.7. Sensilla obscura

The name sensilla obscura (SO) was originally proposed by Cockerill (2005) and has been used in subsequent work on fig pollinating wasps (Li et al., 2009, 2014). They are highly distinctive sensilla grouped dorsally on the pedicel of the pollinator *C. emarginatus* (Table 2; Fig 3A; Fig 4C; Fig. 6A, C). Each SO is flattened and awl-like in shape and strongly tapered to an elongate and slender tip parallel with the antennal surface (Fig. 5G). These sensilla measure $12.24 \pm 1.12 \mu\text{m}$ in length and $2.41 \pm 0.40 \mu\text{m}$ in basal diameter. Between 5 and 6 widely longitudinal grooves line the shaft, which is inserted into an oval socket that is almost equal to the length of the shaft. There are approximately 27 SOs, all of which point toward the head (Fig. 4C).

3.3. Abundance and distribution of female antennal sensilla in the four wasp species

The four species differ significantly in the distribution and abundance of some sensillar types on the antennae (Table 2; Fig. 6). TS occur on the flagellum of the four species but are significantly more abundant in *C. emarginatus* and *Sycophaga* sp. than in *P. longicaudata* and *S. roxburghi* (Table 2; Fig 1; Fig 7). Similarly, MBS are more abundant in *C. emarginatus* and *Sycophaga* sp. than in *P. longicaudata* and *S. roxburghi*. MBS are restricted to the lateral-ventral side of each flagellomere on the funicle and clava. In contrast, the long, rigid ChS-1 present on the flagellum are numerous on *P. longicaudata* and *S. roxburghi*, and none are found on *C. emarginatus* and *Sycophaga* sp. (Table 2; Fig. 7). The short and strong ChS-2 only occur on the lateral-ventral side of the radicle and ventral surface of the pedicel at the scape–pedicel joint, and no major differences in distribution and number were found among the four species. UBS are always located at the apex of the last flagellomere, and a constant number (approximately 6) is found on the antennae of each species (Table 2). MPS are present in all four species. MPS lengths vary according to species, while their width does not vary (Fig. 8). Their size and number are negatively correlated among the four species ($r = -0.96$, $P = 0.02$; Pearson correlation). Smaller MPS sizes, such as lengths of $32.44 \pm 3.52 \mu\text{m}$ on the antennae of *Sycophaga* sp., are associated with higher numbers of MPS ($\chi^2 = 27.809$, $P < 0.001$), thus leading to more ring-like arrangements on the antennae and larger total surface for the smaller MPS ($\chi^2 = 27.152$, $P < 0.001$; Table 2; Fig 3C; Fig 8). The abundance and distribution of BCPS are not significantly different among the four species. TS/ChS-1, MPS, and BCPS always occur concurrently on the same flagellomere, whereas both CoS and SO only occur on the antennae

of the pollinator *C. emarginatus* that enters the receptive figs (Fig. 6). The abundance and distribution as well as the configuration of ST, ChS-1, MBS, MPS, SO, and CoS may indicate the occurrence of morphological adaptations and sensory differentiation among the four species.

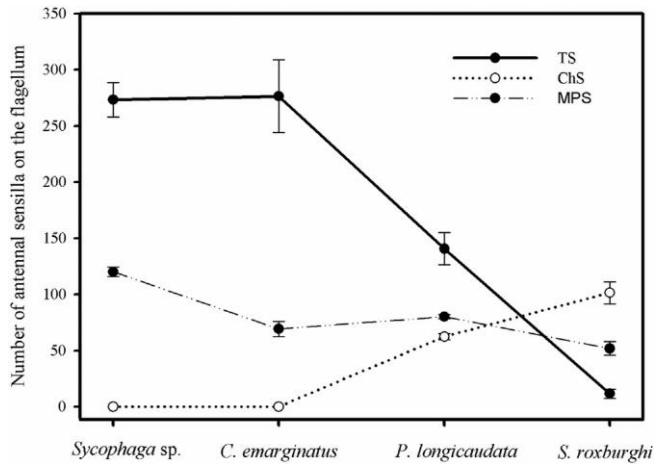


Fig. 7. Number of the three major types of sensilla on the flagellum of females of the four fig wasps associated with *F. auriculata*. TS: trichoid sensilla; MPS: multiporous placoid sensilla; ChS: chaetica sensilla.

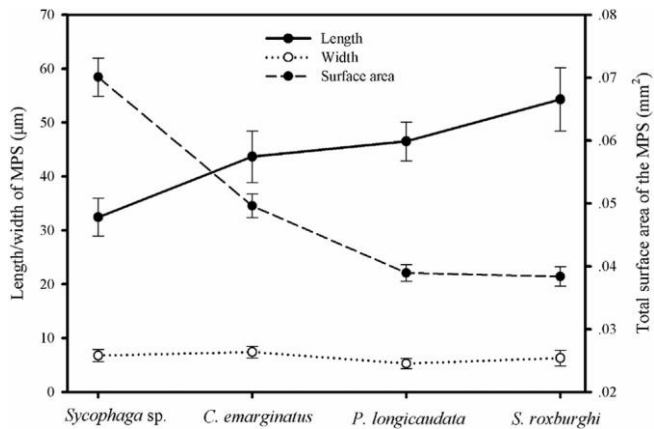


Fig. 8. Measurements and total surface area of multiporous placoid sensilla on the flagellum of females of four fig wasp species associated with *F. auriculata*.

4. Discussion

A wide variety of sensilla have been recorded on the antennae of female parasitoids (Basibuyuk and Quicke, 1999; Isidoro et al., 1996; Polidori and Nieves-Aldrey, 2014; van Baaren et al., 2007), and their detailed structures and configurations can help interpret sensilla function with regard to sensory-guided behaviours, such as host discrimination and exploitation (Das et al., 2011; Isidoro et al., 1996; Ochieng et al., 2000; Roux et al., 2005; Staudacher et al., 2005; van Baaren et al., 2007). There is as yet no standardised naming of sensilla and the same

types of antennal sensilla are subdivided and assigned to different sensilla types by different authors despite presenting similar morphologies and distributions. Therefore, comparisons are difficult and associations between name and function are fuzzy. In this study, we assigned sensilla to different types based on the presence of distinct differences in external appearance and cuticular attachment following the nomenclature used by Basibuyuk and Quicke (1999), Onagbola and Fadamiro (2008), Dweck (2009), and Li et al. (2009, 2014). For example, although hairs had various sizes and were widely distributed on the antennae of the four species (Fig. 4A–D; Fig. 5A–B), they presented similar shafts and basal sockets. Therefore, we used the same name for all of them, i.e. trichoid sensilla, and did not subdivide them again into several subtypes or classify them under other names (Onagbola and Fadamiro, 2008). Chaetica sensilla should reflect the same rigid spines with a flexible basal socket as type 1 and should have a similar appearance and distribution but may vary in size.

Here, we reported 11 different types of female antennal sensilla in four wasp species associated with the figs of *F. auriculata*. These wasps depend largely on species-specific chemicals to discriminate their host figs from other fig species (Hossaert-Mckey et al., 2010; Proffit et al., 2007; Wang et al., 2013). These types of antennal sensilla never occur simultaneously on a single species and their types are consistent with those observed on other hymenopteran parasitoids (Amornsak et al., 1998; Basibuyuk and Quicke, 1999; Ochieng et al., 2000; Onagbola and Fadamiro, 2008; Polidori and Nieves-Aldrey, 2014; Steinbrecht, 1997; van Baaren et al., 2007). The sensilla observed on *C. emarginatus* were similar to those found on the closely related fig pollinating wasp *C. solmsi marchali* (Li et al., 2009; Meng et al., 2016). On the other hand, while the global organisation of sensilla is similar, coeloconic sensilla are absent in the pollinator of *F. curtipes*, *Waterstoniella* sp. (Li et al., 2014). SO are restricted to fig pollinating wasps and always grouped dorsally on the pedicel (Li et al., 2009, 2014; Meng et al., 2016). They have not been observed in other wasp species (Polidori and Nieves-Aldrey, 2014; van Baaren et al., 2007). Analysing the distribution of the different types of sensilla in connection with their morphology and the behaviour of the wasps may provide practical information and theoretical insights into their functions in these fig wasps.

4.1. Putative functions of the different sensilla types

Hair-like TS were abundant on the antennae of the four study species and have been reported on the antennae of nearly all investigated hymenopteran parasitoids (Amornsak et al., 1998; Bleeker et al., 2004; Das et al., 2011; Gao et al., 2007; Li et al., 2009, 2014; Meng et al., 2016; Ochieng et al., 2000; Onagbola and Fadamiro, 2008; Polidori and Nieves-Aldrey, 2014; Romani et al., 2010; Roux et al., 2005; van Baaren et al., 2007). In the four analysed species, the TS occur in various forms and lengths and at different locations, but they always present more longitudinal grooves on the ventral side in the direction of the antennal axis. The sockets of the TS hairs are small, flexible, and asymmetric, thus allowing for the directional movement of the hair; they present the functional morphology of mechanoreceptors (Staudacher et al., 2005).

The absence of apical or peripheral pores and traces of wall pores observed on the TS in the present study further establishes that they are not olfactory receptor (Steinbrecht, 1997). A transmission electron microscopy investigation has further confirmed a mechanosensory function of TS in two pollinating fig wasp species, *C. solmsi marchali* and *Waterstoniella* sp. by showing lack of innervation of the TS body (Li et al., 2009, 2014).

We found two types of non-porous ChS on the antennae of the four species. ChS-1 that were distributed on the flagellum of both *P. longicaudata* and *S. roxburghi* are similar to those described in some braconid *Microplitis* (Gao et al., 2007; Ochieng et al., 2000) and pteromalid *Pteromalus* species (Dweck, 2009; Onagbola and Fadamiro, 2008), while they are absent in pollinating fig wasps (Agaonidae) (Li et al., 2009, 2014; Meng et al., 2016). ChS-1 have been assumed to have a mechanosensory functions because of their socket-like insertions, and because they present non-porous walls as revealed from the SEM images (Gao et al., 2007; Ochieng et al., 2000; Staudacher et al., 2005). Although the location and number of ChS-1 do not vary, their sizes vary even on the same flagellomere, such as on the funicle on *S. roxburghi*. We propose that the various ChS-1 can sense the magnitude of external pressure from the antennal waving frequency and protect the fragile chemoreceptors from damage. ChS-1 are entirely replaced by TS in *C. emarginatus* and *Sycophaga* sp., two species in which the whole length of the flagellum may contact the fig surface.

ChS-2 are widely found on various parasitoids, although they have been given different names, such as Böhm's bristles or hair plates (Romani et al., 2010; Staudacher et al., 2005), aporous type 4 sensilla trichodea (Onagbola and Fadamiro, 2008), and non-porous sensilla chaetica (Dweck, 2009). These small erect sensilla only occur at the level of the radicle and scape–pedicel joint in all species and may function as proprioceptive position detectors that keep the wasps informed of the relative position of the antennal segments (Romani et al., 2010).

The uniporous and multiporous basiconic sensilla recorded in *C. solmsi marchali* and *Waterstoniella* sp. were also present in the four species investigated here (Li et al., 2009, 2014). The morphology and location of the uniporous basiconic sensilla suggest that they correspond to the same category as sensilla previously described under other names such as the fluted BS (Norton and Vinson, 1974), TS with a tip pore (Bleeker et al., 2004), uniporous ChS (Onagbola and Fadamiro, 2008), and uniporous pitted sensilla (Romani et al., 2010). These UBS present an apical pore and emerge above the other sensilla, suggesting that they are involved in contact chemoreception when wasps touch a surface with the tip of their antennae, as has been suggested in many other Hymenoptera (Dweck, 2009; Onagbola and Fadamiro, 2008; Romani et al., 2010; Roux et al., 2005). We noted that after landing on a fig, female wasps of the different species tapped the surface of the fig with the distal part of the antennae bearing UBS, which further suggests a role of these sensilla in contact chemoreception and fig stage-specific discrimination (Proffit et al., 2007). The number of UBS, 6, was remarkably constant among species. The presence of UBS located on the terminal antennal segment seems to be generalised in Chalcidoidea.

Multiporous basiconic sensilla have also been described as curved non-fluted BS (Norton and Vinson, 1974), sensilla basiconica I (Ochieng et al., 2000), TS with wall pores (Bleeker et al., 2004), and multiporous pitted TS (Romani et al., 2010). In general, multiple wall pore sensilla have been assumed to function as olfactory structures (Ochieng et al., 2000; Steinbrecht, 1997; van Baaren et al., 2007). We found numerous pores on the cuticle of multiporous basiconic sensilla under SEM examination (Fig. 5B1), supporting an olfactory function of this type of sensillum. MBS are confined to the lateral-ventral side of the flagellum in the four species, suggesting they are used to detect fig surface chemicals when females contact the fig surface with their antennae (Moore et al., 2000; Wang et al., 2013). In agreement with this speculation, MBS are most abundant in *C. emarginatus* and *Sycophaga* sp., the two species that consistently touch the surface of the fig with the whole length of the flagellum.

Multiporous placoid sensilla are widely distributed on the antennae of chalcid wasps (Barlin and Vinson, 1981; Basibuyuk and Quicke, 1999; Polidori and Nieves-Aldrey, 2014; van Baaren et al., 2007; Ware and Compton, 1992). Usually, MPS are elongate and sausage-shaped and present ring-like arrangements around the flagellomere except for the funicle and the basal flagellomere (F1) (Heraty et al., 2013). Their multiporous surface system indicates that they are olfactory receptors (Bleeker et al., 2004; Steinbrecht, 1997). Ochieng et al. (2000) reported that MPS are dose-dependent sensors that perceive the volatiles of host plants. The exposed surface areas of these sensilla in female fig wasps, including NPFWs in this study, are larger than that of other chalcid female wasps (Barlin and Vinson, 1981; Ware and Compton, 1992) and suggests that the detection of host figs is particularly challenging and involves detecting species-specific chemicals at a distance. However, the specific function and sensitivity of MPS in fig wasp foundresses must be further explored via single-sensillum recordings.

BCPS have been reported in many chalcid wasp species belonging to a diversity of families, and they are referred to as multiporous grooved peg sensilla on the eulophid *Tetrastichus hagenowii* (Ratzeburg, 1852) (Barlin and Vinson, 1981; Ware and Compton, 1992), type I coeloconic sensillum on the braconid *Cotesia* species (Bleeker et al., 2004), type s4 sensilla on the mymaromatoids (Heraty et al., 2013), sensilla ampullaceum on the trichogrammatids (Romani et al., 2010), and peg-like sensillum on the eulophid *Sympiesis sericeicornis* (Nees, 1834) (Meyhöfer et al., 1997). These sensilla, which occur in low numbers on the antennae of all species, are short bulbous pegs with multiporous furrows as revealed by TEM (Li et al., 2009). These sensilla can be olfactory receptors (Barlin and Vinson, 1981; Bleeker et al., 2004; Onagbola and Fadamiro, 2008; Romani et al., 2010; Steinbrecht, 1997), although they may also present mixed functions, such as hygro-, thermo- and mechanoreceptor capabilities (Meyhöfer et al., 1997; van Baaren et al., 2007). Their location in fig-wasps suggest that they may have a role as proprioceptive relative position detectors (Dweck, 2009; Onagbola and Fadamiro, 2008) for the segments of the flagellum.

In other parasitic wasps, the three types of CoS that occur together in *C. emarginatus* are rare and have only been observed in another species belonging to genus *Ceratosolen* (Li et al., 2009).

To our knowledge, this study provides the first record of CoS-1, which does not resemble any CoS on other insects (Polidori and Nieves-Aldrey, 2014; van Baaren et al., 2007; Zacharuk, 1985). The CoS protrusion is strongly grooved along its length, which may be an indicator of surface pores that allow odour molecules to reach receptors (Ameismeier, 1985; Steinbrecht, 1997), suggesting that these sensilla may be involved in volatile perception. However, the low numbers of CoS-1 may restrict the sensory ability (Table 1). The non-porous CoS-2 and CoS-3 are always mixed together. CoS-2 is similar to the auriculate sensillum in the female sphinx moth *Manduca sexta* (Linnaeus, 1763) (Shields and Hildebrand, 1999), the sensilla coeloconica in the mushroom fly *Coboldia fuscipes* (Meigen, 1830) (Zhang et al., 2016), and the CoS-2 in the pollinating fig wasp *C. solmsi marchali* (Li et al., 2009; Meng et al., 2016), whereas CoS-3 resembles the single-walled poreless CoS in some Lepidoptera (Faucheux et al., 2006) and CoS-3 in *C. solmsi marchali* (Li et al., 2009; Meng et al., 2016). To enter into the fig cavity, foundresses always use the spine-like expansion as a tool for lifting the outer ostiolar bracts (Weiblen, 2002, Fig. 2 A4). Therefore, these surface sensilla could intervene as detectors during ostiolar entry. CoS-2 are likely to be involved in proprioceptive functions (Meyhöfer et al., 1997; Staudacher et al., 2005), whereas CoS-3 may sense the pressure of fig bracts (Li et al., 2009; Pophof, 1997).

No equivalent of SO has been reported from non-agaonid wasps (Polidori and Nieves-Aldrey, 2014; van Baaren et al., 2007; Zacharuk, 1985), and they could be generalised in pollinating fig wasps (Cockerill, 2005; Li et al., 2009, 2014; Meng et al., 2016). The shape of SO varies among species. They are leaf-shaped in *C. solmsi marchali* (Li et al., 2009), teardrop-shaped in *Liporrhopalum tentacularis* (Grandi, 1926) (Cockerill, 2005), and are present as trichoid hairs with a bifurcated apex in *Waterstoniella* sp. (Li et al., 2014). Their internal structure is a thick and non-porous wall (Li et al., 2009, 2014). SO are distributed dorsally in a patch on the pedicel and always point toward the wasp's head. Their functions could be to provide a grip on the ostiolar scales similar to the function of the teeth on the mandibular appendages that help the wasps get through the ostiole (Weiblen, 2002).

We may summarise an interpretation of the functional organisation of the female antennae of the study species in the following way. The wasps need proprioceptors enabling them to monitor antenna position. This role is filled by ChS-2 for the basal parts of the antennae and could be filled by BCPS for the flagellum. The principal distance olfactory detection function of the antennae is probably filled by MPS which represent the majority of the receptors that are distributed all along the flagellum. Localised contact chemical stimulation when wasps are tapping the surface of a fig with the tip of their antennae is probably fulfilled by UBS. All along the flagellum, interspersed between the MPS, longer sensilla may deflect obstacles thus protecting BCPS, and allowing the wasps to sense the strength of contact with a surface or an obstacle and allow contact chemical stimulation. These roles are filled by ChS-1, TS and MBS. The well-developed articulated sockets of TS suggest that these sensilla provide finer information on contact than ChS-1. In agreement with this proposition, in *C. emarginatus* and

Sycophaga sp., the two species that contact the surface of the fig with the whole length of the antennae, there are abundant TS and a total lack of ChS-1. The lateral-ventral disposition of MBS suggest that they are involved in contact chemo-stimulation and they are also much more abundant in *C. emarginatus* and *Sycophaga* sp. than in *P. longicaudata* and *S. roxburghi*. Finally, a whole set of sensilla are restricted to the first three antennal segments of the pollinating wasps suggesting that they are associated with various functional aspects of fig-entering behaviour.

Within this general framework, variation is observed. For instance, *Kradibia tentacularis* (Grandi, 1926) pollinating *F. montana* presents exceptionally long antennae and BCPS are present in places that can hardly be linked with a proprioceptor function (Cockerill, 2005). Data on *Waterstoniella* sp. associated with *F. curtipes* shows lack of CoS-1 (Li et al., 2014), a feature that could correlate with the simpler constitution of the tooth-like expansion of the third antennal segment in *Waterstoniella* comparatively to *Ceratosolen*. We observed large variation among species in numbers of sensilla. For instance, in fig-pollinators numbers vary from 738 sensilla including 406 TS in *C. solmsi marchali* (Mancini et al., 2009), through 564 sensilla including 346 TS in *C. emarginatus*, and down to 209 sensilla including 77 TS in *Waterstoniella* sp. (Li et al., 2014).

4.2. Morphological adaptations and differences among four agaonid species

For female pollinating fig wasps, the modified first three antennal segments and especially the tooth on the third antennal segment and the multiple sensillum types on the surface represent conspicuous antennal adaptations that facilitate pollinator passage through the fig ostiole. The challenge is probably largely mechanical, but could involve sensing ostiolar exudates (Machado et al., 2013), ostiolar firmness (Chen et al., 2001), and diverse odours released by glands located in the ostiolar scales (Souza et al., 2015). SO that are grouped dorsally on the pedicel and directed toward the head serve a mechanical function that prevents the wasps from slipping backward when foundresses pass through the ostiolar bracts and may have a function similar to that of the teeth on the mandibular appendage, which the wasps use to pull themselves through the ostiole using similar opening and closing mechanisms (van Noort and Compton, 1996). Interestingly, mandibular appendages and their teeth often present large differences among species, suggesting adaptation to variation in ostiole shape. Similarly, SO present large variation among species. If our hypothesis is true then we may predict limited development of SO in fig pollinating wasps, such as the extant species of genus *Tetrapus*, that loose neither their antennae nor their wings on entry into a fig, because of an easily penetrated ostiole.

The abundance and distribution of the mechanoreceptors such as ST and ChS-1 on the flagellum could also be related to fig surface properties. As the fig develops and grows in size, the surface also shifts from a soft to a hard surface with long surface hair. Hence, an alternative explanation to the higher number of ST present on early-arriving wasp species could be the association with softer figs than for the late-arriving wasp species associated with harder figs. Conversely, ChS-1 are regularly observed in the females of NPFWs, which lay eggs in figs that

are more advanced in development (Fig. 6; Table 2). This result is consistent with what is observed for ST on the antennae of herb- and wood-galling cynipids (Polidori and Nieves-Aldrey, 2014), or female fig wasps in *F. hispida* (Meng et al., 2016), which may indicate that the rigid ChS-1 provide better protection to chemoreceptors below. But this explanation is only applicable to parasites that feed on plant tissue on the outside of the host or have a similar life-history trait (Li et al., 2013; Meng et al., 2016; Polidori and Nieves-Aldrey, 2014).

The variation in the surface coverage area of MPS in the females of the four wasp species could be the result of variation in size and in behaviour. Ware and Compton (1992) reported that there are two distinct adaptative strategies that may increase receptor surface. The first strategy is MPS elongation which increases the receptor-exposed surface area, which in turn enhances olfactory sensitivity. MPS in the late-arriving foundresses, including *P. longicaudata* and *S. roxburghi*, are typically more elongate than those of other females belonging to the same family of wasps (Barlin and Vinson, 1981; Dweck, 2009; Onagbola and Fadamiro, 2008). The second strategy is increasing the number of sensilla and as a result increasing the exposed area by lengthening the antennae and thickening antennal sub-segments. In our case, *Sycophaga* sp. has a larger antenna than the other investigated wasps and presents more rings of MPS with a larger ring arrangement (Fig 3; Fig 8). This feature can be explained by the larger body size of *Sycophaga* sp. compared to the three other species which also translates into larger antennae (Table 1). However, because young figs produce limited quantities of odours (Proffitt et al., 2008), the associated larger MPS surface area of *Sycophaga* sp. may help detect small amounts of fig chemicals. On the other hand among the three similarly-sized wasps, and despite receptive figs producing more odours than other fig stages (Proffitt et al., 2008), the pollinator presented a 1.5 times larger surface area of MPS than the two other NPFWs. We may suggest that the reason is that NPFWs have longer life spans and thus can spend a greater amount of time searching for stage-specific figs in the natural environment (Compton et al., 1994). However, olfactory receptors are extremely sensitive and can be triggered by less than six molecules of host plant volatiles (Angioy et al., 2003), indicating that further study using single-sensillum recordings may provide insights that can explain this adaptation and sensory sensitivity.

In conclusion, females of four fig wasp species associated with figs of *F. auriculata* may use mechanoreceptors and chemoreceptors to locate and discriminate stage-specific figs. In addition to identifying the spine-like expansion and SO related to ostiole entry, this study also revealed that three types of CoS are likely involved in sensing the interior properties of figs. Two mechanosensory sensilla, including TS and ChS-1, occurred on the flagellum, and inconsistent results were observed for species laying their eggs on early soft figs and late hard figs. The chemosensilla involved in host discrimination, i.e., basiconic sensilla, multiporous placoid sensilla and basiconic capitate peg sensilla, present similar morphologies and configurations and provide a sensory infrastructure for our ongoing studies of host discrimination among these four species, including behavioural interpretations and electrophysiological studies. Further studies on the functional morphology of the antennal sensilla of the four foundresses using

transmission electron microscopy coupled with electrophysiological techniques are likely to yield a deeper understanding of the differences in chemical utilization and olfactory sensitivity in the fig wasp community. Comparative studies of antennal structure variation among fig hosts within genera of NPFW and within pollinating figs wasp genera could probably help identify causal ecological factors for inter-specific differences.

Author contributions

PY, ZBL and YQP conceived and designed the study. PY and ZBL performed the experiments and the data analyses. PY and ZBL wrote the first draft of the manuscript after having discussed the results with YQP, DRY and FK. All author contributed to revisions and approved the final manuscript.

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