Arthropod Structure & Development 73 (2023) 101246

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

The spongiform tissue in *Strumigenys* ants contains exocrine glands

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ARTICLE INFO

Article history:
Received 28 October 2022
Received in revised form
1 February 2023
Accepted 2 February 2023
Available online xxx

Handling Editor: Dr G. Scholtz

Keywords:
Spongiform tissue gland
Intersegmental petiole gland
Intersegmental postpetiole gland
Petiole
Postpetiole
Formicidae

ABSTRACT

The insect cuticle is multifunctional and often includes projections used for support, communication or protection. Ants in the genus *Strumigenys* exhibit a peculiar honeycomb-like spongiform tissue that covers their petiole, postpetiole and sometimes also the posterior mesosoma and anterior part of the first gastral segment. The tissue is abundantly developed in workers and queens, and much reduced in males. We found this spongiform tissue is associated with a novel exocrine gland that is made up by class-3 secretory cells that are clustered underneath the major pillars of the cuticular extensions, their associated narrow ducts enter these extensions and open at the surface through small pores. The chemical nature and function of the secretion are still unknown. The honeycomb texture may act in the storage and dispersion of the glandular secretions. In addition to the spongiform tissue gland, the posterior region of the petiole and postpetiole also contain intersegmental petiole and postpetiole glands, of which the ducts open through the intersegmental membrane that forms the connection with the next segment. Future work aimed at identifying the chemicals secreted by these glands will shed light onto the function of these unusual structures.

1. Introduction

The multifunctional characteristics of the cuticle make it a crucial element in the success of insects. Among other functions, it acts as an exoskeleton that provides strength and support, it forms a barrier to protect the internal organs against the outside environment and it prevents desiccation (Nation, 2004). An additional function is its acting as a substrate for various chemicals. This is particularly important in social insects, where cuticular substances are involved in intra- and interspecific communication and relay individual as well as colony-level information about reproductive status and nestmate identity (Breed, 1998; Lahav et al., 1999; Guerrieri et al., 2009).

The multiple functions are reflected in the complexity of the ultrastructural and chemical composition of the various cuticular layers. The cuticular constituents originate from the epidermal cell layer that is situated underneath the cuticle. These cells therefore display a high metabolic activity, that becomes even more prominent when cells differentiate into exocrine gland cells. A direct such modification of the epidermal cells leads to a class-1 gland in which the secretory cells retain their epithelial arrangement; if epidermal cells differentiate through a complex process into bicellular units that are each formed by a secretory cell and its associated duct cell (Sreng and Quennedey, 1976), they become class-3 glands (Noirot and Quennedey, 1974). Especially in social insects, the cuticle is endowed with a multitude of exocrine glands, the secretions of which play a crucial role in various aspects of the organization and communication of the colony (Billen and Morgan, 1998; Vander Meer et al., 1998; Morgan, 2008).

Ant cuticle often displays peculiar structures as spines and projections of which the function is not always clear (Ito et al., 2016). In most *Strumigenys* species, an unusual 'spongiform tissue' (Bolton, 1999) is found that covers large parts of the petiole,

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https://doi.org/10.1016/j.asd.2023.101246
1467-8039/

postpetiole and the first gastral segment symmetrically. In some species, this tissue even extends on the posterior mesosoma, with a massive development (e.g. *Strumigenys philiporum,* Brown, 1988). Apart from *Strumigenys*, such unique and unusual pedicellar formations have only been observed in a more diffuse appearance in the myrmicine genera *Tetheamyrma* and *Dacetinops* (Bolton, 1999). This structure was described as "*formed of areolate and vesiculate, finely involuted and subdivided lamellar chitin*" (Brown, 1953: p.38-39), with considerable differences among species (Bolton, 1999). More recently, Silva and Feitosa (2019) introduced 'areolate processes' as new terminology because insufficient information about features as elasticity or permeability is available, though we prefer to use the traditional name 'spongiform tissue' as this designation only refers to the sponge-like form rather than to any other physical characteristics.

With more than 800 described species, *Strumigenys* represents the third species-rich ant genus (after *Camponotus* and *Pheidole*). They are small, slow-moving predators that mainly feed on collembola and other small arthropods. The genus has a wide distribution in temperate to tropical areas, where the ants live in the soil, leaf litter and rotten wood. Their predatory behaviour follows a ritualized sequence of events that include detection, localization, approach, antennation, attack with fast mandible closure, lifting, stinging, and transport (Dejean, 1986). Some *Strumigenys* species are trap-jaw ants that generate extremely fast snapping movements of the mandibles to catch prey (Larabee and Suarez, 2014; Booher et al., 2021). Apart from a few pioneering SEM and TEM images but without further details by Dejean (1985), no formal description of this spongiform tissue has been published. The function of the peculiar spongiform tissue remains unknown, although a few hypotheses have been formulated such as a possible role in prey attraction (Dejean, 1985) or a protection of the thin waist against seizing by enemies (Wetterer, 2011).

In this study, we examined the morphology and ultrastructure of the spongiform tissue in 19 *Strumigenys* species. We show that this structure is associated with novel glands; its elaborate structure may serve to increase the surface area over which the chemicals produced are sequestered and dispersed.

2. Material and methods

The genus *Strumigenys* is divided in short- and long-mandibulate species, our study included species from both groups (Table 1). Specimens for scanning microscopy (SEM) were dehydrated in 99.5% alcohol and transferred into HMDS (hexamethyldisilazane) for 2 soaks of 30 min each in a covered glass vial. After the second soak, the liquid was allowed to evaporate under a fume hood (Brown, 1993). All specimens were attached to aluminium stubs with double-sided adhesive tape and sputter-coated with gold in a Hitachi E−1010 ion sputter for 60 s and observed under a Hitachi TM3000 tabletop electron microscope.

Three-dimensional surface renderings of *Solenopsis rostrata* were generated using X-ray microtomography (microCT). One worker was fixed in Bouin's solution for 24 h, dehydrated in a graded ethanol series, critical point dried with a 931.GL AutoSamdri Supercritical Point Dryer, and placed in a desiccator for 24 h. The gaster, petiole and postpetiole was then scanned on an Xradia MicroXCT-400 scanner at 25 kV voltage and 5 W power with an exposure time of 8 s. 776 images spanning 180 degrees of the specimen were taken with an exposure time of 8 s. The source-to-sample and detector-to-sample distances were 28.01 mm and 30.03 mm, respectively. Tomographic reconstruction of the scan was performed in Xradia XMReconstructor 8.1. Surface and volume renderings of the spongiform tissue and associated body segments were generated from segmentation performed in Amira 5.4.

For histological and ultrastructural examination, transverse cuts were made in the thorax between the mid- and hindlegs and behind the first gastral segment to detach the petiole and postpetiole region and allow proper penetration for the various chemicals used during tissue processing. Tissues were fixed in cold 2% glutaraldehyde, buffered with 0.05 M Na-cacodylate and 0.15 M saccharose and postfixed in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, tissues were embedded in Araldite and sectioned with a Leica EM UC6 ultramicrotome. Semithin sections of 1 μm were stained with methylene blue and thionin and viewed under an Olympus BX-51 microscope. Thin sections of 70 nm were double stained with uranyl acetate and lead citrate and were examined with a Zeiss EM900 electron microscope. All longitudinal images in this paper are shown with the anterior to the left.

3. Results

The spongiform tissue is located on the posterior part of the mesosoma, on the petiole and postpetiole, and on the first gastral segment. Scanning microscopy shows its intricate honeycomb-like architecture that is made up by three-dimensional extensions of the cuticle. Examination at higher magnification reveals the existence on the cuticular ridges of small round pores with a diameter of approx. 0.5 μm. The spongiform tissue and the associated minute pores are most pronounced in workers (Fig. 1A–C) and queens (Fig. 1D and E). It appears much reduced or absent in males, although some minute pores can still be observed (Fig. 1F and G). We found the spongiform tissue in workers of all 17 species that were examined with SEM, although most are Asian in origin and reflect only a small subset of the many biogeographically restricted species groups (Booher et al., 2021). In most species, it has a similar abundant appearance (Fig. 2A–F), although it can be more reduced as in *Strumigenys benten* (Fig. 2G), *S. membranifera* (Fig. 2H) and *S. rogeri* (Fig. 2I), and rudimentary as in *S. mutica* (Fig. 2J). In contrast, the surface of the spongiform tissue in *S. sauteri* shows round to oval 'windows' of 5–10 μm (Fig. 2M) which is atypical for the species we examined. High magnification observation, however, shows the presence of minute pores in all examined species including *S. mutica* (Fig. 2K) and *S. sauteri* (Fig. 2L). The overall extent of the spongiform tissue in the waist area can be visualized using micro-computed tomography (μCT) scanning (Fig. 3).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.asd.2023.101246

Histological examination illustrates the appearance of the spongiform tissue with thin cuticular extensions that make up a three-dimensional honeycomb structure (Fig. 4). In addition to this merely cuticular component, we found clusters of small class-3 gland cells underneath the major pillars of the entire spongiform tissue (white arrowheads in Fig. 4A–F). We suggest designating these cell clusters as a novel **spongiform tissue gland**. We found the secretory and duct cells in all workers and queens of examined species, but did not see them in the limited material of only 3 males we had available. On sections, the gland cells measure approx. 10 × 5 μm, and are connected with duct cells that enter the branching cuticular extensions of the spongiform tissue (white arrows in Fig. 4E and F). Electron microscopy of the gland cells reveals their end apparatus, of which the occurrence of multiple sections per cell indicate the sinuous trajectory (Fig. 5A and B). The microvilli display a loose arrangement, which creates relatively large spaces in the end apparatus. The central cuticle of the end apparatus contains a discontinuous lining of the epicuticle (grey arrowheads in Fig. 5B and C), which facilitate the transport of secretion into the narrow duct. The cytoplasm contains numerous mitochondria, Golgi apparatus and some lamellar inclusions

Table 1
Survey of the ant species studied with their collection locality and indication of the number of specimens examined with scanning electron microscopy (SEM) and with histology (W = worker, Q = queen, M = male). Figures as "2/1" in the histology/TEM column mean that 2 specimens have been studied histologically, of which 1 also with electron microscopy (TEM).

species		collection locality	SEM			histology/TEM		
			W	Q	M	W	Q	M
short-mandibulate *Strumigenys*	*S. benten*	Nantou County, Taiwan	3					2/1
	S. elegantula	Nantou County, Taiwan	5					
	S. emmae	Tainan City, Taiwan	6	1		1	1/1	
	S. hexamera	Kagawa, Japan				1		
	S. kichijo	Taipei, Taiwan	2			1/1		
	S. leptothrix	Nantou County, Taiwan	4	4				
	S. membranifera	Nantou County, Taiwan	3					
	S. mutica	Nantou County, Taiwan	16	2		3/1	1	1
	S. rostrata	Champaign County, IL, U.S.A.	14			7/2		
	S. sauteri	Nantou County, Taiwan	6	2		9/6		
	S. sydorata	Bogor, Indonesia				1/1	1	
long-mandibulate *Strumigenys*	*S. chuchihensis*	Xinchu County, Taiwan	3			1		
	S. formosensis	Nantou County, Taiwan	5	2	1	4/3		
	S. hispida	Nantou County, Taiwan	6	1	1			
	S. lacunosa	Nantou County, Taiwan	2				1/1	
	S. nanzanensis	Taitung County, Taiwan	4					
	S. orchidensis	Taitung County, Taiwan	4					
	S. rogeri	Nantou County, Taiwan	8	3		1		
	S. solifontis	Nantou County, Taiwan	5	1			1	

(Fig. 5A–C). Endoplasmic reticulum, neither smooth nor rough, was found. Because of the flattened appearance of the secretory cells, their nuclei also appear flattened with a size of approximately 5 × 2 μm (Fig. 5D). The secretion-draining canal of the duct cells has a continuous cuticular lining and an internal diameter of hardly 0.15–0.2 μm. The ducts enter the major cuticular pillars of the spongiform tissue (Fig. 5E and F), the site where they enter the cuticle sometimes being visible as a conspicuous cup-shaped cytoplasmic extension into the cuticle (grey arrow in Fig. 5D). The ducts eventually open at the external surface of the spongiform tissue, where their openings are visible as the small pores that were observed with scanning microscopy (see above).

In addition to the flattened secretory cells of the spongiform tissue gland of which the accompanying duct cells open at the surface of the spongiform tissue, the posterior region of both the petiole and postpetiole also contains a few spherical gland cells with a diameter of 10–15 μm (Figs. 4B and 6). The ducts of these cells have an internal diameter of 0.2–0.25 μm, and open through the intersegmental membranes that connect the petiole with the postpetiole (for the cells situated in the petiole, Figs. 4B, Fig. 6A–D) and the postpetiole with the first gastral segment (for the cells situated in the postpetiole, Fig. 6E–F). Because of the intersegmental opening site, their duct openings are not externally visible. These cells form part of the **intersegmental petiole and postpetiole glands**, that exist in workers (Fig. 6A–C,E), males (Fig. 6D) and queens (Fig. 6F) of all examined species. The gland cells contain numerous mitochondria and have an end apparatus with tightly arranged microvilli (Fig. 6G and H).

Internal tissues in the petiole and postpetiole, apart from the two exocrine glands described above, mainly include muscles, ganglia and nerves, and the oesophagus. None of the muscles, however, is in direct contact with any of these glands. Longitudinal sections reveal the presence of a stridulation apparatus in both queens (Fig. 7A) and workers (Fig. 7B). This is formed by a thin sclerotized scraper at the posterior edge of the postpetiolar tergite and a stridulation file with parallel transverse ridges at approx. 0.5 μm intervals on the anterior margin of the first gastral tergite (Fig. 7B). Although the petiole and postpetiole represent the modified second and third abdominal segments, we only found one ventral ganglion in the petiole. The next ganglion is situated in the first gastral segment, the postpetiole containing only the uninterrupted nerves that connect the petiolar and first gastral ganglia (Fig. 7A).

4. Discussion

The peculiar spongiform tissue that covers major parts of the petiole and postpetiole, and sometimes also the posterior mesosoma and anterior part of the first gastral segment is mainly formed by branched extensions of the cuticle that give it a three-dimensional honeycomb architecture. We found it in all 19 *Strumigenys* species that were examined in this study, albeit much restricted in *S. mutica*. This aberrant appearance in the latter species may be related to its lifestyle as a social parasite of other

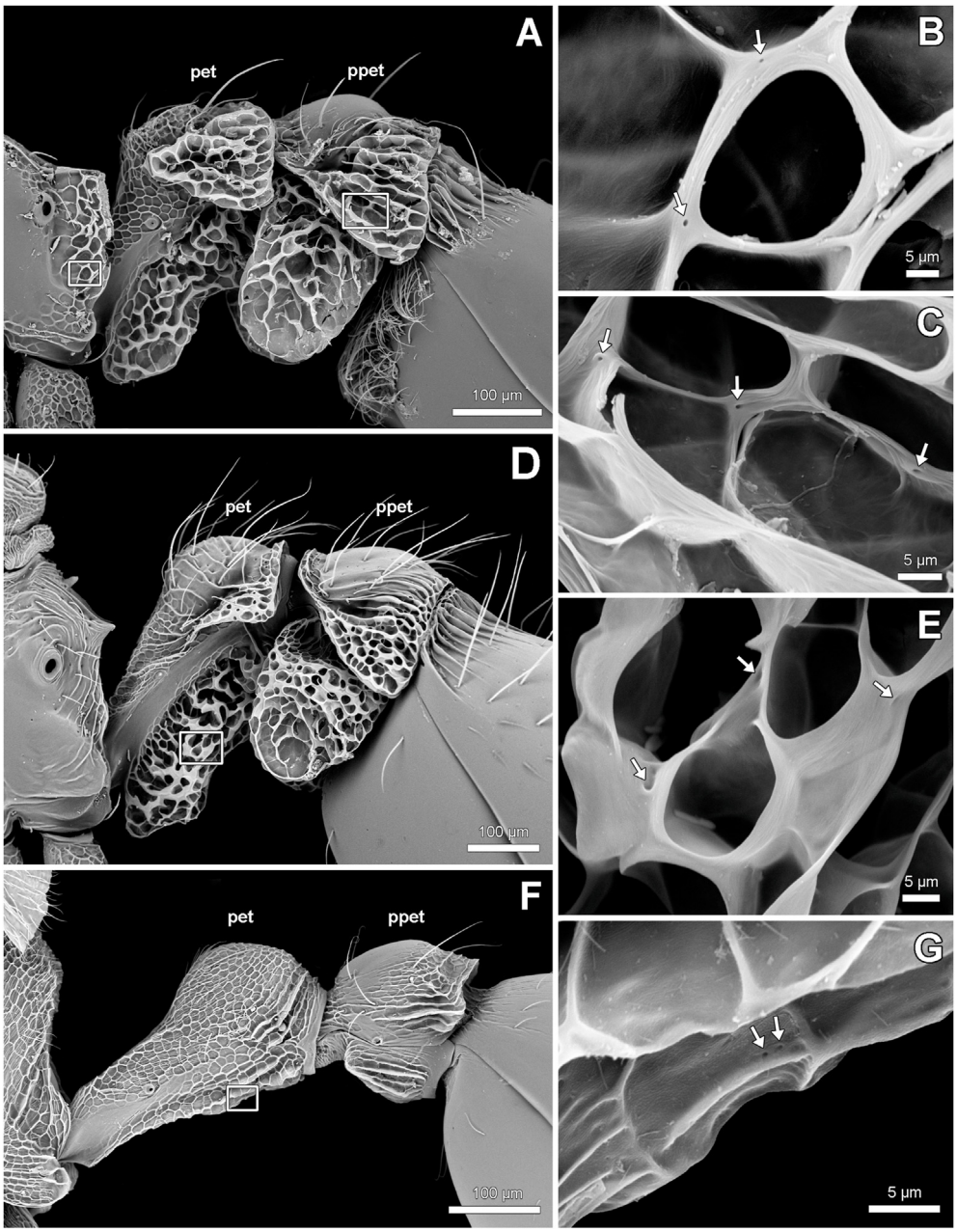

Fig. 1. Profile view scanning micrographs of petiole (pet) and postpetiole (ppet) showing abundant spongiform tissue in *S. hispida* worker (**A**) and *S. leptothrix* queen (**D**), and its rudimentary appearance in *S. formosensis* male (**F**). Right side images show details of enlarged frames, white arrows indicate duct openings on cuticular surface: **B**. Detail of propodeum in *S. hispida* worker. **C**. Detail of lateral postpetiole in *S. hispida* worker. **E**. Detail of ventrolateral petiole in *S. leptothrix* queen. **G**. Detail of ventral petiole in *S. formosensis* male.

Strumigenys species, as we found that *S. mutica* also differs in other anatomical features from congeneric species (Wang et al., 2021a, 2021b). The spongiform tissue is similarly developed in workers and queens, but much less conspicuous in males. Our major discovery was the presence of two exocrine glands associated with this spongiform tissue (Fig. 8):

The **spongiform tissue gland** (orange in Fig. 8) is a novel addition to the exocrine repertoire of social insects. It consists of small flattened class-3 gland cells underneath the major cuticular pillars of the spongiform tissue, their narrow accompanying ducts with a diameter of hardly 0.2 μm penetrate into these pillars and open on the honeycomb surface through minute pores. The gland cells somehow resemble the subepithelial glands that have been described directly underneath the cuticle in several ant groups (Gobin et al., 2003), although the latter appear as scattered individual cells, whereas the cells of the spongiform tissue gland have a clearly clustered appearance. Brown and Wilson (1959, p.291) already speculated that the spongiform tissue is '*sometimes associated with glandular areas*', while Dejean (1985) even provided a few ultrastructural images of gland cells but without further commenting on them. The well-developed end apparatus, mitochondria and Golgi apparatus illustrate the glandular activity. A striking feature is the unusually narrow diameter of the ducts: whereas class-3 gland cells are typically accompanied by ducts with an internal diameter of 0.5–1 μm, regardless of the size of the insect (Billen and Ito, 2018), the ducts of the spongiform tissue

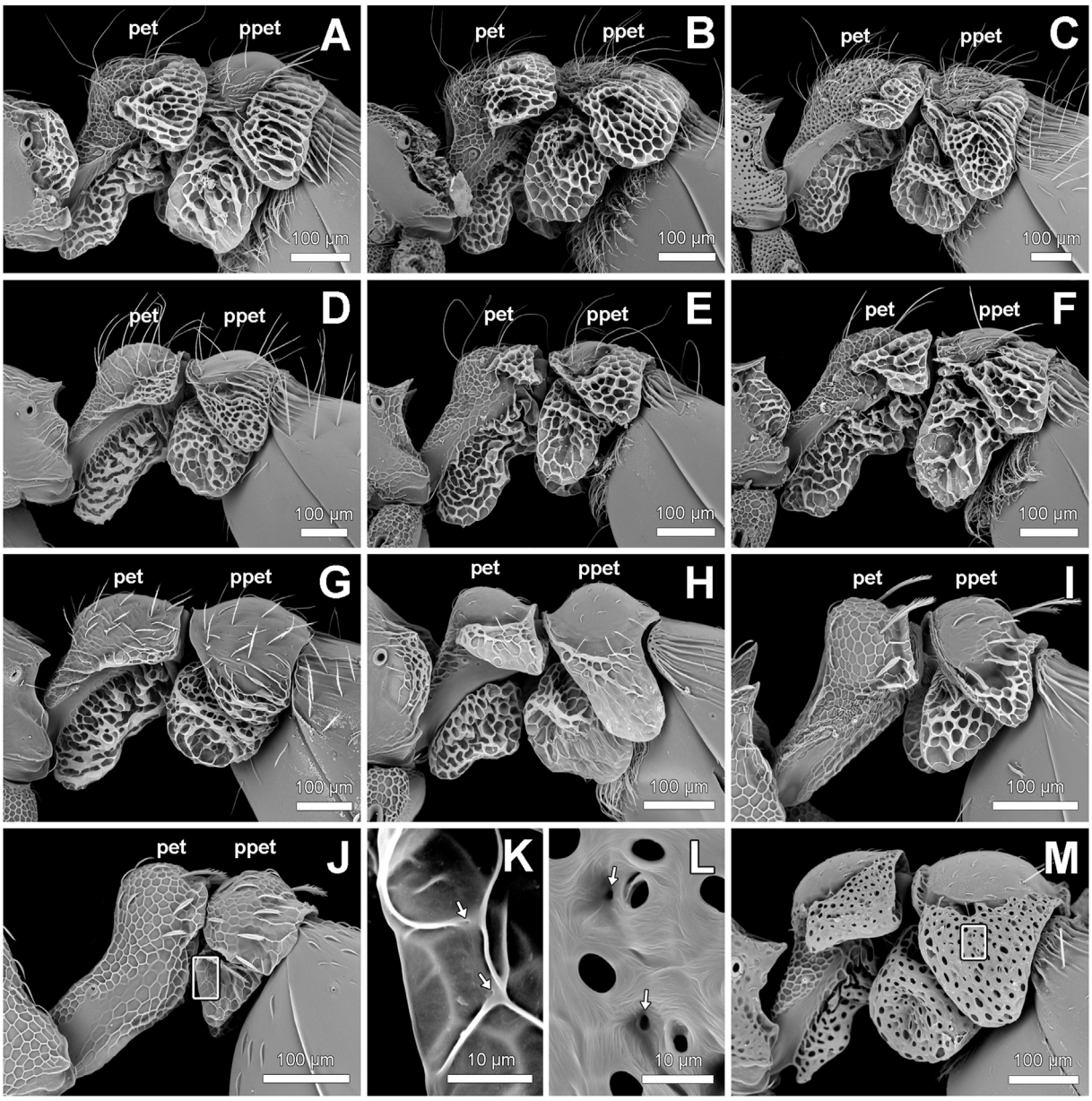

Fig. 2. Profile view scanning micrographs of the petiole (pet) and postpetiole (ppet) showing abundant spongiform tissue in workers of various *Strumigenys* species: *S. chuchihensis* (**A**), *S. kichijo* (**B**), *S. lacunosa* (**C**), *S. leptothrix* (**D**), *S. orchidensis* (**E**), *S. solifontis* (**F**). It appears less developed in *S. benten* (**G**), *S. membranifera* (**H**) and *S. rogeri* (**I**). Note almost absent spongiform tissue in *S. mutica* (**J**) and peculiar appearance in *S. sauteri* (**M**), although enlargement of frame areas in these species does also show minute pores on surface (arrows in **K**,**L**).

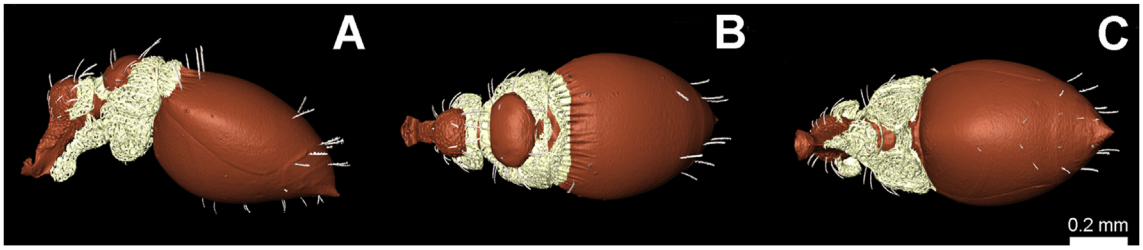

Fig. 3. microCT based surface renderings of the spongiform tissue (yellow) and associated body segments (brown). Lateral (**A**), dorsal (**B**) and ventral view (**C**) of abdomen of *S. rostrata* worker, showing extent of spongiform tissue on petiole, postpetiole and first gastral tergite. See moving animation in Supplementary Video S1.

gland have a diameter of hardly 0.15–0.2 μm. We can only speculate that this may perhaps be related to special physicochemical properties of the secretion and/or with the long trajectory of the ducts on their way from the secretory cells to the exterior. A

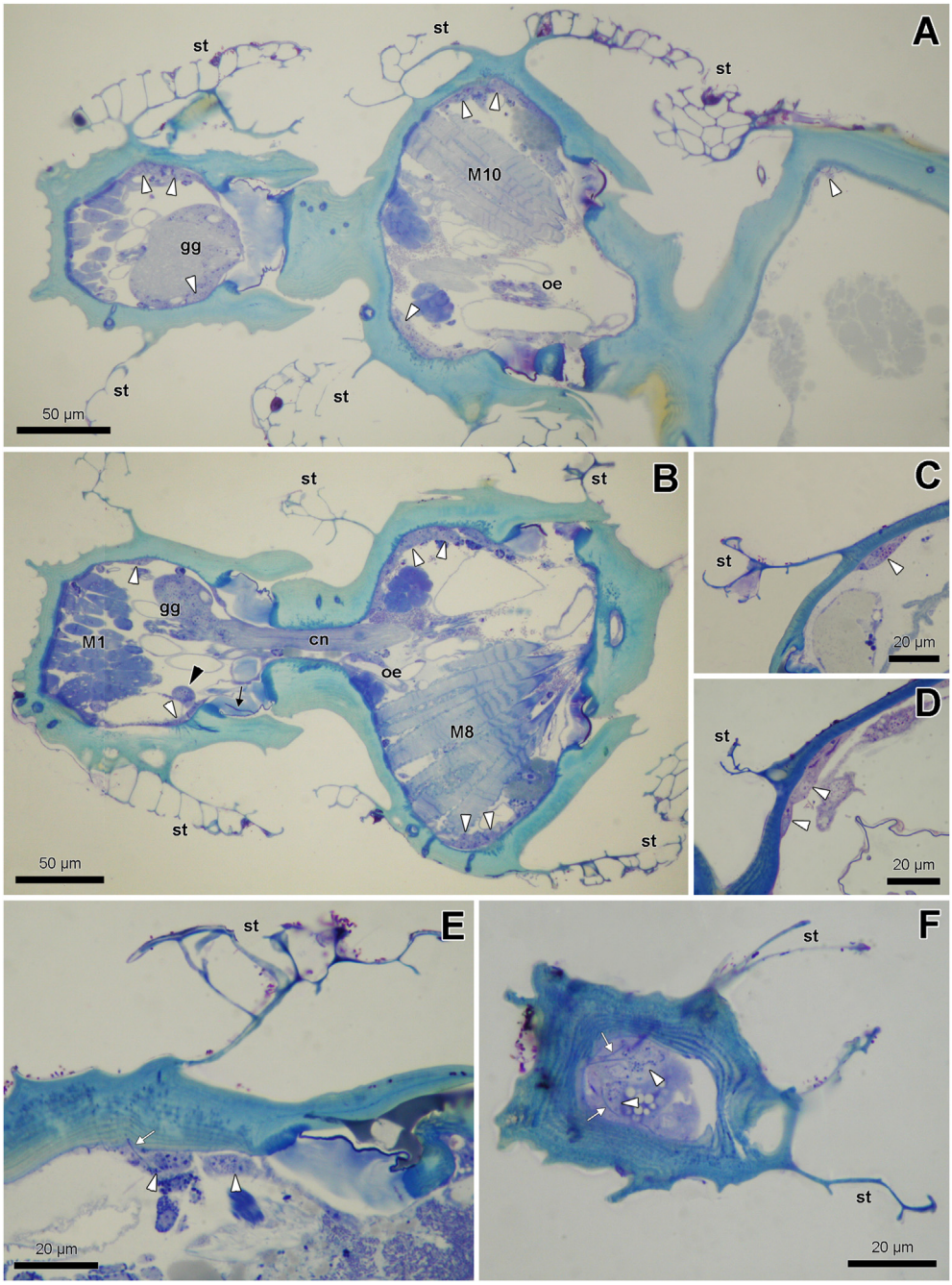

Fig. 4. Semithin sections through the spongiform tissue (st): longitudinal (**A**) and coronal plane section (**B**) of waist area of *S. sauteri* worker, white arrowheads indicate cells of spongiform tissue gland, black arrowhead in B indicates secretory cell of intersegmental petiole gland, black arrow indicates its duct cell. **C**. First gastral tergite of *S. emmae* queen. **D**. First gastral tergite of *S. mutica* worker. **E**. Longitudinal section through petiole of *S. formosensis* worker. **F**. Parasagittal section through petiole of *S. hexamera* worker. cn: connective nerve, gg: ganglion, oe: oesophagus, small white arrows indicate ducts of spongiform tissue gland. M1, M8 and M10 indicate the muscle groups following the numbering system of Hashimoto (1996).

narrower diameter may facilitate to keep secretion liquid during its transportation due to increased capillarity forces.

The **intersegmental petiole and postpetiole glands** (red in Fig. 8) are made up of a few rounded class-3 gland cells in the posterior part of the petiole and postpetiole, with ducts that open through the intersegmental membrane that connects to the next segment. The presence of exocrine glands in the petiolar waist of ants was already noted in *Eciton* queens and workers by Whelden (1963), who described in both the posterior petiole and postpetiole clusters of 6 (minor workers) to 20 class-3 cells (major workers and soldiers) that open through the dorsal intersegmental membrane. Conspicuous glands with clusters of large secretory cells were also found in the petiole and postpetiole of *Protanilla wallacei* (Billen et al., 2013) and *Leptanilla clypeata* (Billen et al., 2022), although these cells have different anatomical characteristics and a different opening site than the intersegmental glands we here described for *Strumigenys*. The function of these intersegmental petiole and postpetiole glands is equally unknown, but may be linked with the production of lubricants to facilitate the movements of the articulating segments (Billen, 2009).

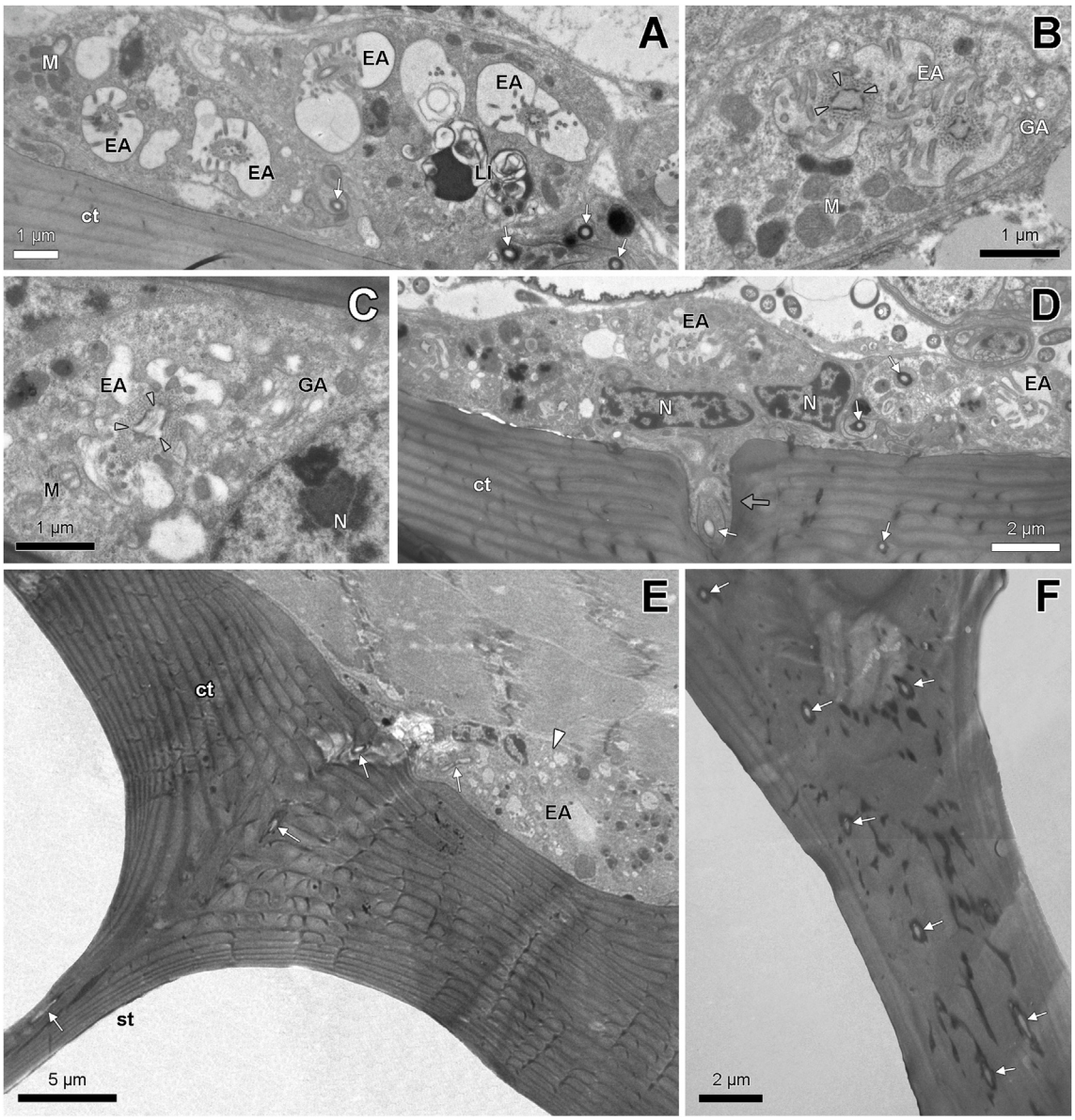

Fig. 5. Electron micrograph details of spongiform tissue gland. **A**. Secretory cell in postpetiole of *S. kichijo* worker showing end apparatus (EA), lamellar inclusions (LI) and mitochondria (M); white arrows indicate ducts. **B**. Detail of end apparatus in *S. sydorata* worker (gaster spongiform tissue), grey arrowheads indicate discontinuous lining of epicuticle. GA: Golgi apparatus. **C**. End apparatus in petiole of *S. sauteri* worker. **D**. Secretory cells in petiole of *S. sauteri* worker. Note cup-shaped extension of cytoplasm into cuticle where duct cell starts transversing the cuticle (grey arrow). **E**. General view of postpetiole in *S. sauteri* worker showing secretory cells (white arrowhead) located underneath cuticular extension of spongiform tissue (st), white arrows show trajectory of ducts into spongiform network. **F**. Detail of cuticular extension of spongiform tissue in *S. sauteri* worker petiole, showing several ducts running through it. ct: cuticle, GA: Golgi apparatus, N: nucleus.

The muscles in both waist segments consist of antagonistic pairs that are mainly responsible for the upward and downward movements by their insertion onto the anterior margins of the next segment: petiolar muscles cause movements of the postpetiole, postpetiolar muscles cause movements of the gaster (see Hashimoto (1996) for more details). Although the spongiform tissue may affect free movements of the waist region, we confirmed the presence of a stridulation apparatus that is formed by a pointed scraper underneath the posterodorsal margin of the postpetiole and a stridulation file of parallel transverse ridges on the anterior part of the first gastral tergite. Postpetiolar muscular action, especially of M8 (retractor of first gastral tergite) and M9 (protractor of first gastral tergite) following Hashimoto (1996) cause back-and-forth movements of the scraper against the stridulation file and hence allow possible sound production in *Strumigenys* ants. Another unexpected finding in this study was the absence of a ganglion in the postpetiole, in spite the latter constitutes the third abdomen segment. We are not aware of any literature data that state whether this represents a common feature for the ant postpetiole, although a preliminary check in a few other species also showed the absence of a postpetiolar ganglion (*Atta sexdens, Protanilla lini, Pseudomyrmex scharpi* and *Solenopsis invicta;* Billen, unpubl. obs.).

The function of the novel spongiform tissue gland remains unknown. However, it is likely that the honeycomb texture of the spongiform tissue may function to increase the surface area for the storage and dispersal of the secretory products. A potential role in prey attraction has been suggested by Dejean (1985) for these

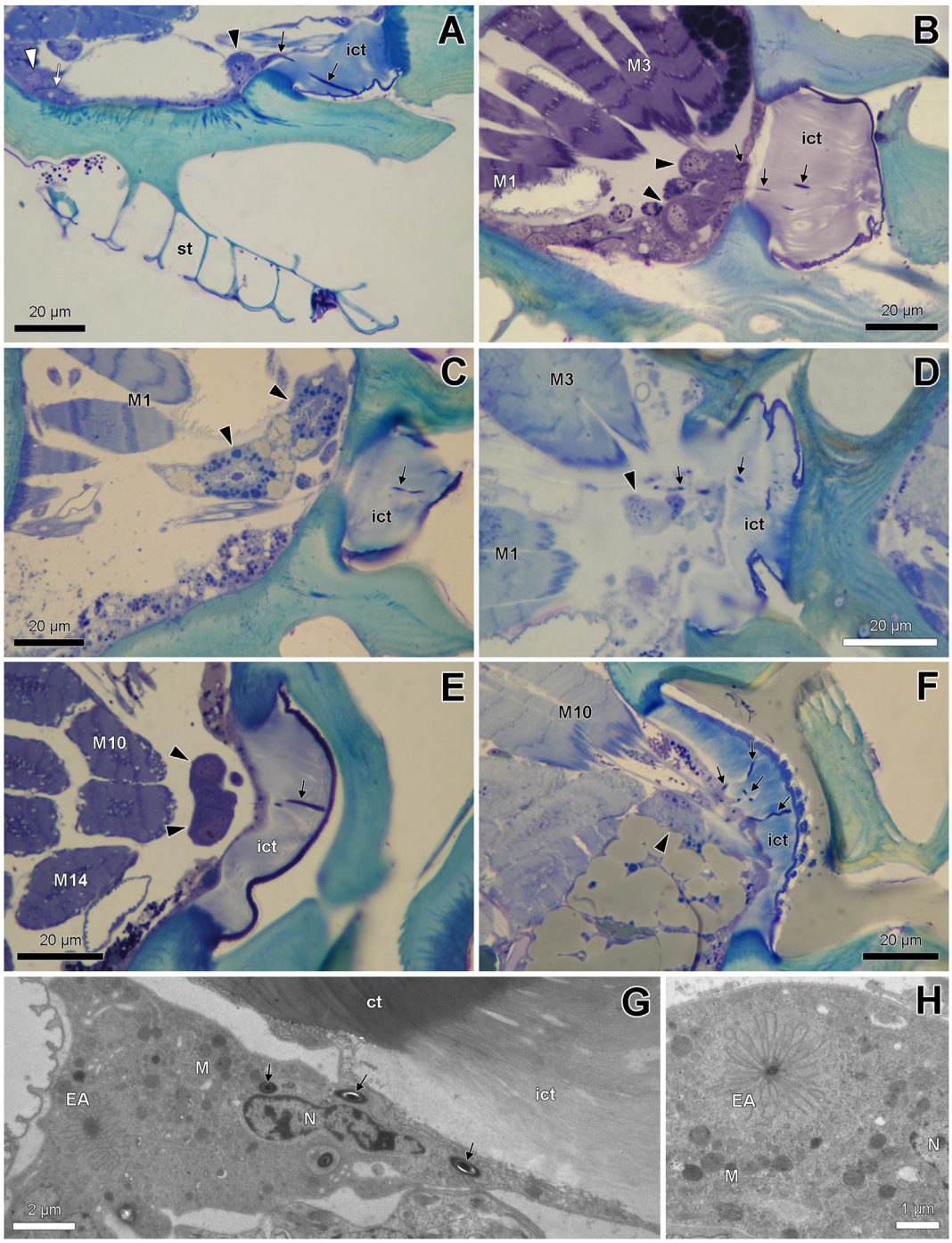

Fig. 6. Secretory cells of intersegmental petiole and postpetiole glands (black arrowheads) and their ducts (black arrows) that open through intersegmental cuticle (ict): **A**. Posterior petiole of *S. sauteri* worker (white arrowhead indicates secretory cell of spongiform tissue gland, white arrow indicates its duct). **B**. Posterior petiole of *S. sauteri* worker. **C**. Posterior petiole of *S. kichijo* worker. **D**. Posterior petiole of *S. mutica* male. **E**. Posterior postpetiole of *S. mutica* worker. **F**. Posterior postpetiole of *S. lacunosa* queen. **G**. Electron micrograph of intersegmental petiole gland cell of *S. sauteri* worker, showing end apparatus (EA) and ducts. **H**. Detail of end apparatus in intersegmental petiole gland of *S. kichijo* worker. ct: cuticle, M: mitochondria, N: nucleus, st: spongiform tissue. M1, M3, M10 and M14 indicate the muscle groups following the numbering system of Hashimoto (1996).

chemicals, although the middle part of the body may not be the most obvious anatomical position for such function. The presence of spongiform tissue in both short- and long-mandibulate species without noticeable difference between groups also indicates that its function may not be related with prey hunting, as these groups differ in their hunting tactics (Masuko, 1984). The absence of rough endoplasmic reticulum indicates that the cells produce a non-proteinaceous, and therefore possibly pheromonal secretion. Since there is no evident musculature or reservoir associated with these glands, it is unlikely that there exists any direct control of secretion emission to the outside. We do not yet know what compounds are secreted. The absence of a reservoir and the small overall volume of the gland considerably compromise the chemical identification of the secretion. With the advance of analytical

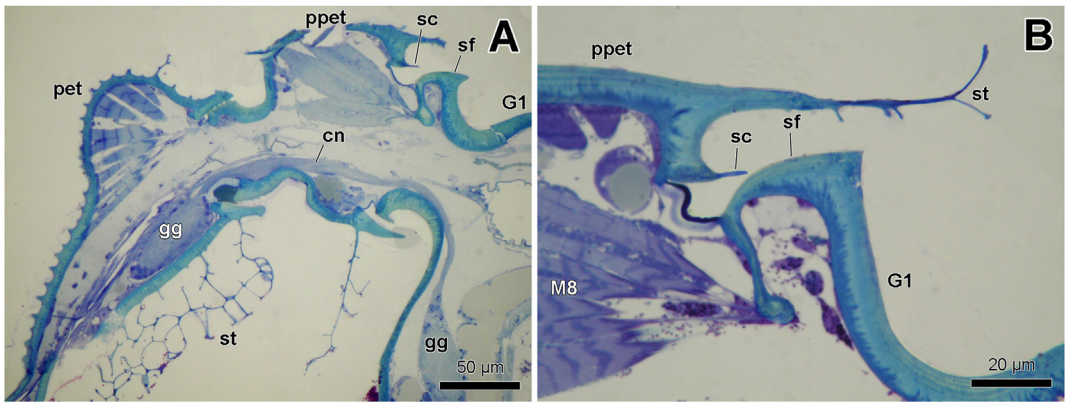

Fig. 7. **A**. Longitudinal section through petiole (pet) and postpetiole (ppet) of *S. emmae* queen. cn indicates nerve that connects ganglia (gg) of petiole and first gastral segment (G1), as no ganglion is present in postpetiole. **B**. Detail of stridulatory file (sf) with fine cuticular ridges and scraper (sc) in *S. formosensis* worker. M8: tergite retractor muscle, st: spongiform tissue.

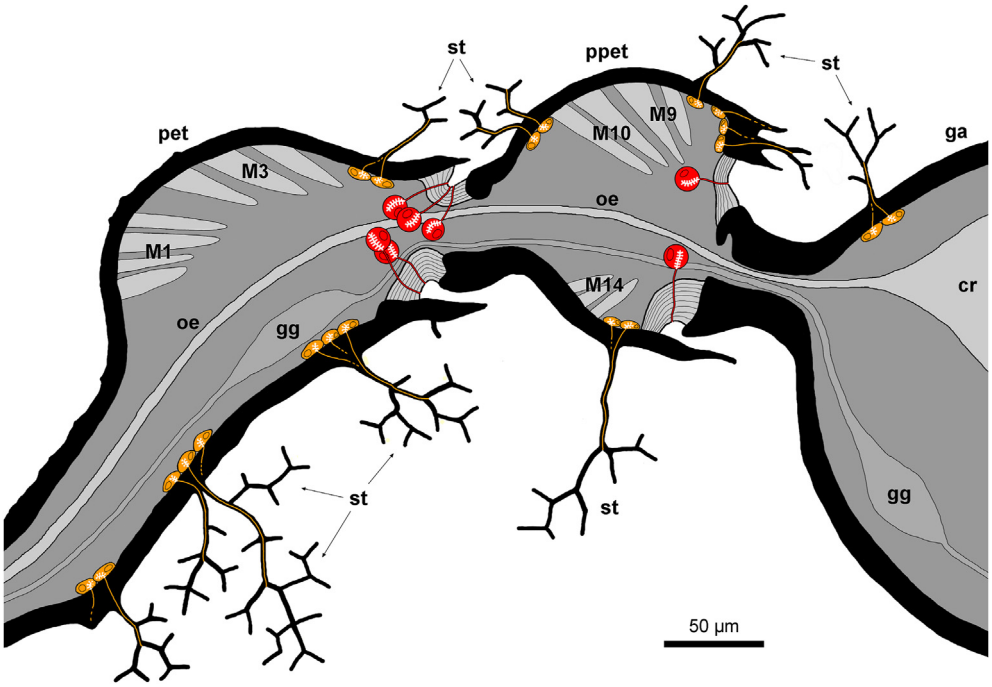

Fig. 8. Schematical presentation of the petiole (pet), postpetiole (ppet) and first gastral segment (ga) showing the distribution of the honeycomb-like spongiform tissue (st) and the associated exocrine glands. The flattened cells of the spongiform tissue gland (orange) are situated underneath the cuticular outgrowths, through which their ducts penetrate to open at the surface. The round cells of the intersegmental petiole and postpetiole glands (red) are situated in the posterior portion of the corresponding segment, their ducts open through the nearby intersegmental membrane. Note the postpetiole does not have a ganglion; the various petiolar and postpetiolar muscle groups (M) are indicated following the numbering system in Hashimoto (1996). cr: crop, gg: ganglion, oe: oesophagus.

technology, however, future work hopefully can focus on the elucidation of the chemical nature of the glandular secretion. For example, Desorption Electrospray Ionization (Takáts et al., 2004) could be used directly on the tissue without sample preparation if it could be focused on a small, specific area. Once the specific chemical compounds produced by the spongiform tissue glands have been identified, we can design a series of bio-assays to determine the function of these secretions and their role in the behavior and ecology of *Strumigenys* ants.

Author statement

Collection of material CCL-SMcG-AVS, scanning microscopy FYC, light and electron microscopy CW-JB, microCT analysis JCG-AVS, manuscript writing CW-JB. All authors approved the final version of the manuscript.

Acknowledgements

We thank An Vandoren for producing the sections for electron microscopy and Jim Nardy for his help with *S. rostrata*. The CT scan of *S. rostrata* was taken using instruments at the Beckman Institute for Advanced Science and Technology at UIUC. We are grateful to the China Scholarship Council (CSC) for a PhD scholarship grant (No. 201906300043) to CW, Ministry of Science and Technology, Taiwan, for a research grant (MOST 109-2621-B-018-001) and the UIUC Department of Entomology for funding.

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