Antennal Sensilla of *Tamarixia radiata* (Hymenoptera: Eulophidae), a Parasitoid of *Diaphorina citri* (Hemiptera: Psyllidae)

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ABSTRACT *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) is an effective idiobiont ectoparasitoid of the psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), vector of the huanglongbing (citrus greening disease) pathogen. We examined the external and functional morphology of the antennal sensilla of adult male and female *T. radiata* by using scanning and transmission electron microscopy, respectively, to gain insights into the behavioral ecology of this parasitoid. The geniculate antennae of male and female *T. radiata* were composed of a long scapula-shaped scape with a basal radicula, a barrel-shaped pedicel, and a long flagellum with a basal ring-like annulus. Five morphologically distinct sensilla, including two types of aporous trichoid sensilla (AST-1 and AST-2), one multiporous trichoid sensillum (MST), one multiporous placoid sensillum (MPS), and one aporous basiconic capitate peg sensillum were identified on the antennae of both sexes. The antennal structures of *T. radiata* were sexually dimorphic. Male antennae consisted of four funicular flagellomeres and possessed a greater number of olfactory MST than female antennae, suggesting their possible function in perception of mate-related volatile cues. Female antennae were characterized by three funicular flagellomeres and a greater number of MPS than male antennae, suggesting their possible function in the perception of host-related volatile cues. The results are discussed in relation to plausible roles of the identified sensilla in mate and host location by this important parasitoid species.

KEY WORDS huanglongbing, citrus greening, antennal morphology, electron microscopy

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) is an effective idiobiont (using killed host for development) ectoparasitoid of immature *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Étienne et al. 2001, McFarland and Hoy 2001). This parasitoid has demonstrated convincing potential as a classical biological control agent of *D. citri* (Aubert and Quilici 1984, Étienne et al. 2001). *D. citri* is an economic citrus pest occurring in tropical Asia and in the New World (Halbert and Manjunath 2004). This psyllid vectors three species of the nonculturable bacterium *Candidatus* Liberibater, responsible for huanglongbing (greening disease) in citrus (Halbert and Manjunath 2004, Bové 2006). Greening invades the tree phloem, causing fruit drop, misshapen bitter and unmarketable fruit, and sometimes results in tree death (Halbert and Manjunath 2004, Bové 2006). This vector species has spread rapidly throughout the state of Florida since its detection in 1998 (Tsai et al. 2000, Halbert et al. 2003). *T. radiata* revived the citrus industry in Reunion Island after its introduction from India in 1978 (Étienne et al. 2001). Also, *T. radiata* became successfully established after its release in Guadeloupe Island, resulting in substantial decline of *D. citri*'s populations within 1 yr of release (Aubert and Quilici 1984, Étienne et al. 2001). *T. radiata* was released in Florida in 1999 and has also established throughout the major citrus-growing regions of the state (Hoy and Nguyen 2001). A single female *T. radiata* can deposit up to 300 eggs over her lifetime (Pluke et al. 2008). Despite *T. radiata*'s reported efficacy as a classical biological control agent of *D. citri,* little is known about its biology. To date, investigations on *T. radiata* have focused on its distribution, parasitization rates, and developmental biology (McFarland and Hoy 2001, Étienne et al. 2001, Michaud 2002, Pluke et al. 2008). To our knowledge, information on the roles of mate-, host- or host habitat-related chemicals for mate or host location is unavailable for this parasitoid species.

The reproductive success of parasitoids is determined by their ability to effectively locate mates, hosts, and to avoid natural enemies and unsuitable environmental conditions (Dicke and Grostal 2001). Semiochemicals are involved in mate (Weseloh 1976, Quicke 1997) and host (Vinson 1991) location by parasitoids (Lewis et al. 1982). Perception of mate- or host-related semiochemicals occurs on specialized sensilla on the antennae of parasitoids (Vinson et al. 1986, Isidoro et al. 1996). The antennal sensilla of hymenopteran parasitoids have been characterized

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using electron microscopic techniques in numerous studies (Olson and Andow 1993, Isidoro et al. 1996, Amornsak et al. 1998, Onagbola and Fadamiro 2008). Some authors have reported considerable sexual dimorphism in structure and types of antennal sensilla, whereas others have reported little or no distinct sexual differences. For example, Navasero and Elzen (1991) and Onagbola and Fadamiro (2008) reported sexual differences in the antennal morphology of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) and *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae) respectively, whereas Pettersson et al. (2001) reported little or no sexual differences in the antennal structure of *Rhopalicus tutela* (Walker) (Hymenoptera: Pteromalidae). The antennal morphology of *T. radiata* including abundance and distribution of sensilla has not been investigated previously. To provide required background information that will facilitate future identification of mate- or host-related semiochemicals for *T. radiata*, we characterized the types, abundance, and distribution of sensilla on its antennae by using standard scanning (Onagbola and Fadamiro 2008) and transmission (Onagbola et al. 2008) electron microscopic techniques. Characterizing the possible functions of *T. radiata*'s antennal sensilla is an important preceding step to achieving our goal of developing an effective attractant for monitoring establishment of this beneficial insect and recruiting its population into citrus groves for improved biological control of *D. citri*.

Materials and Methods

Insect Culture. Newly expanded 'Hamlin' orange [*Citrus sinensis* (L.)] leaf flush infested with *D. citri* was collected from mature trees in an unsprayed grove in Polk Co., FL. The flush was maintained in 40- by 40- by 40-cm Plexiglas cages at 26 ± 1°C, 60 ± 5% RH, and a photoperiod of 14:10 (L:D) h. Collected psyllid nymphs were observed daily for adult emergence. Emerging parasitoids were segregated by sex and reared under the above-mentioned conditions in similar cages containing third-instar *D. citri* nymphs that were obtained from a greenhouse culture described in Wenninger et al. (2008). The *D. citri* culture was maintained on sour orange [*Citrus aurantium* (L.)] and Hamlin orange (*C. sinensis*) seedlings at 27 ± 1°C, 63 ± 2% RH, and a photoperiod of 14:10 (L:D) h. A wasp specimen was sent to Florida Department of Agriculture and Consumer Services (Division of Plant Industry), Gainesville, FL, and identified as *Tamarixia radiata* (Hymenoptera: Eulophidae) (sample E2008-4416-1).

Scanning Electron Microscopy (SEM). Newly emerged adult *T. radiata* of each sex were obtained from our laboratory-reared culture and anesthetized by freezing at −20°C for ≈5 min. Insects were decapitated with a surgical quality steel dissecting knife (Fisher Scientific, Waltham, MA), and their antennae were excised with fine forceps under 40× magnification using a Wild MC3 stereomicroscope (Leica, Heerbrugg, Switzerland). The excised antennae were kept in 70% alcohol for ≈24 h and subjected to a graded alcohol dehydration series by using methods similar to those described by Onagbola and Fadamiro (2008) and Onagbola et al. (2008). Thereafter, the excised antennae were dried in a LADD critical point dryer (LADD Research Industries, Burlington, VT) and were carefully mounted on aluminum stubs with double-sided copper sticky tape. The antennae were sputter coated with gold/palladium (40:60) in a LADD SC-502 high-resolution sputter coater (LADD Research Industries) and then examined in a Kevex S-530 (Hitachi, Tokyo, Japan) SEM operated at 10, 15, or 20kV under ambient laboratory conditions. The sizes of adult male and female *T. radiata*, each flagellar antennomere, and its adhering sensilla were determined under the SEM. Measurements were taken from at least 20 individuals of each sex.

Transmission Electron Microscopy (TEM). TEM was conducted using methods similar to those described previously by Onagbola et al. (2008). *T. radiata* specimens of each sex were anesthetized and decapitated under a stereomicroscope as described above. The isolated parasitoid heads were placed in 3% glutaraldehyde in 0.1 M cacodylate buffer at 6°C for ≈24 h. Subsequently, the specimens were rinsed in the buffer solution and postfixed in 1% osmium tetroxide for ≈2 h. Specimens were later infiltrated in 50, 70, and 100% Spurr's resin for ≈24, 12, and 12 h, respectively, and embedded in pure Spurr's plastic. Specimens were polymerized at 60°C for ≈72 h thereafter. Ultrathin sections of the wasps' antennae were cut with a diamond knife on an ultramicrotome (model 8365, LKB-Huxley Cambridge, United Kingdom) and placed on Gilder grids #200 (Ted Pella Inc., Redding, CA), which were previously coated with 0.5% Formvar (Electron Microscopy Sciences, Fort Washington, PA) solution. Sections were first stabilized in 2% uranyl acetate for ≈15 min. The grids with the affixed ultrathin sections were then submersed in a drop of lead citrate for ≈5 min to regulate the electron density for clearer viewing under the TEM. Sections were rinsed with 2% sodium hydroxide solution followed by excess distilled water before examination under a Morgagni #268 transmission electron microscope (FEI Electron Optics, Einhoven, The Netherlands) at 60 kV.

Calculations and Statistical Analyses. The antennal sensilla on the dorsal and ventral surfaces of male and female *T. radiata* were identified, counted, and measured according to methods described earlier by Onagbola and Fadamiro (2008) and Onagbola et al. (2008). The mean ± SE number of sensillar types per male and female antennomere was determined and the differences between the total number of individual sensillar types on male and female antennae were compared using chi-square analyses. At least 10 *T. radiata* were investigated per sex and at least 20 sensilla of each type and location were examined to calculate average size.

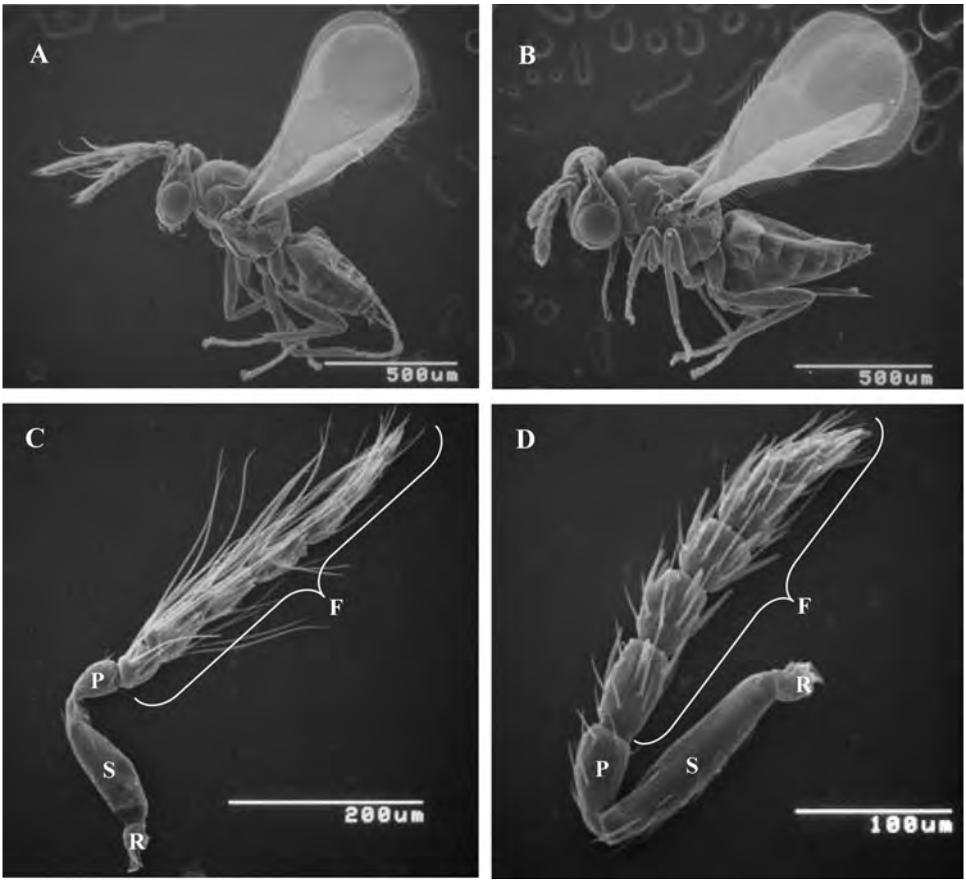

Fig. 1. Scanning electron photomicrographs of adult male (A) and female (B) *T. radiata* and gross morphology of male (C) and female (D) antennae. The antenna of male or female *T. radiata* subdivides into a ball-like radicula, R; at the base of the long scapula-shaped scape, S; the barrel-shaped pedicel, P and the long thread-like flagellum, F.

Results

Gross Antennal Morphology. Scanning electron photomicrographs of adult male (Fig. 1A) and female (Fig. 1B) *T. radiata* and of their respective antennae are presented in Fig. 1. Adult males (0.92 ± 0.002 mm) (mean ± SE) were slightly smaller (body length, measured from the frons to the tip of the abdomen) than females (1.04 ± 0.003 mm) ($n \geq 20$ per sex). The geniculate antennae of male (Fig. 1C) and female (Fig. 1D) *T. radiata* are of the conventional type having a long scapula-shaped scape, S (length, l: ≈200 μm [for both sexes, b]; width, w at the widest portion: ≈70 μm [for male, m], 60 μm [for female, f]) with a ball-like basal radicula, R (l ≈40 μm, w ≈40 μm [m]; l ≈25 μm, w ≈40 μm [f]), a barrel-shaped pedicel, P (l ≈90 μm, w ≈50 μm [b]) and a long flagellum, F (Fig. Am, Af). The flagellum consists of a ring-like basal annulus (l ≈5 μm, w ≈35 μm [b]), a mesal funicle (l ≈390 μm, w ≈55 μm [m]; l ≈190 μm, w ≈60 μm [f]) and a long apical clava (l ≈220 μm, w ≈35 μm [m]; l ≈140 μm, w ≈60 μm [f]), which consists of three antennomeres. The antennal funicle of males and females consists of four and three flagellomeres, respectively. Although females are larger overall, male antennae are ≈1.5 times longer (≈940 μm) than those of females (≈630 μm). Five different types of sensilla are present on the antennae of both sexes.

Types of Antennal Sensilla. Scanning and transmission electron photomicrographs of sensillar types are presented in Figs. 2 and 3, respectively. Type I aporous sensilla trichoidea are characterized by a straight shaft that tapers to a very fine tip (Fig. 2, AST-1). The shafts of these sensilla have fluted surfaces and are inserted into cuticular sockets (Fig. 2). As observed under the TEM, the walls of the AST-1 are thick and grooved (Fig. 3, AST-1). They occur only on the scape and pedicel of males and on the scape, pedicel and proximal (first two funicular antennomeres) portion of the female antennal flagellum. The AST-1 are ≈32–45 μm long and ≈2 μm thick (basal diameter), depending on their location.

The AST-2 are short and peg-like with bent shafts that taper into blunt apices (Fig. 2, AST-2). They are characterized by smooth shaft surfaces and are inserted into cuticular pits. The AST-2 are ≈40–60 μm long and ≈1.2 μm thick. Transverse sections of both the AST-1 and the AST-2 revealed nonporous walls (Fig. 3, AST-1, AST-2).

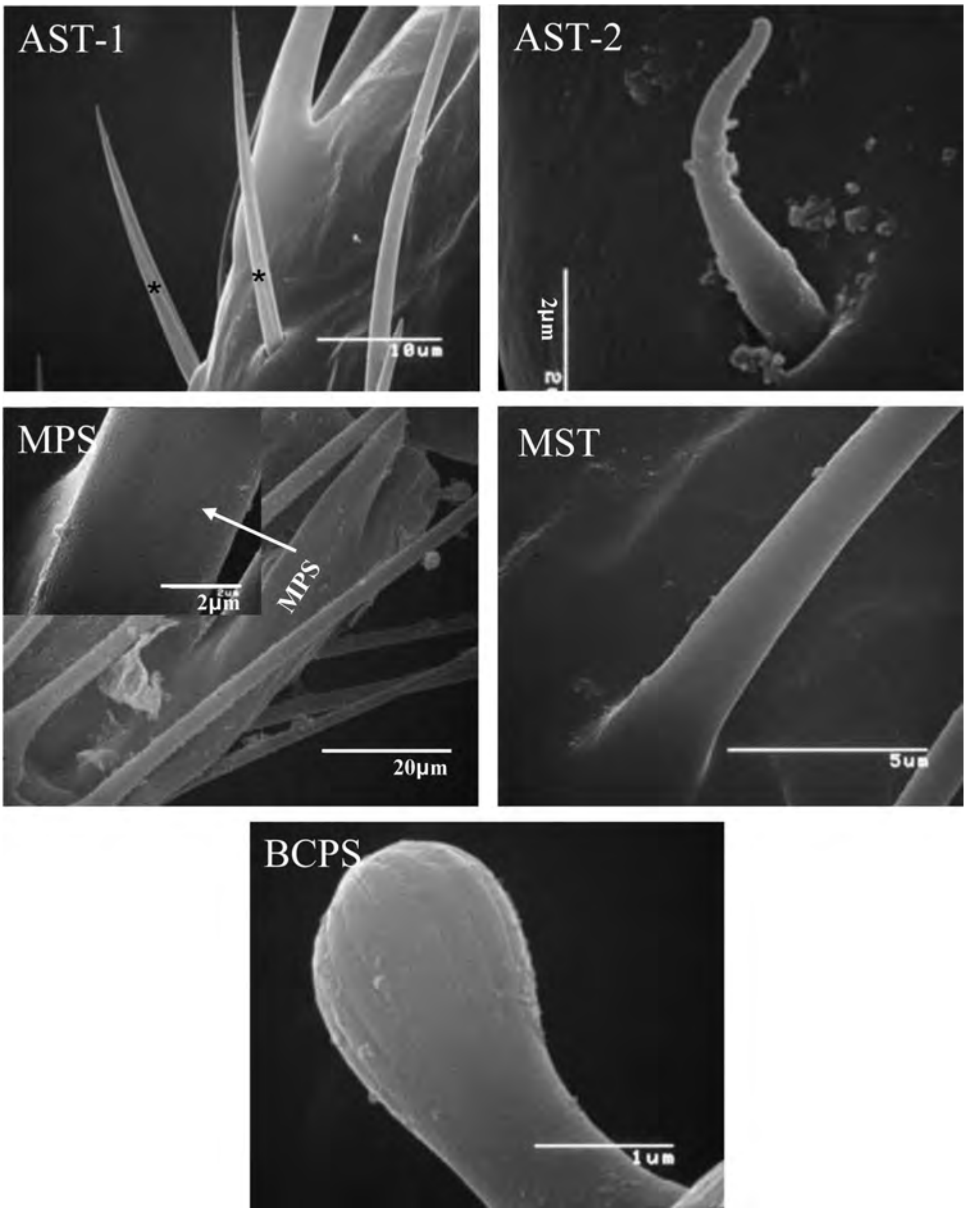

Fig. 2. Scanning electron photomicrographs of the various sensilla on the antennae of male and female *T. radiata.* The figure shows the type I aporous sensilla trichoidea with a grooved shaft surface (AST-1, with asterisks); type II aporous sensilla trichoidea, AST-2; the multiporous placoid sensilla with numerous wall pores, MPS (also magnified to show the presence of wall pores); multiporous sensilla trichoidea, MST; and the basiconic capitate peg sensilla with a grooved bulbous tip, BCPS.

The multiporous placoid sensilla (MPS) on males and females are elongate, plate-like, and are highly conspicuous with numerous wall pores on their sausage-shaped shafts (Fig. 2, MPS). The cuticular wall of the MPS seemed to be nonporous in the TEM photomicrographs (Fig. 3, MPS) given their highly compacted arrangement as revealed by SEM (Fig. 2). The MPS align parallel along the antennal longitudinal axis and are distributed between rows of the multiporous sensilla trichoidea (MST) on each antennal flagellomere of both sexes except for the male's second claval flagellomeres. The MPS arise from the antennal cuticular surface forming an elevated ridge, which protrudes slightly above the antennal surface. In contrast to the MST described above, the MPS are numerically (but not statistically: $\chi^2 = 3.6$, df $= 1$, $P = 0.058$) more abundant on the antennae of females than males (Table 1). They are ≈100 μm long in males and between ≈45 and 50 μm in length in females and are ≈6 μm thick in both sexes. The density of pores on the surface of MPS was ≈20–25 pores per μm^2 (Fig. 2, MPS).

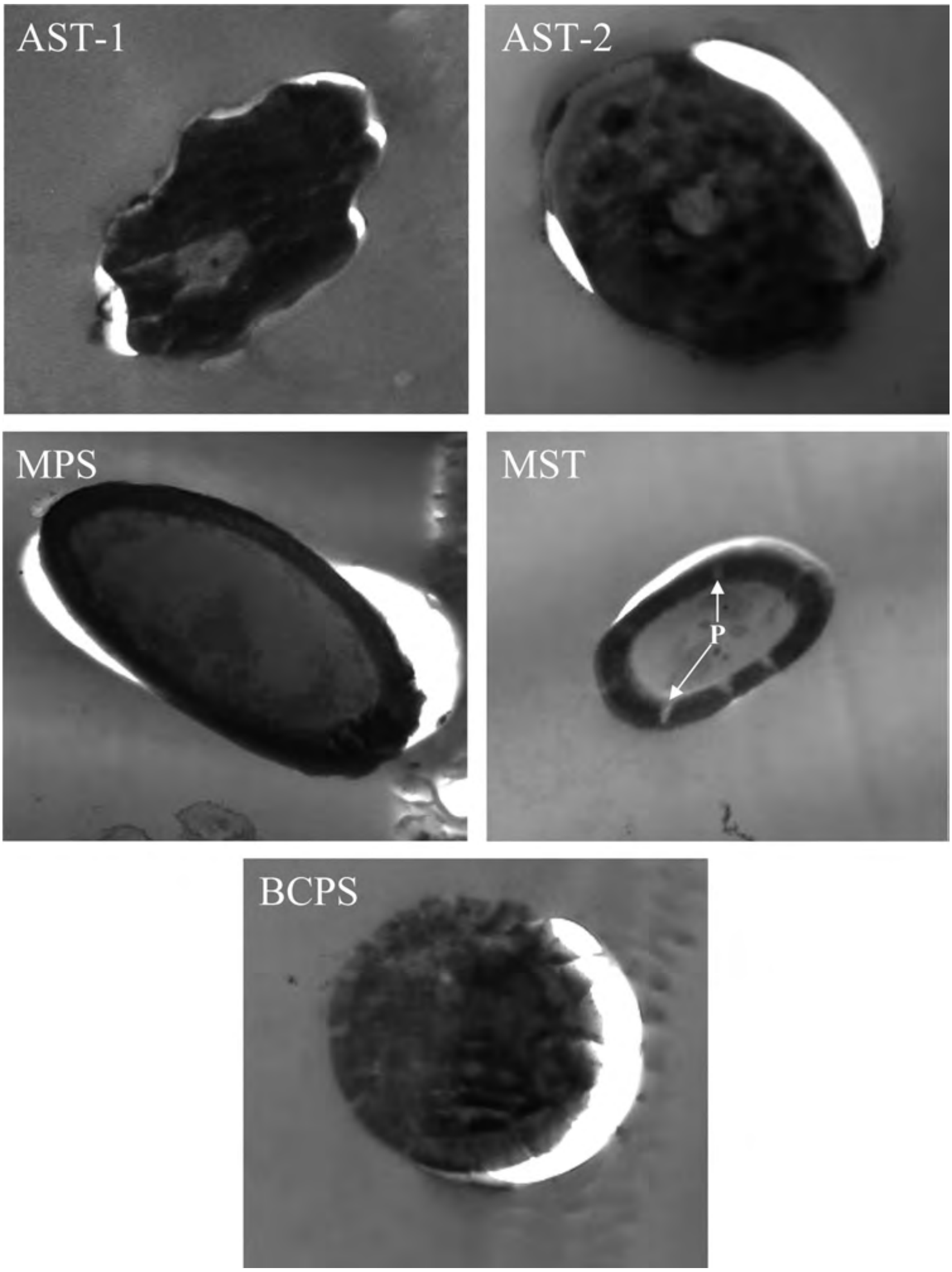

Fig. 3. Transmission electron photomicrographs of the antennal sensilla *T. radiata*. The figure shows the transverse sections of the types I (AST-1) and II (AST-2) aporous sensilla trichoidea, the multiporous placoid sensilla (MPS) and multiporous sensilla trichoidea (MST), and the aporous basiconic capitate peg sensilla (BCPS).

The MST are the most conspicuous and the most abundant sensillar type on the antennae of both male and female *T. radiata* (Fig. 1, M, F; Table 1). They are long and tapering, slightly bulbous at the base and have gradually curved shafts. They arise directly from the antennal cuticular surface and are distributed only on the funicular and claval flagellomeres of the antennae of both sexes. Their shaft surfaces are smooth (Fig. 2, MST) with wall pores (Fig. 3, MST). The MST are more numerous and ≈4 times larger on the antennae of males (Fig. 1M) than females (Fig. 1F). The MST on the antennae of females are ≈71–76 μm long and ≈3.5 μm thick.

The basiconic capitate peg sensilla (BCPS) are short and peg-like with a distinguished, grooved, bulb-like head on a distinct stalk (Fig. 2, BCPS). They are set in shallow cuticular depressions on the distal ends (around the interflagellomere membrane) of both the

Table 1. Distribution and abundance of sensilla on the antennae of male and female *T. radiata*

Sensillar type	Sex	Antennomere						Entire antenna
		Radicula	Scape	Pedicel	Annulus	Funicle	Clava	
AST 1	Male		20.2 ± 0.13	10.4 ± 0.08				30.6 ± 0.16b
	Female		20.3 ± 0.15	16.2 ± 0.06		13.6 ± 0.08		50.1 ± 0.20a
AST 2	Male	10.9 ± 0.07		5.8 ± 0.42				16.7 ± 0.11a
	Female	10.7 ± 0.12		5.9 ± 0.32				16.6 ± 0.12a
MST	Male					43.1 ± 0.18	26.3 ± 0.11	69.4 ± 0.17a
	Female					30.8 ± 0.18	23.3 ± 0.12	54.1 ± 0.17a
MPS	Male					8.0 ± 0.00	6.0 ± 0.00	14.0 ± 0.00a
	Female					12.0 ± 0.00	14.0 ± 0.00	26.0 ± 0.00a
BCPS	Male					10.0 ± 0.00	2.0 ± 0.00	12.0 ± 0.00a
	Female					12.0 ± 0.00	8.0 ± 0.00	20.0 ± 0.00a

Mean ± SE number of sensilla on various antennomeres (flagellum: annulus, funicle, and clava) of the antennae of both sexes of *T. radiata.* Within each sensillar type, means followed by different letters indicate significant differences between males and females ($P < 0.05$; chi-square test).

male and female antennal flagellum. Transverse sectioning of the BCPS revealed no evidence of wall pores suggesting a nonolfactory function (Fig. 3, BCPS).

Abundance and Distribution of Antennal Sensilla. Only one type of sensillum was present on the radicula and scape of *T. radiata*'s antennae. The AST-1 (Fig. 2) is found on the scape and the AST-2 (Fig. 2) on the radicula. The pedicel of the antennae of both sexes bears the AST-1 on its entire cuticular surface and the AST-2 around the basal scape-pedicel elbow joint region. There are ≈10 AST-2 and 20 AST-1 on the radicula and scape of the antennae of both sexes, respectively. There are ≈10 and 16 AST-1 on the antennal pedicel of both males and females whereas, ≈6 AST-2 are present on the antennal pedicel of both sexes (Table 1). The antennal flagellum of both males and females bears three and four sensillar types, respectively. The ring-like annulus, which constitutes the first flagellar antennomere, is devoid of sensilla in both sexes. The AST-1 is absent on the antennal funicle of males but present on that of females (n ≈14), whereas the antennal funicle of both sexes bear the MST, the MPS, and the BCPS (Table 1). There are ≈43, eight, and 10 (male) and ≈31, 12, and 12 (female) MST, MPS, and BCPS, respectively, on *T. radiata*'s antennal funicle. The club-shaped antennal clava also bears ≈26, six, and two (male) and ≈23, 14, and eight (female) MST, MPS, and BCPS, respectively. In general, the AST-1 are significantly more abundant ($\chi^2 = 4.46$, df = 1, $P = 0.035$) on the antennae of females than males (Table 1). In contrast, the abundance of the AST-2 ($\chi^2 = 0.00$, df = 1, $P = 1.000$), MST ($\chi^2 = 1.83$, df = 1, $P = 0.176$), MPS ($\chi^2 = 3.6$, df = 1, $P = 0.058$), and BCPS ($\chi^2 = 2.0$, df = 1, $P = 0.157$) was not significantly different between males and females (Table 1).

Discussion

The current investigation aimed to describe the morphology of the antennal sensilla of *T. radiata* to gain insight into the behavioral ecology of this parasitoid. In general, the morphology and distribution of sensilla are characteristic of parasitic wasps such as *Trichogramma nubilale* (Ertle & Davis) (Hymenoptera: Trichogrammatidae) (Olson and Andow 1993), *R. tutela* (Pettersson et al. 2001), *Cotessia* spp. (Hymenoptera: Braconidae) (Bleeker et al. 2004), and *P. cerealellae* (Onagbola and Fadamiro 2008). The antenna of *T. radiata* is composed of a long, scapula-shaped scape with a basal radicula; a barrel-shaped pedicel; and a long flagellum that subdivides into a ring-like proximal anullus, a mesal funicle, and a club-shaped distal clava (Isidoro et al. 1996, Pettersson et al. 2001, Onagbola and Fadamiro 2008). Five types of sensilla, including the AST-1; the short, peg-like AST-2; the long MST; the plate-like MPS; and the short BCPS, were present on the antennae of both males and females.

Sexual dimorphism of antennal structures occurs commonly among parasitic wasp species (Navasero and Elzen 1991, Onagbola and Fadamiro 2008). Likewise, the antennae of male and female *T. radiata* exhibit considerable sexual dimorphism. The male antenna contains one more funicular flagellomere than that of the female. Also, the MST are nearly four times longer in males than females. However, the overall array and abundance of all antennal sensilla (except for the AST-1) are similar between the sexes as observed in other parasitic wasps such as *R. tutela* (Pettersson et al. 2001). The greater abundance of porous sensilla on male antennae than that of females suggests possible involvement of a female-produced sex pheromone in the behavioral ecology of *T. radiata.*

The AST-1 occurs on the scape and pedicel in both sexes of *T. radiata* but only on the antennal flagellum of females. In general, the AST-1 is more abundant on the antennae of females than males. Information on the presence and distribution of the AST-1 in eulophid wasps is scant (e.g., Barlin et al. 1981). The distribution of the AST-1 on *T. radiata*'s antennae is atypical compared with previous descriptions of parasitic wasp antennal morphology. Only one bare ring-like annulus is present on the flagellum of *T. radiata*'s antenna in contrast to two ring-like annuli with numerous AST-1 reported for other parasitic wasps such as *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) (Wibel et al. 1984) and *P. cerealellae* (Onagbola and Fadamiro 2008). However, sensilla similar to *T. radiata*'s AST-1 have been described on the antennae of other parasitic wasps species. For example, the "aporous sensilla trichoidea" on the antennae of *T. nubilale* (Olson and Andow 1993) and *Microplitis pallidipes* Szepligeti (Hymenoptera: Braconidae) (Gao et al. 2007) are similar to the AST-1 described in this study. The "tactile mechanosensory bristles" on the antennae of *R. tutela* (Walker) (Pettersson et al. 2001) and the aporous type II sensilla trichoidea on the antenna of *P. cerealellae* (Onagbola and Fadamiro 2008) are also similar to the AST-1 on *T. radiata*'s antennae. Numerous authors have suggested that sensilla characterized by morphology similar to *T. radiata*'s AST-1 are mechanosensors (Olson and Andow 1993, Pettersson et al. 2001, Onagbola and Fadamiro 2008). Mechanosensory sensilla detect acoustic/vibrational signals generated by hosts during feeding (Shade et al. 1990) or by some hemipteran hosts during courtship calling behaviors (Tishechkin 2005). Numerous authors have suggested mate finding by acoustic communication among Psylloidea (Tishechkin 1989, 2005; Percy et al. 2006). The minimal sexual dimorphism in the antennal sensilla of *D. citri* (Onagbola et al. 2008) is congruent with the hypothesis that *D. citri* use nonolfactory cues for mate finding. Vibrational signaling as a means of communication between the sexes was recently confirmed for *D. citri* (Wenninger et al. 2009). It is therefore possible that the AST-1 functions in host finding by detecting *D. citri*'s vibrational signals (Wenninger et al. 2009). Although adult *D. citri* are not parasitized, they typically occur among feeding nymphs; thus, adult presence would forecast availability of oviposition sites. Furthermore, the greater abundance of the AST-1 on female antennae corroborates their probable female-specific function of locating psyllid nymphs for egg laying.

The AST-2 are present on the radicula and around the base of the pedicel (at the scape-pedicel joint) on the antennae of both sexes of *T. radiata.* The AST-2 on *T. radiata*'s antennae are similar in location and in structural morphology to the trichoid sensilla on the antennae of *Trichogramma australicum* Girault (Amornsak et al. 1998) and *P. cerealellae* (Onagbola and Fadamiro 2008). The AST-2 were present in similar abundance on the antennae of male and female *T. radiata.* This suggests that the AST-2 are unlikely to have a sex-specific role in the behavior of *T. radiata.* Combined SEM and TEM investigations revealed aporous walls on the AST-2, suggesting a nonolfactory function of these sensilla. However, the location (between two cuticular surfaces) and the finger-like structure of the AST-2 suggest a possible function as proprioceptors for detection of cuticular flexion during antennal movement as has been reported for other insect species like *P. cerealellae* (Onagbola and Fadamiro 2008).

MPS are often the most conspicuous sensilla on the antennae of insect parasitoids (Barlin and Vinson 1981, Amornsak et al. 1998, Pettersson et al. 2001, Bleeker et al. 2004, Onagbola and Fadamiro 2008). Male and female *T. radiata* possess sausage-shaped MPS on their antennae. The type of MPS observed on *T. radiata* occurs commonly on antennae of hymenopteran parasitoids (Barlin and Vinson 1981, Olson and Andow 1993, Pettersson et al. 2001, Bleeker et al. 2004, Onagbola and Fadamiro 2008). Several authors have reported greater abundance of the MPS on the antennae of male parasitoids than on conspecific females (Navasero and Elzen 1991, Bleeker et al. 2004, Gao et al. 2007). However, as observed in this study with *T. radiata,* in some cases the abundance of MPS is greater on the antennae of females than males (Onagbola and Fadamiro 2008). There are approximately twice as many MPS on the antennae of female *T. radiata* compared with males. However, the external morphology and size of the MPS is similar between the sexes. Given that MPS have porous walls and are more abundant on female antennae, their likely function is perception of host-related olfactory cues, which has been suggested previously in other parasitoid species (Steinbrecht 1987, Gao et al. 2007, Onagbola and Fadamiro 2008). Their presence on the antennae of both sexes suggests that MPS not only function in host finding by females but also may function in host habitat selection in males during mate finding.

MST occur on the antennae of both sexes of *T. radiata.* These sensilla are morphologically similar between the sexes but are larger in males than females. The MST on *T. radiata* are morphologically similar and are present in the same relative location compared with MST described in other parasitoid species (Wibel et al. 1984, Olson and Andow 1993, Pettersson et al. 2001, Bleeker et al. 2004, Onagbola and Fadamiro 2008). As has occurred with several sensillar types, the MST have been differently named by various authors. For example, similar sensilla have been termed "sensilla trichoidea" on the antennae of *N. vitripennis* (Wibel et al. 1984) and *T. nubilale* (Olson and Andow 1993). Similar sensilla have also been described as "multiporous sensilla trichoidea" on the antennae of *R. tutela* (Pettersson et al. 2001), two *Cotesia* spp. (Hymenoptera: Braconidae) (Bleeker et al. 2004), and on the antennae of *P. cerealellae* (Onagbola and Fadamiro 2008). Our TEM examination revealed presence of porous walls on MST suggesting a probable olfactory function (Barlin and Vinson 1981, Steinbrecht 1987, Onagbola et al. 2008). The MST are conspicuous (especially on the antennae of males) and are the most abundant sensillar type on the antennae of both sexes of *T. radiata.* They are numerically more abundant on the antennae of males than females, and comprised of twice as much surface area in males than females. Larger surface area may be related to greater ability of collecting and perceiving volatile chemicals (Onagbola and Fadamiro 2008). In general, greater abundance and larger size of MST in males than females suggests their plausible function as detectors of a female-produced sex pheromone as has been reported for other parasitoid species such as *R. tutela* (Pettersson et al. 2001) and *P. cerealellae* (Onagbola and Fadamiro 2008).

BCPS were set in shallow cuticular depressions on the distal ends of antennae of both sexes of *T. radiata.* The BCPS are known to occur on the antennae of other parasitic wasp species (Wibel et al. 1984, Barlin et al. 1981, Olson and Andow 1993, Onagbola and Fadamiro 2008); however, the terminology regarding this structure has been inconsistent among various investigations to date. For example, Barlin et al. (1981) described sensilla similar to the BCPS as "multiporous peg sensilla" on the antennae of the eulophid parasitoid *Tetrastichus hagenowii* (Ratzeburg). Bleeker et al. (2004) and Gao et al. (2007) described similar structures as "coeloconic sensilla" on the antennae of *Cotesia* spp and *M. pallidipes,* respectively. The "pit organs" described by Wcislo (1995) are also morphologically similar to BCPS on antennae of *T. radiata.* BCPS have been reported to function in odor perception (Steinbrecht 1987, Ryan 2002). However, our TEM investigation of this sensillar type revealed aporous walls, suggesting a nonolfactory role as has been observed previously in other species (Wibel et al. 1984, Wcislo 1995, Onagbola and Fadamiro 2008). The specific function of BCPS is difficult to infer given their similar distribution and abundance on males and females of parasitic wasp species (Onagbola and Fadamiro 2008). Based on their location and morphology on *T. radiata,* we suggest that these sensilla may be involved in the detection of hygro- and thermosensory cues as has been reported for similar sensilla in other parasitoid species (Wcislo 1995, Pettersson et al. 2001, Onagbola and Fadamiro 2008).

In summary we have characterized five antennal sensilla on the antennae of male and female *T. radiata.* Observation of their transverse sections by using TEM confirmed the presence of two aporous (likely mechanosensor and proprioceptor functions), two porous (likely odor detector function), and one possible hygro- or thermosensory detector on *T. radiata*'s antennae. The antennae of both sexes exhibit considerable sexual dimorphism. Male antennae are much longer (relative to body length) and possess more multiporous sensilla trichoidea. Semiochemicals are known to play a significant role in parasitoid communication and female-produced sex pheromones have been implicated in mate finding (Weseloh 1976, Quicke 1997). Female-produced sex pheromones are typically long-range male attractants in parasitoid species (Eller et al. 1984, Quicke 1997, Jewett and Carpenter 1999) and mediate subsequent close-range courtship behaviors (Yoshida 1978). Male *T. radiata* have likely evolved larger and more numerous olfactory MST for perception of a female-produced pheromone. In contrast, the antennae of female *T. radiata* possess more MPS and AST-1. The MPS and AST-1 likely serve in detection of host-related olfactory cues in both sexes. Vibrational communication has been recently reported to occur between male and female *D. citri* (Wenninger et al. 2009). Therefore, the mechanosensory AST-1 likely function for detection of psyllid-generated vibrational signals for host finding by females. Characterizing the morphology and the distribution of the various sensilla on the antennae of male and female *T. radiata* will facilitate subsequent electrophysiological investigations of *T. radiata*'s olfactory sensilla. This will enhance our ongoing investigations of the chemical ecology of this parasitoid species with aim of identifying specific attractants for monitoring wasp establishment and for recruiting wasps into commercial orchards for enhanced biological control.

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References Cited

Amornsak, W., B. Cribb, and G. Gordh. 1998. External morphology of antennal sensilla of *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae). Int. J. Insect Morphol. Embryol. 27: 67–82.

Aubert, B., and S. Quilici. 1984. Biological control of the African and Asian citrus psyllids (Homoptera: Psylloidea), through eulophid and encyrtid parasites (Hymenoptera: Chalcidoidea) in Reunion Island, pp. 100–108. *In* S. M. Garnsey, L. W. Timmer, and J. A. Dodds [eds.], Proceedings of the 9th Conference of the International Organization of Citrus Virologists, University of California, 9–13 May 1983, Riverside, CA. University of California, Riverside.

Barlin, M. R., and S. B. Vinson. 1981. Multiporous plate sensilla in antennae of Chalcidoidea (Hymenoptera). Int. J. Insect Morphol. Embryol. 10: 29–42.

Barlin, M. R., S. B. Vinson, and G. L. Piper. 1981. Ultrastructure of the antennal sensilla of the cockroach-egg parasitoid *Tetrastichus hagenowii* (Hymenoptera: Eulophidae). J. Morphol. 168: 97–108.

Bleeker, M.A.K., H. M. Smid, A. C. Aelst, J.J.A. van Loon, and L.E.M. Vet. 2004. Antennal sensilla of two parasitoid wasps: a comparative scanning electron microscopy study. Microsc. Res. Tech. 63: 266–273.

Bové, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88: 7–37.

Dicke, M., and P. Grostal. 2001. Chemical detection of natural enemies by arthropods: an ecological perspective. Annu. Rev. Ecol. Syst. 32: 1–23.

Eller, F. J., R. J. Bartelt, R. L. Jones, and H. M. Kulman. 1984. Ethyl (Z)-9-hexade-canoate a sex pheromone of *Syndipnus rubiginosus,* a sawfly parasitoid. J. Chem. Ecol. 10: 291–300.

Étienne, J., S. Quilici, D. Marival, and A. Franck. 2001. Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) by imported *Tamarixia radiata* (Hymenoptera: Eulophidae). Fruits 56: 307–315.

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

Halbert, S. E., and K. L. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla. Entomol. 87: 330–353.

Halbert, S. E., C. L. Niblett, K. L. Manjunath, R. F. Lee, and L.G. Brown. 2003. Establishment of two new vectors of citrus pathogens in Florida, pp. 1016–1017 *In* Proceedings of the International Society of Citriculture IX Congress, 3–7 December 2000, Orlando, FL. ASHS Press, Alexandria VA.

Hoy, M. A., and R. Nguyen. 2001. Classical biological control of Asian citrus psylla. Citrus Ind. 81: 48–50.

Isidoro, N., F. Bin, S. Colazza, and S. B. Vinson. 1996. Morphology of antennal gustatory sensilla and glands in some parasitoid hymenoptera with hypothesis on their role in sex and host recognition. J. Hymenopt. Res. 5: 206–239.

Jewett, D. K., and J. E. Carpenter. 1999. Chemically-mediated attraction of *Ichneumon* (=*Pterocormus*) *promissorius* (Hymenoptera: Ichneumonidae) males by females. Environ. Entomol. 28: 551–556.

Lewis, W. J., D. A. Nordlund, R. C. Gueldner, P.E.A. Teal, and J. H. Tumlinson. 1982. Kairomones and their use for management of entomophagous insects, XIII. Kairomonal activity for *Trichogramma* spp. of abdominal tips, excretion, and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. J. Chem. Ecol. 8: 1323–1331.

McFarland, C. D., and M. A. Hoy. 2001. Survival of *Diaphorina citri* (Homoptera: Psyllidae), and its two parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarensis* (Hymenoptera: Encyrtidae) under different relative humidities and temperature regimes. Fla. Entomol. 84: 227–233.

Michaud, J. P. 2002. Biological control of Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae), in Florida: a preliminary report. Entomol. News 113: 216–222.

Navasero, R. C., and G. W. Elzen. 1991. Sensilla on the antennae, foretarsi and palpi of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). Proc. Entomol. Soc. Wash. 93: 737–747.

Olson, D. M., and D. A. Andow. 1993. Antennal sensilla of female *Trichogramma nubilale* (Ertle and Davis) (Hymenoptera: Trichogrammatidae) and comparisons with other parasitic hymenoptera. Int. J. Insect Morphol. Embryol. 22: 507–520.

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

Onagbola, E. O., W. L. Meyer, D. Boina, and L. L. Stelinski. 2008. Morphological characterization of the antennal sensilla of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), with reference to their probable functions. Micron 39: 1184–1191.

Percy, D. M., G. S. Taylor, and M. Kenedy. 2006. Psyllid communication: acoustic diversity, mate recognition and phylogenetic signal. Invertebr. Syst. 20: 431–445.

Pettersson, E. M., E. Hallberg, and G. Biggersson. 2001. Evidence for the importance of odour-reception in the parasitoid *Rhopalicus tutela* (Walker) (Hymenoptera: Pteromalidae). J. Appl. Entomol. 125: 293–301.

Pluke, R.W.H., J. A. Quareshi, and P. A. Stansly. 2008. Citrus flushing patterns, *Diaphorina citri* (Hemiptera: Psyllidae) populations and parasitism by *Tamarixia radiata* (Hymenoptera: Eulophidae). Fla. Entomol. 91: 36–42.

Quicke, D.L.J. 1997. Parasitic wasps. Chapman & Hall, London. United Kingdom.

Ryan, M. F. 2002. Insect chemoreception: fundamental and applied. Kluwer Academic Publishers, Amsterdam, The Netherlands.

Shade, R. E., E. S. Furgason, and L. L. Murdock. 1990. Detection of hidden insect infestations by feeding-generated ultrasonic signals. Am. Entomol. 36: 231–234.

Steinbrecht, R. A. 1987. Functional morphology of pheromone-sensitive sensilla, pp. 353–383. *In* G. D. Prestwich and G. J. Blomquist [eds.], Pheromone biochemistry. Academic, Orlando. FL.

Tishechkin, D. Y. 1989. Acoustic signalization of psyllids (Homoptera: Psyllinae) of the Moscow Province. Vestnok Mosc. Univ. 16: 20–24.

Tishechkin, D. Y. 2005. Vibratory communication in Psylloidea (Homoptera), pp 357–363. *In* S. Drosopoulos and M. F. Claridge [eds.], 2006. Insect sounds and communication—physiology, behaviour, ecology and evolution. Taylor & Francis Group, New York.

Tsai, J. H., J. J. Wang, and Y. H. Liu. 2000. Sampling of *Diaphorina citri* (Homoptera: Psyllidae) on orange jessamine in southern Florida. Fla. Entomol. 83: 446–459.

Vinson, S. B. 1991. Chemical signals used by parasitoids. Redia 74: 15–42.

Vinson, S. B., F. Bin, and M. R. Strand. 1986. The role of the antennae and host factors in host selection behavior of *Trissolcus basalis* (Wall.) (Hymenoptera: Scelionidae). Les Colloq. de-I'INRA 43: 267–273.

Wcislo, W. T. 1995. Sensilla numbers and antennal morphology of parasitic and non-parasitic bees (Hymenoptera: Apoidea), Int. J. Insect Morphol. Embryol. 24: 63–81.

Wenninger, E. J., L. L. Stelinski, and D. G. Hall. 2008. Behavioral evidence for a female-produced sex attractant in *Diaphorina citri.* Entomol. Exp. Appl. 128: 450–459.

Wenninger, E. J., D. G. Hall, and R. W. Mankin. 2009. Vibrational communication between the sexes in *Diaphorina citri* (Hemiptera: Psyllidae). Ann. Entomol. Soc. Am. 102: 547–555.

Weseloh, R. M. 1976. Dofour's gland: source of sex pheromone in a hymenopterous parasitoid. Science (Wash., D.C.) 193: 695–697.

Wibel, R. G., J. D. Cassidy, H. E. Buhse, Jr., M. R. Cummings, V. P. Bindokas, J. Charlesworth, D. L. Baumgartner. 1984. Scanning electron microscopy of antennal sense organs of *Nasonia vitripennis* (Hymenoptera: Pteromalidae). Trans. Am. Microsc. Soc. 103: 329–340.

Yoshida, S. 1978. Behaviour in males in relation to the female sex pheromone in the parasitic wasp, *Anisoptera calandrae* (Hymenoptera: Pteromalidae). Entomol. Exp. Appl. 23: 152–162.

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