

Sex-specific antennal sensory system in the ant *Camponotus japonicus*: structure and distribution of sensilla on the flagellum

Aki Nakanishi · Hiroshi Nishino · Hidehiro Watanabe ·
Fumio Yokohari · Michiko Nishikawa

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Abstract The antennae are a critically important component of the ant's highly elaborated chemical communication systems. However, our understanding of the organization of the sensory systems on the antennae of ants, from peripheral receptors to central and output systems, is poorly understood. Consequently, we have used scanning electron and confocal laser microscopy to create virtually complete maps of the structure, numbers of sensory neurons, and distribution patterns of all types of external sensilla on the antennal flagellum of all types of colony members of the carpenter ant *Camponotus japonicus*. Based on the outer cuticular structures, the sensilla have been classified into seven types: coelocapitular, coeloconic, ampullaceal, basiconic, trichoid-I, trichoid-II, and chaetic sensilla. Retrograde staining of antennal nerves has enabled us to count the number of sensory neurons housed in the different types of sensilla: three in a coelocapitular sensillum, three in a coeloconic sensillum, one in an ampullaceal sensillum, over 130 in a basiconic sensillum, 50–60 in a trichoid-I sensillum, and 8–9 in a trichoid-II sensillum. The basiconic

sensilla, which are cuticular hydrocarbon-receptive in the ant, are present in workers and unmated queens but absent in males. Coelocapitular sensilla (putatively hygro- and thermoreceptive) have been newly identified in this study. Coelocapitular, coeloconic, and ampullaceal sensilla form clusters and show biased distributions on flagellar segments of antennae in all colony members. The total numbers of sensilla per flagellum are about 9000 in unmated queens, 7500 in workers, and 6000 in males. This is the first report presenting comprehensive sensillar maps of antennae in ants.

Keywords Sensillar map · Chemoreceptor · Hygro- and thermoreceptor · Cuticular hydrocarbon · Social insect · Carpenter ant, *Camponotus japonicus* (Insecta)

Introduction

Ants heavily depend on chemical communication for their social lives and have well-developed antennal chemosensory systems. Colonies of ants consist of non-reproductive females (workers) and reproductive males and females (queens). These colony members play sex- or cast-specific roles throughout their social lives to maintain their colonies and communicate with each other by using chemical signals, the so-called pheromones (Hölldobler and Wilson 1990). Mechanisms for nestmate recognition in ants, in which many kinds of cuticular hydrocarbons are involved, may be related to complex processes in neural networks (Mintzer 1982; Hölldobler and Wilson 1990; Hara 2003; Lucas et al. 2005; Ozaki et al. 2005; Brandstätter et al. 2008). Recent studies have started to reveal the molecular basis underlying social behavior in ants (Lucas and Sokolowski 2009). However, the compre-

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A. Nakanishi · H. Watanabe · F. Yokohari · M. Nishikawa (✉)
Department of Earth System Science, Fukuoka University,
Fukuoka 814-0180, Japan
e-mail: michiko@fukuoka-u.ac.jp

H. Nishino
Research Institute for Electronic Science, Hokkaido University,
Sapporo 060-0812, Japan

hensive organizations of neural networks for antennal chemosensory systems, from input signals to efferent outputs, have yet to be revealed in these social insects.

Antennae of insects are major sense organs that signal environmental information by means of a variety of sensillum filters. Antennal sensilla consist of a cuticular apparatus, sensory neurons, and auxiliary cells (Altner and Prillinger 1980; Steinbrecht 1997; Keil 1999). Physiological and morphological studies have revealed functional properties of sensilla in insects. The outer cuticular apparatus of sensilla is specialized depending on the sensory modalities that they process. Thus, chemoreceptive sensilla, hygro/thermoreceptive sensilla, and mechanoreceptive sensilla can, to some extent, be discerned based on their outer cuticular structures (Altner and Prillinger 1980; Steinbrecht 1997; Keil 1999).

Axons of chemosensory neurons responsive to volatile chemicals project to antennal lobe glomeruli in the brain. Sensory neurons expressing the same odorant receptor converge onto single glomeruli. Thus, each glomerulus acts as a functional unit to process a limited spectrum of odorants (Gao et al. 2000; Vosshall et al. 2000). For example, in males of moths and cockroaches, a large number of sensory neurons responsive to female sex pheromones converge onto a single large glomerulus (macroglomerulus) or a set of large glomeruli (macroglomerular complex). The olfactory glomeruli are thus “chemotopically” organized in the antennal lobe.

Recent studies in holometabolous and hemimetabolous insects have revealed some degree of topographic interaction between sensilla and glomeruli. Some types of antennal sensilla are not distributed uniformly over the surface of the antennae. In fruit flies, some types of chemosensory sensilla are clustered in particular regions of the antenna (Vosshall et al. 1999; Couto et al. 2005). In cockroaches and honeybees, hygro- and thermoreceptive sensilla form an orderly array around the circumference of the flagellum (Yokohari 1999; Nishino et al. 2009). Thus, the locations of the different types of sensilla can be mapped to different regions of the glomerular clusters in the antennal lobe (Couto et al. 2005; Nishino et al. 2009). Female sex-pheromone-receptive afferents are arranged in an orderly array within the macroglomerular complex, based on the three-dimensional locations of their somata in the antenna of the cockroach (Nishino and Mizunami 2007). Some degree of somatotopic organization of receptor axons is also evident in the ordinary glomeruli of honeybees (Pareto 1972; Nishino et al. 2009) and cockroaches (Nishino and Mizunami 2006). In cockroaches, such somatotopic organization of olfactory afferents appears to be utilized by interneurons having local receptive fields on the antenna (Hösl 1990; Nishino and Mizunami 2007). Knowledge of the distribution patterns of

sensory neurons and their numbers on antennae is thus important if we are to understand the neural pathways underlying the elaborate chemical communication seen in ants.

The structure and distribution of antennal sensilla have been reported in ants (Dumpe 1972b; Hashimoto 1990; Renthal et al. 2003). Furthermore, in different ant species, the physiological functions of sensory neurons in individual sensilla have previously been elucidated by bioassay experiments or by electrophysiological recordings: alarm substance-receptive neurons in the trichoid curvata sensilla of the black garden ant (Dumpe 1972a), a mechanoreceptive neuron in a campaniform sensillum or in a chaetic sensillum of the black garden ant (Dumpe 1972a), a carbon-dioxide-receptive neuron in an ampullaceous sensillum of the leaf-cutting ant (Kleineidam et al. 2000), cuticular hydrocarbon-receptive neurons in the basiconic sensilla of the carpenter ant (Ozaki et al. 2005), and a cold-receptive neuron in a coeloconic sensillum of the leaf-cutting ant (Ruchty et al. 2009). These morphological and physiological studies of antennae contribute to our understanding of the neural mechanisms underlying chemical communication systems in ants. Comprehensive antennal sensillar mapping is however needed as a first step in analyzing the signal input channels of antennal sensory systems. Carpenter ants, which are common ant species and have relatively large brains, have been useful laboratory animals for electrophysiological studies (Galizia et al. 1999; Ozaki et al. 2005; Yamagata et al. 2005, 2006; Zube et al. 2008) and neuroanatomical studies (Gronenberg et al. 1996; Ehmer and Gronenberg 2004; Kleineidam et al. 2005; Tsuji et al. 2007; Nishikawa et al. 2008; Zube and Rossler 2008; Mysore et al. 2009). Furthermore, the antennal sensilla for nestmate recognition have previously been identified by electrophysiological and biochemical studies in the ant (Ozaki et al. 2005).

In this study, we focus on antennal chemosensory systems in ants and establish a comprehensive sensillar map for antennal flagella in all colony members of the ant *Camponotus japonicus*.

Materials and methods

Animals

Ants (*Camponotus japonicus*) were caught around a nest on the campus of Fukuoka University during the mating season in spring from April to May. Small-sized workers, alated females (unmated queens), and males were collected and then subjected to morphological and histological analyses.

Scanning electron microscopy

Isolated antennae were immersed in 70% acetone, ultrasonically cleaned, hydrated in distilled water, immersed in a 4% osmic acid (OsO_4) solution for 2 h, dehydrated in a graded acetone series from 70% to 100%, air-dried, and coated with platinum-palladium by using a Hitachi E-1030 ion sputter. Observations were made with a Hitachi S-4100 field emission scanning electron microscope (FE-SEM). In order to map all types of sensilla on all flagellar segments, the specimens were pasted on aluminum plates with the long axes of the antennae standing vertically. Micrographs of the entire aspect around each flagellum were then made into montages by using image-processing software (Adobe Photoshop CS2, Adobe Systems, San Jose, Calif., USA). The respective types of sensilla were marked with different colors and shapes on the montages by using image-processing software (Adobe Illustrator CS2, Adobe Systems). Care was taken to eliminate overlapping or missing areas on the montages of antennal flagellar surfaces for our mapping procedures. Such mappings were performed on all flagellar segments in all colony members.

Retrograde staining of sensory neurons in flagella

Ants were cold-anesthetized on ice for 5 min and decapitated. The heads, facing up, were fixed to a beeswax plate with insect pins. The antennae were displaced laterally and embedded in modeling clay at the distal region. Retrograde fills (staining somata of sensory neurons) with fluorescent dyes were performed. Before staining, a borosilicate microelectrode (internal diameter: 0.57 mm) was pulled and narrowed by using a laser puller (Sutter Instrument, Novato, Calif., USA) and then fractured at a point such that the tip would loosely fit the chosen nerve. Immediately before staining, the microelectrode was filled with rhodamine dextran with biotin (3,000 MW, lysine-fixable, Microruby, D7162; Invitrogen, Eugene, Ore., USA) or fluorescein dextran with biotin (3,000 MW, lysine-fixable, Microemerald, D7156; Invitrogen). No obvious difference in the staining pattern produced by using either dextran dye was found. Axons of sensory neurons housed in sensilla on the dorsal surface and ventral surface of the flagellum form the dorsal nerve and ventral nerve, respectively. To map sensory afferents to their respective antennal nerves, retrograde double-labeling was used. The two antennal nerves were exposed by removing the cuticle surface of the scape and sectioned at the middle region of the scape. The two microelectrodes filled with either dye were positioned near the dissected nerves by maneuvering modeling clay attached to each electrode. The distal cut

ends of the nerves were placed separately into the two tapered glass electrodes containing either dye. After each specimen had been incubated in a dark and moist chamber at 4°C for 48 h, the antennae were cut from the head. One side of the cuticle on the flagellum was sliced off by using a thin blade so that the nerves and sensory neurons would remain in the flagellar lumen. The flagellum partially stripped of cuticle was fixed with 4% formaldehyde solution at 4°C for 6 h. It was then dehydrated in an ascending ethanol series and cleared in methyl salicylate.

Confocal microscopy and image processing

In the whole-mount preparation of an antenna filled with methyl salicylate, sensory neurons of sensilla were viewed on the cuticular-removed side of the flagellum by means of confocal microscopy (LSM-510; Carl Zeiss, Jena, Germany). Sensory neurons labeled by fluorescein were visualized by using an argon laser with a band-pass filter (505–530 nm), whereas those labeled by rhodamine were visualized by using a helium-neon laser with a long-pass filter (<560 nm). To determine the cuticular structures of sensilla, light-microscopic photomicrographs obtained by using differential interference contrast optics were overlaid on optical sections taken simultaneously. In most cases, retrograde filling of antennal nerves stained sensory neurons but not auxiliary cells. Dendrites innervating the cuticular apparatus of the sensilla could be observed in most cases. As a result, we could identify both the cuticular apparatus and somata of sensory neurons in a sensillum in the flagellar segment. Serial optical sections were acquired at intervals of 0.5–1 μm . All images were taken at a resolution of 1024×1024 pixels and were saved as TIFF files. Short image stacks were reconstructed two-dimensionally by using image-processing software attached to the LSM image browser and further processed by Adobe Photoshop CS2 to adjust brightness and contrast.

Three-dimensional reconstructions of sensory neurons and outer cuticular structures of sensilla

Series of TIFF images were imported to image-processing software (Amira; TGC, Berlin, Germany) and analyzed. Cuticular structures of the sensilla and somata of sensory neurons in sensilla were manually outlined for each optical slice and were three-dimensionally reconstructed. On the reconstructed images, the shapes of the cuticular structures and the numbers of somata in the sensilla were profiled by using image-processing software (Amira).

Results

Sensillar mapping

The antenna of the ant consists of a scape, a pedicel, and ten flagellar segments in workers and unmated queens, whereas males have eleven flagellar segments (Fig. 1). The orientations of the antennae follow those of the body axis. Figure 1 shows the anterior side view of the right half of an ant head with the antennal flagella pointing downward so that the dorsal surfaces of the antennae face forward (Fig. 1a–c), whereas Fig. 1d–f shows the corresponding ventral surfaces.

We regarded the joint membranes between the scape and pedicel as the medial side of the antenna (Fig. 1). The four aspects, viz., dorsal, medial, ventral, and lateral, around the antennal flagellum were marked approximately on the montages. In the antennae of all colony members, the round segments and slightly curved flagellar segments made it difficult to apply longitudinal or transverse orientations along the lengths of flagellar segments on montages. Nevertheless, the distribution patterns of sensilla

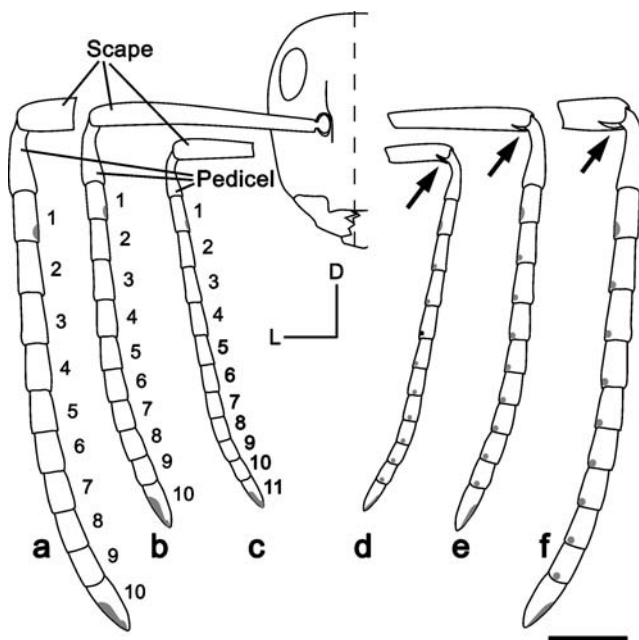


Fig. 1 Antennae of the ant *Camponotus japonicus*. Schematic drawings were based on scanning electron micrographs of antennae in all colony members (D dorsal, L lateral). The right half of a worker's head is represented in anterior view, whereas the antennae of all colony members are represented in dorsal and ventral side views. a–c Dorsal side views of antennae. d–f Ventral side views of antennae. Antennae consist of a scape, a pedicel, and 10 flagellar segments (1–10) in an unmated queen (a, f) and a worker (b, e) and 11 flagellar segments (1–11) in a male (c, d). The joint (arrows) between a scape and a pedicel is a marker for the medial side of the antenna in all colony members. Gray zones on flagellar segments indicate cluster regions of coelocapitular, coeloconic, and ampullaceous sensilla (a–f). Bar 0.5 mm

over the flagellar segments faithfully represented the real antenna. Since the cuticular structures of the ampullaceous and coeloconic sensilla were totally embedded in the lumen of the flagellum, only small apertures of these sensilla could be found on the antennal surface in observations by scanning electron microscopy (Fig. 2a, see also below). Therefore, these sensilla were assigned to either type according to the aperture sizes on the antennal surface. The diameters of the apertures in ampullaceous sensilla were 0.8–1.0 μm , whereas those of coeloconic sensilla were 1.2–1.5 μm (Fig. 2a).

Antennal flagellum sizes and segment numbers

Each flagellar segment from proximal to distal was numbered consecutively from 1 to 10 or 11 (Fig. 1). Morphometric measurements showed that unmated queens had the largest antennae, and that males had the smallest: the entire antennal length was about 6 mm in workers, about 7 mm in unmated queens, and about 5 mm in males. The entire flagellar length was about 3.2 mm in workers, about 3.8 mm in unmated queens, and about 2.4 mm in males. The maximum diameters of flagella measured at the base of the flagellum were about 150 μm in workers, about 190 μm in unmated queens, and about 120 μm in males. Compared with other segments, the distalmost flagellar segments in all colony members were not round in cross section but were somewhat ellipsoidal and slightly flat with respect to the ventro-dorsal axis of the flagellum. The gray zones on the flagellar segments in Fig. 1 represent schematically the cluster area of coelocapitular, coeloconic, and ampullaceous sensilla.

Morphological features of sensilla on the flagellum

Based on the morphological features of the cuticular apparatus of the sensilla, flagellar sensilla were classified into seven types: coelocapitular, coeloconic, ampullaceous, basiconic, trichoid-I, trichoid-II, and chaetic sensilla (Table 1). Since one type of chaetic sensillum was readily distinguishable from other types, based on its unique appearance and frequent occurrence close together with basiconic sensilla, we called this type chaetic-A sensilla and examined their locations separately from other types of chaetic sensilla (Table 1).

A coelocapitular sensillum had a peg in a pit inside the flagellum (Fig. 2a–c). The mushroom-like structure of the peg about 2 μm in diameter protruded from the central hole of an oval-shaped shallow depression about 5 μm in diameter on the antennal surface (Fig. 2a, b). The aperture of the pit was observed as a narrow gap surrounding the protrusion of the peg (Fig. 2b) in the shallow depression of the antennal surface. No pore was seen in the mushroom-

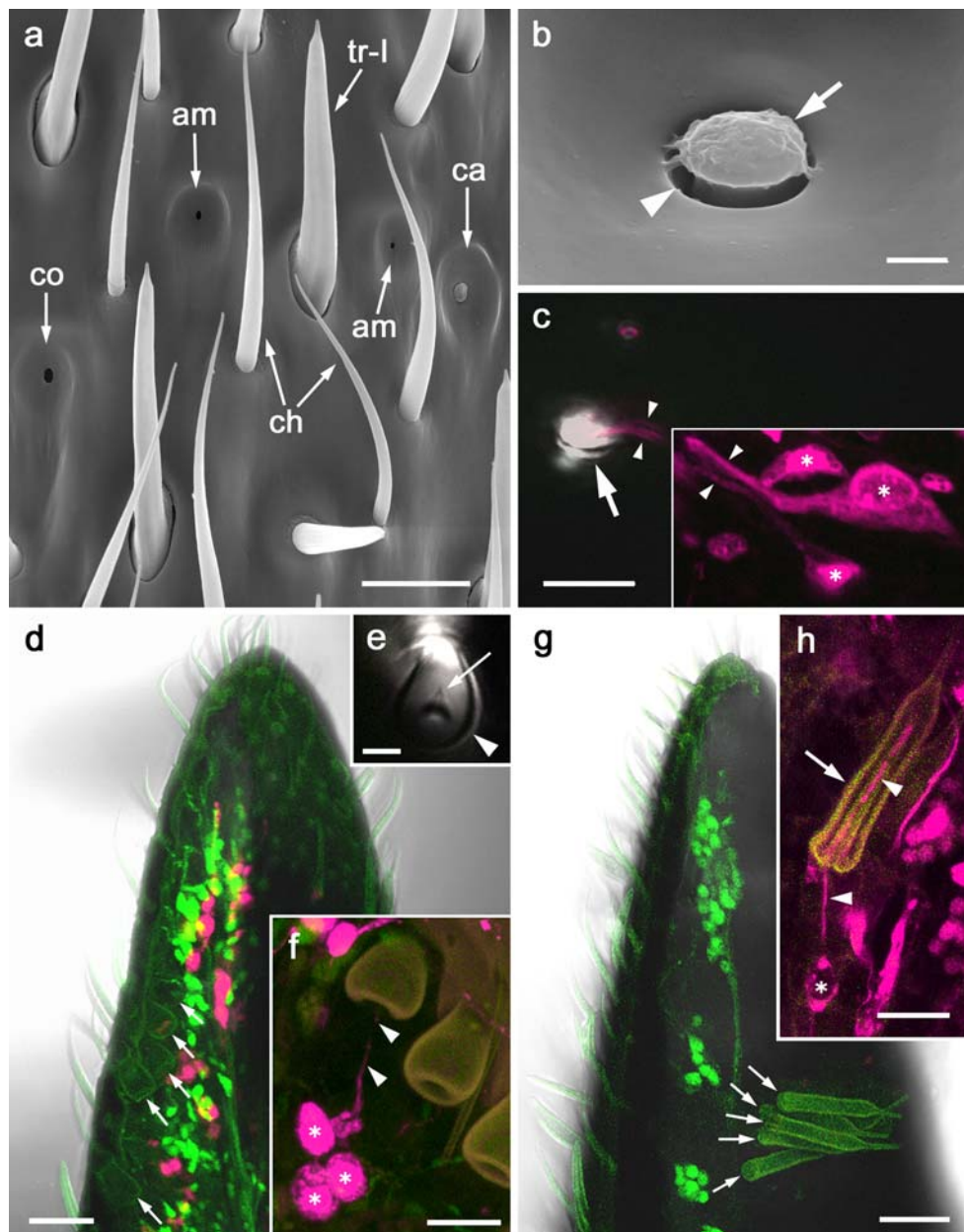


Fig. 2 Structures of coelocapitular, coeloconic, and ampullaceous sensilla. Scanning electron micrographs (**a**, **b**) and confocal laser scanning micrographs (**c**–**h**). **a** Coelocapitular (*ca*), coeloconic (*co*), and ampullaceous sensilla (*am*) are located close to each other. On the antennal flagellar surface, only apertures of coeloconic and ampullaceous sensilla are observed. Diameters of apertures in coeloconic sensilla are $>1.2\ \mu\text{m}$ and, in ampullaceous sensilla, $<1\ \mu\text{m}$. Chaetic sensilla (*ch*) and trichoid-I sensillum (*tr-I*) are also observed on the flagellar segment. Bar $10\ \mu\text{m}$. **b** Coelocapitular sensillum exhibiting a cuticular apparatus with a mushroom-like protrusion (*arrow*) in a pit (*arrowhead*) on the antennal surface. Bar $1\ \mu\text{m}$. **c** The cuticular apparatus of the sensillum (*arrow*) appears relatively thin and transparent in the flagellar lumen in confocal laser scanning micrographs. Three dendrites (*between arrowheads*) innervate the cuticular apparatus of the sensillum. *Inset*: Three somata (*asterisks*) with three dendrites (*between arrowheads*) of the same sensillum, each at a different depth in the flagellar lumen. Bar $10\ \mu\text{m}$. **d** In the most distal flagellar segment, coeloconic sensilla (*arrows*) are distributed close to each other. Somata of sensory neurons are heavily stained with two

dyes: rhodamine (*pink*) and fluorescein (*green*). Cuticular structures of the antenna are not stained by dyes but appear colored because of the strong contrast adjustment. Bar $20\ \mu\text{m}$. **e** The cuticular apparatus of a coeloconic sensillum in the flagellar lumen consists of a small cone-shaped peg (*arrow*) in a gourd-like pit (*arrowhead*). Bar $5\ \mu\text{m}$. **f** A coeloconic sensillum houses three sensory neurons with three somata (*asterisks*) and dendrites (*arrowheads*) innervating a cuticular apparatus. Non-stained cuticular apparatus of the coeloconic sensilla in the lumen appear *yellowish green* because of the strong contrast adjustment. Bar $10\ \mu\text{m}$. **g** A cluster of ampullaceous sensilla (*arrows*) in the lumen of the most distal flagellar segment. Somata of sensory neurons in the flagellar lumen are strongly stained *green*. The non-stained cuticular apparatus of the ampullaceous sensilla in the lumen appears *light green* because of the strong contrast adjustment. Bar $20\ \mu\text{m}$. **h** An ampullaceous sensillum houses a single sensory neuron with a soma (*asterisk*) and dendrites (*arrowheads*) innervating a long cone-shaped peg in a cylindrical pit (*arrow*). Non-stained cuticular apparatus in the lumen appears *yellow* because of the strong contrast adjustment. Bar $10\ \mu\text{m}$

Table 1 Numbers of sensilla on all flagellar segments ($F1-F10$) in all types of colony members of *Camponotus* (*Neurons* numbers of sensory neurons in each type of sensilla, n number of sensilla examined). Two individual sensilla (#1, #2) from each colony member were examined. According to the locations of sensilla on the montages of scanning electron micrographs, the numbers of respective sensillum types were counted on each flagellar segment for all colony repertoires

Sensilla type	Coelocapitular		Coeloconic		Ampullaceal		Basiconic		Chaetic-A		Trichoid-I		Trichoid-II		Chaetic		Σ	
Neurons	3 ($n=7$)		3 ($n=9$)		1 ($n=10$)		130 ($n=6$)		4–6 ($n=9$)		50–60 ($n=8$)		8–9 ($n=7$)					
Individual sensillum	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2
Workers																		
F1	6	7	3	1	2	5	5	3	8	7	3	8	1	4	630	679	658	714
F2	0	0	1	1	0	1	7	10	9	12	19	27	6	5	748	835	790	891
F3	0	1	2	1	1	1	10	9	11	10	33	35	8	12	666	713	731	782
F4	1	0	1	1	0	1	10	10	11	12	42	54	11	14	586	639	662	731
F5	1	1	1	2	1	0	15	13	14	14	52	60	14	20	584	586	682	696
F6	1	1	1	2	0	1	14	15	13	14	67	71	15	24	525	512	636	640
F7	1	1	1	3	1	1	20	22	16	18	78	83	18	29	551	510	686	667
F8	1	1	2	3	2	1	20	21	19	19	88	91	23	39	456	422	611	597
F9	2	1	2	5	2	0	27	27	20	22	97	92	32	44	494	473	676	664
F10	6	6	10	10	8	11	50	58	58	62	168	167	51	69	763	883	1114	1266
Σ	19	19	24	29	17	22	178	188	179	190	647	688	179	260	6003	6252	7246	7648
Unmated queens																		
F1	9	9	3	3	2	2	6	3	12	11	11	14	0	3	1022	742	1065	787
F2	0	0	2	1	0	2	8	7	11	8	29	29	7	11	932	925	989	983
F3	2	1	2	0	0	1	8	9	13	10	51	75	11	23	898	750	985	869
F4	1	1	1	1	1	3	11	11	14	13	81	81	16	20	755	694	880	824
F5	1	1	2	1	0	3	12	7	15	14	77	78	21	23	664	600	792	727
F6	1	1	1	2	0	1	20	19	17	13	111	110	24	29	677	530	851	705
F7	1	1	3	2	1	2	23	26	18	17	112	101	34	22	580	563	772	734
F8	0	1	1	2	3	1	26	39	20	21	137	125	43	35	609	538	839	762
F9	1	1	2	3	2	2	29	20	20	13	139	124	39	44	613	411	845	618
F10	5	7	11	12	9	8	55	54	70	60	243	172	67	58	1168	808	1628	1179
Σ	21	23	28	27	18	25	198	195	210	180	991	909	262	268	7918	6561	9646	8188
Males																		
F1	6	5	2	2	1	4	0	0	8	8	4	0	0	0	614	538	635	557
F2	0	1	1	2	0	3	0	0	6	11	27	16	3	1	649	688	686	722
F3	0	1	1	2	1	1	0	0	6	7	37	30	5	3	477	623	527	667
F4	1	0	1	1	1	3	0	0	9	6	60	62	11	9	454	544	537	625
F5	0	1	0	2	0	0	0	0	9	8	59	67	11	11	416	437	495	526
F6	1	0	2	1	1	1	0	0	7	7	72	76	11	13	451	367	545	465
F7	1	0	2	1	1	1	0	0	10	9	85	82	14	15	417	328	530	436
F8	1	1	2	1	2	1	0	0	12	14	96	80	16	18	328	277	457	392
F9	1	0	2	1	1	0	0	0	14	13	117	88	23	23	316	298	474	423
F10	1	0	2	2	2	0	0	0	13	14	100	88	16	13	300	323	434	440
F11	4	5	5	2	2	2	0	0	33	31	94	89	16	7	514	564	668	700
Σ	16	14	20	17	12	16	0	0	127	128	751	678	126	113	4936	4987	5988	5939

like structure (Fig. 2b). The cuticular apparatus of the sensillum appeared relatively thin and transparent in the flagellar lumen (Fig. 2c). The sensillum housed three sensory neurons with large somata about 10 μm in diameter (Fig. 2c).

The coeloconic sensillum had a small aperture about 1.2 μm in diameter at the center of an oval-shaped shallow depression about 5 μm in diameter on the antennal surface (Fig. 2a). The entire cuticular structure of the sensillum was embedded in the flagellar lumen (Fig. 2d). In the flagellar lumen, the sensillum had a short cone-shaped peg about 5 μm in length and about 3 μm in diameter at the peg base (Fig. 2e) at the bottom of a gourd-shaped double-pit about 10 μm in diameter (Fig. 2e) connecting to a small aperture on the antennal surface (Fig. 2a). Each coeloconic sensillum housed three sensory neurons with large somata about 8 μm in diameter (Fig. 2f).

The ampullaceous sensillum had a small aperture less than 1 μm in diameter at the center of an oval-shaped shallow depression about 5 μm in diameter (Fig. 2a) on the antennal surface, and the entire cuticular structure was embedded in the flagellar lumen (Fig. 2g). In the flagellar lumen, the sensillum had a long peg about 25 μm in length and 3 μm in diameter at the peg base in a deep cylindrical pit about 30 μm long and 8 μm in diameter (Fig. 2h) connecting with a narrow duct about 1.2 μm in diameter and more than 30 μm in length to a small pit (not shown) subjacent to a small aperture on the antennal surface (Fig. 2a). An ampullaceous sensillum housed only a single sensory neuron with a large soma about 8 μm in diameter (Fig. 2h).

The basiconic sensillum was a relatively short and thick thumb-shaped sensillum about 20 μm long and 5 μm wide at half its height (Fig. 3a). Many pores perforated the outer side of the cuticular surface furthest from the antennal trunk (Fig. 3b) but not on the other side of the sensillum facing the antennal trunk (not shown). The base of the sensillum was about 6 μm in diameter and appeared to have the structure of a flexible joystick (Fig. 3a). A gap was observed between the base of the sensillum and the antennal surface (Fig. 3a). The sensillum had over 130 sensory neurons with somata each 2–3 μm in diameter (Fig. 3e).

One chaetic-A sensillum (Fig. 3a) appeared to be a bristle about 7 μm in length and about 3 μm in base diameter and tapered gradually toward the tip vicinity and sharply at an apical area (Fig. 3a). The sensillum had several thin longitudinal striae on its side wall (Fig. 3c), a single terminal pore (Fig. 3d), and a narrow circular gap directly surrounding its base (Fig. 3a). The sensillum tended to incline slightly toward the antennal base in contrast with other types of sensilla, which usually inclined toward the antennal tip (Fig. 3a). The sensillum housed 4–6 sensory neurons with somata each 3–5 μm in diameter (Fig. 3f).

The trichoid-I sensillum had a thick sword-shaped cuticular apparatus of about 30 μm in length (Fig. 4a). The sensillum was straight but extremely inclined along the antennal trunk toward the distal sides of the longitudinal antennae (Fig. 4a, e). On both sides of the sword, pores were aligned in oblique parallel rows along the cuticular surface (Fig. 4b). The base of the sensillum was elliptical and about 6 μm in its major axis (Fig. 4a). A narrow gap was present between the base of the sensillum and the antennal surface (Fig. 4a). The sensillum had 50–60 sensory neurons with somata about 3–5 μm in diameter (arrow, Fig. 4e).

The trichoid-II sensillum was a thin flat hair about 70 μm in length that mostly curved toward the antennal trunk and with a relatively sharp apical tip lacking a terminal pore (Fig. 4c). The sensillum had many pores irregularly distributed along the center of both sides (Fig. 4d). The base of the sensillum was relatively round with a diameter about 3 μm (Fig. 4c). A narrow gap lay between the base of the sensillum and the antennal surface (Fig. 4c). The sensillum had 8–9 sensory neurons with somata about 3–5 μm in diameter (arrow, Fig. 4f).

In our classification, all bristle-shaped sensilla with single narrow circular gaps around the bases were included in a single chaetic sensillar type, except for chaetic-A sensilla (Table 1, Fig. 2a). Thus, various subtypes of chaetic sensilla fell into this category irrespective of whether a terminal pore was present and regardless of the length, thickness, and side-wall sculpture. These sensilla were distributed all around the flagellar segments (Fig. 2a). A narrow gap around the base of the sensillum on the antennal surface was observed in most of the sensilla (Fig. 2a).

Numbers and distributions of different types of sensilla on flagellar segments

Maps of all sensilla types were generated from the montages of scanning electron micrographs for the flagellum in all colony members. These revealed sex-specific sensilla in the ant: basiconic sensilla were observed in workers and unmated queens, but not in males (Table 1, Fig. 5). Other types of sensilla were observed in all ants (Table 1, Fig. 5).

The numbers of all types of sensilla were counted for each of the flagellar segments in all ants (Table 1). Total numbers of sensilla on the flagellum were about 7500 in workers, about 9000 in unmated queens, and about 6000 in males (Table 1). Sensillar maps for workers are schematically presented based on the montages of scanning electron micrographs (see Electronic Supplementary Material, Figs. S1, S2, S3, S4, S5, S6, S7, S8, S9, S10). The largest number of sensilla was on the distalmost flagellar segments

Fig. 3 Structures of basiconic and chaetic-A sensilla. Scanning electron micrographs (a–d) and confocal laser scanning micrographs (e, f). **a** A basiconic sensillum (*ba*) is a thick thumb-shaped hair about 20 μm in length with a narrow gap (*large arrowhead*) between the base of the sensillum and antennal surface. A chaetic-A sensillum (*ch-A*) is a bristle-shaped hair about 20 μm long with a narrow gap (*small arrowhead*) between the base of the sensillum and antennal surface. Chaetic-A sensilla are tilted considerably toward the proximal direction of the antenna compared with the other sensilla. Paired basiconic and chaetic-A sensilla are frequently observed proximally and distally, respectively. Bar 5 μm . **b** Numerous perforations appear on the outer surface of a basiconic sensillum, opposite the antennal trunk. Bar 0.5 μm . **c** Several longitudinal striae occur along the cuticular surface of the chaetic-A sensillum. Bar 0.5 μm . **d** A terminal pore at the tip of the chaetic-A sensillum. Bar 0.2 μm . **e** A basiconic sensillum with more than 130 somata (*arrow*) of sensory neurons with dendrites (*arrowheads*) innervating the cuticular apparatus of the sensillum. *Inset*: Same sensillum (*ba*) at a different depth. Bar 10 μm . **f** Four somata (*arrow*) of sensory neurons in a chaetic-A sensillum extend dendrites (*arrowheads*) that innervate the cuticular apparatus of the sensillum. *Inset*: Same sensillum (*ch-A*) at a different depth. About 70% of chaetic-A (*ch-A*) and basiconic (*ba*) sensilla are located close to each other distally and proximally, respectively. Bar 10 μm

in workers and unmated queens or on the second segments in males (Table 1, Figs. 6, 7, 8). The second largest numbers of sensilla were on the second flagellar segments in workers and unmated queens and on the 11th segments in males (Table 1). In the middle to proximal segments, all sensilla except for chaetic sensilla showed sparse distributions (Table 1, Figs. 6, 7, 8). We assigned ampullaceal or coeloconic sensilla into groups depending on the diameters of their apertures as seen in scanning electron micrographs (Figs. 2a, 5d–f). In confocal observations of the flagellar lumina with retrogradely stained neurons, the numbers of these sensilla corresponded closely to those seen in scanning electron-microscope observations (Table 1, Figs. 2, 5, 6, 7, 8). Coelocapitular, coeloconic, and ampullaceal sensilla were closely located to each other and made clusters, which formed an orderly array around the circumference of the flagellum from the medial side on proximal segments to the lateral side on distal segments in all ants (see Electronic Supplementary Material, Figs. S1, S5, S6, S7, S8).

Coelocapitular sensilla These numbered, in all antennal flagella, about 19 in workers, 22 in unmated queens, and 15 in males, or about 0.3% of the total sensilla (Table 1). More than 50% of the coelocapitular sensilla were distributed on the first and the most distal flagellar segments in all ants (Table 1, Figs. 5, 6, 7, 8). The coelocapitular sensilla were clustered on the apical tips of the lateral to ventral sides of the distal most flagellar segments in all ants (Figs. 5, 6, 7, 8). In the second to ninth flagellar segments in workers and unmated queens or in the tenth in males, the sensilla were clustered on the ventral to medial sides of the segments (Figs. 1, 6, 7, 8). The sensilla were closely spaced, clustered

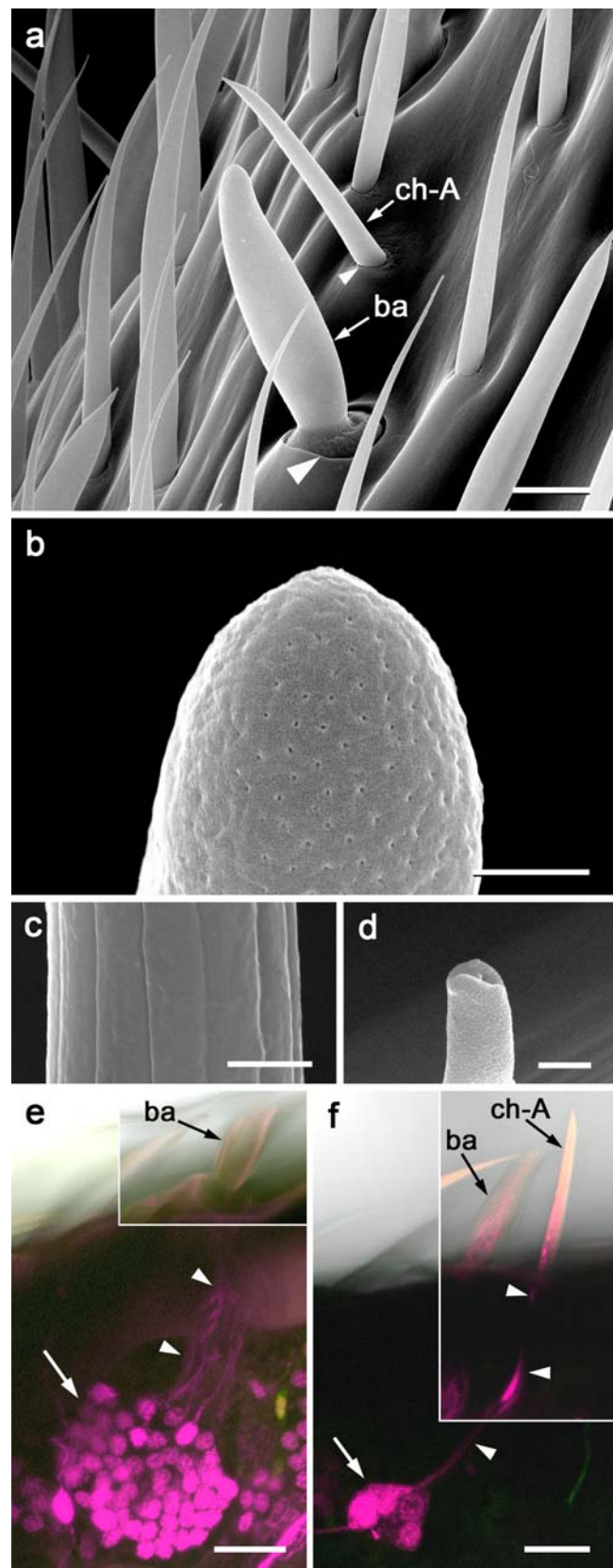


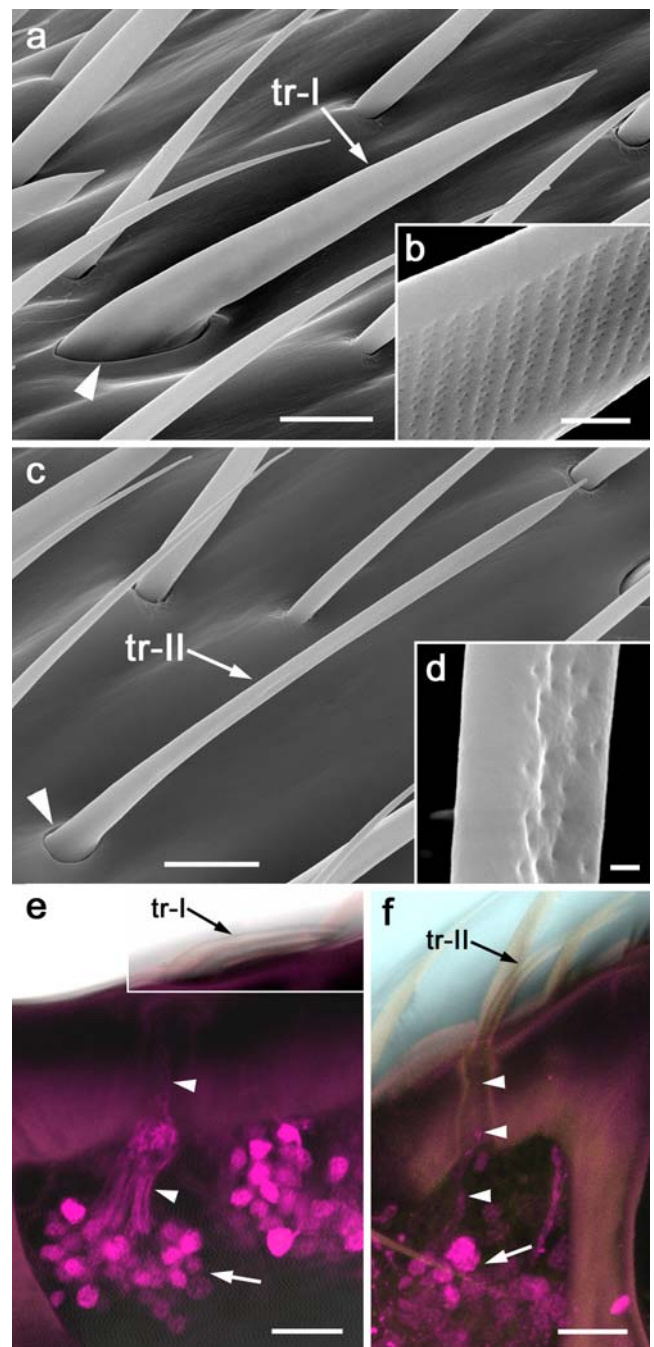
Fig. 4 Structures of trichoid-I and -II sensilla. Scanning electron micrographs (**a–d**) and confocal micrographs of the sensilla (**e, f**). **a** Trichoid-I sensillum (*tr-I*) with a thick sword-shaped and extremely inclined sensillum about 30 μm in length with a narrow gap around the oval-shaped base of the sensillum (arrowhead). Bar 5 μm . **b** Numerous perforations form the thin furrows aligned obliquely along both sides of the cuticular surface of the sensillum. Bar 0.5 μm . **c** Trichoid-II sensillum (*tr-II*) with a long thin curved hair-like shape more than 30 μm in length with a narrow gap (arrowhead) around the base of the sensillum. Bar 5 μm . **d** Many pores are distributed randomly on both sides of the sensillum. Bar 0.2 μm . **e** Trichoid-I sensillum with more than 50 sensory neurons having somata (arrow) and dendrites (arrowheads) innervating the cuticular apparatus of the sensillum. Inset Outer cuticular structure of the same sensillum (*tr-I*) at a different depth. Bar 10 μm . **f** Trichoid-II sensillum (*tr-II*) with nine sensory neurons having somata (arrow) and dendrites (arrowheads) innervating the cuticular apparatus of the sensillum. Bar 10 μm

on the ventral to medial sides, and densely on the medial sides of the first flagellar segments in all ants (Figs. 6, 7, 8).

Coeloconic sensilla These numbered about 27 in workers, 28 in unmated queens, and 19 in males, or about 0.3% of the total sensilla (Table 1). About half of the coeloconic sensilla were distributed in the most distal flagellar segments of workers and unmated queens (Table 1, Figs. 5, 6, 7), with relatively few sensilla on these segments in males (Table 1, Figs. 5, 8). The coeloconic sensilla were also closely spaced and clustered on the medial side of the first flagellar segments in all ants (Figs. 5, 6, 7, 8). In the second to ninth flagellar segments in workers and unmated queens or in the tenth segment in males, the sensilla were clustered on the ventral to lateral sides of the segments (Figs. 6, 7, 8). In the most distal flagellar segment, they were clustered on the lateral side of the segment in all ants (Figs. 5, 6, 7, 8).

Ampullaceal sensilla The number of these, in all antennal flagella, was about 20 in workers, 22 in unmated queens, and 14 in males, or about 0.2% of the total sensilla (Table 1). About half were located in the most distal flagellar segments in workers and unmated queens (Table 1, Figs. 5, 6, 7, 8). The ampullaceal sensilla were also clustered on the medial side of the first flagellar segments in all ants (Figs. 5, 6, 7, 8). In the second to ninth flagellar segments in workers and unmated queens or in the tenth segment in males, the sensilla were clustered on the ventral side of the segments (Figs. 5, 6, 7, 8). In the most distal flagellar segments, the sensilla were clustered on the lateral side of the segments in all ants (Figs. 2, 5, 6, 7, 8).

Basiconic sensilla These numbered about 180 in workers and 200 in unmated queens, or about 2% of total sensilla (Table 1). About 30%–40% of the basiconic sensilla were distributed on the most distal flagellar segments (Table 1, Figs. 5, 6, 7). The basiconic sensilla were not clustered but were most densely distributed on the medial sides, with



more on the dorsal sides and fewer on the lateral and ventral sides of flagellar segments in workers and unmated queens (Figs. 5a, b, 6, 7). No basiconic sensilla were found in the antennae of males (Figs. 5c, 8). In workers and unmated queens, the largest number of sensilla was on the most distal segments and gradually decreased toward the proximal segments (Table 1, Figs. 5, 6, 7, 8).

Chaetic-A sensilla The total numbers of these, in all antennal flagella, were about 180 in workers, 200 in unmated queens, and 130 in males, or about 2% of the

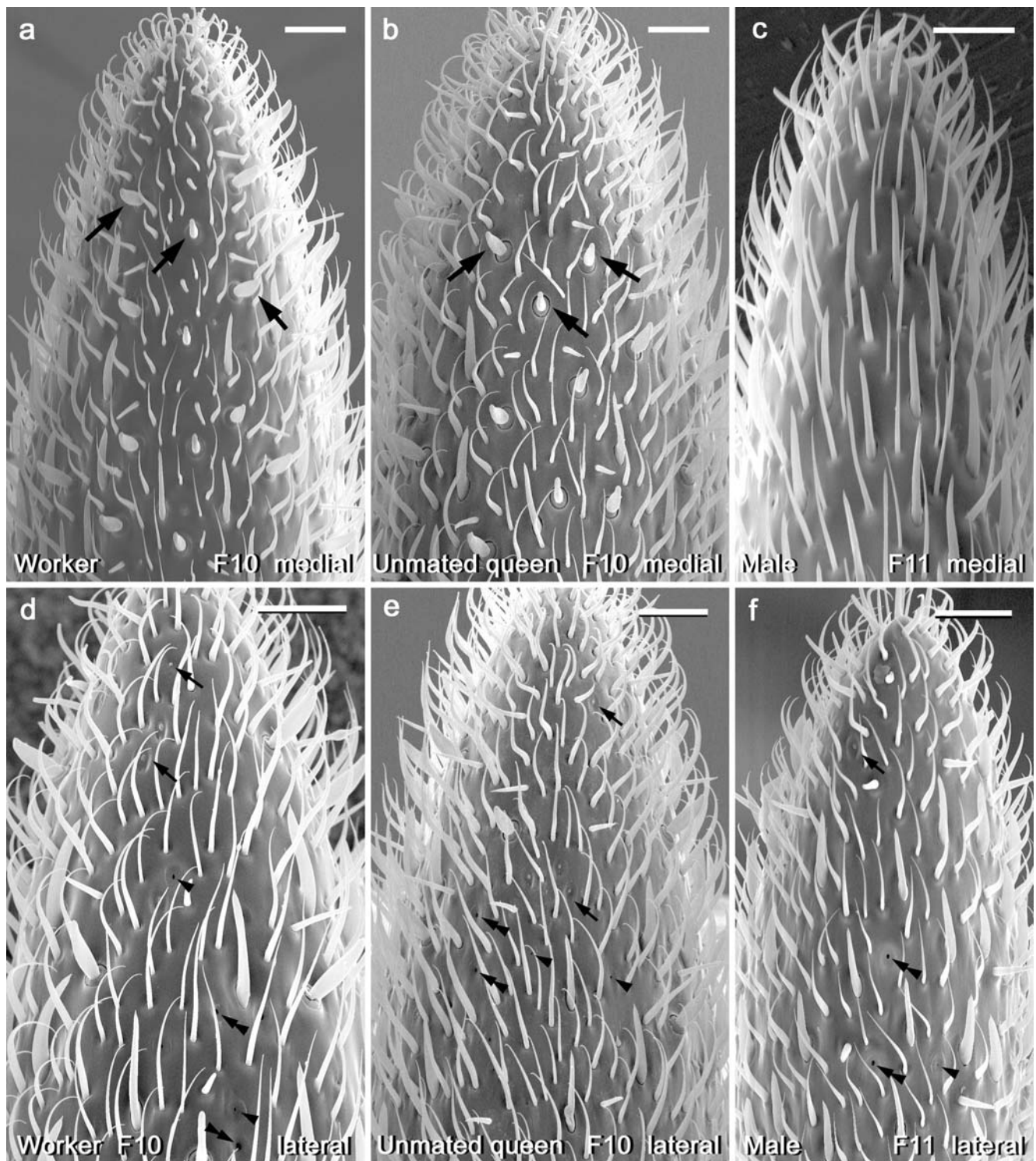


Fig. 5 Scanning electron micrographs of the most distal flagellar segments of a worker (**a**, **d**), unmated queen (**b**, **e**), and male (**c**, **f**) ant. **a–c** Medial sides of the segments. Female-specific basiconic sensilla (*arrows*) are densely distributed in a worker (**a**) and an unmated queen (**b**) but not in a male (**c**). **d–f** Lateral sides of the segments in all

colony members have clusters of coelocapitular (*arrows*), coeloconic (*double-arrowheads*), and ampullaceous sensilla (*single-arrowheads*). Basiconic sensilla are sparsely distributed on the lateral sides of the distalmost flagellar segments in a worker (**d**) and an unmated queen (**e**). Bar 20 μ m

Fig. 6 Representation of sensillar distributions on the three flagellar segments in the left antenna of a worker. **a–d** Tenth-most distal flagellar segment. **e–h** Fifth segment. **i–l** First proximal most segment. The sensillar mapping of four aspects of each flagellar segment are shown: dorsal (**a, e, i**), medial (**b, f, j**), ventral (**c, g, k**), and lateral sides (**d, h, l**). All types of sensilla are most densely distributed on the tenth segment (**a–d**). Most types of sensilla other than chaetic sensilla gradually decrease in number from the distal to proximal segments (**e–l**). Only coelocapitular sensilla are densely distributed on the ventro-lateral side of the first proximal most flagellar segment (**j, k**). Coelocapitular, coeloconic, and ampullaceous sensilla are clustered on each segment, and these clusters are lined on the segments from the lateral side of distal segments (**a**) via the ventral side of middle segments (**f**) to the ventro-medial side of proximal segments (**k**). Basiconic sensilla are most densely distributed on the dorso-medial side of the tenth segment (**c**) but are very sparsely distributed on proximal segments (**i–l**). Since each map is shown schematically, based on montages of scanning electron micrographs, the sizes of segments are somewhat different from their real lengths in this and subsequent figures. *Bottom* Explanation of sensilla types. Bar 50 μ m

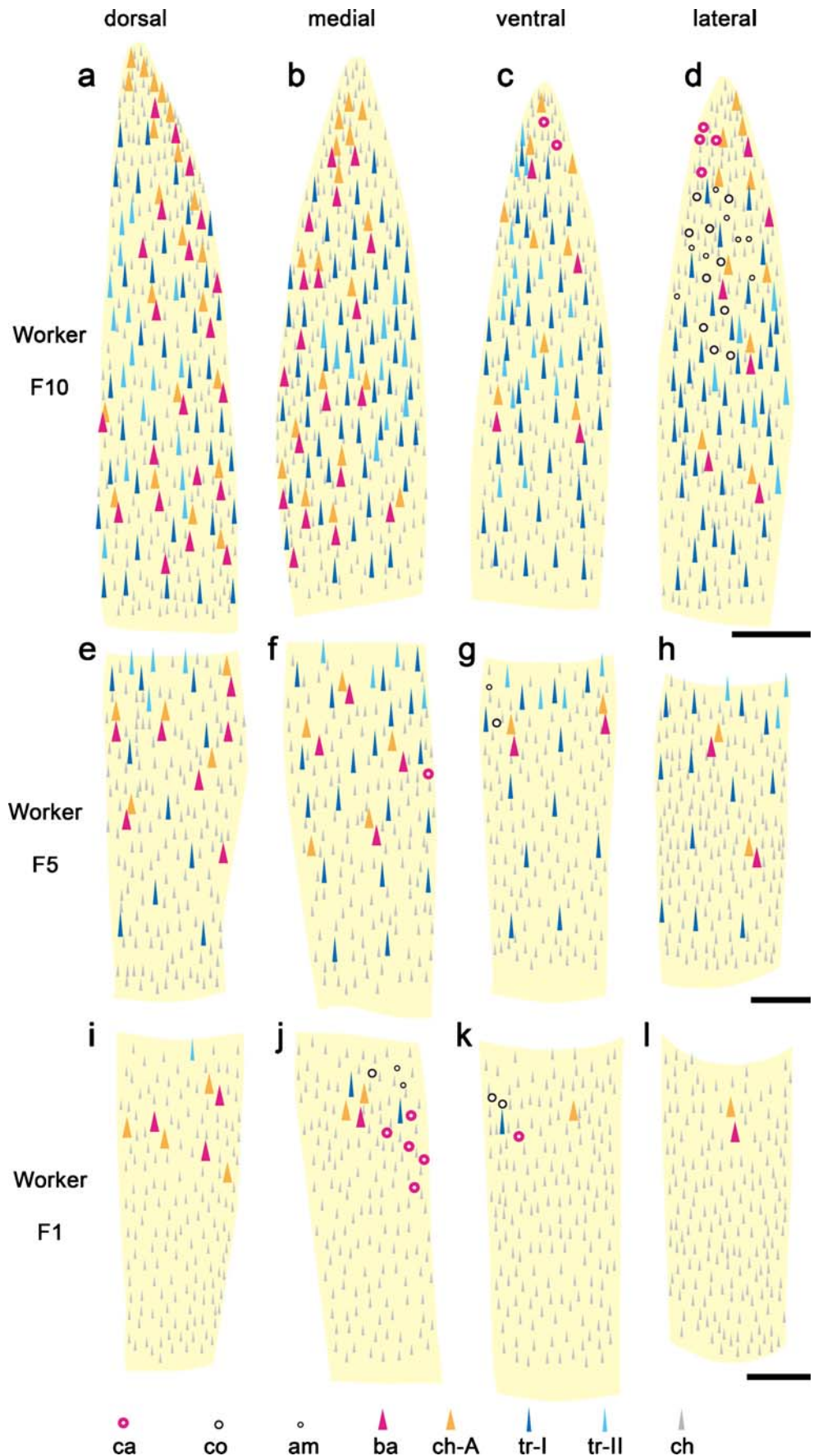


Fig. 7 Maps of sensillar distributions arranged as in Fig. 6, but for the three flagellar segments in the left antenna of an unmated queen. Sensillar maps from four aspects of each flagellar segment are shown: dorsal (a, e, i), medial (b, f, j), ventral (c, g, k) and lateral (d, h, l). All types of sensilla are most densely distributed on the tenth distal most flagellar segment (a–d). Most types of sensilla other than chaetic sensilla gradually decrease in number from distal to proximal (e–l). Only coelocapitular sensilla are densely distributed on the medial side of the first proximal most flagellar segment (k). Coelocapitular, coeloconic, and ampullaceous sensilla are clustered on each segment, and these clusters are aligned from the ventro-lateral side of distal segments (a) via the ventral side of middle segments (f) to the ventro-medial side of proximal segments (k). Basiconic sensilla are most densely distributed on the medial side of the tenth segment (c) but are distributed sparsely on proximal segments (i–l). *Bottom* Explanation of sensilla types. *Bar* 50 μ m

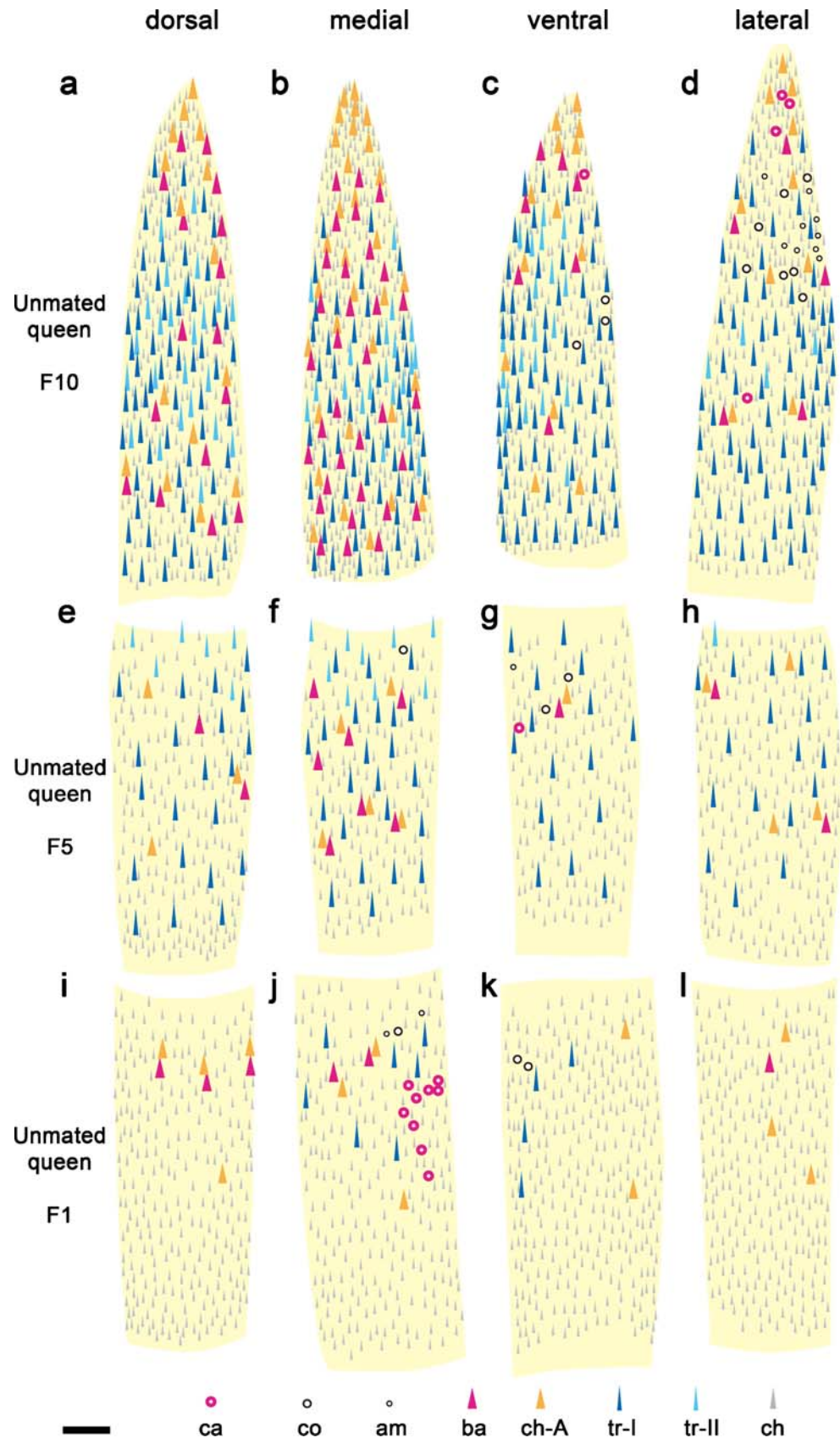
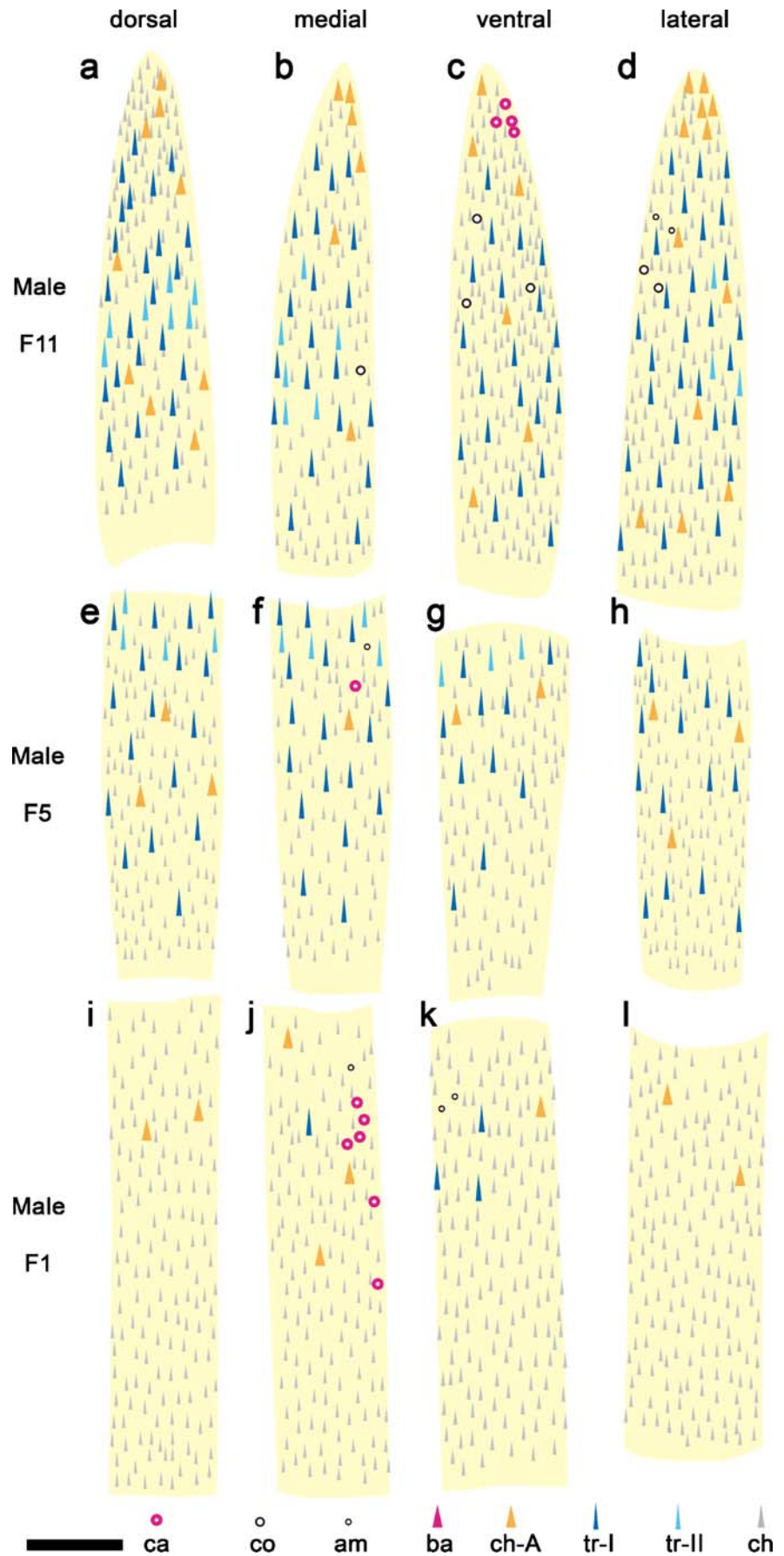


Fig. 8 Maps of sensillar distributions arranged as in Fig. 6, but for the three flagellar segments in the left antenna of a male. The 11th flagellar segment (a–d) is most distal, the fifth (e–h) is middle, and the first (i–l) is most proximal. All types of sensilla are most densely distributed on the 11th distal most flagellar segment (a–d). Most types of sensilla other than chaetic sensilla gradually decrease in number from distal to proximal segments (e–l). Only coelocapitular sensilla are densely distributed on the ventral side of the first proximal-most flagellar segment (k). Coelocapitular, coeloconic, and ampullaceous sensilla are clustered on each segment, and these clusters are lined on the segments from the ventro-lateral side of distal segments (a) via the medial side of middle segments (f) to the medial side of proximal segments (k). Female-specific basiconic sensilla are completely lacking on all segments in male flagella. *Bottom* Explanaton of sensilla types. *Bar* 50 μ m



total sensilla (Table 1). About 30% were distributed on the most distal flagellar segments in all ants (Table 1, Figs. 5, 6, 7, 8). About 70% of the chaetic-A sensilla on the flagellum were located just distal to the basiconic sensilla (Fig. 3). The chaetic-A sensilla were not clustered but were most densely distributed on the medial sides, with more on the dorsal sides and fewer on the lateral and ventral sides of the flagellar segments (Figs. 5, 6, 7, 8). Most were on the distal most segment and gradually decreased in the proximal segments (Table 1, Figs. 5, 6, 7, 8). The conspicuous feature of this sensillum type, i.e., its tilting proximally on the antennae, enabled us to use it as a conspicuous landmark to map other sensilla.

Trichoid-I sensilla These numbered, in all antennal flagella, about 660 in workers, 950 in unmated queens, and 710 in males, or about 10% of the total sensilla (Table 1). Although the numbers of other types of sensilla in males were lower than those in workers, the trichoid-I sensilla in males were more numerous (Table 1, Figs. 5, 6, 7). About a quarter were distributed on the most distal flagellar segments in workers and unmated queens (Table 1, Figs. 5, 6, 7). Almost the same numbers of sensilla were distributed on segments 8–11 in males (Table 1, Figs. 5, 8). Most sensilla were on the distal-most segments, gradually decreasing in the proximal segments in all ants (Table 1, Figs. 5, 6, 7, 8). They were not clustered but were uniformly distributed around each flagellar segment (Figs. 5, 6, 7, 8).

Trichoid-II sensilla These totaled about 220 in workers, 265 in unmated queens, and 120 in males, or about 3% of the total sensilla (Table 1). About a quarter were distributed on the most distal flagellar segments in workers and unmated queens (Table 1, Figs. 5, 6). The trichoid-II sensilla were not clustered but were uniformly distributed on the dorsal to ventral via medial sides of flagellar segments and were sparsely distributed on the lateral sides of the segments in all ants (Figs. 5, 6, 7, 8). The largest number of sensilla was found on the most distal segments, gradually decreasing in the more proximal segments in all ants (Figs. 5, 6, 7, 8). The sensilla were distributed on the middle regions of the most distal segments and at the distal margin of the segments on other segments (Figs. 5, 6, 7, 8).

Chaetic sensilla This sensillum type was most numerous, totaling about 6000 in workers, over 7000 in unmated queens, and about 5000 in males, or about 80% of the total sensilla (Table 1). About 15% of the sensilla were distributed on the most distal flagellar segments in workers and unmated queens (Table 1, Figs. 5, 6, 7), where they were densely distributed, gradually decreasing in density toward the middle segments, but increasing again toward the proximal segments in all ants (Table 1, Figs. 5, 6, 7, 8).

The chaetic sensilla other than chaetic-A sensilla were not clustered but were distributed uniformly around each flagellar segment in all ants.

Not only the numbers, but also their distribution patterns for the respective sensillum types in each flagellar segment were different between females and males. Little difference was observed in the numbers of sensilla between individuals in the respective colony members, but the distribution patterns were almost the same (Table 1, Figs. 6, 7, and 8).

Discussion

Sexual dimorphism and sexual specificity in antennae of the ant

The organization of antennal segments in the ant is similar to that in another well-known social hymenoptera, the honeybee *Apis mellifera*: a scape, a pedicel, ten flagellar segments in workers and eleven flagellar segments in drones (Esslen and Kaissling 1976). Unmated queens also have ten flagellar segments and the largest antennae in the ant. The differences in the numbers of flagellar segments result in sexually dimorphic antennae in the ant and honeybee. However, a great difference is found in the male antennae of the two species. The antennae of honeybee drones are larger than those of workers, whereas the antennae of ant males are thin but almost the same length as those of workers. These antennal sizes correlate with the numbers of antennal sensilla: about 9000 flagellar sensilla in unmated queens, about 7500 flagellar sensilla in workers, and about 6000 flagellar sensilla in males. The total numbers of flagellar sensilla in the ant are the largest among Formicidae species reported so far (Dumpe 1972b; Hashimoto 1990; Renthal et al. 2003). In honeybees, the total numbers of flagellar sensilla are about 20000 in a drone and 6000 in a worker (Esslen and Kaissling 1976). No such data exist for queens, but sexually dimorphic antennae are also observed in honeybees.

The sexual dimorphism is also evident in sex-pheromone-receptive sensilla, which are generally much more abundant in males (pheromone receivers) than in females (pheromone emitters; Schaller 1978; Boeckh and Tolbert 1993; Hansson 1999; Gemenio and Schal 2004). Female sex-pheromone-receptive sensilla have been identified in the antennae of moths and cockroaches (Boeckh and Tolbert 1993; Hansson 1999). In moth species, the extraordinarily different numbers of sensilla between the sexes are associated with the obviously different appearances of the antennae (Keil 1999). In cockroach species, although the antennae in both sexes appear to be almost the same, males have indeed many more sensilla than do

females (Schaller 1978; Boeckh and Tolbert 1993; Hansson 1999; Gemenio and Schal 2004). The numerous sex-pheromone-receptive neurons in insect antennae also innervate sexually dimorphic antennal lobes, with the presence of a macroglomerular complex in males of moths or cockroaches (Boeckh and Tolbert 1993; Hansson 1999). Sensory neurons responding to the queen pheromone component are found in pore plate (placoid) sensilla of the honeybee drone (Kaissling and Renner 1968), which also has an extraordinarily large macroglomerular complex in the antennal lobe (Arnold et al. 1985). The numbers of pore plate sensilla in honeybees are much larger in drones than in workers (Esslen and Kaissling 1976), and hence, this type of sensillum might be a candidate for the female sex-pheromone-receptive sensillum in honeybees. Although the hair-like trichoid curvata sensilla and the pore plate sensilla in Hymenoptera have different appearances, their morphological and physiological profiles are similar: both types of sensilla have the cuticular apparatus of many lined perforations, an encircling ledge, and delicate cuticular ledges reinforcing the perforated plate and respond to a wide range of organic compounds including various pheromones (Dumpe 1972a; Walther 1979; Martini and Schmidt 1984; Martini 1986; Hashimoto 1990; Renthal et al. 2003). A macroglomerulus is also found in the antennal lobe of *Camponotus* males (Nishikawa et al. 2008), which have larger numbers of trichoid-I sensilla than workers. This suggests that female sex-pheromone-receptive neurons are also located in or among the trichoid-I sensilla of the ant.

On the other hand, basiconic sensilla are completely lacking in males of the ant, an absence previously reported in fire ants (Renthal et al. 2003). The basiconic sensilla in honeybees are also female-specific and completely lacking in drones (Esslen and Kaissling 1976; Nishino et al. 2009). Basiconic sensilla are thus female-specific and morphologically different from the candidate for female sex-pheromone-receptive sensilla as described above. The absence of female-specific sensilla in males might also be associated with the sex-specific antennae in the ant and with the remarkable decrease in the numbers of antennal lobe glomeruli in males (Nishikawa et al. 2008). Since over 130 sensory neurons have been newly found in the basiconic sensillum in this study, at least 130 glomeruli might be missing in the male antennal lobe, if individual sensory neurons express distinct olfactory receptors (Couto et al. 2005).

Morphological and functional properties of antennal sensilla

Antennal sensillar types in Formicidae have been morphologically classified based on the appearances of their

sensilla (Dumpe 1972b; Hashimoto 1990; Renthal et al. 2003). Seven types of flagellar sensilla are distinguished in *Lasius fuliginosus*: chaetic, trichoid, trichoid curvata, basiconic, coeloconic, ampullaceous, and campaniform sensilla (Dumpe 1972b). In this study of *Camponotus japonicus*, flagellar sensilla have also been classified into seven types: ampullaceous, coeloconic, coelocapitular, basiconic, trichoid-I, trichoid-II, and chaetic sensilla. We have not observed campaniform sensilla such as those described previously (Dumpe 1972b), but we have found coelocapitular sensilla on the flagella of the ant. Based on cuticular wall structures of these antennal sensilla, we speculate that the functional properties of sensilla are as follows: sensilla with wall pores are olfactory, sensilla with tip pores are gustatory, and sensilla without pores are either mechanosensitive or thermo/hygrosensitive (Altner and Prillinger 1980; Keil 1999). Wall structures of sensilla observed by scanning electron microscopy also suggest, to some extent, the functional properties of the sensilla. We can thus now propose the functional properties of the seven types of sensilla reported in this study.

The coelocapitular sensillum newly found in this investigation of the ant probably corresponds to the coelocapitular sensillum identified as a hygro- and thermo-receptive sensillum in the honeybee (Yokohari et al. 1982). The appearance of the sensillum obviously differs from that of campaniform sensilla in the pedicel of the ant. Since the cuticular structure of the unperforated mushroom-like protrusion of the sensillum in the ant is similar to that in the honeybee, the sensillum in the ant might participate in hygro- and thermo-reception. The sensillum in the honeybee contains a moist-receptive neuron, a dry-receptive neuron, and a cold-receptive neuron (Yokohari et al. 1982). According to our confocal observations, the sensillum has three sensory neurons, which may be moist-, dry-, and cold-receptive neurons, as in honeybees. In addition, the campaniform sensilla in the flagellum of the ant *Lasius fuliginosus* (Dumpe 1972b) and the honeybee *Apis mellifera* (Esslen and Kaissling 1976) might correspond to the coelocapitular sensilla.

The coeloconic sensillum, which has a cone-shaped peg in a pit in the flagellar lumen and has three sensory neurons, seems to be morphologically the same as those in other ant species (Dumpe 1972b; Hashimoto 1990; Renthal et al. 2003; Ruchty et al. 2009). In the red imported fire ant, the coeloconic sensillum appears to have a chemoreceptor function because of its tip pore (Renthal et al. 2003). An electrophysiological and ultrastructural study in the leaf-cutting ant has shown that the sensilla are thermo-sensitive and have a grooved and double-walled peg without pores on the cuticular surface. The fine structure of coeloconic sensilla in leaf-cutting ants is similar to that of thermo- and hygroreceptive sensilla in the honeybee

(Yokohari 1983). Coeloconic types of thermo- and hygro-receptive sensilla have also been reported in locusts, honeybees, crickets, and fruit flies (Yokohari 1999; Yao et al. 2005). Cockroach antennae possess two types of thermo-receptive sensilla: one is a thermo- and hygro-receptive sensillum, a so-called capitular or no-pored sensillum, and the other is an olfactory and thermoreceptive sensillum with wall pores and a grooved and double-walled cuticular apparatus (Schafer and Sanchez 1973; Yokohari 1976; Schaller 1978; Altner and Prillinger 1980; Schaller 1982; Nishikawa et al. 1992). However, the function of the coeloconic sensillum in the ant is still unknown. Electrophysiological and fine structural investigations are clearly needed.

The ampullaceous sensillum, which has a long peg in a pit in the flagellar lumen and a single sensory neuron, is probably the same type as in other ant species (Dumpeert 1972b; Hashimoto 1990; Kleineidam et al. 2000). In leaf-cutting ants, an electrophysiological and ultrastructural study has shown that the sensilla are CO₂-sensitive and have many dendritic branches in a grooved peg with pores (Kleineidam et al. 2000). The ampullaceous sensilla in the honeybee are also CO₂-sensitive (Lacher 1964). Although the sensilla have not been examined physiologically in this study, those in ants may be CO₂-sensitive, as in the leaf-cutting ant (Kleineidam et al. 2000).

Each of the ampullaceous, coeloconic, and coelocapitular sensilla has a pit subjacent to the cuticular surface of the antenna. Similar structures have been shown in other ant species (Dumpeert 1972b).

The basiconic sensillum of *Camponotus* corresponds to the basiconic sensillum previously reported in this species (Ozaki et al. 2005) and in other ant species (Dumpeert 1972b; Renthal et al. 2003). A morphological and physiological study has shown that the sensilla are cuticular-hydrocarbon-sensitive and have about 200 sensory neurons (Ozaki et al. 2005). Our confocal observations show that the sensilla contain over 130 sensory neurons. Such a large number of sensory neurons may correspond to reception of many kinds of cuticular hydrocarbons that have to be resolved for nestmate discrimination in the ant (Mintzer 1982; Hölldobler and Wilson 1990; Hara 2003; Ozaki et al. 2005; Brandstaetter et al. 2008). The numerous pores distributed on the wall of basiconic sensilla indicate their olfactory function (Altner and Prillinger 1980; Keil 1999). However, in female parasitic wasps, gustatory features in such sensilla have been reported for host recognition (Isidoro et al. 2001). Intriguingly, the pores in the basiconic sensillum of the red imported fire ant are distributed not all around a sensillum, but only on one side of a sensillum (Renthal et al. 2003). This side seems to make easy contact with objects in the environment. Basiconic sensilla in the ant also have gustatory features used for nestmate recognition

(Ozaki et al. 2005). Therefore, basiconic sensilla might have capabilities for both contact and volatile chemoreception. The sensilla may, as a result, play a significant role related to female-specific tasks in the social lives of the ant.

The trichoid-I sensillum in the ant, which is extremely inclined and with numerous perforations on the cuticular apparatus, is the same type as a trichoid curvata sensillum in other ant species (Dumpeert 1972b; Hashimoto 1990; Renthal et al. 2003). The hair-like trichoid curvata sensilla and the plate-like pore plate sensilla in other Hymenoptera may be regarded as homologs (Martini and Schmidt 1984; Martini 1986; Hashimoto 1990; Renthal et al. 2003). The sensory neurons in a pore plate sensillum number 12–22 in honeybees (Nishino et al. 2009), 9–23 in wasps (Martini and Schmidt 1984; Martini 1986), and 20–30 in the trichoid curvata sensillum of ants (Dumpeert 1972a). More than 50 sensory neurons have been counted here in a trichoid-I sensillum. Such a large number of sensory neurons suggest that these sensilla have a wide range of olfactory spectra. Electrophysiological analysis in ants has revealed that trichoid curvata sensilla respond to stimulation with alarm substances, by which the sensory neurons can be divided into 10 reaction groups, with almost no overlap (Dumpeert 1972a). Although the outer cuticular structures of all trichoid curvata sensilla in ants are exactly the same and are numerous distributed in flagella, the combinations of olfactory neurons are probably different and, as for the pore plate sensilla in honeybees (Dumpeert 1972a; Getz and Akers 1993; Kelber et al. 2006), divided into different groups. The sensilla in the ant may also have alarm-substance-receptive neurons.

The trichoid-II sensillum found in this study is probably the same type as the trichoid sensillum in other ant species (Dumpeert 1972b). Since no physiological studies have been performed on this sensillum, its function is still unknown. However, it seems to be an olfactory sensillum because of the presence of many pores on the cuticular surface of the sensillum.

The chaetic-A sensillum in *Camponotus* corresponds to one of the chaetic sensilla in other ant species (Dumpeert 1972b; Hashimoto 1990). The sensillum has a terminal pore at the tip and lacks pores in striae on the cuticular surface, suggesting that it has a contact-chemosensory function (Altner and Prillinger 1980). Furthermore, the sensilla point in the opposite direction to the other sensilla, facing the distal end of the antenna, so that the terminal tips of the sensilla could make contact with external objects more easily than other sensilla.

Other types of chaetic sensilla in this study have been roughly divided into two types with or without a terminal pore. Those with a terminal pore might be contact chemoreceptive sensilla, whereas those without might be mechanoreceptive sensilla. Detailed observations of termi-

nal pores have not been carried out in this study. Consequently, the number and distribution of the sensilla includes both types.

A campaniform sensillum has been reported to contain a mechanoreceptive neuron in ants (Dumpe 1972b). Usually, campaniform sensilla or marginal sensilla in insect antennae are not distributed in the middle regions of flagellar segments but are restricted to the distal and marginal regions of the pedicel or flagellar segments (Schafer and Sanchez 1973; Toh 1977). A distinct structure of campaniform sensilla has been observed around the distal margin of the pedicel of antennae in all colony members in this study, but campaniform-sensillum-like structures have not been seen in the flagellar segments of the ant. Hence, their number and distribution on flagellar segments are not reported in this study.

Characteristic distribution patterns of flagellar sensilla

The locations of the various types of sensilla on antennae have been mapped in the different regions of glomerular clusters in the antennal lobe (Couto et al. 2005; Nishino et al. 2009). In the honeybee antenna, coelocapitular, coeloconic, and ampullaceous sensilla cluster and localize to the distal region of the anterior and posterior sides of each flagellar segment (Esslen and Kaissling 1976; Nishino et al. 2009). Axons of their sensory neurons exclusively converge upon the ventral antennal nerve and terminate in particular glomerular groups in the antennal lobe (Nishino et al. 2009). In the antenna of the ant, as in that of the honeybee, coelocapitular, coeloconic, and ampullaceous sensilla also form clusters and an orderly array around the circumference of the flagellum. Differential retrograde dye-filling of antennal nerves in this study has revealed that these sensory neurons are exclusively stained with the same color as the ventral nerve. Thus, axons of these sensory neurons exclusively belong to the ventral antennal nerve, and thus, a topographical relationship between antennal sensilla and antennal lobe glomeruli might exist in the ant, as in the honeybee.

The coelocapitular, coeloconic, and ampullaceous sensilla are distributed on most flagellar segments in the ant, whereas in the honeybee, they are not distributed on the first and second segments (Esslen and Kaissling 1976; Nishino et al. 2009). The coelocapitular sensilla are most densely distributed on the first flagellar segments in *Camponotus* members. Crickets also have the coeloconic type of hygro- and thermoreceptive sensilla distributed around the circumference of the flagellum (Itoh et al. 1984; Nishikawa et al. 1985). In all these insects, therefore, the sensilla seem to have biased distributions that are species-specific. In cockroaches, the capitular sensilla, which are probably functionally identical to coelocapitular or coelo-

conic sensilla in ants, honeybees, or crickets, are distributed along the longitudinal lines of the flagella (Tominaga and Yokohari 1982; Nishikawa et al. 1992), but other types of sensilla are distributed evenly around the flagella. Moreover, most of the sensilla in cockroaches are densely distributed on the middle segments of the flagellum (Schaller 1978). The particular patterns of sensillar distribution cannot yet be related to function but probably reflect the particular behavioral patterns or habitats of the individual insects.

The female-specific basiconic sensilla in the ant do not form clusters but show a biased distribution on flagellar segments in workers and unmated queens. The region of densely distributed sensilla on the flagellum seems well placed to make contact with external objects. Such a distribution pattern has also been suggested to serve a contact-chemosensory function. The numbers of basiconic sensilla are almost the same as those of chaetic-A sensilla on the flagellum, and both are densely distributed on the medial side of the most distal segment. About 70% of basiconic sensilla are paired with chaetic-A sensilla, which have been used as a landmark sensillum for the sensillum maps in this study.

Trichoid-I sensilla are the second most numerous among the seven types mapped here and are distributed uniformly around the circumference of each flagellar segment, although more densely on distal segments and less densely on proximal segments. In the honeybee, the worker flagellum has a pore plate sensillum-free zone, whereas the same zone of the drone has a sparse distribution of pore plate sensilla (Esslen and Kaissling 1976; Nishino et al. 2009). Such a sensillum-free zone has not been observed for trichoid-I sensilla in any of our ant colony members. Although males have thin and short antennal flagella compared with those in ant workers, they nevertheless have a relatively large number of trichoid-I sensilla.

The characteristics of sensillum distribution patterns may be reflected in the topographical projection of sensory neurons to the antennal lobes and may thus influence the somatotopic or chemotopic organizations of glomeruli. Thus, the sensillar maps presented here provide essential information for the functional analysis of the antennal sensory system. This is the first report of the comprehensive mapping of antennal flagellar sensilla in all colony members of the ant. It should provide a good basis for studying the neural mechanisms of chemical communication in these social insects.

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