RESEARCH ARTICLE

Morphology and ultrastructure of the infrabuccal pocket in *Camponotus japonicus* Mayr (Hymenoptera: Formicidae)

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Abstract

The infrabuccal pocket (IBP) is a peculiar structure in ants whose detailed morphology and function still remain poorly known in most species, despite first being described more than a century ago. This paper describes the morphology of the IBP in *Camponotus japonicus* using light and electron microscopy. The IBP occurs in workers, queens and males, with similar anatomical characteristics in each caste. The size and shape of the IBP are changeable depending on the degree it is filled due to a daily pattern of pellet ejecting. Dissection data show that the IBP is usually empty in the morning and full by mid-day. The long ornamented hairs covering the inner wall of the prepharynx and the anterior part of the IBP form an effective filtering system for solid particles. The pocket wall thickens posteriorly, possibly providing special bending and stretching properties. The cuticle-lined epithelium around the entire pocket is formed by a uniform cell layer without glandular function, but also giving it mechanical strength. The structural features as described are in agreement with the need for filtering solid particles that form part of the diet of this ant species.

Keywords *Camponotus* ants · Infrabuccal pocket · Mouthparts · Food filtration · Microscopy

Introduction

Ants are a successful group of social insects, with different species varying dramatically in colony size from as few as some tens to several millions of individuals (Hölldobler and Wilson 1990). Although ant colonies represent a superorganism due to the interplay between their members, each single individual ant is equipped with the anatomical infrastructure for efficient food uptake. Food collection at the colony level is enabled by division of labour with forager workers collecting the food and distributing it throughout the colony via trophallaxis.

The digestive tract of each individual ant is formed by highly specialized organs for the uptake of food particles, the assimilation of the nutritive substances contained in the food, and the elimination of the recalcitrant elements and wastes. When ants initially collect food particles in their mouth, the infrabuccal pocket (IBP) serves as a filtering device that allows only fluids and sufficiently small particles to move further into the digestive tube (Bailey 1920; Eisner and Happ 1962; Gotwald 1969).

The IBP is an unpaired ventral sac-like outgrowth of the hypopharynx in the preoral cavity (Gotwald 1969; Richter et al. 2019), that is also well-developed in wasps (Edwards 1980). In honeybees, the IBP occurs as an inconspicuous infolding of the hypopharynx without a particular function (Snodgrass 1956), while it is absent in termites (J. Šobotník pers. comm.). In ants, its structure and function were described by several early myrmecologists (Janet 1895;

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Bailey 1920; Eisner and Happ 1962). The IBP mainly acts as an effective filtering device, and its filtration capacity varies according to species and worker size (Eisner and Happ 1962; Quinlan and Cherrett 1978; Glancey et al. 1981). The pioneer study by Eisner and Happ (1962) in *Camponotus pennsylvanicus*, studying workers fed with differently sized corundum particles, elegantly documented how the IBP prevented particles larger than 150 μm to enter the digestive tract. Eelen et al. (2004) described a novel glandular epithelium lining the infrabuccal cavity and anterior pharynx in both workers and queens of the pharaoh's ant *Monomorium pharaonis*, with action as lubricant, digestion, filtering solid food particles, or grooming among the possible functions of this glandular differentiation of the IBP (Eelen et al. 2004). In the fungus-growing ants (Attini), the IBP plays a special function as carrier of a mycelium of the fungus garden by founding gynes (Hölldobler and Wilson 1990; Mueller et al. 2001). A similar situation has been recently reported for plant-inhabiting *Azteca* ants, of which the alate queens carry fungus material in their IBP that probably ensures the fungal transmission to the host plant (Mayer et al. 2018). The IBP can also be used to prevent the invasion and spread of microbial parasites such as the specific fungus garden parasite *Escovopsis* (Little et al. 2003, 2006).

Camponotus japonicus is one of the most common and widespread ant species in China, and is a natural enemy for many forest pests (Wu and Wang 1995). The species is usually monogynous but can have up to 3 queens with colonies that can reach a colony size of over 4000 workers (Wang et al. 1991). Their main diet consists of honeydew collected from aphids and small arthropod preys (Wu and Wang 1995). Given the presence of solid food in the *C. japonicus* diet, we here aim to test the hypothesis that the IBP is adapted for the filtering of such solid material. To test this hypothesis, we examined the morphology and ultrastructure of the IBP of the various castes of *C. japonicus* using light (LM) and electron microscopy (SEM and TEM), aiming to illustrate the filtering features of the IBP in this species. Our study complements the behavioural work on *C. pennsylvanicus* (Eisner and Happ 1962) and the gross anatomical work on *C. modoc* (Hansen et al. 1999), which will hopefully enable comparative studies and argumentations on the evolution of the IBP as a filtering structure.

Materials and methods

Ant material

Minor and major workers, alate queens and males of *C. japonicus* were collected in May and June of 2018 and 2019 on the campus of Northwest A&F University, Yangling, Shaanxi Province, China. Prior to their preparation

for microscopy, the ants were placed in a refrigerator for 10 min to reduce their activity, and were then dissected under a Leica EZ4HD microscope.

Dissection data of IBP filling status

To assess the filling degree of the IBP in minor and major workers, additional ants were collected from five field colonies from the same locality in the morning (between 8 and 9 am; $N = 38$ minor, 37 major), noon (between 1 and 2 pm; $N = 21$ minor, 13 major) and evening (between 6 and 7 pm; $N = 22$ minor, 10 major) on days with warm weather when the ants were fully active ($> 25 °C$). The ants were dissected and the filling status of their IBP examined. When empty, the IBP is transparent and, therefore, hardly or not visible at all during dissection; when full, it appears turgid, with irregular shapes when it is partly filled.

Light microscopy (LM)

The heads of three individuals of each caste were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Postfixation was carried out 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 (Wetzlar, Germany). Serial semithin sections with a thickness of 1 μm were stained with a 0.1% solution of methylene blue and thionin. Sections were viewed under an Olympus BX-51 microscope (Tokyo, Japan), equipped with an Olympus Camedia C-3040 Zoom digital camera (Tokyo, Japan). Images were analysed using the Olympus DP-Soft programme. Longitudinal section figures in this paper are shown with the anterior to the left.

Transmission electron microscopy (TEM)

Ten IBPs (five minor and five major workers) were carefully excised from the head, torn open, and fixed in 2.5% glutaraldehyde for 12 h at 4 °C and then transferred into 1% osmium tetroxide (pH 7.2). After dehydration in a graded acetone series, samples were infiltrated in a 1:1 mixture of acetone and propylene oxide, pure propylene oxide, and a 1:1 mixture of propylene oxide and Araldite® before embedding in pure Araldite®. The samples were sectioned with a Leica-Ultracut microtome (Wetzlar, Germany), and double-stained 80-nm-thin sections were examined with a Hitachi HT770 transmission electron microscope (Tokyo, Japan).

Scanning electron microscopy (SEM)

The heads of five major workers were cut longitudinally to expose the IBP. The IBPs of ten other workers (five minor

and five major) were carefully excised and torn open to reveal the interior surface. Samples were fixed in 2.5% glutaraldehyde (in phosphate-buffered saline, 0.1 M, pH 7.2). After four rinses (20 min each) in this PBS-buffer, the samples were dehydrated in an ethanol series and dried in 100% acetone. The samples were coated with gold after critical point-drying, then observed and photographed under a Hitachi S-3400N scanning electron microscope (Tokyo, Japan).

Results

When ingested, food particles are expected to pass through an initial filtering system formed by the labium before they reach the IBP. The *C. japonicus* labium contains an intricate system with parallel rows of fine hairs with a length of approx. 10 µm and a width of 1 µm. The hairs have a curved distal end that points posteriorly (Fig. 1a–c).

The IBP is situated underneath the anterior part of the pharynx, where it appears as a sac-like outgrowth of the hypopharynx. The pocket is found in minor and major workers, queens (alate as well as dealate) and males, with a similar structural appearance in each caste (see Fig. 4a–d). The size of the pocket is changeable depending on the degree it is filled. The full pocket in minor workers reaches a diameter of 649.0 ± 93.7 µm ($n = 10$), in major workers it measures 853.1 ± 90.6 µm ($n = 8$). Precise measurements of the empty

IBP could not be made as it is almost transparent, wrinkled and is easily torn apart during dissections (Fig. 2b). Dissection data of minor and major workers showed that the IBP was empty in more than 80% of the workers in the morning, whereas it was full in the majority of the individuals at noon and in the evening (Table 1). The full IBP appears as a turgid sand-coloured sac (Fig. 2a), while in the empty condition it is transparent and wrinkled (Fig. 2b). The contents of the filled IBP appear as a disorderly mass of material, that together forms a pellet (Fig. 2c, d). Although most of the contents can hardly be recognized, it also includes isolated cuticular inclusions that represent remnants of ingested arthropods.

The anterior part of the IBP is connected to the pharynx with dorsal and ventral valve-like structures that play a role in the opening and closing of the pocket. The dorsal roof of the prepharynx forms a downward anterior lip that channels incoming food into the IBP before it reaches the pharynx (Figs. 3a, b, 4a–d). The anterior portion of the IBP is separated from the pharynx by a hairy dorsal lip that forms a dorso-anterior closing structure for the IBP (Fig. 4a–d). This dorsal lip has a ventral equivalent which is formed by an upward lobe of the labium and the hypopharynx. This ventral lip acts as the ventro-anterior closing device (Fig. 4a–d), with a surface of small posteriorly pointing combs with 3-µm-long hairs (Fig. 3c).

The internal surface of the IBP reveals a characteristic pattern of cuticular scales, of which the appearance
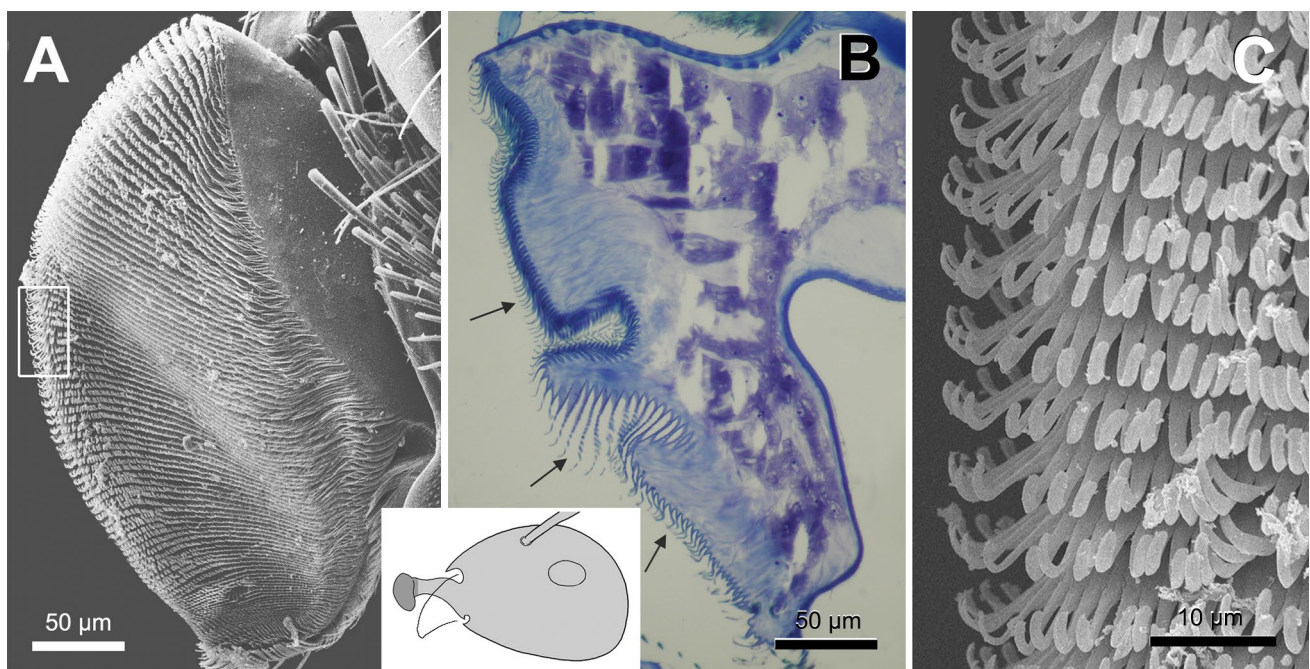

Fig. 1 Images of the labium of major workers. **a** Scanning micrograph overview of the labium. **b** Semithin section through labium showing regular pattern of small hairs (arrows). **c** Detail of framed part in **a**. The inset shows a profile view of a worker head with extended labium, the darker grey part is shown in **a** and **b**
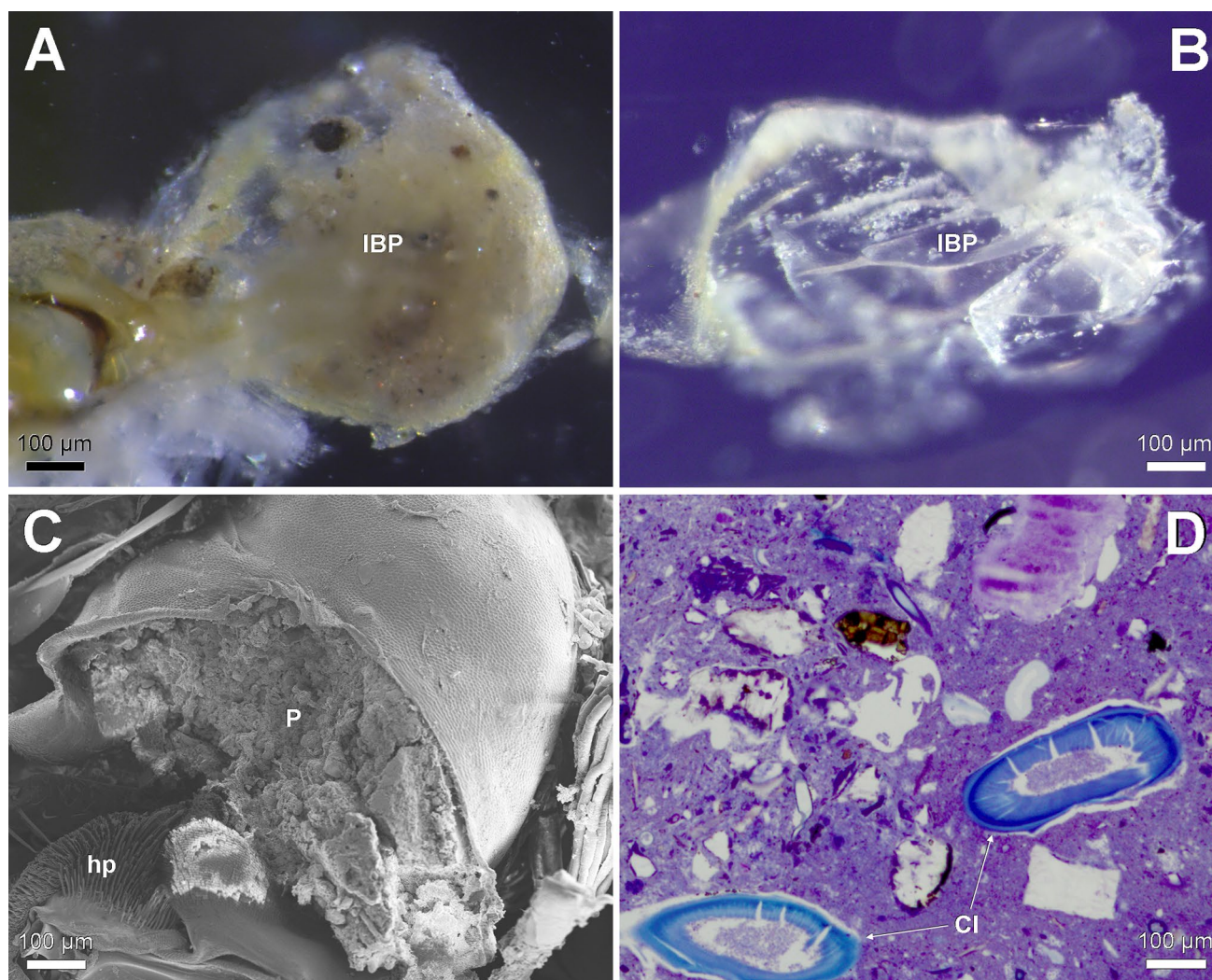
Fig. 2 Dissection images and contents of the IBP. **a** Turgid full IBP (minor worker). **b** Transparent and wrinkled empty IBP (minor worker). **c** Scanning electron micrograph of partly torn open full IBP of major worker showing exposed pellet contents (P) and the hypopharyngeal plate (hp). **d** Semithin section of disorderly contents of full IBP of minor worker. Note cuticular inclusions (CI) that represent remnants of ingested arthropods. **a** and **b** by Lili Chang, **d** by Wenjing Xu

Table 1 Dissection data of minor and major workers collected in the morning, noon and evening, showing the numbers and percentages of ants with empty vs. filled IBP (when empty, the IBP is transparent and, therefore, is hardly or not visible, the IBP was considered full when it appeared turgid)

Time	Caste	Total number	Empty		Full	
			Number	Percentage (%)	Number	Percentage (%)
8.00–9.00	Minor worker	38	33	86.8	5	13.2
	Major worker	37	30	81.1	7	18.9
13.00–14.00	Minor worker	21	2	9.5	19	90.5
	Major worker	13	0	0.0	13	100.0
18.00–19.00	Minor worker	22	1	4.5	21	95.5
	Major worker	10	2	20.0	8	80.0

changes from anterior to posterior. The anterior part of the IBP, at the level of the dorsal lip, is very hairy (Fig. 3d). This results from the occurrence of many fork-shaped scales with 6–9 tine-like filtering hairs, that have a length of 10–15 µm and are directed posteriorly. The central part of the IBP is internally covered with polygonal scales with 4–6 posteriorly oriented hairs that have a length of 4–6 µm (Fig. 3e). The scales are separated
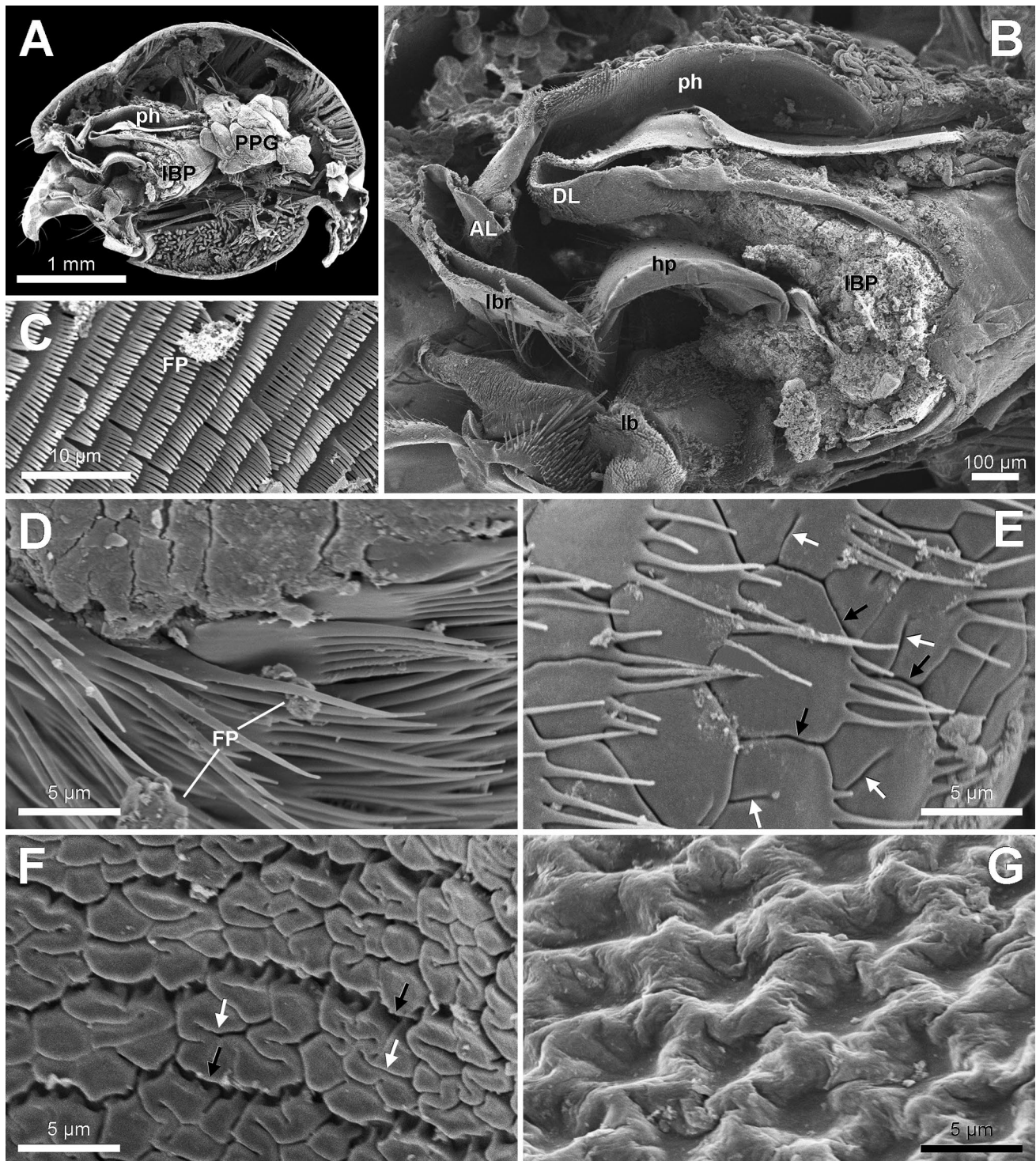
Fig. 3 Scanning electron micrographs showing the location and details of the IBP in major workers. **a** Inside view of longitudinally split head showing IBP positioned ventrally of the pharynx (ph) and the postpharyngeal gland (PPG). **b** Detail view of the IBP with the anterior (AL) and dorsal lip (DL), *hp* hypopharyngeal plate, *lb* labium, *lbr* labrum. **c** Detail of filtering hairs of ventral lip. **d** Close-up of scales with posteriorly pointing tine-like filtering hairs on dorsal lip (note food particles (FP) stuck by the hairs). **e** More posteriorly in the IBP, the filtering hairs become fewer and shorter. Note grooves between adjacent scales (black arrows) and grooves in central part of scales (white arrows). **f** Scales without hairs in the most posterior portion of the IBP, with grooves separating adjacent scales (black arrows) and star-shaped grooves on surface of scales (white arrows). **g** External appearance of the IBP showing a reticulate pattern of polygonal surfaces lined with thickened margins
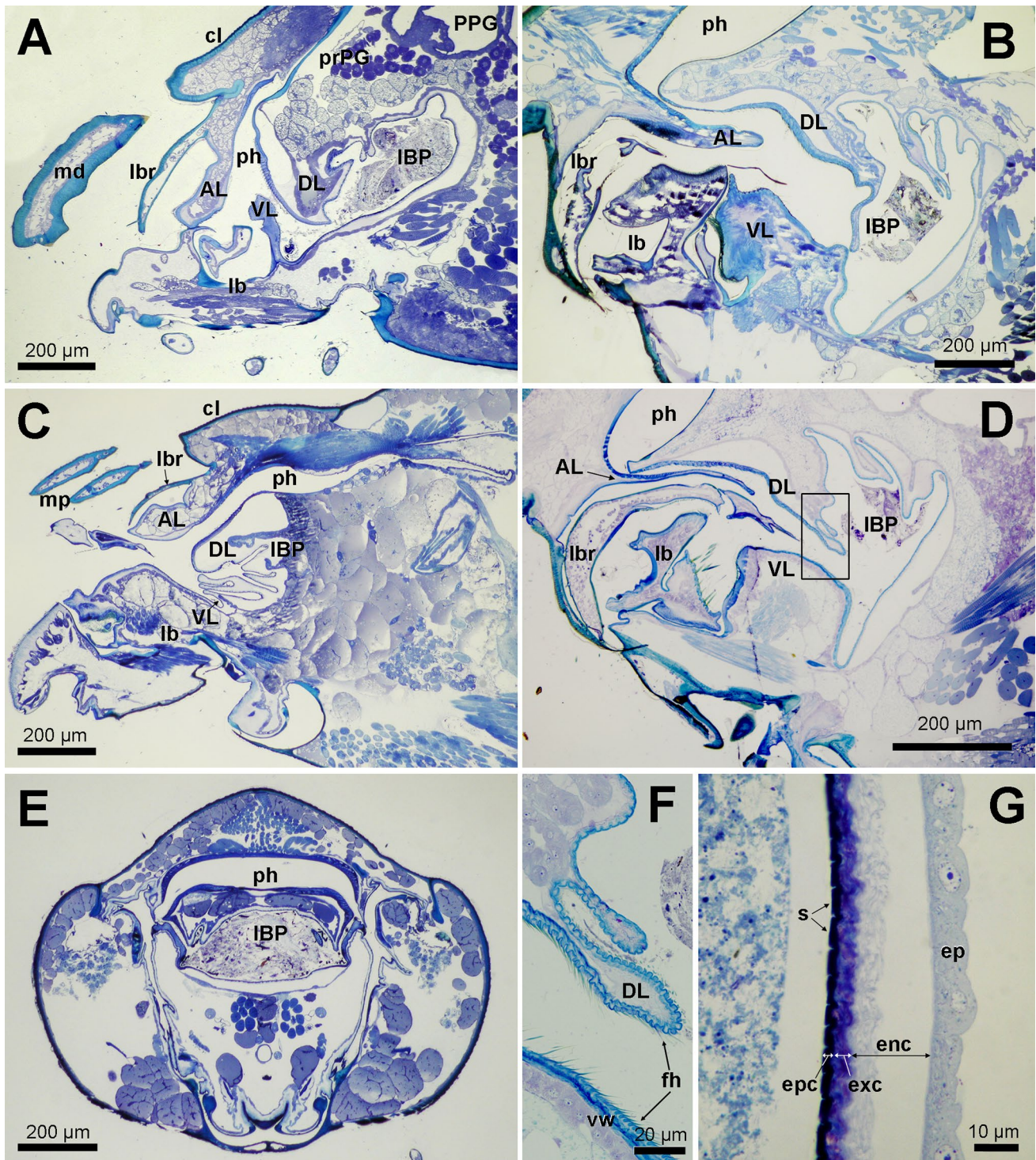
Fig. 4 Semithin histological sections through the head and details of the IBP. **a–d** Longitudinal sections through the head of a minor worker (**a**), major worker (**b**), male (**c**) and alate queen (**d**; framed part is enlarged in F), showing the IBP with the complicated occurrence of an anterior (AL), dorsal (DL) and ventral lip (VL) in the anterior part, that separate the IBP from the pharynx (ph). **e** Transverse section through male head with filled IBP. Note extended wall of filled IBP (**a**, **e**) compared with wrinkled wall in empty (**c**) or slightly filled IBP (**b**, **d**). **f** Longitudinal section through dorsal lip and ventral wall (vw) of IBP in alate queen, showing the long posteriorly directed filtering hairs (fh). **g** Detail of posterior wall of IBP in major worker with single epidermis (ep) and multi-layered cuticle (enc: endocuticle, exc: exocuticle, epc: epicuticle). *cl* clypeus, *lb* labium, *lbr* labrum, *md* mandible, *mp* mouthparts, *prPG* propharyngeal gland, *PPG* postpharyngeal gland, *s* scales

by grooves, with shorter grooves appearing on the central surface of each scale (see black, respectively, white arrows in Fig. 3e). In the most posterior distal part of the IBP, the scales are wrinkled without any hairs. The scales have a diameter of 6.4 ± 0.3 μm ($n = 10$), and are separated by broad grooves of 0.5–1 μm, while narrow star-shaped grooves of 0.2 μm mark the central scale surface (Fig. 3f). The external surface of the IBP shows a reticulate pattern of polygonal areas that are lined with thickened margins (Fig. 3g, see also Fig. 2c). These surfaces have a diameter of 6.3 ± 0.4 μm ($n = 10$), and probably correspond with the internal appearance of the scales (compare Fig. 3f, g).

Semithin sections reveal the histological organization of the IBP, with a mostly straight wall lining in the full IBP (Fig. 4a, e) and a wrinkled wall in the empty IBP (Fig. 4b–d). The wall of the IBP is essentially formed by an epidermis with a cuticular lining (Fig. 4g). The lip structures and the anterior portion of the IBP clearly show the cuticular scales bearing long posteriorly pointing filtering hairs (Fig. 4f), while the posterior part of the IBP only shows scales without any hairs. The cuticle has a thickness of around 10–12 μm in the anterior part up to 40 μm posteriorly. It is made up of a dark external epicuticle of 1 μm thickness, an exocuticle of medium electron density and a thickness of 3–4 μm, and a pale endocuticle with a variable thickness between 5 and 35 μm (Fig. 4a–d, g). The thickness of the endocuticle in all inspected ants considerably increases in the distal part of the IBP (Fig. 4a–d). In all castes, the epithelium has a thickness around 20 μm anteriorly at the level of the dorsal lip, but is elsewhere restricted to 6–10 μm (Fig. 4g). We did not find any muscles associated with the IBP.

Ultrastructural examination of the cuticular scales shows an electron-dense epicuticular layer with a thickness of 1 μm (Fig. 5a–c). The scales are separated by grooves at the luminal side, which give the cuticular surface an irregular outline. In the anterior part of the IBP, these grooves are up to 2 μm deep (Fig. 5a), whereas in the posterior part shallow grooves around 1 μm in depth separate the scales (black arrows in Fig. 5b) and smaller grooves occur on the top surface of the scale (white arrows in Fig. 5b). A pale and fibrillar endocuticle of irregular thickness forms the major part of the cuticular wall of the IBP. The hairy scales in the anterior part of the IBP are mainly epicuticular outgrowths (Fig. 5a). Numerous round to oval structures of 1–3 μm are encountered inside the IBP (Fig. 5b), with a morphology resembling the one of yeast cells. The cells of the epidermal wall have a cytoplasm that is poor in organelles, with irregular microvillar differentiations of the apical cell membrane (Fig. 5d–e). The apical part of the lateral cell membranes shows obvious interdigitations that are reinforced with septate junctions (Fig. 5e).

Discussion

Ants represent an insect group with a diverse diet (Blüthgen and Feldhaar 2009; Nelsen et al. 2018), with various anatomical adaptations for the uptake and ingestion of food. One of these is the IBP that among other functions is known to act as a filtering device to prevent solid particles to enter the digestive tract. Janet (1895) was the first to study its morphology in ants and wasps, and speculated that its main function was filtering of solid particles to prevent obstruction of the digestive tract. Although a comparative study of the IBP in social insects is lacking, its main action as a filtering device has been postulated as its function of origin in ants (Eisner and Happ 1962). Its development appears linked to diets containing solid particles such as arthropod prey, as the IBP is well developed in prey-feeding ants and wasps (Edwards 1980), whereas it is vestigial in honeybees (Snodgrass 1956) and absent in termites (J. Šobotník pers. comm.). The filtering function in ants was experimentally documented in *C. pennsylvanicus*, *Acromyrmex octospinosus* and *Solenopsis invicta* by providing the ants with food containing particles of different sizes and examining the uptake of these particles into the digestive tract after feeding (Eisner and Happ 1962; Quinlan and Cherrett 1978; Glancey et al. 1981). For leaf-cutting ants, the IBP performs a special additional function as carrier of a mycelium of the fungus garden by the founding gyne (Hölldobler and Wilson 1990; Mueller et al. 2001; Little et al. 2003) or as a specialized sterilization device by workers to kill spores of the garden parasite (Little et al. 2006). For most ants, however, the detailed morphology and function of the IBP remain unclear.

In the present paper, we investigated the morphology and ultrastructure of the IBP in *C. japonicus*, from which we can conclude it forms an efficient filtering mechanism. Our main findings can be summarized as follows:

1. The IBP is present in workers, queens and males, with similar anatomical characteristics in the various castes. Contrary to most other ants, males of *Camponotus* have a rather high longevity as they are produced in autumn, and only mate after hibernation the next spring (Wang and Wu 1992; Hansen and Klotz 2005). During this period, they probably ingest food, which makes a functional IBP plausible. The size and shape of the IBP varies according to the degree it is filled, with a daily pattern of pellet ejecting, that results in the IBP being usually empty in the morning and full by mid-day and in the evening. Similar daily changes have been reported for *Acromyrmex octospinosus* (Febvay and Kermarrec 1981) and *Ectomomyrmex javanus* (Wang et al. 2018). The interdigitating lateral membranes of the lining epi-
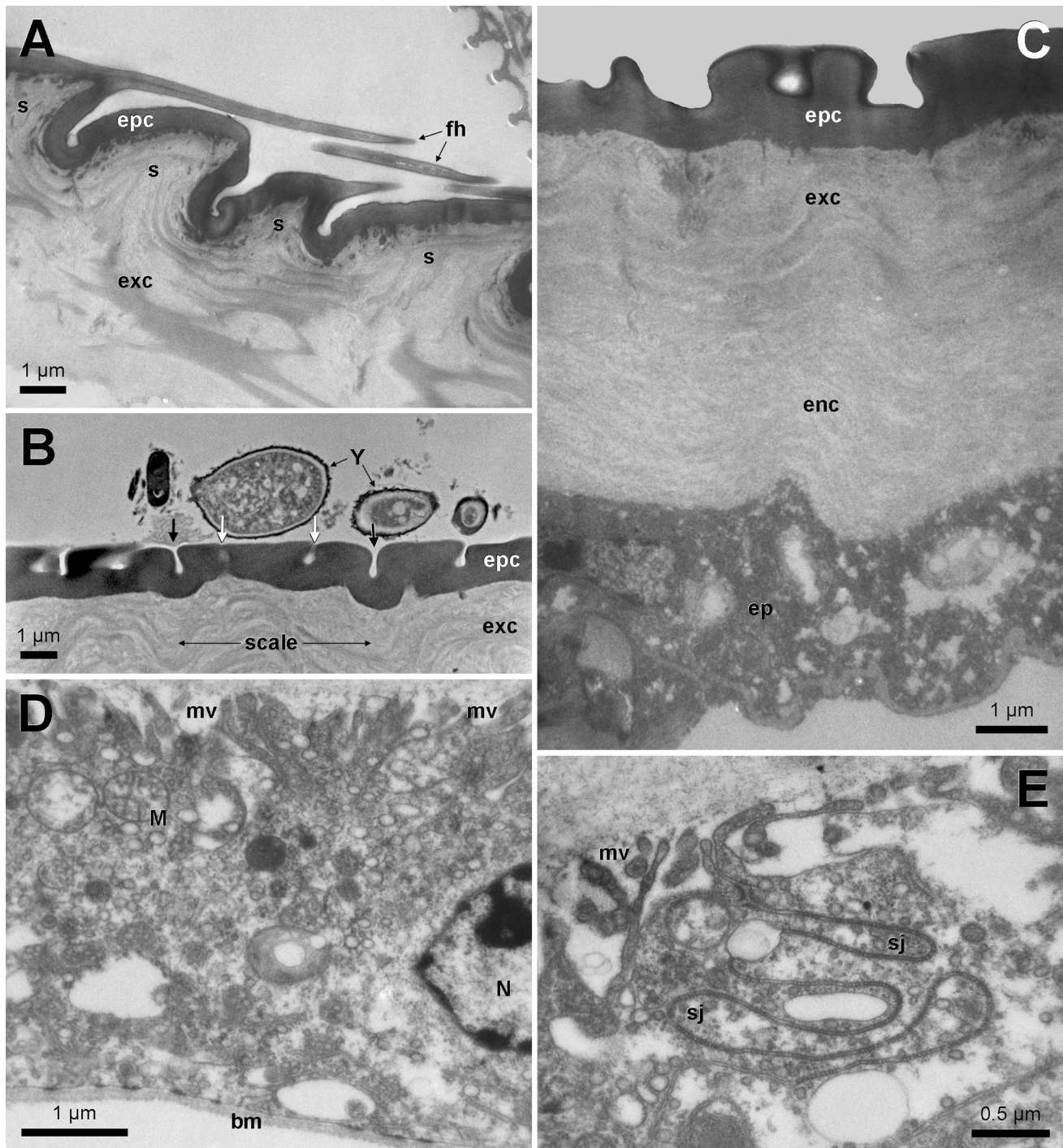
Fig. 5 Transmission electron micrographs of the IBP wall. **a** Cuticular scales (s) with epicuticular filtering hairs (fh) in the anterior part of IBP of minor worker. **b** Cuticular scales without hairs and yeast cells (Y) in posterior region of IBP of minor worker. Adjacent scales are separated by deeper grooves (black arrows), more shallow grooves occur on upper scale surface (white arrows). **c** Posterior wall of IBP in minor worker (enc: endocuticle, ep: epidermis, epc: epicuticle, exc: exocuticle). **d** Epidermis of IBP wall in major worker, showing irregular apical microvilli (mv) and cytoplasm with few organelles. **e** Detail of apical intercellular contacts with clear interdigitations and septate junctions (sj). *bm* basement membrane, *M* mitochondria, *N* nucleus

dermal cells, that also occur in *E. javanus* (Wang et al. 2018), provide the mechanical strength to allow the frequent size changes of the IBP. The luminal surface of the

scales towards the posterior shows a gradually more pronounced appearance of grooves. We assume that these grooves also give more flexibility to the scales, that is

needed given the daily size and shape changes, and that are most pronounced in the posterior distal part of the IBP.

2. The downward extension of the prepharynx and the upward extension of the labium with the hypopharyngeal plate form a dorsal and ventral valve-like structure, respectively, which plays a role to control the opening and closing of the pocket. As the IBP is not associated with any muscular supply, the emptying of its contents could be achieved through changes in hemolymph pressure in the head capsule or through movements of the mouthparts. Similar examples of indirect muscular action resulting in hemolymph pressure and subsequent discharge of substances have been documented for the metapleural gland of *Crematogaster inflata* (Billen et al. 2011) and the mandibular gland of *Camponotus* sp. 'exploding ants' (Davidson et al. 2012).

3. The internal surface of the IBP reveals a characteristic pattern of scales, of which the appearance changes from anterior to posterior. In the anterior part, we found long bushy hairs that are posteriorly directed, which provide an efficient filter mechanism to prevent large solid food particles to enter the digestive tube (stuck particles can be seen in Fig. 3c, d). The number and length of the filtering hairs gradually diminish posteriorly, similar to the IBP of the carnivorous ant *Ectomomyrmex javanus* (Wang et al. 2018). Minor differences were found in the appearance of the hairs of both species, with *E. javanus* having shorter filtering hairs with a consistent length (Wang et al. 2018). It remains unknown, however, whether the morphology of the filtering hairs may be related with the ants' feeding habit.

4. The direction of the filtering hairs is in agreement with the movements of the contents of the IBP, which have been elegantly illustrated for *Camponotus modoc* by Hansen et al. (1999). Both *Camponotus japonicus* and *Ectomomyrmex javanus*, therefore, show the IBP-hairs directed posteriorly (see Fig. 3 horizontally inverted of Wang et al. 2018). Hansen et al. (1999) described how ingested food undergoes an initial filtering through the maxillary and labial hairy combs before it enters the IBP. The flow of food particles follows a roughly circular route ventrally into the pocket, to leave it dorsally (see Fig. 5 of Hansen et al. 1999). Also in *Camponotus japonicus* (as well as *E. javanus*), the posteriorly directed filtering hairs facilitate the moving of the food into the IBP, but only allow liquid and sufficiently small particles to move out of the pocket and enter the pharynx. Further in the digestive tract of *Camponotus* sp., the specialized sepalous proventriculus is adapted for the processing of liquid food (Eisner and Happ 1962; Cook and Davidson 2006). The filtering ability of the IBP also applies to the possibility to filter bacteria, as the diversity of bacteria in the midgut is significantly lower than in the IBP (Zhang et al. 2018).

5. Apart from its thickening in the region of the dorsal lip, the epithelial lining of the IBP wall is rather thin with epithelial cells containing a cytoplasm poor in organelles, which could be expected to not have a glandular function. Some other ant species have the epithelium of their IBP wall differentiated into a glandular epidermis, such as *Monomorium pharaonis,* which has the epithelium lining the dorsal side of the pocket obviously thickened (Eelen et al. 2004), while the entire pocket wall in *Protanilla wallacei* is thickened (Billen et al. 2013). The IBP of other species, such as *Ectomomyrmex javanus* (Wang et al. 2018), does not have any glandular function. The reason for the occurrence of such interspecific differences so far remains unclear. As the opening of the labial gland is situated in close proximity to the IBP, it may be possible that salivary gland enzymes impregnate the ingested food, thus promoting a first step digestion in the IBP (Febvay and Kermarrec 1981).

6. Numerous cells showing the general ultrastructural features of yeasts (Osumi 1998) occur inside the IBP. One yeast strain (similar to *Wickerhamiella* sp. with 97% identity) has been isolated from the IBP of *C. japonicus* with 96.7% isolation frequency (Zhang et al. 2018). Similar cells were also observed in the IBP of the carnivorous ant *E. javanus* (Wang et al. 2018). Hansen et al. (1999) also isolated 1 yeast colony from the IBP of *Camponotus modoc* and 13 different colonies in *C. herculeanus*. Mankowski and Morrell (2004) have isolated *Debaryomyces polymorphus* from the IBP of *C. vicinus,* and predicted that these yeasts can be used as nutrient source for the ants or as digestive enzyme providers in the metabolic process to help food digestion and decomposition. Pagnocca et al. (2008) isolated three yeast strains from the IBP of *Atta capiguara* and *A. laevigata*. Also fungal spores and particles of plant tissue have been found in the IBP-pellets of several ant species with different diets and nesting sites (Bailey 1920), and in some species the pellets can even be fed to the larvae (Wheeler and Bailey 1920). The function and origin of such fungal and yeast presence among the IBP contents remains unknown, and will need further examination.

In conclusion, our data provide a histological and ultrastructural documentation of the IBP in *Camponotus japonicus*, that complement the behavioural (Eisner and Happ 1962) and gross anatomical studies (Hansen et al. 1999) made on the IBP of other *Camponotus* species. The omnivorous diet of many *Camponotus* spp., including the intake of solid food particles such as prey, requires a functional filtering device to prevent larger food particles out of the

digestive tract, and reject them as pellets. The anatomical and ultrastructural features of the IBP provide the ants with such filtering device. Our observations also showed a clear daily pattern of pellet ejection, that deserves more attention in future studies.

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