Types of Antennal Sensilla of Three *Pseudacteon* Species (Diptera: Phoridae) Females That Parasitize Red Imported Fire Ants (*Solenopsis invicta*) (Hymenoptera: Formicidae)

by

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ABSTRACT

While antenna is the main organ for insect to accept the external chemical signals, the antennal sensilla that are diverse in structure and function form the insect receptors in chemical communication. Since a variety of *Pseudacteon* species are important natural enemies of the red imported fire ant, *Solenopsis invicta* Buren (. *invicta*), to elucidate the types of *Pseudacteon* sensilla will promote the study and understanding of the selection behavior of *Pseudacteon* in parasitizing . *invicta*. This study has used scanning electron microscope (SEM) to observe and investigate the female's antennal sensilla of three *Pseudacteon* species, the *Pseudacteon* (P.) *litoralis*, *P. obtusus*, and P. *tricuspis*, and demonstrated that there are four types of sensilla, the trichoid, basiconic, coeloconic, and chaetic sensilla, on their antennal flagellum. Among them, the former three are common in all three species, with trichoid sensillum as mostly abundant, while the chaetic sensillum exists only in the antennae of *P. obtusus*. The trichoid sensilla exhibit significant interspecies variations and are further classified into two subtypes based on the presence or absence of protrusions, the surface of which contains different shades of groove-like or irregular punctate structures. The basiconic sensilla resemble short spines with densely porous structures on the surface and are in the length of 7.3-9.8 μm and the width of 1.3-1.6 μm, upright or slightly bent. The coeloconic sensilla are irregularly formed in the middle and base of the flagellum, without surface pores; each coeloconic sensillum has eight finger-like folds in unequal lengths, while the end of the fold resembles a blunt cone. The chaetic sensilla enlarge at the base, possess multiple fold-like structures and fine-tipped ends, and are approximately 5 μm in length.

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Keywords: Fir ant; Natural enemy; *Solenopsis invicta*; *Pseudacteon*; Antennal sensilla

INTRODUCTION

. *invicta* is one of the internationally recognized and dangerous invasive species. It originally distributes in the Parana river basin of South America but later on continuously spreads to and jeopardizes other countries including United States, Australia, China, etc. (Zeng et al. 2005a, Zeng et al. 2005b, Callcott 2002, Callcott & Collins 1996, Nattrass & Vanderwoude 2001). Because this pest threatens the local agriculture and forestry, public safety, human health, and biodiversity, the affected countries and regions have paid serious attentions to its prevention and control (Lofgren et al. 1975, Porter & Savignano 1990, Tschinkel et al. 1995, Wojcik et al. 2001). An important factor to restrain the . *invicta* population is their original natural enemies in South America, among which *Pseudacteon* is the most potential parasitoid and has received the most in-depth studies (Borgmeier & Prado 1975, Fowler et al. 1995, Orr et al. 1995, Porter 1998a, Porter 1998b, Porter 2000).

Fire ant-attacking *Pseudacteon* exhibits specificity (Borgmeier 1962, Porter 1998a, Porter & Alonso 1999, Porter 2000). Since the fly is small in size, and the oviposition is quick, it is difficult to investigate the relationship between the ovipositor and the host-locating mechanism. *Pseudacteon* locates fire ants likely by pursuing their chemical odors (Borgmeier 1922, Porter et al. 1995). Therefore, to study the *Pseudacteon*'s antennal sensilla may help answering this question. Each *Pseudacteon* species usually parasitizes ant of certain fixed range of body size (Morrison et al. 1997). The present study has investigated three *Pseudacteon* species, the P. *tricuspis*, P. *litoralis*, and *P. obtusus*. The former two have already been used for field release to control the fire ants, while the latter one is also under preparation to be released to the field (Lu et al. 2008; Gilbert & Patrock 2002). This study has observed and investigated the female antennal sensilla of these three *Pseudacteon* species, aiming to provide theoretical basis for better understanding of the insect chemosensory system, the relationship between insect antennal sensilla and their behavioral responses, as well as the chemical links between insects and natural enemy, and to provide scientific basis for the development of new measures for . *invicta* prevention, as well as technical support for future morphological screening of other *Pseudacteon* species to apply in the biological control of . *invicta*.

MATERIALS AND METHODS

The insect samples

The alcohol-impregnated specimens of *P. tricuspis*, *P. litoralis*, and *P. obtusus* were generous gifts from Dr. Sanford D. Porter (Center for Medical, Agricultural, and Veterinary Entomology, USA), and were kept in the refrigerator with -18 for test.

Specimen preparation for Scanning electron microscopy (SEM)

Female fly was singled out with a fine brush, rinsed three times in 0.1 mol/L phosphate buffer (pH 7.0), 20 min each time, added with mixed solution of 2.5% glutaraldehyde and 2% polyformaldehyde, and incubated at 4 °C for 24 h. The specimen was then rinsed three times in phosphate buffer for 30 min each time, added with 1% osmium tetroxide solution, and incubated at 4 °C for 24 h. The sample was rinsed three times with phosphate buffer for 10 min each time, dehydrated in 30% (v/v), 50%, 70%, 80%, and 90% alcohol, respectively, each for 10 min, and then dehydrated in absolute ethanol three times for 10 min each time. The specimen was finally treated with tert-butanol for three times, 10 min each time and freeze-dried in a freeze dryer (JFD-310 Freeze Drying Device). The gold/palladium was sputtered using an JFC-1600 Auto Fine Coater. The specimen was fitted with conductive adhesive under stereoscope and observed with SEM (JMS-6360, Japan Electron Optics Laboratory Co., Ltd, Japan). The SEM Micrographs of the antennae and sensilla of the female flies were taken and observed. The antennal sensilla types among the females of three species phorid flies were compared.

Terminology

In this study, the antennal morphology and sensilla types were described and classified according to the terminologies and the Snodgrass classification presented by Schneider (1964) and Zacharuk (1985).

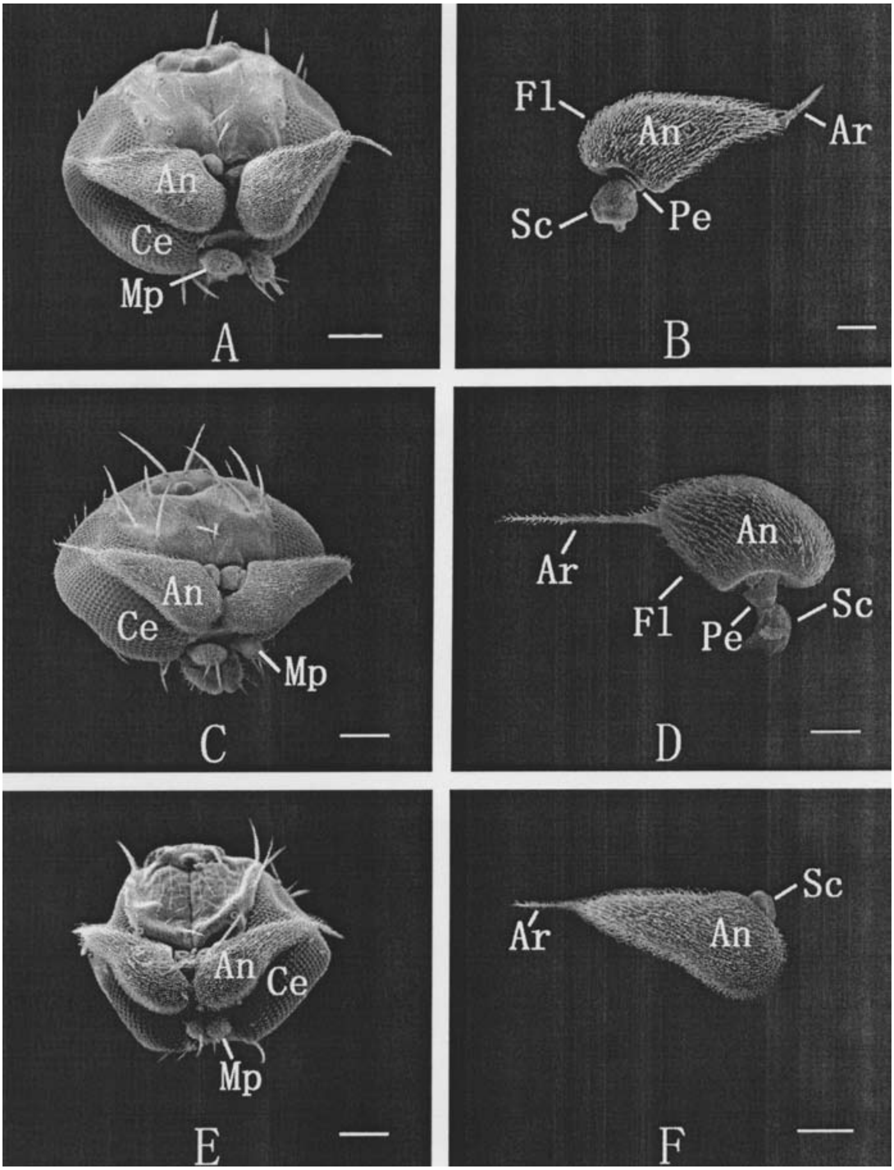

Fig.1 SEM micrographs of heads and antenna of the decapitating phorid flies females *P. litoralis*, *P. obtusus* and *P. tricuspis*
(A) P.*litoralis* head; (B) P.*litoralis* antenna; (C) *P. obtusus* head; (D) *P. obtusus* antenna; (E) P. *tricuspis* head; (F) P. *tricuspis* antenna. An, Antenna; Ce, compound eyes; Mp, maxillary palp; Ar, Arista; Pe, Pedicel; Fl, Flagellum; Sc, Scape. Scale bars: (A, C, and E) 100 µm; (B, D, and F) 50 µm.

RESULTS

Antennal morphology of female Pseudacteon

The *Pseudacteon* antennae present at the front end of the two compound eyes, with a length of 198-337 μm (Figure 1A, C, and E). Each antenna comprises three segments, the scape, the pedicel, and the flagellum. Among them, the shortest one is pedicel, hiding under the flagellum. The flagellum enlarges in the female *Pseudacteon*, and its end has an arista in the length of ~170 μm.

Scape and pedicel

There are no sensilla on the scape and pedicel in *Pseudacteon*. The scape is in a shape of flat cylinder, covered by dense micro-hairs, while the pedicel is relatively small with its end buried into the depression of the flagellum (Figure 1B, D, and F).

Flagellum

The flagellum forms the most important segment of *Pseudacteon* antenna

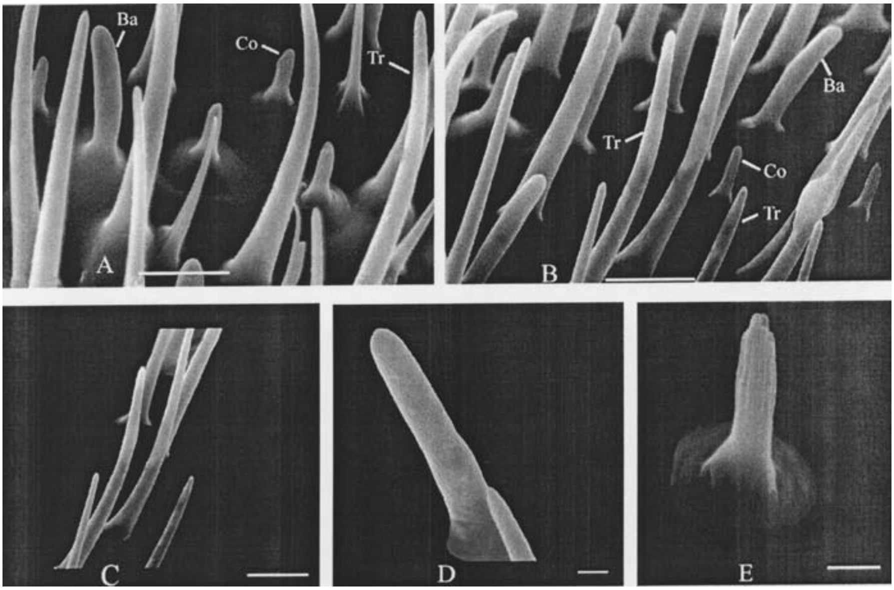

Fig.2 SEM micrographs of antennal sensilla of the decapitating phorid fly P. *litoralis* female (A and B) antennal sensilla on flagellum: (Ba) sensilla basiconica, (Co) sensilla coeloconica, and (Tr) sensilla trichodea; (C) sensilla trichodea; (D) sensilla basiconic; (E) sensilla coeloconica. Scale bars: (A , B and D) 5μm; (C and E) 1μm.

and is covered by large amount of sensilla. The length of sensilla increases gradually from the flagellar base to its end. The sensillum directions are all towards the end, which makes the flagellum looks very supple. There are four types of sensilla observed in these three *Pseudacteon* species. P. *tricuspis* and P. *litoralis* share three types of sensilla, the trichoid, basiconic, and coeloconic sensilla, while all four types of sensilla are found in *P. obtusus*, i.e., the trichoid, basiconic, coeloconic, and chaetic sensilla. However, the morphology of trichoid sensilla is significantly differentiated and varies evidently among the three species. The chaetic sensillum exists only in *P. obtusus* (Figure 3I and J).

Trichoid sensillum

The trichoid sensillum is the mostly abundant sensillum in these three observed *Pseudacteon* species. They densely cover almost the end of the antennal flagellum, with the diameter of 1.1-1.3 μm (Fig.2 A, B and C, Fig.3A and B, Fig.4 A). Under high magnification, it is observed that the surface of sensilla trichodea has different shades of groove-like structure or irregular punctate structures(Fig.3 C and D, Fig.4 B).

Basiconic sensillum

SEM results show that the number of basiconic sensilla ranks second right after that of trichoid sensilla. The shape of basiconic sensillum resembles a short spine, and its surface is densely covered by pore-like structures, which stand vertical or slightly curved on the flagellum (Fig. 2 D, 3 E and F, and 4 E and F). The basiconic sensillum has a length of 7.3-9.8 μm and a width of 1.3-1.6 μm.

Coeloconic sensillum

Among the four types of sensilla, the morphology of coeloconic sensilla manifests least variations among the three species. On the antennal flagellum of the female flies, coeloconic sensilla distribute randomly at the base and in the middle; there are no coeloconic sensilla observed at the end of flagellum. The coeloconic sensillum rises from a circular cavity, with a finger-like fold structure, in the length of 2.7-3.4 μm and the diameter of 0.8-1.1 μm. SEM indicates that each coeloconic sensillum possesses eight finger-like folds, with

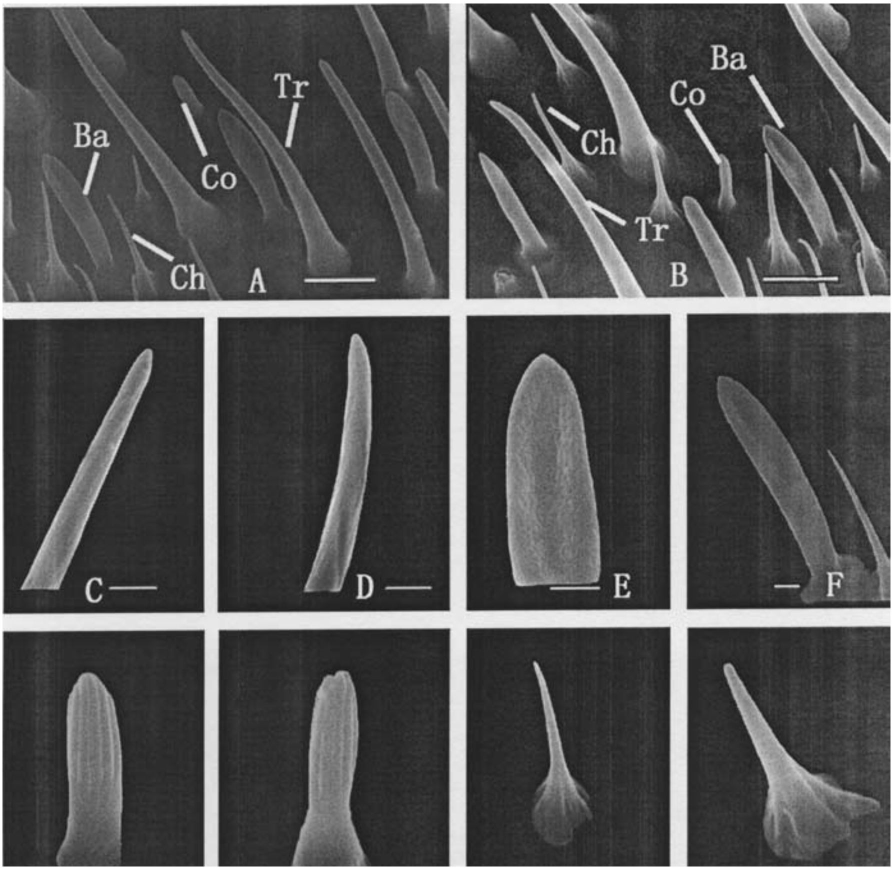

Fig.3 SEM micrographs of antennal sensilla of female decapitating phorid fly *P. obtusus* (A and B) antennal sensilla on flagellum: (Ba) sensilla basiconica, (Ch) sensilla chaetica, (Co) sensilla coeloconica, (Tr) sensilla trichodea; (C and D) sensilla trichodea; (E and F) sensilla basiconica; (G and H) sensilla coeloconica; (I and J) sensilla chaetica. Scale bars: (A and B) 5μm; (C, D, E, F, G, H, I, and J) 1μm.

unequal lengths, the diameters of which are not tapering along the growth direction of the sensilla (Figure 2 E, 3 G and H, 4 C and D).

Chaetic sensillum

The chaetic sensillum is only observed on the flagellum of *P. obtusus* (Figure 3 I and J). The chaetic sensilla stand upright like thorns, and their base also has multiple fold structures. However, these folds are not as obvious as those of coeloconic sensilla. They only exist at the base of the sensilla and disappear

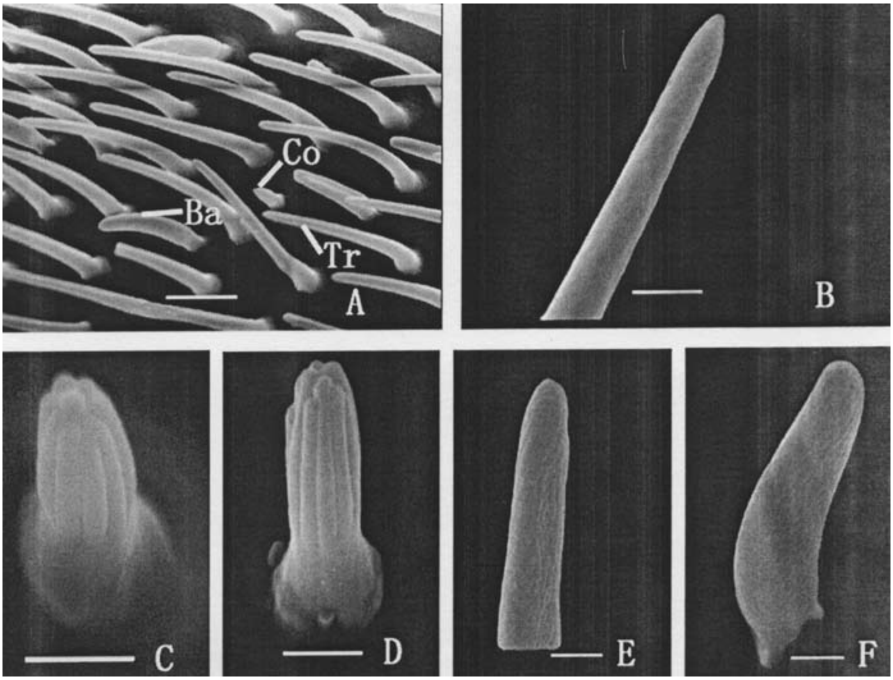

Fig.4. SEM micrographs of antennal sensilla of female decapitating phorid fly P. *tricuspis* (A) antennal sensilla on flagellum: (Ba) sensilla basiconica, (Co) sensilla coeloconica, and (Tr) sensilla trichodea; (B) sensilla trichodea; (C and D) sensilla coeloconica; (E and F) sensilla basiconica. Scale bars: (A) 5μm; (B, C, D, E, and F) 1μm.

along the growth direction above the base portion. The sensillum chaeticum is about 5 μm long and tapered gradually towards the end.

Arista

The end of antennal flagellum of female *Pseudacteon* tapers gradually and forms a wheat awn-like arista. Each aristae comprises three segments. The two segments close to the base are relatively thick, densely covered with micro-hairs and spines, and with irregular long grooves. The third segment is a long thin strip with dense micro-hairs and the similar grooves (Fig. 5 B).

DISCUSSION

Sensillum is a part of specialized epidermis on the insect body wall and a important structure to respond to the surrounding environment and a variety

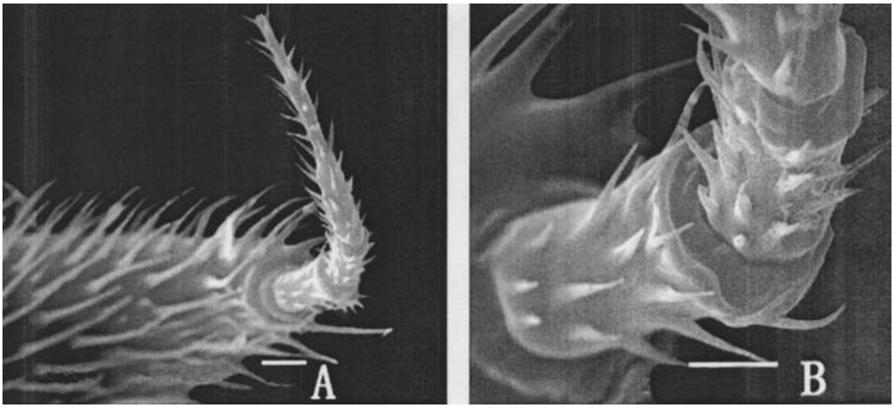

Fig.5. SEM micrographs of antennal arista of female decapitating phorid fly P. *litoralis*
(A) antennal arista; (B) two coxae and one apical segment of antennal arista. Scale bars:(A) 10μm; (B) 5μm.

of internal stimuli. It is the information receiving device for insect body to perceive the internal and external environments, to make chemical communication, and in company with the nervous system to control and regulate the insect behavior (Yu 2007). In consistence with the observation of Chen & Fadamiro (2008), the antennal sensilla of female *Pseudacteon* present only on the flagellum.

Sensilla trichodea are the dominant type of antennal sensilla in both quantity and distribution. The trichoid sensilla were classified into short, medium, and long three subtypes according to their lengths (Chen & Fadamiro 2008). However, our SEM results indicate that the length of trichoid sensilla varies greatly, and it is difficult to divide them into subtypes based on mere length. According to Yu (2007), this type of sensilla may have dual functions to act as both mechanical and sense of touch receptors. *Pseudacteon* hovers over the . *invicta* colony, locks the target, and quickly dives and inserts an egg into the worker ant's thorax region. During this process, the trichoid sensilla likely play an important role. The speed of female *Pseudacteon*'s oviposition attack is quick, merely lasting 0.1 to 1.0 second (Borgmeier 1922, Porter *et al.* 1995). Therefore, female *Pseudacteon* needs more sensitive and abundant mechanical as well as sense of touch receptors. The structure of trichoid sensilla in these three *Pseudacteon* species is less variable and only forms two subtypes according to the presence of surface protrusions, which may be evolved from the fly's long parasitic life and may have increased the area of receptors and hence facilitated the perception of host information.

The surface of *Pseudacteon* basiconic sensilla is porous. This type of sensilla had olfactory function and, sometimes, mechanoreceptive function, too (Na et al. 2008). Basiconic sensillum with such structure is also called type II basiconic sensillum. Their walls are thin and rich in nerve cells, mainly stimulated by general odorant from the outside world (e.g., the plant odor, and the odor from natural enemies) (Na et al. 2008). The number of basiconic sensilla is only after that of trichoid sensilla. They may function, in assistance to the trichoid sensilla, to identify and determine the host information, via the perception of chemical odor.

Coeloconic sensilla are the least in female *Pseudacteon*. Studies have shown that this type of sensilla is located in the abdomen and outer side of the antennal flagellum, and stimulated by carbon dioxide, humidity, plant odors, etc (Na et al. 2008). Therefore, *Pseudacteon*'s coeloconic sensilla may perform the similar functions.

The chaetic sensillum only exists on the flagellum of *P. obtusus*. Its length (~5 μm) is only longer than that of coeloconic sensilla. Each *Pseudacteon* species generally parasitizes ants in certain range of body size. P. *tricuspis* attacks medium to large fire ant workers, and P. *litoralis* attacks medium-large to large fire ant workers. No chaetic sensilla are observed on both of these species, implying that the function of chaetic sensilla is related to the identification of body type of the host, which warrants further studies.

The arista observed in this study are all located in the end of antennal flagellum, which differs from the reported aristae location in Diptera flies (Sukontason et al. 2004, Sukontason et al. 2007). There are no sensilla observed on the arista of these three *Pseudacteon* species, implying that arista may not relate to the chemical responses.

In summary, this study has observed and compared the differences in morphology, number, and distribution of female antennal sensilla in three *Pseudacteon* species. The two major types of sensilla, the trichoid and basiconic sensilla, are most likely related to the perception of chemical information, suggesting a close relationship between the chemical odor and the parasitic behavior of *Pseudacteon*. The results of this study provide theoretical basis for further exploring the relationship between the sensillum types of parasitic *Pseudacteon* and the mechanism of locating host . *invicta*, and for further in-depth understanding of the neurobehavior of these important . *invicta*-parasitic *Pseudacteon* species, as well as provide scientific basis for developing new means to biologically control . *invicta*.

ACKNOWLEDGMENTS

We would like to thank Sanford D Porter for kindly furnishing the specimen. Our study was supported by National Basic Research Program of China (Award# 2009CB119200) and National Natural Science Foundation of China (Award# 30900942).

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