# Antennal sensilla of the decapitating phorid fly, *Pseudacteon tricuspis* (Diptera: Phoridae)

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## Abstract

*Pseudacteon tricuspis* Borgmeier is a parasitic decapitating phorid fly (Diptera: Phoridae), which has been released in the southern United States in the last decade for biological control of invasive imported fire ants, *Solenopsis* spp. In a previous study, we demonstrated that *P. tricuspis* uses fire ant semiochemicals to locate host worker ants. To provide a solid background in support of our research on mechanisms of host location and olfaction in phorid flies, we studied the morphology of the antennal sensilla of both sexes of *P. tricuspis* using scanning and transmission electron microscopy. Antennae of *P. tricuspis* show strong sexual dimorphism in structure and shape: the female has a feathered arista which is located distally on the flagellum and has three sub-segments that bear small spinules of microtrichia. The first two antennal segments, scape and pedicel, bear no sensillum, but were densely covered by microtrichia. Three major types of sensilla were found on the flagellum (funicle) of both sexes: sensilla trichodea, sensilla basiconica, and sensilla coeloconica. Two of these, s. trichodea and s. basiconica were differentiated into three (short, medium, and long) and two (blunt-tip and sharp-tip) subtypes, respectively, for a total of six morphologically different sensilla subtypes. Ultrastructural studies revealed multiple wall pores on s. trichodea and s. basiconica, possibly suggesting chemoreceptory functions. The third and least abundant sensilla type, s. coeloconica, had no wall pores and may function as thermo-hygroreceptors. With the exception of the long subtype of s. trichodea which was recorded only on the male antennae, no other marked sexual differences were recorded in the number and distribution of antennal sensilla in *P. tricuspis*. These results are discussed in relation to the host location behavior of *P. tricuspis*, and could facilitate future studies on the neurobiology of olfaction in decapitating phorid flies.
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## 1. Introduction

Decapitating phorid (humpbacked) flies, *Pseudacteon* spp. (Diptera: Phoridae) are highly specific parasitoids of imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae) (Porter et al., 1995; Morrison, 2000). After initial successful introduction and subsequent mass rearing in Florida in the 1990s, two species, *P. tricuspis* Borgmeier and *P. curvatus* Borgmeier, have since been continuously released in many parts of southern United States for biological control of imported fire ants (Porter, 2000; Graham et al., 2003; Vogt and Streett, 2003).

Olfaction has been suggested as the long range cue used by *Pseudacteon* phorid flies to locate host fire ants (Gilbert and Morrison, 1997; Orr et al., 1997; Porter, 1998), but very little research has been conducted on the mechanisms of host location. In a recent study, we provided the first direct evidence of semiochemical-mediated attraction of *Pseudacteon* species to fire ant host odor and demonstrated behavioral and electroantennogram (EAG) responses of both sexes of *P. tricuspis* to red imported fire ant (*Solenopsis invicta* Buren) odor stimuli (Chen and Fadamiro, 2007).

As most olfactory sensilla are located on the antennae of insects (Hallberg and Hansson, 1999; Gullan and Cranston, 2000), a detailed study of the antennal sensilla is necessary to better understand host location mechanisms in *Pseudacteon* phorid flies. Although numerous studies have characterized antennal sensilla of various species of Hymenopteran parasitoids (Norton and Vinson, 1974; Navasero and Elzen, 1991; Olson and Andow, 1993; Ochieng et al., 2000; Pettersson et al., 2001; Bleeker et al., 2004) and non-parasitic Dipterans (Sutcliffe et al., 1990; Pfeil et al., 1994; Shanbhag et al., 1999; Fernandes et al., 2004; Sukontason et al., 2004, 2005),

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relatively little information exists on the antennal sensilla of Dipteran parasitoids (Giangiuliani et al., 1994; Rahal et al., 1996) and virtually none for parasitic phorid flies.

The aim of the present study was to investigate the antennal sensory structures of *P. tricuspis* with the goal of identifying and characterizing sensilla types involved in chemoreception. We present here the first examination of the morphology, abundance, and distribution of antennal sensilla in male and female *P. tricuspis* and discuss their probable roles in the behavior and ecology of the parasitoid. It is anticipated that this study will facilitate future research on the electrophysiology and neurobiology of olfaction in decapitating phorid flies.

## 2. Materials and methods

### 2.1. Insects

*P. tricuspis* were reared on workers of red imported fire ant, *S. invicta* at the fire ant rearing facility of the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA as previously described (Porter et al., 1997). Parasitized fire ant worker heads were held in a plastic jar (25 cm × 13 cm) with a lid until emergence. Newly emerged flies were separated by sex and maintained in 6 cm diameter Petri dishes at $25 \pm 1$ °C, $70 \pm 5$% RH, and L14:D10 h. Sugar solution (25% sucrose) and water were provided in the Petri dishes as previously described (Chen et al., 2005). Adult phorid flies processed for electron microscopy were 2-day-old. Prior to preparation for microscopy, flies were first anesthetized by chilling for ∼15 min at 4 °C.

### 2.2. Scanning electron microscopy (SEM)

Preparation for SEM was modified after the techniques previously described by Sukontason et al. (2003, 2007). Phorid flies of both sexes (≈20 per sex) were first pre-fixed in 2.5% glutaraldehyde mixed with phosphate buffer solution (PBS) at a pH of 7.4 at 4 °C for 24 h followed by postfixation in 1% osmium tetroxide for 24 h. For some specimens, an antenna was excised under a stereomicroscope (National Microscope, Model DC 3-420, Meiji, Japan) at 40× prior to pre-fixation. Specimens were then rinsed with PBS and dehydrated in a graded ethanol series of 30, 50, 70, 80, 90, 95%, and absolute ethanol, in each case for 12 h. This dehydration process was followed by critical-point drying. The specimens were then mounted to double-sticky tapes on aluminum stubs, sputter-coated with gold (in a Pelco SC-7 auto-sputter coater), and examined with a DSM 940 SEM (Carl Zeiss, Jena, Germany). Micrographs were taken of the antennae and sensilla, and the dimensions of the sensilla were measured. Abundance and the distribution of the antennal sensilla types were compared between males and females.

### 2.3. Transmission electron microscopy (TEM)

In general, procedures for TEM were similar to those described above for SEM. After dehydration in graded ethanol series (as described above), specimens were placed in acetone for 12 h and then transferred into a graded series of resin:acetone mixtures at ratios 1:3, 1:1, and 3:1, with the specimens remaining in each mixture at 4 °C for 24 h. The specimens were then embedded in Spurr's epoxy resin and incubated at 70 °C for 24 h. Ultrathin sections were cut with a diamond knife on a Leica Ultracut T ultramicrotome (Leica Microsystems, Vienna, Austria), stained with uranyl acetate and lead citrate, and viewed with a Zeiss EM 10C 10CR TEM (Carl Zeiss, Jena, Germany).

## 3. Results

### 3.1. Terminology

The terminologies and nomenclatures used to describe antennal morphology and classify sensilla types in this study follow those used for other Dipterans by Zacharuk (1985), Shanbhag et al. (1999), and Sukontason et al. (2004). Classification of sensilla types is based on morphological characters revealed by SEM/TEM (such as presence and number of pores) and comparison with illustrations in published literature on Dipterans.

### 3.2. General description of antenna of P. tricuspis

Adult *P. tricuspis* of both sexes bear a pair of antennae located frontally between the large compound eyes (Fig. 1A and B). Each antenna consists of a proximal scape, pedicel, and distal flagellum (3rd antennal segment also referred to as funiculus). In females, the flagellum is reduced, with its tip forming the feathered arista (Fig. 1B and D), which is absent in males (Fig. 1A and C). The length (mean ± S.D.) of the antenna is not significantly different (*t*-test, $P = 0.077$) between males (≈394.6 ± 53.3 μm; $n = 5$; Fig. 1C) and females (≈335.9 ± 35.4 μm, including the arista; $n = 5$; Fig. 1D). However, the width (mean ± S.D.) of the antenna is significantly shorter (*t*-test, $P = 0.036$) in males (≈101.2 ± 4.7 μm, $n = 5$) than in females (≈112.7 ± 9.1 μm, $n = 5$). Unlike in most Dipterans (see for instance, Fernandes et al., 2004; Sukontason et al., 2004, 2007), the pedicel is the shortest segment of the antenna of *P. tricuspis* and is concealed under the flagellum (Fig. 1E–G). In this species, only the flagellum bears sensilla. The distribution of the sensilla types varies by angle of view: most sensilla types are more abundant on the dorsal side than on the ventral side.

### 3.3. Scape and pedicel

The first two antennal segments (scape and pedicel) of *P. tricuspis* bear no sensillum, but a long bristle occurs on the pedicel (Fig. 1F and G). The cuticular surface of the scape and pedicel is densely covered by small spinules of microtrichia, which are small hair-like structures with sharp-pointed tips (Fig. 1H and I).

### 3.4. Flagellum

The flagellum is the most important antennal segment of *P. tricuspis*, on which numerous sensilla are found. It is densely
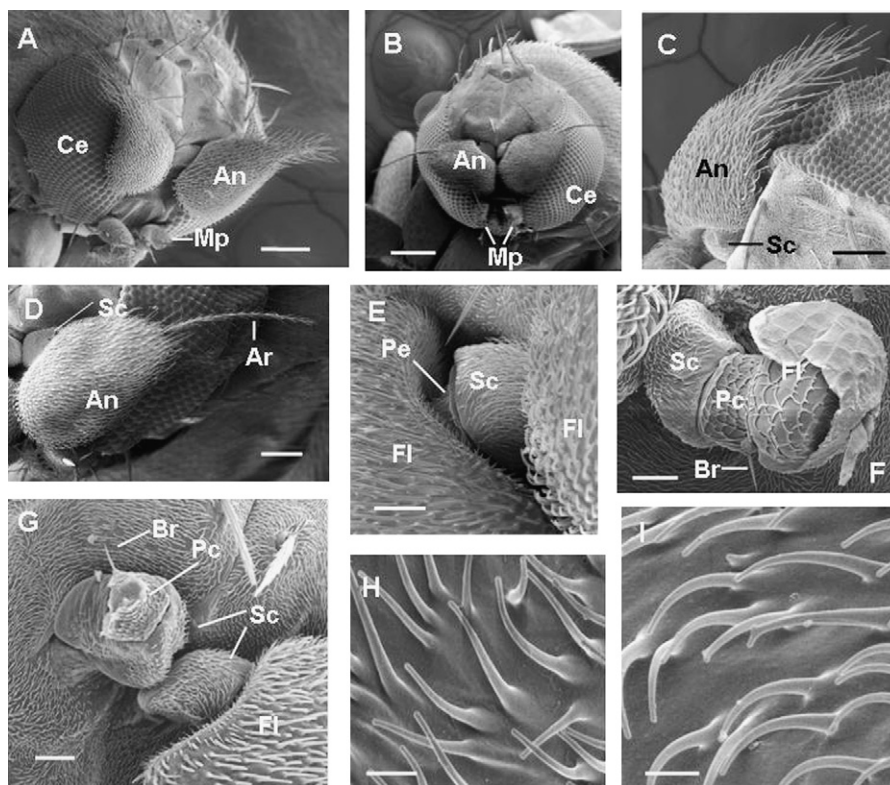
Fig. 1. SEM micrographs of adult *P. tricuspis*: (A) male head; (B) female head; (C) male antenna; (D) female antenna; (E) antenna showing concealed pedicel; (F) male antenna (with the flagellum removed) showing enlarged scape and shortened pedicel; (G) female antenna (with the flagellum removed) showing enlarged scape and shortened pedicel; (H) microtrichia on the scape of a male antenna; (I) microtrichia on the scape of a female antenna. An, Antenna; Ce, compound eyes; Mp, maxillary palp; Sc, scape; Ar, arista; Pe, pedicel; Fl, flagellum; Br, bristle. Scale bars: (A) 100 μm; (B) 125 μm; (C and D) 50 μm; (E–G) 20 μm; (H and I) 2 μm.

covered with microtrichia, which gradually diminished in density from the base to the tip (Fig. 2A). Three major types of sensilla were observed on the flagellum of male and female *P. tricuspis*: s. trichodea, s. basiconica, and s. coeloconica (Fig. 2A and B). All sensilla are oriented in a direction to the tip of antenna giving the flagellum a velvety appearance.

*3.4.1. Sensilla trichodea*

Sensilla trichodea were the most numerous sensilla type found on the flagellum of *P. tricuspis*. They are densely distributed over the dorsal surface, but rarely found on the proximal part of the ventral surface. On the basis of hair length, three subtypes of s. trichodea were recorded on the flagellum of males: short (10–15 μm long), medium (20–30 μm long), and long (>40 μm long) s. trichodea (Table 1). However, only the short and medium subtypes occurred on the flagellum of females. In general, a gradual increase in the length of s. trichodea from the base to the distal end of the flagellum was recorded, particularly in males. The mean width (measured at half-length) of the short, medium, and long s. trichodea is ≈1.1, 1.3, and 1.3 μm, respectively (Table 1). The long subtype was found only on the tip of the flagellum of males (Fig. 2C). Each s. trichodeum arises from a conspicuous base, and gradually tapers to a pointed tip (Fig. 2). These sensilla tend to occur in parallel to the length of the flagellum with a characteristic middle curvature that enables easy identification under low magnification.

Under high magnification, s. trichodea can be seen as having a grooved cuticular surface with many pores penetrating the
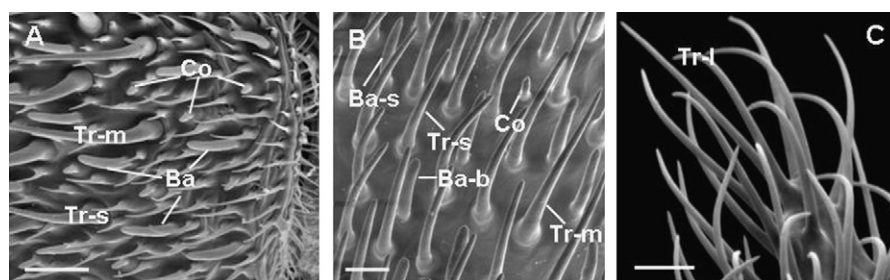


Fig. 2. SEM micrographs showing features on the surface of the flagellum of *P. tricuspis*. (A) Promixal part of the flagellum; (B) middle part showing three types of sensilla; (C) the tip of a male antenna showing long s. trichodea; Tr-s, short subtype of s. trichodea; Tr-m, medium subtype of s. trichodea; Tr-l, long subtype of s. trichodea; Ba-b, blunt-tip s. basiconica; Ba-s, sharp-tip s. basiconica; Co, s. coeloconica. Scale bars: (A) 10 μm; (B) 5 μm; (C) 10 μm.

Table 1
Morphometric data of sensilla types found on the flagellum of male and female *P. tricuspis*

| Sensillum type | | Hair length[a] ($\mu$m $\pm$ S.D.) | Hair width[a,b] ($\mu$m $\pm$ S.D.) | Pores per $\mu$m$^2$ | $\approx$Pore size (nm) | No. of sensillum (mean $\pm$ S.D.)[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Dorsal view | | Lateral view | |
| | | | | | | Male | Female | Male | Female |
| S. trichodea | Short | 12.7 $\pm$ 1.8 | 1.1 $\pm$ 0.1 | 18 | 4 | 175 $\pm$ 1 | 204 $\pm$ 38 | 44 $\pm$ 4 | 79 $\pm$ 11 |
| | Medium | 23.4 $\pm$ 2.8 | 1.3 $\pm$ 0.2 | | | 88 $\pm$ 2 | 89 $\pm$ 23 | 69 $\pm$ 8 | 86 $\pm$ 16 |
| | Long[d] | 53.6 $\pm$ 9.4 | 1.3 $\pm$ 0.1 | | | 30 $\pm$ 4 | – | 25 $\pm$ 0 | – |
| S. basiconica | Blunt-tip | 8.0 $\pm$ 0.9 | 1.6 $\pm$ 0.1 | 42 | 13 | 64 $\pm$ 11 | 57 $\pm$ 14 | 45 $\pm$ 10 | 69 $\pm$ 11 |
| | Sharp-tip | 8.4 $\pm$ 01.2 | 1.3 $\pm$ 0.2 | 20 | 8 | 7 $\pm$ 0 | 10 $\pm$ 1 | 6 $\pm$ 3 | 10 $\pm$ 2 |
| S. coeloconica | | 2.4 $\pm$ 0.1 | 1.1 $\pm$ 0.1 | – | – | 16 $\pm$ 1 | 25 $\pm$ 4 | 22 $\pm$ 2 | 15 $\pm$ 2 |

[a] $n$ = 10 antennae (5 per sex).
[b] Diameter measured at half-length.
[c] 2 antennae per sex per view; values are number of sensilla counted on one side of the flagellum.
[d] Male antennae only ($n$ = 5), not present on the female antennae.

wall inside these grooves (Fig. 3A). These pores occur at a density of $\approx$18 pores $\mu$m$^{-2}$ at half-length, as estimated by SEM. Proceeding distally, the pore density gradually declines. All the pores are arranged in a random pattern. However, pores are absent near the base of hair shaft. Based on TEM sections, the outer pore canal is $\leq$10 nm wide and 5–10 nm deep, and widens into a relatively small pore kettle $\approx$100 nm across (Fig. 4A–C). The cuticular walls of the sensilla are thick ranging from 210 nm basally to 120 nm at the tip (Fig. 4A–C). There appear to be two neurons associated with each s. trichodea. In general, the dendrites of the neurons extend unbranched into the sensillum lumen (Fig. 4B and C).

### 3.4.2. Sensilla basiconica

Like s. trichodea, the density of s. basiconica is greater on the dorsal side of the flagellum than on the ventral side. Two subtypes of s. basiconica were recorded on the flagellum of male and female *P. tricuspis*: blunt-tip and sharp-tip s. basiconica (Fig. 3B and D). The blunt-tip subtype bears stem-like, blunt-tipped shafts, whereas the sharp-tip subtype gradually tapers to a sharp-tip distally. The length and width at the base of the sharp-tip subtype (8.4 $\mu$m in length and 1.3 $\mu$m in width) are similar to that of the blunt-tip subtype (Table 1). Generally, most pegs of these sensilla do not curve, but some may appear slightly curved distally. Both subtypes are evenly
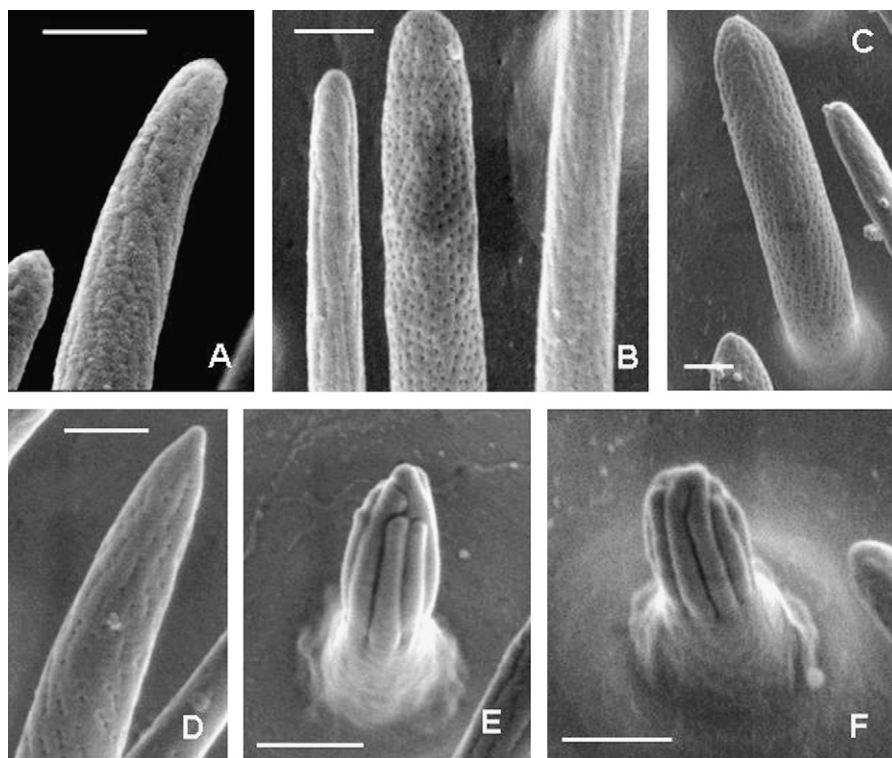


Fig. 3. High-resolution SEM micrographs of sensilla types on the flagellum of *P. tricuspis*. (A) s. trichodea; (B and C) blunt-tip s. basiconica; (D) sharp-tip s. basiconica; (E) s. coeloconica on the female antenna; (F) s. coeloconica on the male antenna. Scale bars: (A–F) 1 $\mu$m.
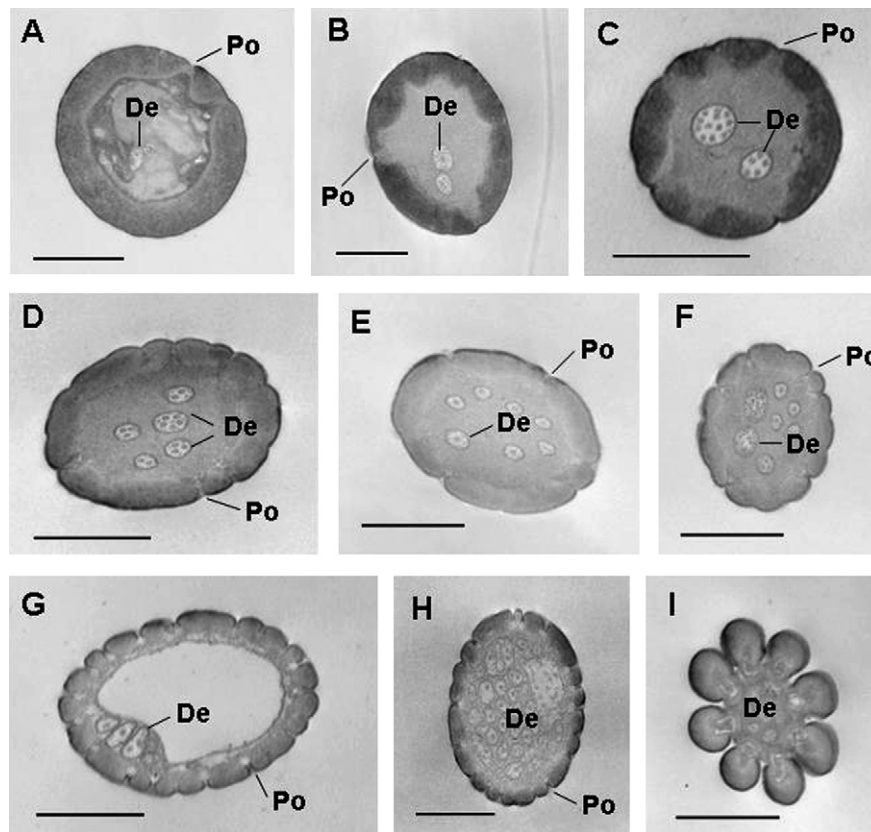
Fig. 4. TEM cross-sections of various types of sensilla on the flagellum of *P. tricuspis*. (A–C) Cross-section at the proximal base (A), midregion (B), and tip (C) of s. trichodea; (D–F) cross-section at the midregion (D and E), and tip (F) of sharp-tip s. basiconica; (G) cross-section at the base of blunt-tip s. basiconica showing dendritic branching; (H) cross-section at the midregion of blunt-tip s. basiconica showing numerous dendritic branches; (I) cross-section of s. coeloconica showing eight finger-like projections and six dendritic branches; De, dendrite; Po, pore. Scale bars: (A–I) 500 nm.

distributed over the proximal end of the flagellum and can be easily differentiated under low magnification from s. trichodea, based on the relatively shorter peg length and wider width. The sharp-tip subtype is far less abundant than the blunt-tip subtype in both sexes (Table 1).

The cuticular wall of the sharp-tip subtype (80–140 nm) is slightly thicker than that of the blunt-tip subtype (50–100 nm). The whole cuticular wall of s. basiconica seta is penetrated by numerous pores. These pores are uniformly distributed over the surface of the sensillum in the blunt-tip subtype, occurring at a density of $\approx 42$ pores/$\mu m^2$ (measured at half-length; Table 1). Pores on the sharp-tip subtype are arranged in linear arrays (rows) and are much fewer than those on the blunt-tip subtype, occurring at a density of $\approx 20$ pores/$\mu m^2$ (measured at half-length; Table 1). Like s. trichodea pores, each pore of s. basiconica widens just below the cuticular surface to form a pore kettle. However, this kettle, which is nearly 20 nm across, is much smaller than that of s. trichodea (Fig. 4D–H). Fewer dendritic branches were observed in the sharp-tip subtype than in the blunt-tip subtype (Fig. 4D–H).

### 3.4.3. Sensilla coeloconica

Sensilla coeloconica are the shortest and fewest sensilla type found on the flagellum of male and female *P. tricuspis*. They are scattered irregularly on the whole surface of the flagellum and arise from slightly depressed individual pits (Fig. 3E and F).

There are $\approx 20$ s. coeloconica on each side of the flagellum of both sexes (Table 1). The s. coeloconica on the surface of the male antenna are $\approx 2.7$ $\mu m$ long, while those on the female antenna are slightly shorter. The diameter is $\approx 0.9$ $\mu m$ at the base and 1.1 $\mu m$ at half-length, tapering to $\approx 0.3$ $\mu m$ towards the tip in males (Fig. 3E). However, these sensilla do not taper from base to tip in females (Fig. 3F). The shaft of a s. coeloconicum is longitudinally grooved and appears to be made up of closely apposed cuticular finger-like projections, as reported for *Drosophila* antennae by Shanbhag et al. (1999). We observed about eight such fingers, which are of varying length. The diameters of these fingers appear to be relatively uniform along the length of an individual finger and among different fingers. TEM sections also confirmed eight fingers per sensillum (Fig. 4I). We observed six dendritic branches and some tubular structure between cuticular fingers and central lumen.

### 3.5. Arista

The tip of the flagellum of the female antenna is reduced, forming the feathered arista (Fig. 5A). The entire arista is about 170 $\mu m$ long and 2–10 $\mu m$ in diameter. Each arista consists of two short basal segments and one long distal segment that gradually taper toward the tip. The surface of the arista is covered by microtrichia (Fig. 5B–D). TEM sections revealed no
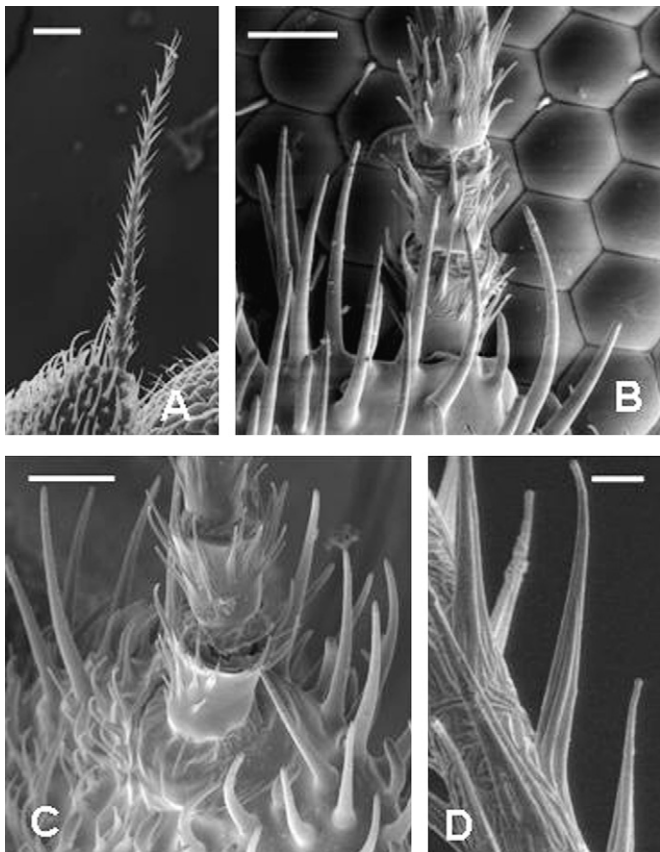
Fig. 5. SEM micrograph of the arista of *P. tricuspis*. (A) Whole arista; (B) dorsal view of the proximal part (base); (C) ventral view of the proximal part; (D) microtrichia on the 3rd segment of the arista. Scale bars: (A) 20 μm; (B–C) 10 μm; (D) 2 μm.
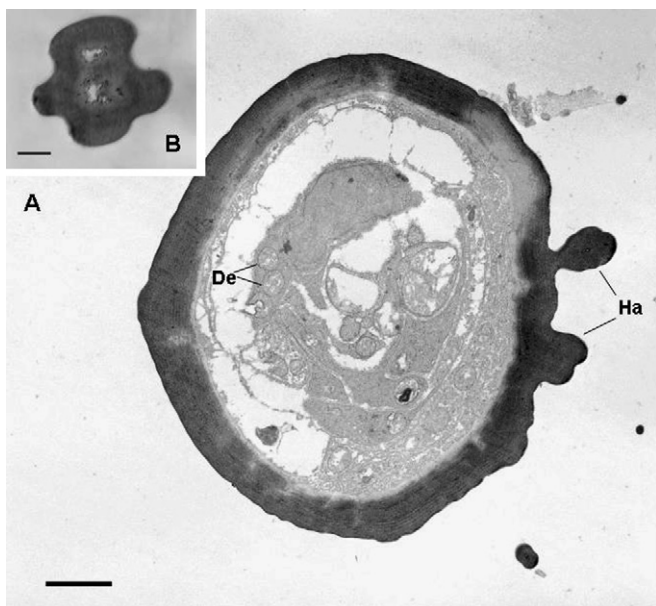


Fig. 6. TEM cross-section of the arista of *P. tricuspis*. (A) Whole arista; (B) arista hair (microtrichia); De, dendrite; Ha, aristal hair. Scale bars: (A) 1 μm; (B) 200 nm.

pores on the arista or arista hairs (microtrichia), but many dendrites can be seen in the arista haemolymph space (Fig. 6A and B).

## 4. Discussion

The results of the present study, which is the first to characterize antennal sensilla of parasitic phorid flies (Diptera: Phoridae), are generally in conformity with those previously reported for other Dipteran species (Sutcliffe et al., 1990; Giangiuliani et al., 1994; Pfeil et al., 1994; Rahal et al., 1996; Shanbhag et al., 1999; Fernandes et al., 2004; Sukontason et al., 2004, 2005, 2007), but with some key exceptions. The size, shape, and morphology of the antennae of *P. tricuspis*, including the presence of only three antennal segments, are typical of most flies in the Cyclorrhapha suborder (Shanbhag et al., 1999). However, the very short and barely conspicuous size of the pedicel of *P. tricuspis*, which is concealed under the flagellum, may be a morphological characteristic of the family Phoridae, as previously reported in the non-parasitic phorids *Megaselia halterata* (Wood) (Pfeil et al., 1994) and *M. scalaris* (Loew) (Sukontason et al., 2005).

The sexual dimorphism in the shape of the antennae of *P. tricuspis* may represent a key difference between phorid flies and several other Dipteran species. In female *P. tricuspis*, the tip of the flagellum is reduced, forming the feathered arista, which is absent in males. Aristate antennae are known to occur in both sexes of several species in the order Diptera including *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Shanbhag et al., 1999), *Dermatobia hominis* (L.) (Diptera: Cuterebridae) (Fernandes et al., 2002), and *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) (Fernandes et al., 2004). In their SEM study, Sukontason et al. (2004) reported aristate antennae on both sexes of six species of flies belonging to the families Calliphoridae, Sarcophagidae, and Muscidae. The reason for the absence of an arista on the antenna of male *P. tricuspis* is unclear. However, based on previous studies which reported absence of olfactory sensilla on the arista of several Dipteran species (Dethier et al., 1963; Ross and Anderson, 1987), it is unlikely that the evolution of this form of antennal sexual dimorphism in *P. tricuspis* is related to chemoreception. This has also been confirmed in the present study in which no olfactory sensillum was found on the arista of female *P. tricuspis*, and in a previous electrophysiological study which showed no significant difference in the EAG response of female *P. tricuspis* with or without the arista to imported fire ant (*S. invicta*) odor (unpublished data). Furthermore, the lack of a mechanical stimulus-transducing apparatus and pores in the aristal wall rules out a mechano- or chemoreceptive function for the arista of female *P. tricuspis*, as inferred also for *D. melanogaster* (Foelix et al., 1989). Sayeed and Benzer (1996) proposed a hygroreceptory function for the arista of *D. melanogaster*. Sexual differences in the shape of the antennae of *P. tricuspis* may constitute an alternative criterion for distinguishing between the sexes, which at present are differentiated by presence/absence of the sclerotized female ovipositor (Porter, 1998). The location of the arista on the

antennae of female phorid flies may represent yet another form of divergence from most other flies. The arista of female *P. tricuspis* is located at the tip of the flagellum, whereas in the many other Dipteran species, the arista originates from the posterior surface near the lateral edge of the flagellum (Rahal et al., 1996; Shanbhag et al., 1999; Sukontason et al., 2004), or is located dorso-laterally on the flagellum (Sukontason et al., 2007).

We recorded no sensillum on the scape and pedicel (one long bristle occurred on the pedicel) of both sexes of *P. tricuspis*. This is in contrast to previous reports of bristle sensilla on the scape and pedicel of many Dipteran species including *Delia radicum* (L.) (Diptera: Anthomyiidae) (Ross and Anderson, 1987), *Trichopoda pennipes* F. (Diptera: Tachinidae) (Giangiuliani et al., 1994), and *C. hominivorax* (Fernandes et al., 2004). In addition, s. trichodea have been reported on the scape and pedicel of six fly species in the families Calliphoridae, Sarcophagidae, and Muscidae (Sukontason et al., 2004). Sukontason et al. (2007) also reported s. trichodea on the scape and an unidentified sensilla type on the pedicel of *Hydrotaea chalcogaster* (Wiedemann) (Diptera: Muscidae). Microtrichia, as observed on the scape and pedicel of *P. tricuspis*, were frequently designated by non-innervated spinules, spines, or trichomes in other fly species (Shanbhag et al., 1999; Stocker, 2001; Fernandes et al., 2002).

Three main types of sensilla were recorded on the flagellum of both sexes of *P. tricuspis*, similar to those reported for other Dipteran species (Sutcliffe et al., 1990; Giangiuliani et al., 1994; Rahal et al., 1996; Shanbhag et al., 1999; Fernandes et al., 2004; Sukontason et al., 2004, 2007). Two of the three main types, s. trichodea and s. basiconica are differentiated into three and two subtypes, respectively, for a total of six morphologically different sensilla subtypes. Sensilla trichodea was the most abundant type observed on the antennae of both sexes of *P. tricuspis*, as reported also for some other Dipteran species (Ross and Anderson, 1987; Rahal et al., 1996; Fernandes et al., 2004). In contrast, Giangiuliani et al. (1994) found no s. trichodea on the antennae of the tachinid fly, *T. pennipes*, on which s. basiconica were the most numerous. Similarly, s. basiconica were the most common sensilla recorded on the antennae of both sexes of the six fly species studied by Sukontason et al. (2004). The walls of s. trichodea are penetrated by pores, as previously reported for *D. melanogaster* (Stocker, 1994; Riesgo-Escovar et al., 1997; Shanbhag et al., 1999). Traditionally, a mechanoreceptor function for s. trichodea has been proposed in many insects including the parasitoid wasp, *Microplitis croceipes* (Cresson) (Ochieng et al., 2000), human bot fly, *D. hominis* (Fernandes et al., 2002), and red imported fire ant, *S. invicta* (Renthal et al., 2003). In contrast, Shanbhag et al. (1999) classified s. trichodea as single-walled wall pore sensilla based on the relatively low number of pores and the short diameter of the outer pore canal, and proposed an olfactory function for this sensilla type. Several authors have also proposed or demonstrated olfactory function for s. trichodea in *Drosophila* (Clyne et al., 1997; Riesgo-Escovar et al., 1997), including confirmation of pheromone sensitivity using electrophysiological studies (Clyne et al.,

1999). Given the abundance and distribution of s. trichodea on the flagellum of *P. tricuspis* and the fact that they are perforated by small pores, it is possible that this sensilla type may actually play a role in chemoreception. In that case, the long subtype, which is present only on the male antennae, may function in the reception of female-related odorants such as sex pheromones, which are yet to be identified for *P. tricuspis*.

The two subtypes of s. basiconica recorded for *P. tricuspis* are generally similar to the subtypes of the same sensilla reported for other Dipteran flies, although up to three main subtypes were identified in some studies (Rahal et al., 1996; Shanbhag et al., 1999; Fernandes et al., 2004). The blunt-tip and sharp-tip subtypes described in this study are similar to the basiconic sensilla type 1 and type 2, respectively, reported for *Pseudoperichaeta nigrolineata* Walker (Diptera: Tachinidae) (Rahal et al., 1996). The multiporous pitted structure along the entire surface of the s. basiconica of *P. tricuspis* resembles that of the typical wall pore feature of this sensilla type reported on the antennae of various insect species such as *P. nigrolineata* (Rahal et al., 1996), *D. melanogaster* (Shanbhag et al., 1999), *Phoracantha semipunctata* F. (Coleoptera: Cerambycidae) (Lopes et al., 2002), and *C. hominivorax* (Fernandes et al., 2004). The presentation of s. basiconica with thin walls, high-pore density and dendritic branches suggest an olfactory function (Shanbhag et al., 1999), which has also been verified by the use of electrophysiological recordings in *D. melanogaster* (Siddiqi, 1991) and *P. semipunctata* (Lopes et al., 2002).

The s. coeloconica is the most morphologically distinct and least abundant sensilla type on the flagellum of *P. tricuspis*. They are easily distinguishable from other sensilla types by their short length and fundamentally different shape (Shanbhag et al., 1999). These finger-like sensilla are similar in structure to the double-walled s. coeloconica reported for *D. melanogaster* (Riesgo-Escovar et al., 1997; Shanbhag et al., 1999), to the "pit pegs" reported for *D. hominis* (Fernandes et al., 2002), and to a thick and non-porous wall reported for *M. scalaris* (Sukontason et al., 2005). However, unlike in *D. hominis* (Fernandes et al., 2002), the s. coeloconica on the flagellum of *P. tricuspis* are not located in individual pits but arose from the antennal surface like other sensilla types, as reported also for *D. melanogaster* (Riesgo-Escovar et al., 1997; Shanbhag et al., 1999). Sensilla coeloconica of *P. tricuspis* are not differentiated into subtypes. In contrast, two subtypes of s. coeloconica were reported for *D. melanogaster* (Shanbhag et al., 1999), while Fernandes et al. (2004) reported five subtypes for *C. hominivorax*. The absence of wall pores on the s. coeloconica of male and female *P. tricuspis* suggests that they are unlikely to function as chemoreceptors. Using electrophysiological bioassays, Schneider and Steinbrecht (1968) demonstrated response of s. coeloconica on antennae of several insect species to $CO_2$, temperature, and humidity. Also, Shanbhag et al. (1995) ascribed a thermo-hygroreceptory function to the s. coeloconica found on the sacculus of the flagellum of *D. melanogaster*. However, chemoreceptory functions have also been demonstrated for the multiporous s. coeloconica recorded on the antennae of several Dipteran species including *D. melanogaster* (Clyne et al., 1997; Shanbhag et al., 1999) and *D. hominis*

(Fernandes et al., 2002). Our data suggest that the aporous s. coeloconica of *P. tricuspis* possibly may function as thermo-hygroreceptors.

With the exception of the long subtype of s. trichodea which was recorded only on the male antennae, no other marked sexual differences were recorded in the distribution and abundance of sensilla on the antennae of *P. tricuspis*, possibly confirming the results of a previous study showing only slight differences in the EAG response of male and female *P. tricuspis* to host fire ant odor (Chen and Fadamiro, 2007). The estimated number of sensilla on the flagellum of both sexes was similar: ≈380 sensilla were counted on the dorsal side of a male flagellum versus ≈385 sensilla recorded per female flagellum under the same view (Table 1). The degree of sexual dimorphism in the abundance and distribution of antennal sensilla varies significantly within the order Diptera. On the one hand, no marked sexual differences were found in the diversity and distribution of antennal sensilla of several species including *Hydrotaea irritans* (Fallén) (Been et al., 1988), *T. pennipes* (Giangiuliani et al., 1994), and five of the six fly species studied by Sukontason et al. (2004). Moderate sexual differences in sensilla number and distribution have been reported for some species including *Ceratitis capitata* Wied (Mayo et al., 1987), *D. melanogaster* (Shanbhag et al., 1999), and *D. hominis* (Fernandes et al., 2002). In contrast, Stocker (2001) reported marked sexual differences for some strains of *D. melanogaster*, in which females can have up to 24% more basiconic sensilla and 32% less trichoid sensilla than males. Marked sexual dimorphism in typology, topography, and quantity of antennal sensilla was recorded also for *C. hominivorax* (Fernandes et al., 2004). Other authors have also reported significant sexual differences in the number of funicular sensory pits for many Dipteran species, in particular flies in the family Muscidae (Slifer and Sekhon, 1964; White and Bay, 1980; Been et al., 1988; Sukontason et al., 2004); however, we did not detect presence of sensory pits on the antennae of *P. tricuspis*.

In conclusion, this study has identified and characterized the distribution of different sensilla types in *P. tricuspis*, including the two types (s. trichodea and s. basiconica), which are likely involved in chemoreception. The presence of both sensilla types in significant numbers on the antennae of males and females probably suggests the importance of chemoreception in the behavioral ecology of this decapitating phorid fly species. Future functional antennal morphology and electrophysiological studies are needed to confirm the proposed functions of the three sensilla types identified in this study. These results will provide necessary background information for our ongoing studies of host location mechanisms and neuroethology of olfaction in phorid fly parasitoids of imported fire ants.

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