

Morphology and ultrastructure of the infrabuccal pocket in *Strumigenys* ants

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Abstract

The morphology of the infrabuccal pocket has been studied with light and electron microscopy (SEM, TEM) in 19 species of *Strumigenys* ants. The structural organization is similar in workers, queens and males, and supports the involvement of the pocket in the filtration of food particles before they can enter the digestive tract. A carpet of posteriorly oriented bristle hairs on the hypopharynx first guide ingested food into the pocket, where large solid particles are compacted into a pellet that will be regurgitated. The remaining products enter the digestive tract through a filtering wall of parallel hair combs lining the pharynx interior that are directed against the food flow. This mechanical filtering allows only liquids and sufficiently small food particles to enter the digestive system. The wall of the infrabuccal pocket is differentiated into a conspicuous glandular epithelium, of which the ultrastructural characteristics can be understood as an adaptation against the frequent shape changes of the pocket. The gland elaborates a non-proteinaceous secretion, although its functional significance still remains unknown.

1. Introduction

Ants and other social insects cooperate as a giant superorganism with an ingenious social organization based on an amazing communication system (Hölldobler and Wilson, 1990). Each individual of the society represents an almost robotic contribution to the colony and also functions as an efficient unit with its own organismal structures. Food uptake and digestion are examples of functions that each individual has to deal with. Ant diet can vary considerably among species, ranging from strictly vegetarian species (e.g. leaf-cutting ants: Weber, 1972) or ferocious generalist predators (e.g. army ants: Gotwald, 1995), to omnivorous species (e.g. wood ants: Domisch et al., 2009) and specialist predators (e.g. *Discothyrea* and *Proceratium* that feed on arthropod eggs: Masuko, 2020).

Regardless of their diet, adult ants and aculeate Hymenoptera in general feed on liquid food only as solid particles cannot pass through the proventriculus because of the petiolar constriction (Cassill et al., 2005; Csata and Dussutour, 2019). In an elegant pioneering study in which they fed *Camponotus pennsylvanicus* workers with corundum particles of varying diameter, Eisner and Happ (1962) showed how the infrabuccal pocket acted as an efficient filter system that does not allow particles larger than 150 μm to enter the digestive tract. In similar studies, Quinlan and Cherrett (1978) found that in *Acromyrmex octospinosus* leaf-cutting ants particles as small as 10 μm are efficiently filtered by the infrabuccal pocket, while Glancey et al. (1981) found that this filtering capacity in *Solenopsis invicta* fire ants is as low as 0.88 μm and therefore makes the use of solid baits in this species limited. Structural analysis of the infrabuccal pocket has revealed the complexity of this organ with numerous filtering hairs and scales in *Acromyrmex octospinosus* (Febvay and Kermarrec, 1981), *Camponotus modoc* (Hansen et al., 1999), *Ectomyrmex javanus* (Wang et al., 2018) and *Camponotus japonicus* (Wang et al., 2019). These studies also showed that the contents of the pocket accumulated as pellets that are regurgitated on a daily basis. Hansen et al. (1999) and Mankowski et al. (2004, 2021) noted the presence of bacterial and yeast cells in the infrabuccal pocket of *Camponotus modoc* and *Camponotus vicinus*.

In the pharaoh's ant *Monomorium pharaonis* (Eelen et al., 2004) and *Protanilla wallacei* (Billen et al., 2013), the wall of the pocket and the adjacent anterior pharynx are lined with glandular epithelium, while in most other species, this is not the case. The function of this glandular differentiation of the infrabuccal pocket wall still remains unclear.

As part of a larger study examining a survey study of the exocrine system in the ant genus *Strumigenys*, the infrabuccal pocket of these ants also raised our interest. With more than 800 described species, these small predatory ants, that mainly feed on collembola, long formed the most speciose genus of the myrmicine tribe Dacetini (Bolton, 1999; Baroni Urbani and de Andrade, 2007). Recently this group was synonymized within the enlarged tribe Attini based on molecular evidence (Ward et al., 2015). *Strumigenys* ants are characterized by the presence of a number of exocrine glands that are exclusive to this genus. Bolton (1999) claimed them to have a set of five such glands, of which the existence of four could be confirmed: the apicofemoral and apicotibial glands in the legs (Billen et al., 2000), the ventral scape gland (Wang et al., 2021a) and the basimandibular gland (Wang et al., 2021c). Only the presumed 'mesopleural gland' was found to not exist (Wang et al., 2021b). In the present paper, we describe the infrabuccal pocket and its associated glandular structure in workers, queens and males of 19 *Strumigenys* species using light and electron microscopy.

2. Material and methods

Strumigenys ants were collected from queenright laboratory nests that were maintained at National Changhua University of Education, Taiwan. The artificial nests consisted of a round plastic tray 21.5 cm in diameter with a plaster of Paris floor to provide moisture, and covered with a lid. Ants were fed with *Cyphoderus albinus* springtails twice a week. Non-Taiwanese species were collected from natural nests (see Table 1 for survey of material examined). The anterior part of the head was cut off and fixed in cold 2% glutaraldehyde (buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose), followed by postfixation in 2% osmium tetroxide. Tissues were dehydrated in a graded acetone series, embedded in Araldite and sectioned with a Leica EM UC6 ultramicrotome. Semithin sections of 1 μm thickness were stained with methylene blue and thionin and examined using an Olympus BX-51 microscope, thin sections of 70 nm were double stained with uranyl acetate and lead citrate and examined using a Zeiss EM900 electron microscope. Heads of *Strumigenys formosensis* and *S. solifontis* workers were longitudinally split with a sharp razorblade, critical point dried in a Balzers CPD 030 instrument and mounted with double adhesive tape on aluminium stubs for examination under a JEOL JSM-6360 scanning microscope. All longitudinal views are shown with the anterior to the left.

3. Results

3.1 Initial food selection by mouthparts

The first contact between an insect and its potential food happens at the mouthparts (Fig. 1A,E). For catching their prey, *Strumigenys* ants are equipped with snatching mandibles, that can be rather short ('short-mandibulate species', see Table 1) or elongate trap-jaw mandibles ('long-mandibulate species', see Table 1). The maxillae are equipped with long stipites, while the most active part of the labium in food uptake is the glossa. In workers, queens and males, the latter unpaired structure consists of a retractable pad which is ornamented with 10-15 parallel combs containing numerous upward directed hairs of 2-3 μm long (Fig. 1B-D). When extended (as in Fig. 1F,H and 4A,C), the distance between the hairy combs is approx. 4-5 μm , while this is approx. 2 μm when retracted (as in Fig. 1G and 4B).

3.2 Temporary food storage in infrabuccal pocket

Food particles that pass the first screening by the mouthparts move into the oral cavity, where they are guided by the epi- and hypopharynx towards the infrabuccal pocket which essentially is a ventral outgrowth of the pharynx. The pocket has a diameter around 40 μm , which is flexible and can expand up to 70-80 μm when it is filled. The cuticular surface, especially of the hypopharynx, is particularly developed and consists of 20 μm long upright bristle hairs covering the distal upper surface hypopharynx. This is followed by a carpet of posteriorly directed hairs on the upper surface of the central and proximal hypopharynx. Similar hairs also cover the surface of the dorsal lip between the pharynx and the infrabuccal pouch (Fig. 2A,B); on microscopy sections, this dorsal lip appears as a triangular boundary that separates the upper infrabuccal pouch from the pharynx (Figs 5A-C). The posteriorly directed hairs on the dorsal lip and hypopharynx appear in microscopy sections as 3 μm long scales (Fig. 3A-C). These scales show a transitional tine pattern: upon entering the infrabuccal pocket, each scale displays 5-10 shorter tines with a length around 1 μm , and more posteriorly only smooth polygonal scales with a diameter around 5 μm remain to cover

the surface of the pocket (Fig. 2C). Food particles to be ingested have a short stay inside the infrabuccal pocket. Upon moving through the buccal tube, they must pass through a filtering wall of parallel hairy ridges on the dorsal lip (Fig. 2D) and pharynx (Figs 2E,3D) that are directed against the flow of the incoming food. Histological observation of all examined species illustrates a similar anatomical organization of the pharynx and infrabuccal pocket in workers (Fig. 4A,B), queens (Fig. 4C) and males (Fig. 4D).

3.3 Infrabuccal pocket gland

Histological sections also revealed a thickening of the infrabuccal pocket wall into a conspicuous glandular epithelium of 10-15 μm thick, whereas the epithelium lining the tegumental tissues elsewhere in the oral cavity hardly reached 2 μm (Fig. 5). Such glandular differentiation was found in workers (Fig. 5A,C,D), queens (Fig. 5B,E,F) and males (Fig. 4D), and occurred regardless of how full the infrabuccal pocket was (Fig. 5E,F). The glandular differentiation of the epithelium partly continues along the posterior part of the proximal hypopharynx (Fig. 3A,B). Longitudinal sections illustrate how the dorsal and anterior parts of the infrabuccal pocket can be closed by the dorsal lip and the central portion of the flexible hypopharynx (Fig. 5A-C). On transverse sections, the upper part of the pocket lumen which is near the dorsal lip appears Y-shaped (Fig. 5D-E). The infrabuccal pocket is not associated with any muscular supply.

Ultrastructural examination of the infrabuccal pocket wall shows the cylindrical cells of the monolayered glandular epithelium with round to oval nuclei that are mainly located in the basal portion of the cells (Fig. 6A). The cuticular inner lining of the wall near the pocket entrance displays posteriorly oriented scales (Fig. 6B), while the main part of the pocket is covered by smooth polygonal scales that are separated from each other by an apical groove (Fig. 6C; note this transition in the appearance of the scales in Fig. 2C). The cuticle contains an electron-dense outer epicuticle, an irregular exocuticle and a thick electron-clear inner endocuticle (Fig. 6B,C). The apical cell membrane of the gland cells is differentiated into a conspicuous layer of 0.5-1 μm long microvilli, while the lateral cell contacts typically show an apical desmosome followed by a winding region of septate junctions (Fig. 6C-F). In the lower region of the epithelium, lateral cell membranes of neighbouring cells appear without any special modifications (Fig. 6D,E). The cytoplasm of the cells contains smooth endoplasmic reticulum, numerous mitochondria and an occasional Golgi apparatus (Fig. 6F-H). Rough endoplasmic reticulum, however, is not present. The basal cell membrane rests upon a thin amorphous basement membrane of 50 nm and displays numerous invaginations that penetrate up to 3 μm into the cells (Fig. 6I).

4. Discussion

The structural characteristics of the mouthparts, infrabuccal pocket and pharynx in workers, queens and males of all examined *Strumigenys* species illustrate a very efficient filtering system for potential food particles. This morphological similarity regardless of sex, caste, or mandibular classification (short- vs long-mandibulate *Strumigenys* species) shows that the infrabuccal pocket has a similar function at the individual level. Particles that get through initial selection by the mouthparts find a direct route into the infrabuccal pocket by the posteriorly directed brush hairs on the dorsal lip and the proximal hypopharynx. From the pocket, valuable nutrients will continue their journey into the digestive tract while waste material is compacted into a pellet. It has been observed that such pellets are spit out on a daily basis in *Acromyrmex octospinosus* (Febvay and Kermarrec, 1981) as well as in *Ectomomyrmex javanus* (Wang et al., 2018) and *Camponotus javanus* (Wang et al., 2019).

Ingested food particles, however, upon entering through the buccal tube, have to move against a dense layer of bristle hairs that cover the dorsal lip and the pharynx lumen. They are thus subject to a thorough filtering that only allows liquids and sufficiently small solid particles to move on through the digestive tract (Eisner and Happ, 1962). The suggested trajectory of the food particles is nicely illustrated in figure 5 of Hansen et al. (1999), including a ventral influx into the infrabuccal chamber, an upward move in its posterior portion, while the contents leave the pocket again along its dorsal part (Hansen et al., 1999). The arrangement and appearance of the bristle hairs as reported here for *Strumigenys* is in agreement with that described in the few other ant species for which such information is available (*Acromyrmex octospinosus*: Febvay and Kermarrec, 1981; *Ectomomyrmex javanus*: Wang et al., 2018; *Camponotus modoc* and *C. japonicus*: Hansen et al., 1999; Wang et al., 2019). Only small detail differences exist such as the shorter scale hairs at the pocket entrance that measure 1 μm and entirely smooth polygonal scales that line the inner pocket surface in *Strumigenys*, whereas in *C. japonicus*, the scale hairs have a length of 4-6 μm and the polygonal scales are wrinkled with a more complex groove system (Wang et al., 2019). The structural similarity of the infrabuccal pocket in workers, queens and males supports its function to filter ingested food particles, as this represents a common digestive prerequisite for each individual ant. The absence of any muscles indicates that the pocket activities are indirectly regulated by changes in hemolymph pressure and/or by the movements of nearby muscles or organs.

Besides the mechanical function of filtering food particles, other functions have been attributed to the infrabuccal pocket in other ant species. The best-known example are the founding queens of *Atta* leaf-cutting ants that during their nuptial flight carry a piece of the fungus garden from their maternal nest in their infrabuccal pocket as an inoculum to start a new fungus garden at the new nest site (Weber, 1972). Similar examples of fungiculture exist in founding queens of plant-inhabiting ants from various ant lineages as *Crematogaster*, *Tetraponera* and *Azteca* ants, that equally carry fungus material in their infrabuccal pocket during colony foundation (Baker et al., 2017; Mayer et al., 2018). Fungus-growing attine ants also use their infrabuccal pockets to selectively remove the virulent parasite *Escovopsis* from their gardens, and some basal attine lineages even have a specialized worker caste that is in charge of the construction and management of the infrabuccal pellets (Little et al., 2003). Other authors have reported the presence of bacteria and yeasts from infrabuccal pockets of various ant species. These microorganisms were found at higher levels in the pocket compared to nest material and soil outside of nests, although their potential role in the digestion of nutrients or population immunity remains unknown although two of the studies indicated a nutritional role of an infrabuccal yeast in *Camponotus vicinus* (Mankowski and Morrell, 2004; Pagnocca et al., 2008; Mankowski et al., 2021). Recent studies in two predatory ponerine species (Zheng et al., 2021) and species that feed on honeydew (Zheng et al., 2022) showed that the bacterial composition in the infrabuccal pocket is related with diet. As no information exists on the presence of microorganisms in the infrabuccal pocket of *Strumigenys*, this could form an interesting study topic in future.

A striking characteristic of all examined *Strumigenys* species is the presence, in workers, queens and males, of a clear thickening of the infrabuccal pocket wall into a glandular epithelium ('class-1' following the gland classification system of Noirot and Quennedey, 1974). The glandular nature of the epithelium is supported by the well-developed apical microvilli and basal invaginations, that represent surface-increasing modifications that allow an increased transportation of metabolites. The cytoplasmic composition with numerous mitochondria and smooth endoplasmic reticulum, together with the absence of rough endoplasmic reticulum, is indicative for the elaboration of a non-proteinaceous secretion (Noirot and Quennedey, 1974; Billen and Morgan, 1998). These ultrastructural characteristics were also found in *Monomorium pharaonis*, and exclude an eventual digestive

function (Eelen et al., 2004). The intercellular contacts display a typical arrangement with an apical desmosome, followed by a sinuous region with septate junctions and more basally undifferentiated cell junctions. Similar differentiations also occur in the epithelial lining of the infrabuccal pocket of *Camponotus japonicus*, and can be regarded as a strengthening adaptation of the cells to the frequent shape changes the pocket undergoes (Wang et al., 2019).

The functional significance of the glandular differentiation of the infrabuccal pocket wall is not clear. *Strumigenys* shares this feature with *Monomorium pharaonis* (Eelen et al., 2004) and *Protanilla wallacei* (Billen et al., 2013). From own unpublished observations on almost 30 species representing 10 ant subfamilies, we know that the infrabuccal pocket also has a clear epithelial gland only in *Aphaenogaster senilis*, *Discothyrea sauteri*, *Leptanilla clypeata* and *Pseudomyrmex schuppi*). It remains unclear whether this reflects any link with the ants' diet, as the dietary spectrum of these species covers a wide range: *Strumigenys* are mainly predators on collembola, *Discothyrea* feed on arthropod eggs (Masuko, 2020), *Leptanilla* hunt for geophilomorph centipedes (Ito and Yamane, 2020) and *Protanilla* for japygid diplurans (Ito et al., 2021). Conversely, *Monomorium pharaonis* and *Aphaenogaster senilis* are omnivorous. Although the predatory lifestyle of 4 of the above genera may be suggestive, a glandular differentiation of the infrabuccal pocket epithelium is completely lacking in *Proceratium japonicum* (although this species has a diet that is very similar to *Discothyrea*), and this is also the case in generalist predators as *Dorylus orientalis* and *Labidus praedator* (Billen, unpubl. obs.). The occurrence of the infrabuccal gland in all examined species of *Strumigenys* therefore remains enigmatic, and adds to the peculiar interest this genus offers for exocrine gland research (Bolton, 1999; Wang et al., 2021a,b,c).

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Table 1. Ant material with collection locality and number of workers (W), queens (Q) and males (M) examined in this study using scanning microscopy (SEM) and histological sections (figures as “4/1” mean that 4 specimens have been studied using histological techniques, 1 of these with TEM).

species		Collection locality	SEM			histology/TEM		
			W	Q	M	W	Q	M
short-mandibulate <i>Strumigenys</i>	<i>S. benten</i>	Nantou County, Taiwan				2		3
	<i>S. canina</i>	Kobe, Japan				2		
	<i>S. elegantula</i>	Nantou County, Taiwan				1/1		
	<i>S. emmae</i>	Tainan City, Taiwan				3		
	<i>S. hexamera</i>	Kagawa, Japan				4/1		
	<i>S. kichijo</i>	Taipei, Taiwan				1/1		
	<i>S. leptothrix</i>	Nantou County, Taiwan				1		
	<i>S. mutica</i>	Nantou County, Taiwan				4/1	2	1/1
	<i>S. sauteri</i>	Nantou County, Taiwan				3	2	
long-mandibulate <i>Strumigenys</i>	<i>S. chuchihensis</i>	Xinchu County, Taiwan				1		
	<i>S. formosensis</i>	Nantou County, Taiwan	9			5		
	<i>S. hispida</i>	Nantou County, Taiwan				4/3		
	<i>S. koningsbergeri</i>	Bogor, Indonesia				1/1		
	<i>S. lacunosa</i>	Nantou County, Taiwan					1/1	
	<i>S. liukueiensis</i>	Nantou County, Taiwan					4/1	
	<i>S. minutula</i>	Pingtung County, Taiwan	4			1/1		
	<i>S. perplexa</i>	Clyde Mountain, NSW, Australia				1/1		
	<i>S. rogeri</i>	Nantou County, Taiwan					4/1	
	<i>S. solifontis</i>	Nantou County, Taiwan	6			5		

Figure legends

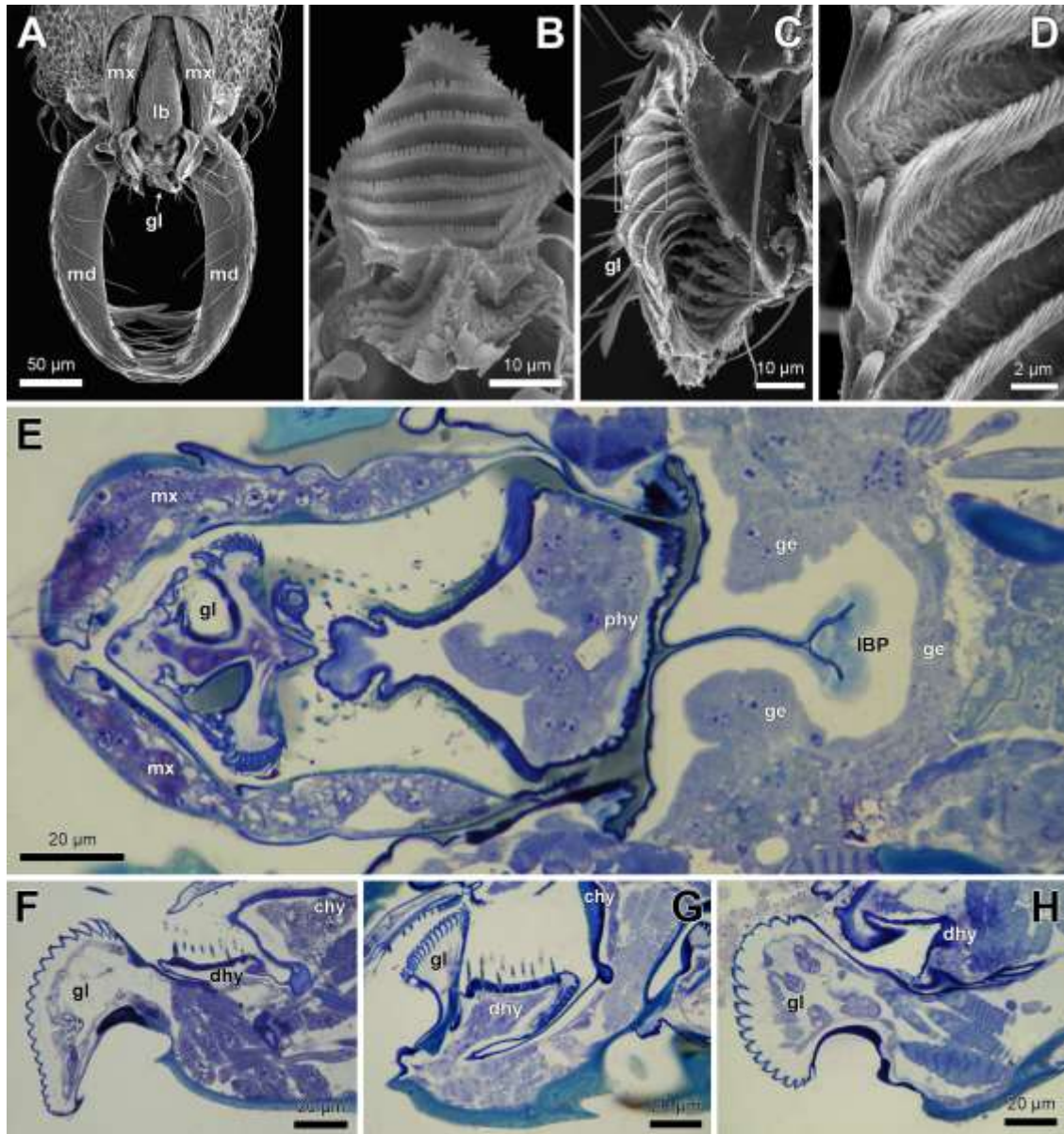


Fig. 1. A-D, scanning micrographs: **A.** Mouthparts of *S. minutula* worker in ventral view, **B.** Glossa of *S. solifontis* worker in anteroventral view. **C.** Lateral view of partly cut glossa of *S. formosensis* worker, **D.** enlarged detail view of framed area in C. E-H, semithin sections: **E.** Frontal section through mouthparts and infrabuccal pocket (IBP) of *S. chuchihensis* worker, note glandular epithelium (ge) lining wall of IBP. **F.** Longitudinal sections of labium with extended glossa of *S. canina* worker, **G.** Retracted glossa of *S. kichijo* worker, **H.** Extended glossa of *S. benten* male. chy: central hypopharynx, dhy: distal hypopharynx, gl: glossa, lb: labium, md: mandible, mx: maxilla, phy: proximal hypopharynx.

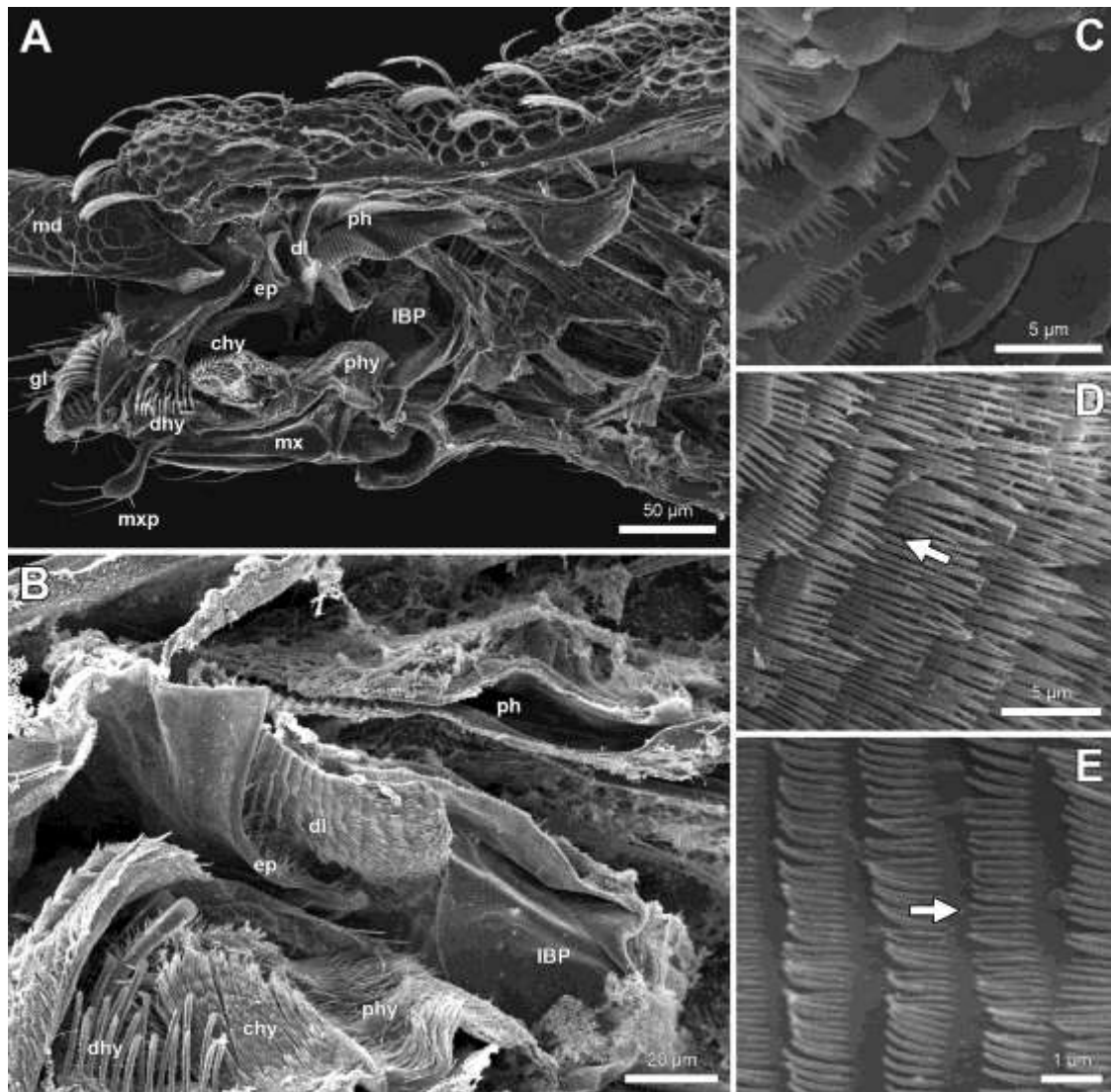


Fig. 2. Scanning micrograph internal views from longitudinally split anterior heads. **A,B.** *S. formosensis* worker, showing survey of mouthparts and location of infrabuccal pocket. **C.** Detail of inner anteroventral wall of infrabuccal pocket in *S. solifontis* worker. **D.** Detail of dorsal lip surface in *S. formosensis* worker. **E.** Detail of inner pharynx wall in *S. solifontis* worker. chy: central hypopharynx, dhy: distal hypopharynx, dl: dorsal lip, ep: epipharynx, gl: glossa, IBP: infrabuccal pocket, md: mandible, mx: maxilla, mxp: maxillary palp, ph: pharynx, phy: proximal hypopharynx, white arrows indicate direction of ingested food flow.

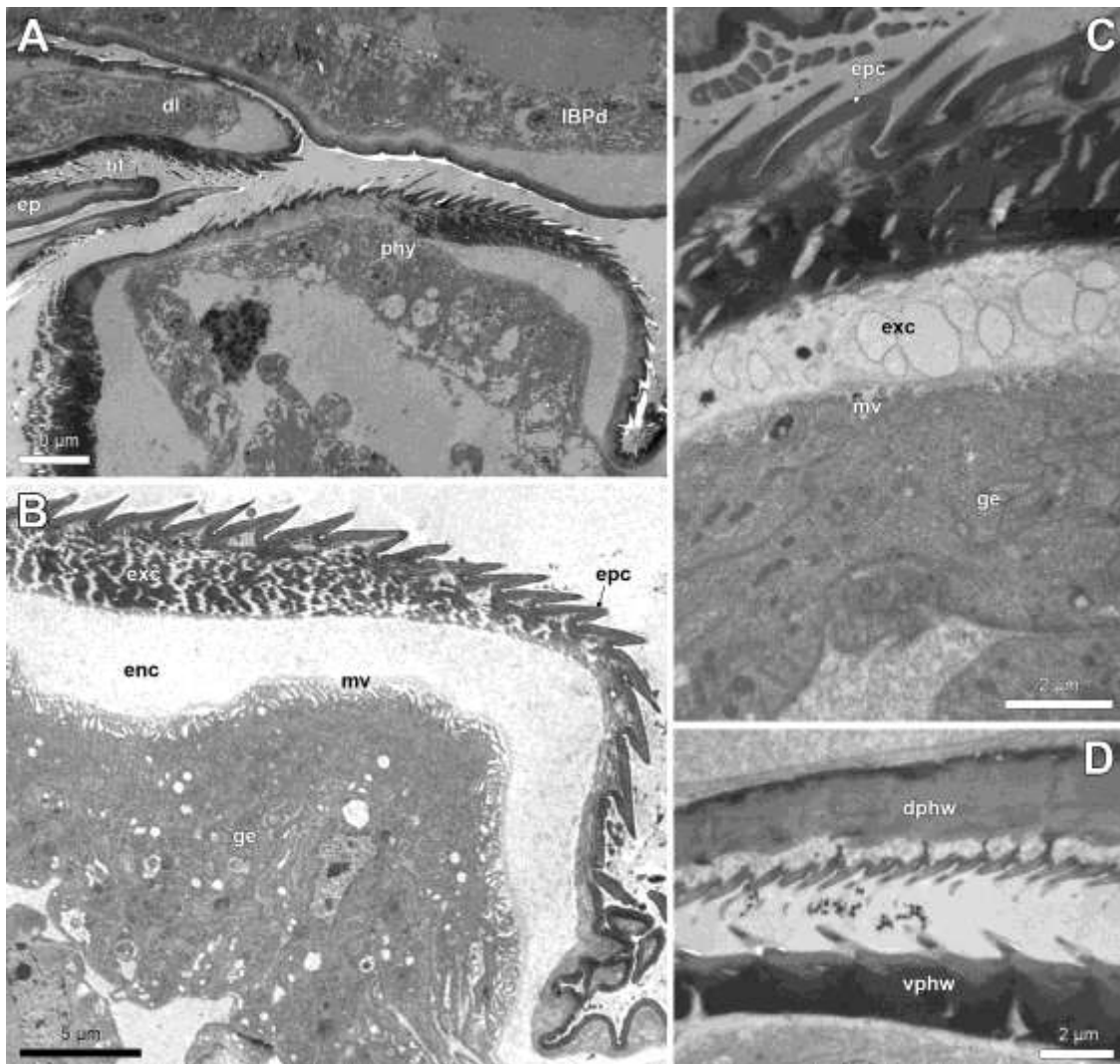


Fig. 3. Electron micrographs of proximal hypopharynx (phy) and pharynx. **A.** Junction between pharynx, proximal hypopharynx and infrabuccal pocket entrance of *S. lacunosa* queen. **B.** Proximal hypopharynx of *S. perplexa* worker. Note transition from hairy scales to smooth scales at IBP entrance in lower right corner. **C.** Detail of proximal hypopharynx of *S. minutula* worker. **D.** Anteriorly directed bristle hairs in pharynx of *S. liukueiensis* queen. bt: buccal tube, dhy: distal hypopharynx, dl: dorsal lip, dphw: dorsal pharynx wall, enc: endocuticle, ep: epipharynx, epc: epicuticle, exc: exocuticle, ge: glandular epithelium, IBPd: infrabuccal pocket dorsal wall, mv: microvilli, vphw: ventral pharynx wall.

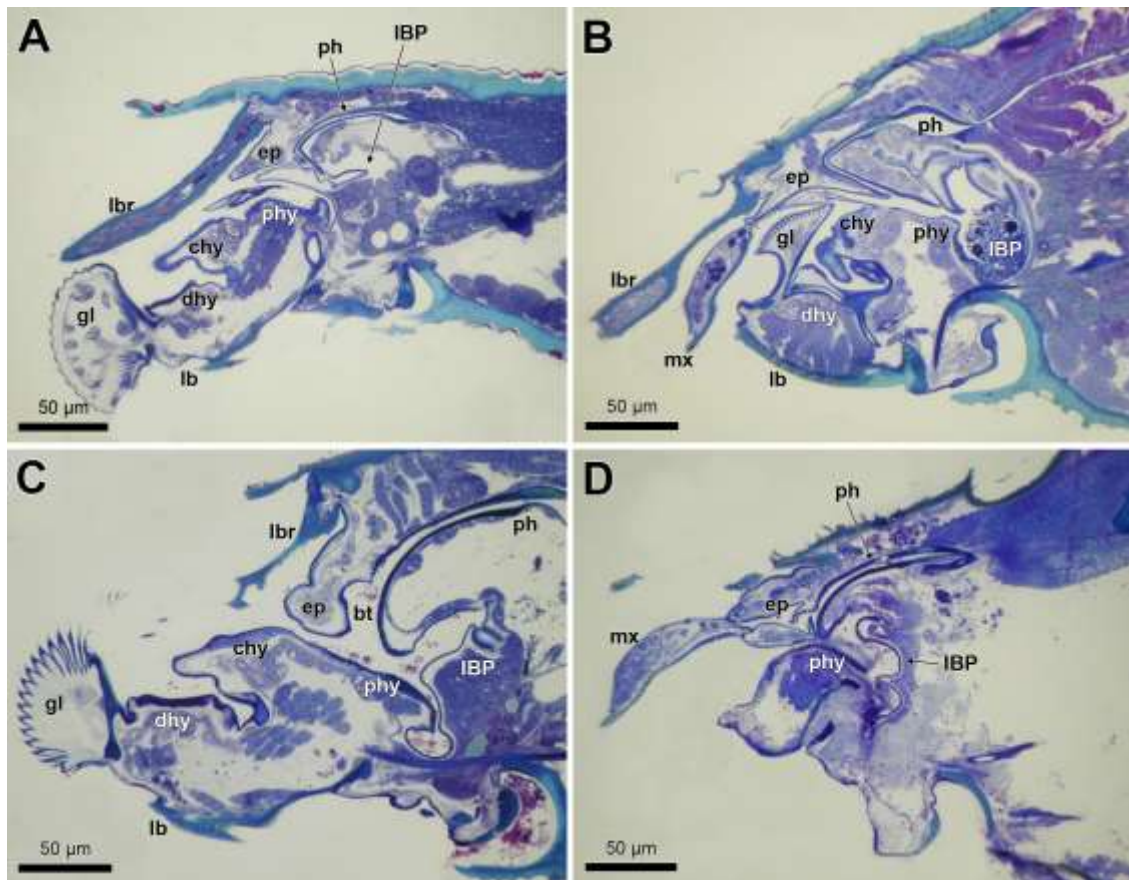


Fig. 4. Semithin longitudinal sections along head midline of anterior head with labium extended (A,C) or retracted (B), and with empty (A,D), partly filled (C) or filled infrabuccal pocket (B). **A.** *S. canina* worker, **B.** *S. hexamera* worker, **C.** *S. mutica* queen, **D.** *S. mutica* male. bt: buccal tube, chy: central hypopharynx, dhy: distal hypopharynx, ep: epipharynx, gl: glossa, IBP: infrabuccal pocket, lb: labium, lbr: labrum, mx: maxilla, ph: pharynx, phy: proximal hypopharynx.

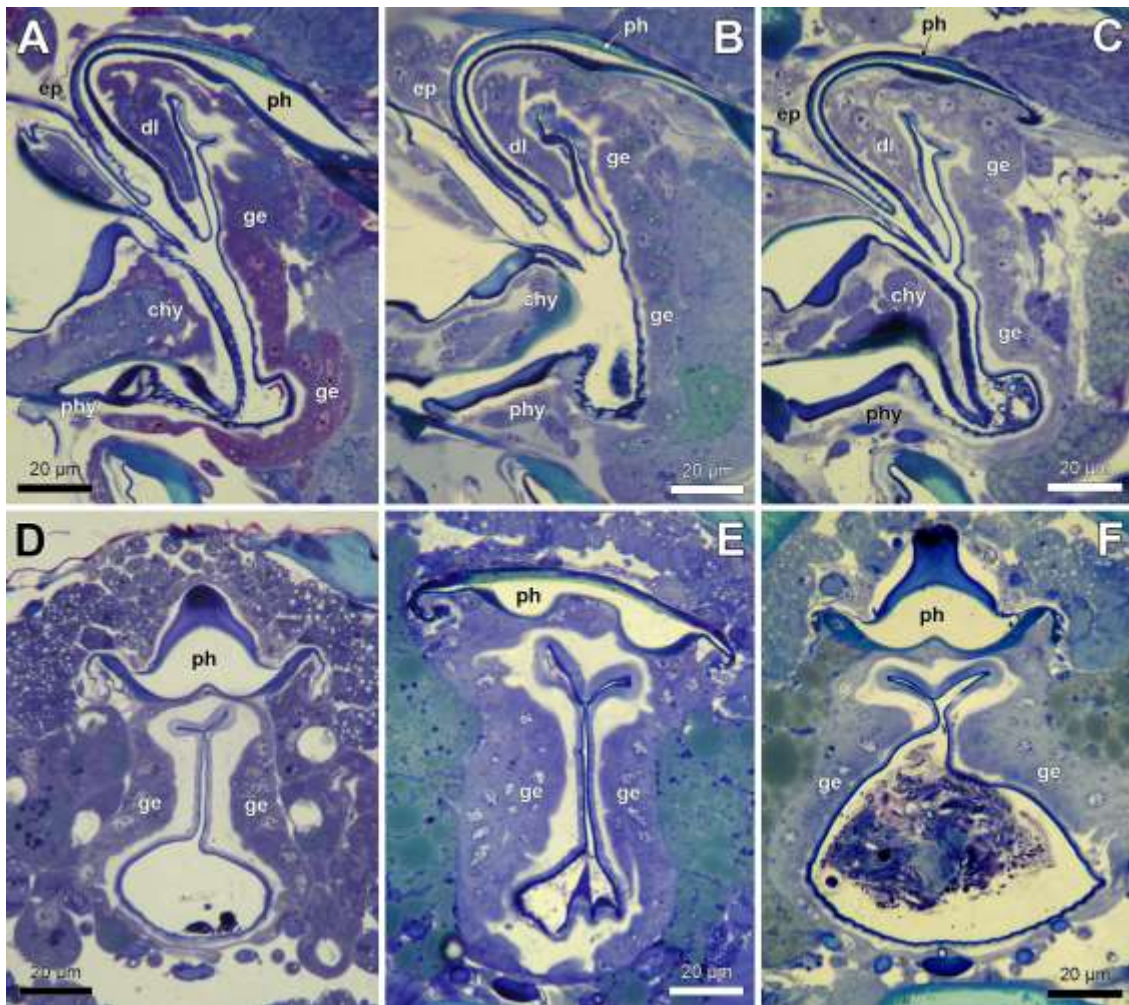


Fig. 5. Semithin section details of infrabuccal pocket in longitudinal (A. *S. perplexa* worker, B. *S. liukueiensis* queen, C. *S. hexamera* worker) and transverse view (D. *S. canina* worker, E. *S. sauteri* queen with empty IBP, F. *S. sauteri* queen with full IBP), showing presence of conspicuous glandular epithelium (ge). chy: central hypopharynx, dhy: distal hypopharynx, dl: dorsal lip, ep: epipharynx, ph: pharynx, phy: proximal hypopharynx.

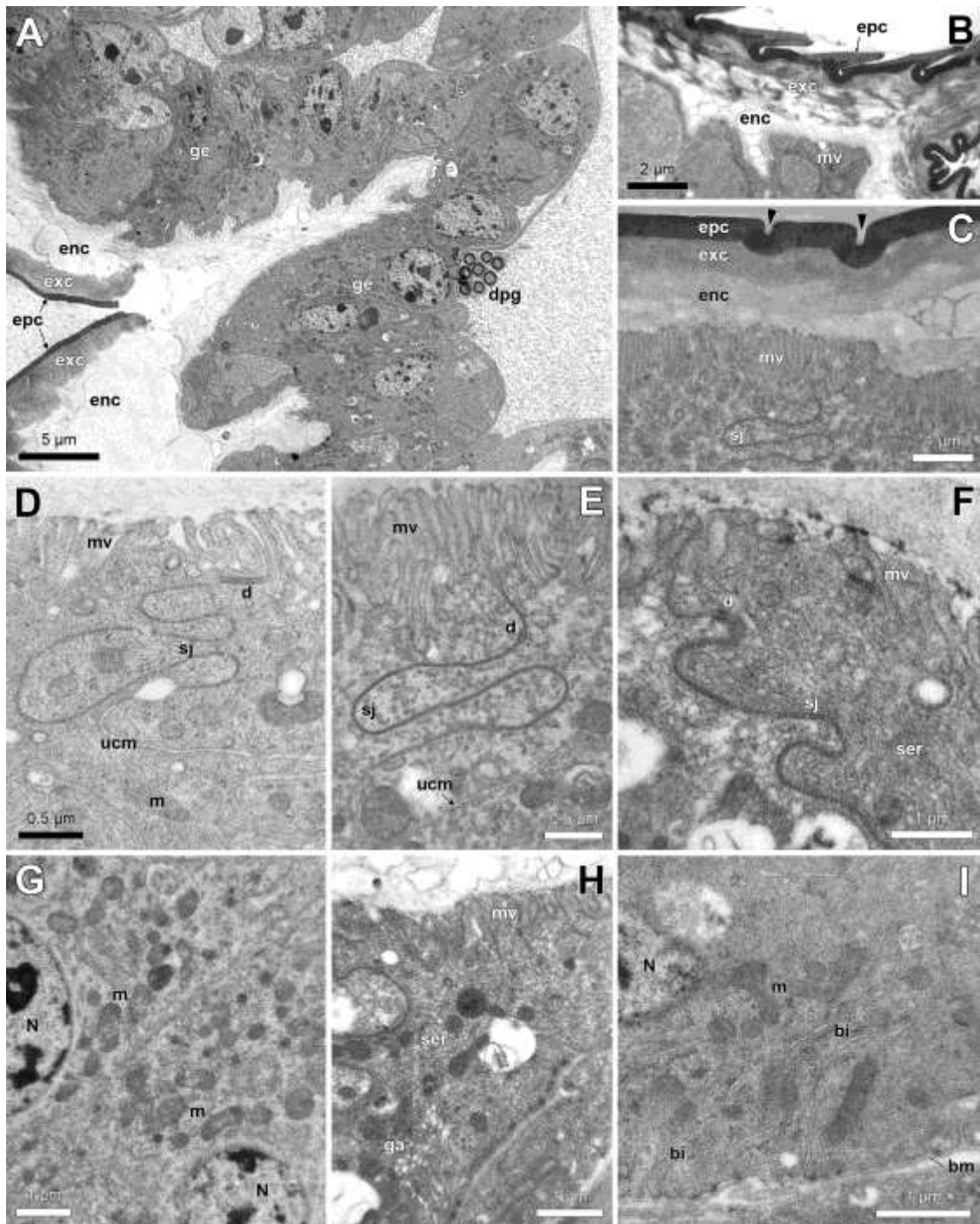


Fig. 6. Electron micrographs of glandular epithelium (ge). **A.** Survey view in *S. elegantula* worker. **B.** Posteriorly oriented hairy scale of proximal hypopharynx of *S. minutula* worker. **C.** Smooth scales lining ventral wall of infrabuccal pocket of *S. hispidula* worker, arrowheads indicate grooves between scales. **D.** Apical junction between gland cells in *S. mutica* worker. **E.** Apical junction between gland cells in *S. hispidula* worker. **F.** Apical junction between gland cells in *S. konigsbergeri* worker. **G.** Central cytoplasm with mitochondria (m) in *S. hispidula* worker. **H.** Apical cytoplasm with smooth endoplasmic reticulum (ser) and Golgi apparatus (ga) in *S. minutula* worker. **I.** Basal cytoplasm with mitochondria and basal invaginations in *S. kichijo* worker (bi: basal invaginations, bm: basement membrane). d: desmosome, dpg: ducts prepharyngeal gland, enc: endocuticle, epc: epicuticle, exc: exocuticle, ga: Golgi apparatus, mv: microvilli, N: nucleus, ser: smooth endoplasmic reticulum, sj: septate junction, ucm: unmodified cell membrane.