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Short communication

Morphology of a novel glandular epithelium lining the infrabuccal cavity in the ant *Monomorium pharaonis* (Hymenoptera, Formicidae)

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Abstract

A novel glandular epithelium lining the infrabuccal cavity and anterior pharynx is described in both workers and queens of the pharaoh's ant *Monomorium pharaonis*. The infrabuccal cavity, connected with the buccal tube, forms a ventral outgrowth of the anterior pharynx, and as such displays the tegumental lining with a cuticle and an epithelial layer. In its dorsal region, the cavity's epithelium reaches a thickness of approx. 11–12 μm in both workers and queens, which is considerably thicker than the epithelium lining the rest of the infrabuccal cavity. Also the possible role of the infrabuccal gland is discussed.

Keywords: Ultrastructure; Exocrine glands; Ants

1. Introduction

Social insects, and ants in particular, display an overwhelming variety of exocrine glands, which for the greater part is linked with the social organization and communication system that characterize the functioning of their colonies (Hölldobler and Wilson, 1990; Billen and Morgan, 1998). Exocrine glands are found in all body parts, including the legs and antennae, and perform a wide variety of functions. Several glands occur in association with the mouthparts, such as mandibular and intramandibular glands (Schoeters and Billen, 1994; Billen and Espadaler, 2002; Grasso et al., 2004), pro- and postpharyngeal glands (Delage-Darchen, 1976; Billen and Peusens, 1984), maxillary glands (Beck, 1972), as well as labial glands (Gama and Cruz Landim, 1982). We here report on a novel glandular differentiation of part of the epithelial lining of the infrabuccal cavity in workers and queens of the pharaoh's ant *Monomorium pharaonis*.

2. Material and methods

Workers and queens of *M. pharaonis* were obtained from stock colonies maintained at the University of Copenhagen. The anterior part of the head was fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose, and postfixed in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, the heads were embedded in Araldite and sectioned with a Reichert Ultracut E microtome. Semithin 1 μm sections were stained with methylene blue and thionin and viewed in a Zeiss Axioskop microscope, double stained 70 nm thin sections were examined in a Zeiss EM900 electron microscope.

3. Results

The infrabuccal cavity is an unpaired invagination in the wall of the hypopharynx (Fig. 1). It is situated right in front of the anterior pharynx, which is the functional mouth that functions as a suction pump, i.e. the cibarium. The entrance of the invagination is protected by a lobe, or lip, hanging down—the hypopharyngeal lobe according to Snodgrass

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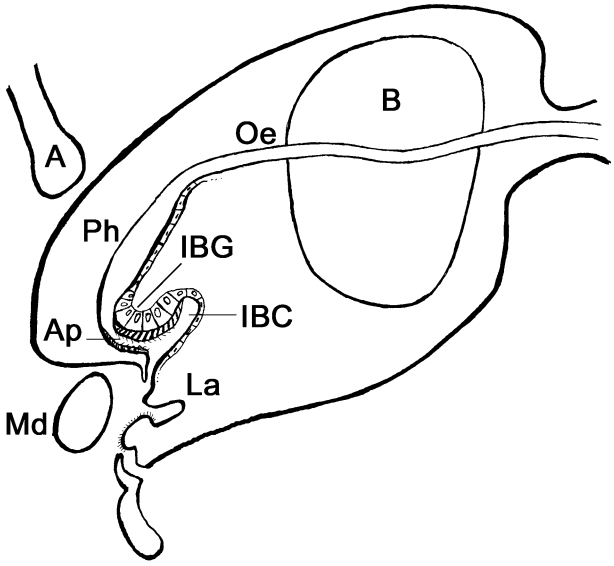


Fig. 1. Schematical longitudinal section through the ant head, showing the position of the infrabuccal cavity (IBC) and its glandular epithelium (IBG). A, antenna; Ap, anterior pharynx; B, brain; La, labium; Md, mandible; Oe, oesophagus; Ph, pharynx.

(1956). The epithelium lining the dorsal side of this cavity together with that of its continuation lining the ventral wall of the anterior pharynx is considerably thickened (Figs. 1 and 2(A) and (B)). Secretory cells of the propharyngeal gland touch the glandular epithelium at several places (Fig. 2(A) and (B)). The cuticle lining the anterior pharynx has a thickness of approx. 3 μm, and shows alternating dense and less dense parts. Its apical part displays a grater-like microsculpture with protrusions that may act as a filter for the incoming food (Fig. 2(C)). The epithelium at the dorsal side of the infrabuccal cavity is lined with a normal layer of cuticle.

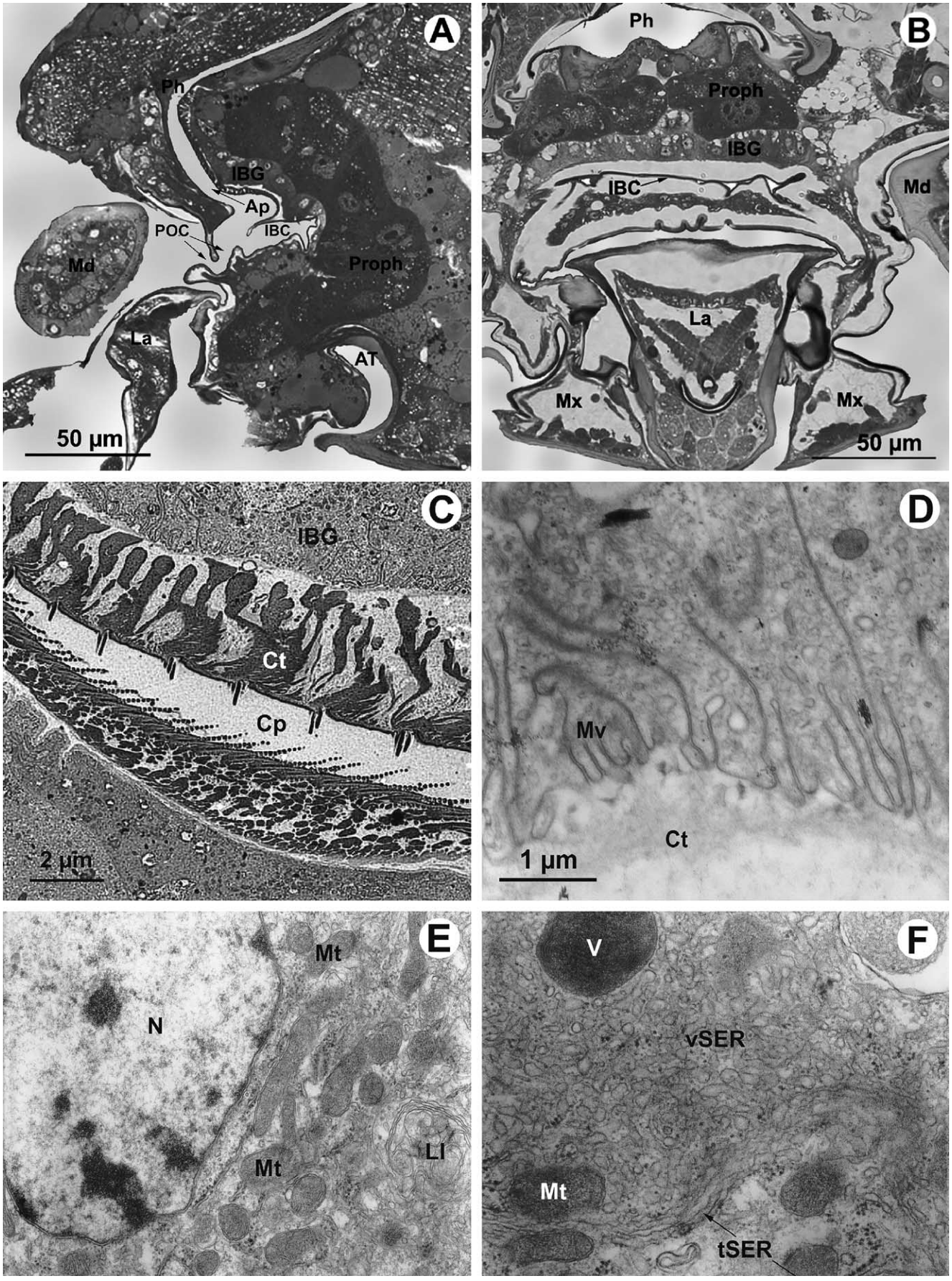
The glandular epithelium reaches a thickness of approx. 11.3 ± 1.61 μm in workers ($n=11$) and approx. 12.3 ± 2.6 μm in queens ($n=26$). The other part of the infrabuccal cavity is lined with a thin epithelium with a thickness of approx. 2.8 ± 0.8 μm in workers and approx. 2.4 ± 0.6 μm in queens. The width of the epithelial gland cells is approx. 8 μm in both workers and queens. The cells of the glandular epithelium have a centrally located oval nucleus with a size of approx. 7×5 μm^2. The apical cell membrane is differentiated into an irregular microvillar border (Fig. 2(D)), while the basal cell membrane shows a fairly regular topography with only a few invaginations. The cytoplasm contains numerous mitochondria, which are mostly concentrated near the cell nucleus (Fig. 2(E)). We observe a well developed smooth endoplasmic reticulum (SER) which is spread throughout the cell (Fig. 2(F)). This endoplasmic reticulum is divided in a vesicular part and a tubular part, with the vesicular form most abundant (Fig. 2(F)). Granular endoplasmic reticulum was only found in very small amounts and is sparsely distributed. Also lipid droplets, electron-dense vesicles and lamellar inclusions are distributed over the glandular cell (Fig. 2(D) and (F)).

4. Discussion

The existence of the infrabuccal cavity or pocket in ants was already noted by Janet (1898). The function of this structure is best known in the fungus-growing ants (Attini). Before starting her mating flight, the colony founding gyne carries parts of the mycelium of the fungus garden into her infrabuccal cavity. After mating, the queen searches a good nest place and expels the contents of the infrabuccal cavity into her excavated nest, where a new fungus garden can be started (Weber, 1982; Hölldobler and Wilson, 1990; Mueller et al., 2001). This infrabuccal cavity is also part of the mechanism that leaf-cutter ants use to prevent the invasion and spread of general microbial parasites and the specific fungus-garden parasite *Escovopsis* (Little et al., 2003). In the non-fungus growing ant species the mouthparts and the infrabuccal cavity are known to be used for cleaning the cuticle of the ants of dirt and microorganisms when grooming themselves, their nestmates, brood and eggs. The debris and other solid material is deposited in the infrabuccal cavity, from where it is actively ejected as a compressed pellet (Hölldobler and Wilson, 1990). The mouthparts, infrabuccal cavity, and the filter device in the anterior pharynx act in concert to filter solids from the material consumed during feeding and grooming (Cannon, 1998). In the lower hymenopterans the infrabuccal cavity is known to masticate pollen (Vilhelmsen, 1996).

The present paper is the first report on the glandular differentiation of the wall of this infrabuccal cavity. The glandular epithelium corresponds to type I following the classification of Noirot and Quennedey (1974), in which the glandular cells are arranged in a simple, monolayered epithelium. Earlier papers describing the cephalic exocrine glands in ants never mention this thickened epithelium of the infrabuccal cavity (Pavan and Ronchetti, 1955 for *Linepithema humile*; Bausenwein, 1960 for the *Formica rufa*-group; Kürschner, 1971 for *Formica pratensis*), even not in a paper on *M. pharaonis* itself (Baiocco and da

Fig. 2. Semithin section through the anterior part of the head of a *M. pharaonis* worker (A, longitudinal) and queen (B, transverse section), showing the location of the infrabuccal gland (IBG). (C–F) Electron micrographs of the infrabuccal gland, (C) cuticular microsculpture of the anterior pharynx in a queen (oral cavity to the right), (D) detail of apical region in a queen, and (E–F): details of the cytoplasm of the worker, Ap, anterior pharynx; AT, anterior tentorium; Cp, cuticular protrusions; Ct, cuticle; IBC, infrabuccal cavity; La, labium; LI, lamellar inclusions; Md, mandible; Mt, mitochondria; Mv, microvilli; Mx, maxilla; N, nucleus; Ph, pharynx; POC, pre-oral cavity; Proph, propharyngeal gland; tSER, tubular smooth endoplasmic reticulum; V, dense vesicles; vSER, vesicular smooth endoplasmic reticulum.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Cunha, 1992). Every individual of *M. pharaonis* that we investigated showed the thickened epithelium at the same position and this both for workers and queens. Also Snodgrass (1956), who reports on the existence of an infrabuccal sac in the honeybee, did not mention the presence of such glandular epithelium. Landolt and Akre (1979) found four new exocrine glands related to the mouthparts in some social Vespidae, but none of them agreed with our glandular epithelium. An epithelial gland was reported between the labium and the first ventral segment in larvae of the leaf-cutting ant *Acromyrmex octospinosus* (Kermarrec and Febvay, 1985), which therefore is not homologous to the glandular epithelium we describe.

The function of the novel infrabuccal gland epithelium in *M. pharaonis* remains unknown at present, with a number of speculative possibilities: (1) the epithelium could produce lubricant substances that facilitate the movements of food particles passing through the pharynx; (2) a digestive role could be considered as the gland lines part of the anterior alimentary tract where incoming food passes; (3) the anterior pharynx has been reported as a system that prevents small solid particles to block the alimentary tract by filtering them out. *Camponotus americanus* has thus been reported to filter particles larger than 150 μm (Eisner and Happ, 1962) where *Acromyrmex octospinosus* filters particles larger than 10 μm (Quinlan and Cherrett, 1978). *Solenopsis invicta* can even filter particles as small as 0.88 μm (Glancey et al., 1980). The cuticular microsculpture we found in *M. pharaonis* can be interpreted in this context. In the infrabuccal cavity the sequestered particles become compacted into a pellet, which can be fed to the larvae in some species (Wheeler and Bailey, 1920; Glancey et al., 1980). It is remarkable that we have never observed pellets, or any signs of debris, in the infrabuccal cavity of queens in spite of histological investigations of the heads of more than 100 queens of different ages and mating status; Secretions from the glandular epithelium could play a role in predigestion of such pellets. Febvay and Kermarrec (1981), describing the infrabuccal filter system of *Acromyrmex octospinosus*, overlooked the presence of the glandular epithelium, and postulated the labial gland secretion might contribute in the degradation of pellets that are stored in the infrabuccal cavity. A function of this gland in producing digestive substances, however, may be questioned for the novel epithelium, as there is only a very limited presence of granular endoplasmic reticulum, which would be expected for the elaboration of digestive enzymes (Noirot and Quennedey, 1974; Billen and Morgan, 1998). Moreover, as *M. pharaonis* queens are known to obtain their food in a liquid form, mainly from larval secretions (Børgesen, 1989), a function for the novel epithelium in producing pellet-degrading enzymes would not make sense in the queen caste; (4) finally, it is noteworthy, that pharaoh's ant queens spend a large part of the time grooming themselves—as do the workers. The protruding infrabuccal gland which may function as a kind of an inner tongue, is placed in a position that makes it possible for the ant—directly or indirectly—to place the infrabuccal gland products on any body part of herself or other colony members when grooming (Basibuyuk and Quicke, 1997). The presence of a well-developed smooth endoplasmic reticulum is indicative for production of hydrocarbons, which may have potential functions as lubricants and/or which may facilitate trophallaxis. The oily substance which may be added to all body parts during frequent grooming can function as a cleaning agent or as a disinfectant—but it may also be that the function of the gland has more to do with recognition of sex, caste, and/or reproductive status—probably in concert with other glands, producing substances to the parfume of the cuticular hydrocarbons of ants.

More research will definitely be needed to find out about the exact function of this novel glandular tissue.

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