Morphology of the exocrine glands associated with the maxillolabial complex in the ant *Camponotus japonicus* Mayr, 1866 (Hymenoptera: Formicidae)

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Abstract

In this study, we investigated the morphological characteristics of three glands that are associated with the labium and the maxillae in the female castes as well as males of the ant *Camponotus japonicus* Mayr, 1866 by using serial semithin sections and scanning electron microscopy (SEM). We propose to name these glands as the postmentum base gland, the stipes base gland and the cardo base gland. The cardo base gland represents what was previously known as the maxillary gland, the two other glands are novel findings. They all belong to class-3 glands and have no reservoir for the storage of secretion. Their morphology is similar among the studied categories (major worker, minor worker, queen and male). The postmentum base gland is made up by 10-15 secretory units that open through the articulation membrane on both sides of the base of the labial postmentum with no significant differences among castes. The stipes base gland is formed by a cluster of secretory units that open at the articulation membrane between cardo and stipes. This gland is much larger in major workers (almost 140 cells) than in the other castes (approx. 50-60 cells). The cardo base gland opens through the membrane that connects the cardo and stipes to the head capsule. The number of cells of this gland is much larger in major workers and queens (approx. 190 resp. 150) than in minor workers and males (approx. 45 resp. 40). No information is yet available on the function of the glands.

Introduction

Uptaking food and perceiving the environment are among the most important things for any animal species. In insects, the head is of crucial importance to accomplish these functions, for which it is equipped with several exocrine glands that produce the active secretions. The three major cephalic glands in ants are the mandibular, prepharyngeal and pharyngeal glands (following the revised designation of gland names as recently recommended by Richter et al. 2019, 2020). Of these, the paired mandibular glands are commonly involved in the alarm-defence system (Hölldobler and Wilson 1990), the paired prepharyngeal glands produce digestive enzymes (Ayre 1967), and the glove-shaped pharyngeal gland plays a major role in nestmate recognition (D'Ettorre 2013). Besides these major glands, the head capsule in ants may also contain smaller exocrine structures. Examples include various intramandibular glands (Billen and Espadaler 2002 for *Pyramica membranifera*; Billen *et al.* 2013 for *Protanilla wallacei*; Billen and Delsinne 2014 for *Tatuidris tatusia*; Billen and Al-Khalifa 2016 for *Brachyponera sennaarensis*), an infrabuccal cavity gland (Eelen et al. 2004 for *Monomorium pharaonis*) or a stipes epithelial gland (Billen et al. 2013 for *Protanilla wallacei*). In this paper, we are focusing on the small glands associated with the maxillolabial complex. In several species of formicine ants, a number of detailed anatomical descriptions have been published by German authors half a century ago (Otto 1958 and Bausenwein 1960 for *Formica rufa*; Emmert 1966, 1968 and Kürschner 1971 for *Formica pratensis*; Beck 1972 for *Formica sanguinea* and *Polyergus rufescens*).

Camponotus japonicus is a widespread and open-field formicine ant species in east Asia that can be easily collected (Liu *et al.* 2001). It is important in forest as well as agricultural pest control (Wu and Wang, 1995). The species is monogynous and polymorphic, a mature nest usually including several hundreds to thousands of individuals comprising major and minor workers, a

queen and seasonally also alate gynes and males. The ants' body size is 0.6 to 1.3 cm, hence it can be dissected comfortably under the microscope and used for structural and functional studies. As we are interested in the general biology of this ant species, we here describe three pairs of smaller glands in the head capsule of the different female castes as well as males: the postmentum base gland, the stipes base gland and the cardo base gland. Of these, the postmentum base and stipes base glands are exocrine structures that have never been described before in any ant species.

Materials and methods

Ant material

Nests of *C. japonicus*, including major (head width: 3.50-3.85 mm, body length: 12.35-14.90 mm) and minor workers (head width: 1.25-1.60 mm, body length: 7.05-7.45 mm), queens and males, were collected from the edge of the fields and forests in Yangling, Shaanxi province, China. More than 40 individuals from 10 colonies have been used for this study. The ants were refrigerated at -20℃ for around 10 minutes to make them less active, and then dissected under a Leica EZ4HD microscope in Ringer's physiological solution.

Light microscopy

The heads of 5 males, 4 minor workers, 3 major workers and 2 queens were separated by making a transverse cut at the level of the compound eyes to allow the various chemicals used during tissue processing to enter the head capsule. The heads were fixed with 2.5% cold glutaraldehyde buffered in 0.1 M Na-cacodylate (pH 7.2) for 12 h, postfixed with 2% cold osmium tetroxide for 2 h, and dehydrated in a graded acetone series. After embedding in Araldite, the samples were sectioned in longitudinal or transverse direction with a Leica EM UC6 ultramicrotome at a thickness of 1 or 2 µm and stained with methylene blue (1%). Serial semithin sections were examined with an Olympus BX-43 system microscope connected to an Olympus DP25 camera.

As secretory cells of all these three glands are spherical cells, cell counts were made as follows: for each gland, we first determined cell diameter by measuring the size of the largest cells on sections. We subsequently counted the number of all secretory cells that appear on serial sections at an interval distance equivalent to this average cell diameter. As the interval was set as the average cell diameter, the cumulative number of cells represents the total number of cells of the gland, as each cell will be hit once during this interval distance. Longitudinal section images are shown with the anterior pointing to the left. To compare differences of glandular cell numbers among castes, a Mann-Whitney U-test followed by the Benjamini-Hochberg method using False Discovery Rate calculation was operated in R studio. We used the corrected p-values to indicate the significance of difference.

Scanning electron microscopy (SEM)

Of 24 individuals (6 individuals of each female caste and 6 males) the dorsal portion of the head capsule was removed to expose the internal tissues for SEM-observation. The head parts were fixed in 2.5% cold glutaraldehyde with 0.2 M Na-phosphate (pH 7.2) for 12 h, dehydrated in a graded ethanol series and transferred to isoamyl acetate. The samples were critical-point dried with an Emitech K850 instrument. A Hitachi E-1045 sputtering device was used for gold-coating. Observation and recording were done under a Hitachi S4800 scanning electron microscope.

Results

A combination of SEM and observation of serial semithin sections revealed the existence of three pairs of 'smaller glands' that are associated with the maxillolabial complex in major and minor workers, queens as well as males (Fig. 1). These are the postmentum base gland (indicated with large grey arrows on the figures), the stipes base gland (large white arrows), and the cardo base gland (large black arrows). They all belong to class-3 glands following the standard classification by Noirot and Quennedey (1974), in which glands are made up of a number of bicellular units, each unit comprising a secretory cell and its accompanying duct cell. The junction of both cells is formed by an end apparatus that represents a device for draining the secretory products from the secretory cell into the duct cell. None of the three glands has a reservoir for the storage of secretion as the duct cells open through the articulation membranes that connect the mouthparts.

Postmentum base gland

The postmentum base gland is formed by a glandular cluster at each side of the base of the labial postmentum (Fig. 2B-D). The duct cells of the secretory units open through the articulation membrane that connects the base of the postmentum with the stipes (Fig. 3A-C). The spherical secretory cells are largest in queens (14.48 ± 0.96 μm), followed by minor workers, males and major workers (Table 1). The difference of cell numbers among castes is not significant (P > 0.05) (Tables 1 and 2). This gland is by far the smallest of the glands described in this paper with only 10-15 secretory cells at each side, and on cross sections appears nearest to the body midline. On longitudinal sections, it has the most anterior position of the three glands. Because of the low number of cells and especially their hidden position near the base of the labium, the postmentum base gland could not be seen with SEM.

Stipes base gland

The paired stipes base gland at each side consists of a round cluster of spherical secretory cells (Fig. 2A, 4A). The slender duct cells open through the articulation membrane between the stipes and cardo (Fig. 4B-D). On cross as well as longitudinal sections, this gland occupies an intermediate position between the more mesad postmentum base gland and the more laterad cardo base gland, and is located mesially of muscle M17 (M. tentoriocardinalis, that connects the anterior tentorial arm and the mesal side of the cardo - see Richter et al. 2019) (Fig. 2B). Although we found no clear differences in the general anatomy and the cell size between categories, the number of cells of the stipes base gland of major workers is significantly higher than in the other categories (P < 0.01, Tables 1 and 2).

Cardo base gland

The compact cell cluster that makes up the cardo base gland at each side is located near the proximal part of the cardo (Fig. 2A, 5A). The ducts open through the articulation membrane that connects the cardo and stipes to the head capsule (Fig. 5B-D). On cross sections, this gland has the most lateral position of the three glands, and corresponds with the maxillary gland in earlier publications (see under Discussion). Contrary to the stipes base gland, it is located laterad of muscle M17 (M. tentoriocardinalis; see Richter et al. 2019) (Fig. 2B). On longitudinal sections, this

gland occupies the most posterior position of the three glands. Cell size in the cardo base gland is fairly similar in all categories, but cell number is significantly larger in major workers and queens than in minor workers and males (P < 0.01, Tables 1 and 2).

Discussion

The mouthparts of ants represent one of the most complicated parts of the body with various interconnected and articulating sclerotized elements. A very elegant study of the cephalic anatomy of *Wasmannia affinis* workers (Richter et al. 2019) provides a detailed account of the various mouthparts, the tentorial elements of the endoskeleton and the associated musculature. This paper moreover updates the nomenclature for the large exocrine glands in the head with prepharyngeal (previously propharyngeal) and pharyngeal (previously postpharyngeal) glands. The maxillary gland, which is often listed as a smaller cephalic gland in ants, does not exist in this myrmicine species (Richter et al. 2019). This indicates that these glands have either been overlooked previously due to their small size, or that they are a feature of only a few ant species including *C. japonicus*. Only detailed observations of the gland inventory of more ant species can solve this question and reveal the evolutionary history of these novel glands. In our survey of the glands in *C. japonicus*, however, we described three paired smaller glands that are associated with the maxillolabial complex, and that occur in all three female castes studied as well as in males. None of these glands was reported in the old survey study of the cephalic glands of *C. pennsylvanicus* (Forbes and McFarlane 1961).

The novel **postmentum base gland** is by far the smallest of the three glands, of which the ducts open through the articulation membrane between the base of the labial postmentum and the stipes. This gland has never been described before, although it may be misleading in old papers reporting on cephalic glands in *Formica* to find the suggestive names of gular gland (Meinert 1860) and tongue gland (Otto 1958). The figures in these publications, however, indicate that the gland described is actually equivalent to the cardo base gland (see further).

The **stipes base gland** equally represents a novel gland for ants. It is a fairly large gland, especially in the major workers that have approx. 140 secretory cells at each side. The ducts open through the articulation membrane that connects the maxillary cardo and stipes. This location has not been reported previously as a gland site, although Boonen and Billen (2016) for *Monomorium pharaonis* (Linnaeus, 1758) mentioned that *"occasionally, secretory cells are found medially to the tentorial arm"*. This comment provided a preliminary indication for the existence of our novel stipes base gland. This location at the mesal side of the anterior tentorial arm and muscle M17 (Richter et al. 2019) clearly differs from the position of the cardo base gland, which is situated at the other, more lateral side of these useful anatomical landmarks (see also Fig. 1).

The **cardo base gland** is relatively large, especially in the major workers and queens, with almost 200 and 150 secretory cells at each side, respectively. The ducts open through the articulation membrane that links the cardo and stipes to the head capsule. This location makes this gland equivalent to what has been described previously under various names: Meinert (1861) referred to it as the gular gland, while Otto (1958) described it as the tongue base gland and Kürschner (1971) as the lingual gland. Bausenwein (1960) renamed it as the maxillary base gland, which is anatomically correct as the cardo indeed is the most proximal part of the maxilla. The detailed paper by Beck (1972) shortened the name to maxillary gland, although it may seem confusing in his figure 7 that this gland opens through the membrane that connects the head

capsule directly to the stipes. This can be understood as the cardo is a merely cuticular piece that does not allow any hemolymph to move further from the head capsule through the cardo into the stipes. The stipes therefore has a further membranous connection directly with the head capsule to ensure its oxygen and nutrition supply (Richter, pers. comm.). It is through this membrane that Beck's maxillary gland opens. As the novel stipes base gland is equally associated with the maxilla, however, the name 'maxillary gland' has become too vague. Additional confusion has been created in the literature as several authors used the name 'maxillary gland' when describing the actual prepharyngeal gland (Bausenwein 1960; Forbes and McFarlane 1961; Kürschner 1971; Phillips and Vinson 1980; Toledo 1967; Gama and Cruz Landim 1982). We therefore propose to rename the 'maxillary (base) gland' more precisely as 'cardo base gland', which is also in line with the other novel glands names 'postmentum base gland' and 'stipes base gland'. As mentioned, the cardo base gland is located laterad of the M. tentoriocardinalis (M17), whereas the stipes base gland occurs mesad of this muscle as the most distinctive character (Fig. 1). Hölldobler (2016) published a detailed study on the exocrine system of legionary ants in which he also described the 'maxillary gland' in *Eciton*. This probably also represents the cardo base gland, although it is difficult to judge as the figures in this paper are longitudinal sections that are more difficult to interpret.

Conclusion

Our study illustrates the complicated nature of the maxillolabial complex in the head of ants, it adds two new discoveries to the already very extensive exocrine repertoire of ants, and attempts to update and clarify the confusing nomenclature of what was previously known as the maxillary gland. The function of the three glands associated with these mouthparts at present is speculative. Future examination with electron microscopy may shed light on the eventual function following the cytoplasmic composition. A mechanical function of providing lubricants to the several articulations between the cuticular elements of the mouthparts may be plausible, as the glands are found in all female castes as well as in males. For the larger stipes base gland and cardo base gland, additional functions can be expected considering the size of the gland, especially in the major workers and queens. Although these glands are not structurally linked with the digestive tract as such, their secretion may play a role in initial digestion as the mouthparts are obviously involved in food uptake and processing. As the postmentum base gland and stipes base gland are first described here, only detailed observations of the gland inventory of more ant species can reveal the evolutionary history of such novel glands.

Acknowledgements

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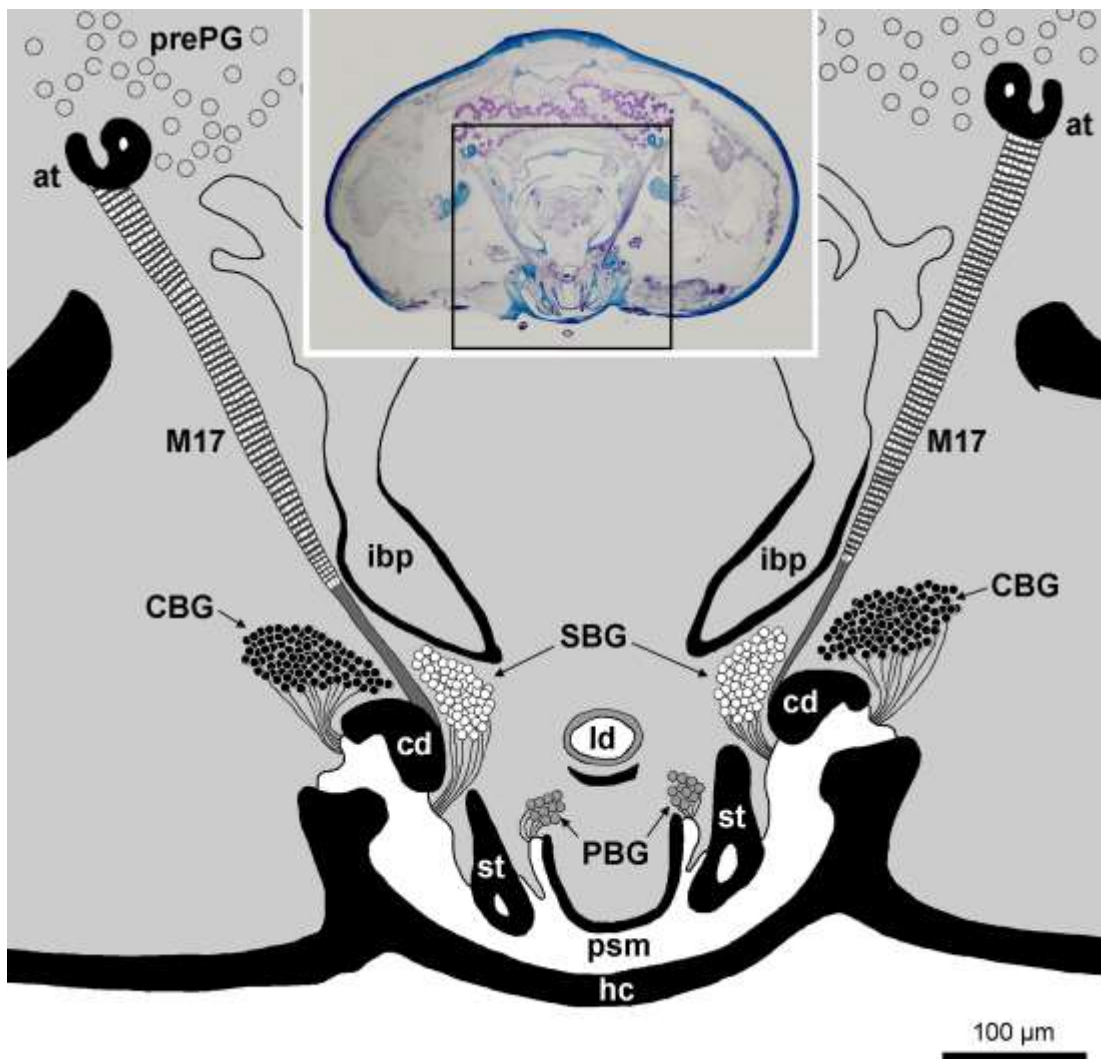
Fig. 1. Schematical drawing indicating the location of the three glands of the maxillolabial complex (CBG: cardo base gland, PBG: postmentum base gland, SBG: stipes base gland), the inset section image shows the area of the drawing part (we provide the schematical drawing for clarity as the 3 glands do not ideally appear together on the same histological section). at: anterior tentorium arm, cd: cardo, hc: head capsule, ibp: infrabuccal pocket, ld: labial gland duct, M17: M. tentoriocardinalis, prePG: prepharyngeal gland, psm: postmentum, st: stipes.

Fig. 2. Location of the three glands associated with the maxillolabial complex of *Camponotus japonicus.* **A**. Scanning micrograph of major worker. **B-D**. Transverse semithin sections showing the glands. **B**. Major worker. **C**. Queen. **D**. Male. cd: cardo, hc: head capsule, ibp: infrabuccal pocket, lb: labium, mx: maxilla, ld: labial gland duct, mf: muscle fibers, st: stipes, big black arrow: cardo base gland, big gray arrow: postmentum base gland, big white arrow: stipes base gland, small arrows: duct cells, arrowheads: M17 (M. tentoriocardinalis).
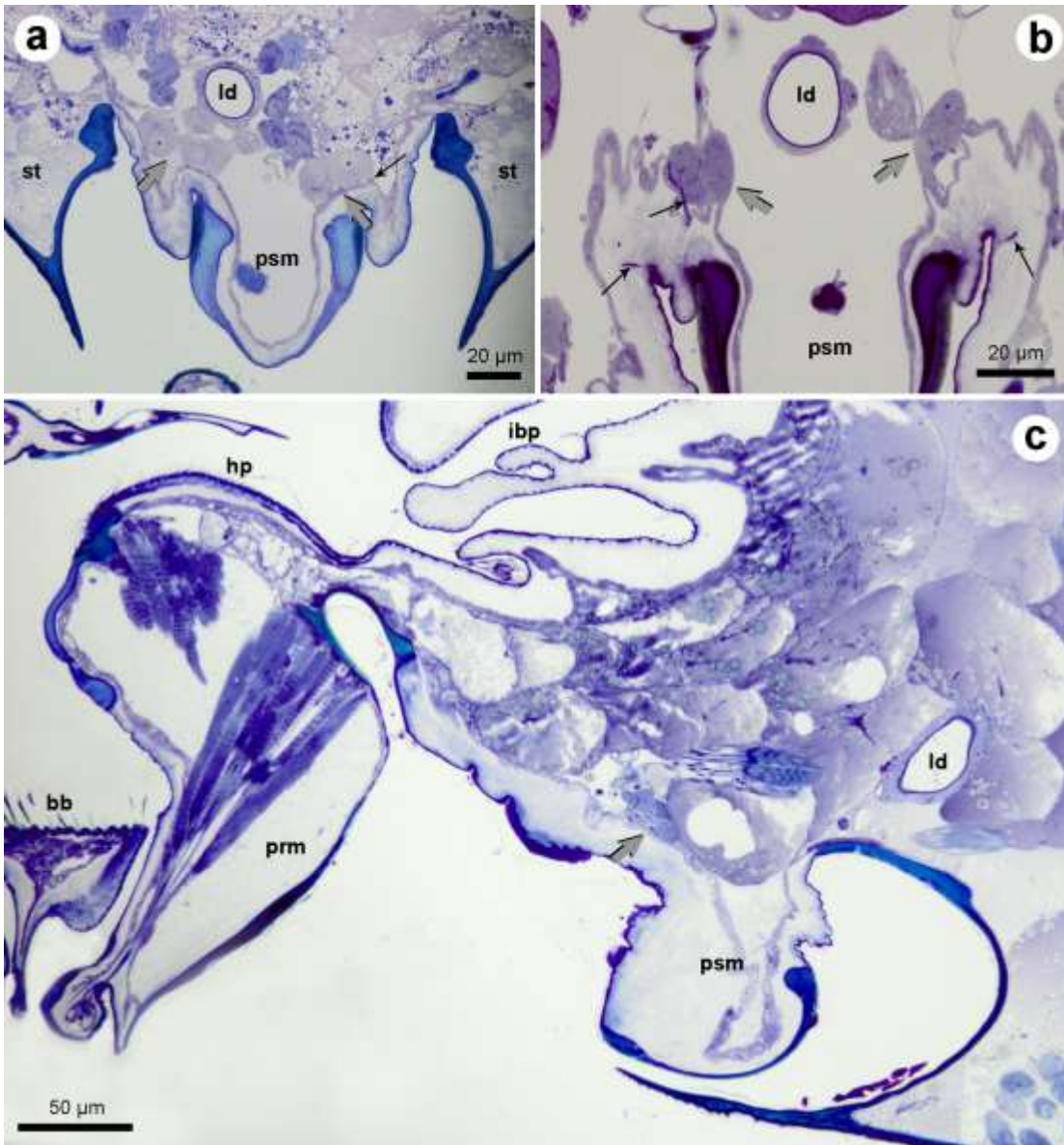
Fig. 3. Cross sections through labium base gland of minor worker (**A**) and male (**B**). **C**. Longitudinal section through the labium of major worker. hp: hypopharynx, ibp: infrabuccal pocket, ld: labial gland duct, prm: prementum, psm: postmentum, bb: basiparaglossal brush, st: stipes, big gray arrow: labium base gland, small arrows: duct cells.
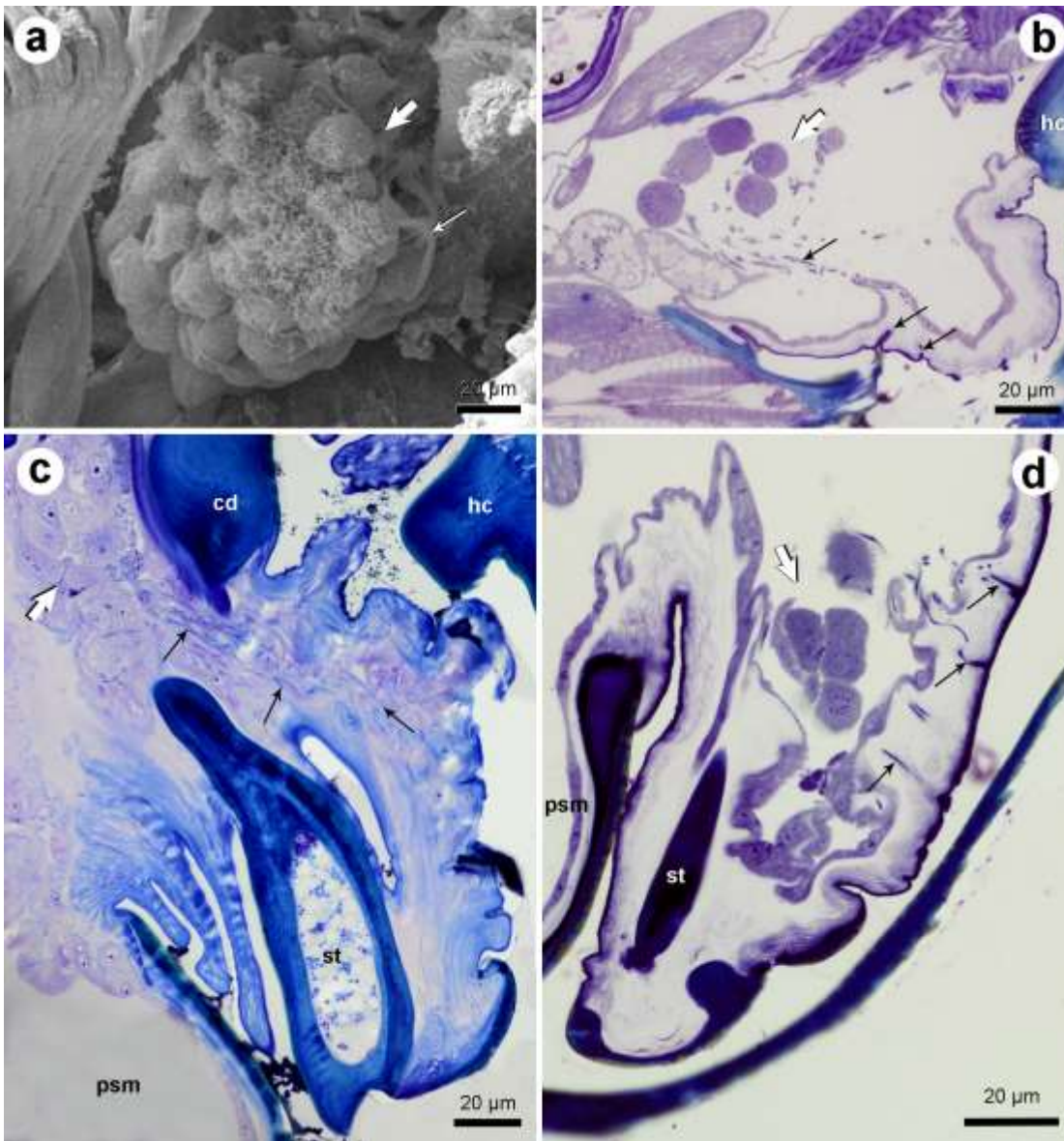
Fig. 4. Stipes base gland. **A**. Scanning micrograph of the stipes base gland in major worker. **B**. Longitudinal section through stipes base gland in minor worker. **C** and **D**. Cross sections through stipes base gland of queen (**C**) and male (**D**). Cd: cardo, HC: head capsule, Lb: labium, St: stipes, big white arrow: stipes base gland, small arrows: duct cells.
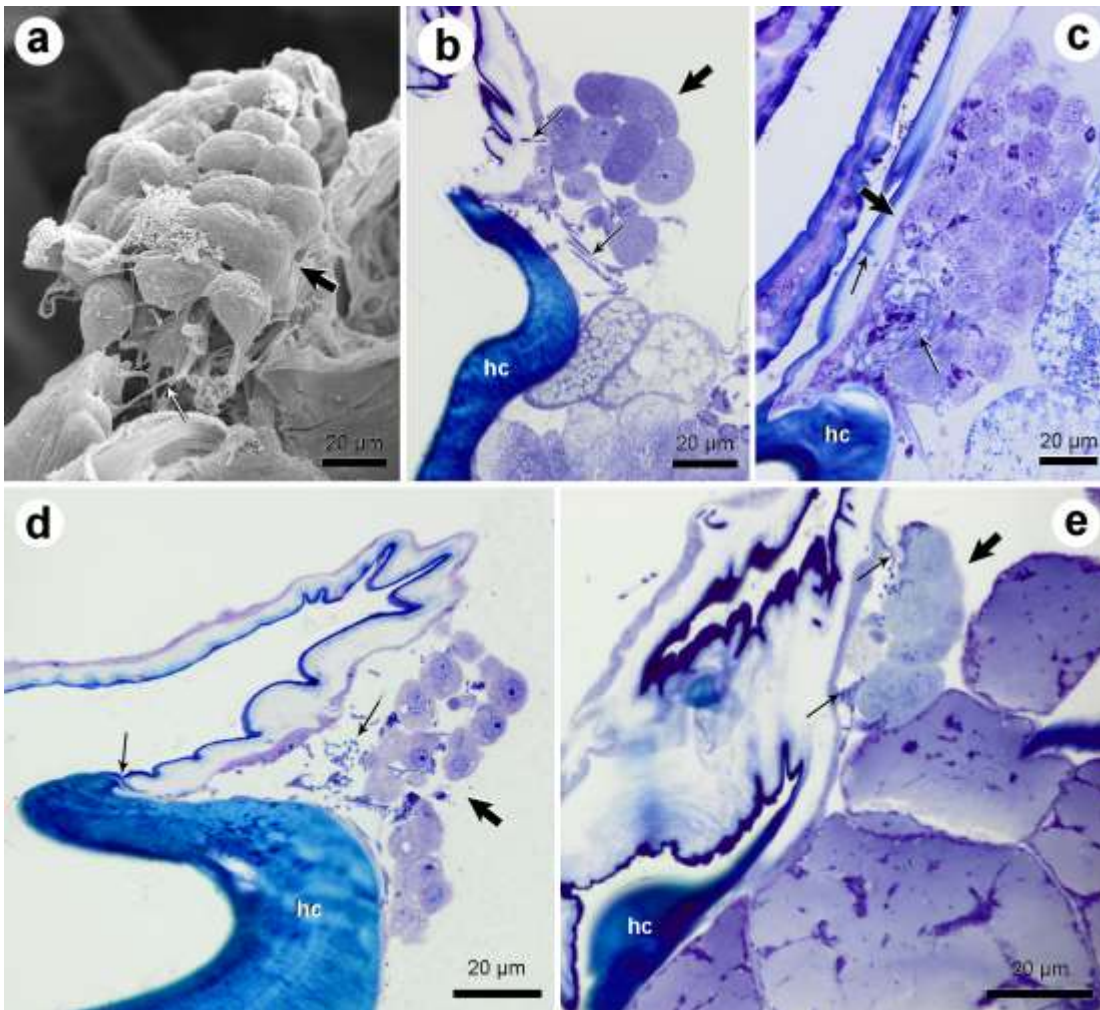
Fig. 5. Cardo base gland. **A**. Scanning micrograph in major worker. **B**. Longitudinal section in minor worker. **C-E**. Cross sections through cardo base gland of queen (**C**), major worker (**D**) and male (**E**). HC: head capsule, big black arrow: stipes base gland, small arrows: duct cells.

Table 1. Summary of gland cell diameter and cell number of the three glands in the three female castes and the males of *Camponotus japonicus*

Glands	Castes	Diameter (μm)	Cell number
Postmentum base gland	Minor worker	13.99±0.84	9.5±1.69
	Major worker	10.92±0.49	14.25±6.13
	Queen	14.48±0.96	14.25±1.5
	Male	11.35±1.04	12.25±1.83
Stipes base gland	Minor worker	13.26±0.54	47.5±11.6
	Major worker	10.92±0.27	139.25±16.17
	Queen	14.7±0.42	61.25±14.82
	Male	11.75±0.59	58.25±13.57
Cardo base gland	Minor worker	13.72±0.91	44.25±6.2
	Major worker	11.18±0.39	191.5±51.11
	Queen	13.37±0.5	146.75±30.96
	Male	11.06±0.45	38.11±7.61

Table 2. P-values of caste-related differences based on secretory cell numbers of the three glands in *C. japonicus* (p-value<0.01 indicates significant differences between two castes; w=minor worker, W=major worker, Q=queen, M=male)

Glands	M vs Q	M vs w	M vs W	Q vs w	Q vs W	w vs W
Postmentum base gland	0.2084	0.0688	0.7318	0.4752	0.5598	0.3814
Stipes base gland	1	0.2486	0.0195*	0.1103	0.0571	0.0195*
Cardo base gland	0.0097**	0.0986	0.0058**	0.0097**	0.1143	0.0058**