The antenna cleaner gland in *Messor rufitarsis* (Hymenoptera, Formicidae)

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**Abstract.** TEM and SEM analysis of the tibio-tarsal antennal cleaner in *Messor rufitarsis* reveals a pore region on the surface of the basitarsus. These pores are originating from a cluster of highly prismatic epidermal cells that fill the anterior hemolymph space of the leg almost completely. Within the cytoplasm of the cells, basal labyrinth, nucleus/rough endoplasmic reticulum, 'secondary lysosomes', a lot of smooth endoplasmic reticulum and distal microvilli regions can be distinguished indicating that the cells are 'class I' gland cells. Their secretion appears to be transported to the antennal cleaner apparatus via a subcuticular space and pores. It is suggested that the secretion might be used for cleaning of the antenna and/or chemical communication. SEM examination of six additional species indicates that an antennal cleaner gland might be present throughout the ants.

**Keywords:** Epidermal gland, ants, anterior legs, basitarsus, ultrastructure

**Introduction**

Numerous exocrine glands have been described in social hymenopterans, and much attention has been paid to those functioning in the context of chemical communication. Apart from this, epithelial glands of various functions are found as in other insects (reviews in Hölldobler and Wilson, 1990; Billen, 1990, 1991). In this paper, a gland has been analyzed that is connected to the antenna cleaning apparatus in the tibio-tarsal region of the forelegs. This cleaning apparatus is found in nearly all Hymenoptera (Gennerich, 1922; Schönnitzer, 1986; Schönnitzer and Lawitzky, 1987; Francoeur and Loiselle, 1988). A specialization of the antenna cleaner is found in Formicidae, where already Janet (1894) described a 'bande poreuse' with a putative gland function that is not present in other Hymenoptera. A fine structural examination of this region was thus required to verify the glandular character of the cells. We have therefore examined the antenna cleaner gland of *Messor rufitarsis* (Myrmicinae) with both, the scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In addition, we have examined the cleaning apparatus of six other formicid species with the SEM in order to find out if the presence of the gland is a common character of all Formicidae. A previous account of our findings is given in Schönnitzer and Dott (1989).

**Materials and methods**

Workers of *Messor rufitarsis* (Fabricius, 1804) were kept in colonies in a climatic chamber (20°C, 80–90% humidity) and fed with honey, fruits and flies. The ants were collected close to Verona, Italy.

For TEM analysis, animals were anaesthetized with CO₂ and dissected in chilled fixative as follows. The head was cut off, and the body was dissected between pro- and mesothorax. Thus, the first thoracic segment could be used for handling of the specimens. In addition the articles were removed. The specimens were fixed for 2 h (0.25% glutaraldehyde, 2% paraformaldehyde, 5% DMSO, 0.1 M cacodylate buffer pH 7.2; Curtis et al., 1987), rinsed in buffer and postfixed in OsO₄ in buffer. After dehydration, specimens were embedded in Durcupan ACM. Ultrathin sections were double-stained.
with uranyl acetate and lead citrate and examined in Zeiss EM 9 or EM 10 electron microscopes. Semi thin sections (thickness: 1–4 μm) were stained with methylene blue according to Andres (1965).

In addition, Myrmica laevisnoidis, Tetramorium guineense, Atta sexdens (Myrmicinae), Paraponera clavata, Ectatomma tuberculatum (Ponerinae), and Myrmecia gulosa (Myrmecinae) were inspected by SEM. For this, the legs were dissected, ultrasound-cleaned in 70% ethanol, dehydrated through a graded ethanol series, transferred to ether, air dried, mounted, sputtered with gold on a Bio Rad SC 510, and investigated with a JSM 35 CF (Jeols) at 10 kV.

Results

Gross morphology of the basitarsus

The basitarsus of Messor rufitarsis is about 0.5 μm long and has a diameter of 54–60 μm. The proximal part of the basitarsus of the foreleg is part of the tibio-tarsal antenna cleaner (Schönitzer and Lawitzky, 1987; Francoeur and Loiselle, 1988). The latter is formed by a spur which is inserted at the distal end of the tibia, and a shallow notch, a slight depression of the basitarsus including a row of straight hairs (the comb, Fig. 1a). These hairs are directed posteriorly. The anterior side of the notch is covered with many paddle-shaped hairs. Between these hairs and the comb there is a specialized area of cuticle with fissures and holes (‘bande poreuse’ sensu Janet, 1894; Fig. 1). This area extends parallel over the whole length of the comb. The pore area is approximately 180 μm long and 7 μm wide. In Messor, approximately 63 holes per 100 μm² are found with a diameter of about 0.2 μm. The fissures are about 0.05 μm broad.

Beneath the cuticle, which is about 10 μm thick, a thin layer of epidermal cells is found surrounding the inner lumen of the basitarsus. A septum divides this lumen into two separate haemolymph spaces. The smaller compartment contains a cuticular process (diameter: 5 μm) with a small, electron-lucent lumen and a relatively wide cuticle passing all along the basitarsus (in this region, Markl, 1966, has identified a tendon; see also Sutcliffe and McIver, 1987). Within the larger compartment two nerves, a trachea, and the gland cells are present. Below the ‘bande poreuse’, the gland fills the major part of the basitarsus, considerably restricting the haemolymph compartment distally and proximally. The two nerves contain about 120–200 axonal profiles. They probably correspond to the two main branches of nerve IN10 as described by Markl (1966).

Fine structure of the gland

The cell cluster proximal to the pore region is composed of a single layer of highly prismatic cells showing fine structural details indicative of a secretory function (see below, and Fig. 3). All the cells belong to the same type, and specialized canal cells are lacking. Therefore the gland corresponds to class I after the terminology of Noirot and Quennedey (1974, 1991). In cross-sections of the basitarsus, 10–20 gland cells can be seen in general, and the gland includes altogether 500–700 cells. The cells are of a slender shape (width: 2–5 μm). From the basal lamina to the apical end below the cuticle, they have a length of approximately 25 μm. Neighboring gland cells have straight cell borders in their basal region with an intracellular cleft of 0.01–0.03 μm (Figs. 2c,f, 3). In the apical region, the cell borders form numerous loops with septate and tight junctions. Apicobasally projecting bundles of microtubules are frequent in the cell border region.

In the basal region of the cells, adjacent to the basal lamina, the cell membranes show numerous deep infoldings forming a basal labyrinth (Fig. 2g). Within this labyrinth, the extracellular cleft measures constantly about 0.04 μm. The basal cytoplasm of the secretory cells contains numerous vesicles with a lumen of low electron density. In some of the cells, smooth ER and glycogen granules are very frequent. In addition, the basal labyrinth contains microtubules, mitochondria, and electron-dense vesicles.

Distal to the basal labyrinth, the nuclei are found in the basal third of the gland cells (Fig. 2e,f). A Golgi apparatus is often situated in close vicinity of the nucleus. These Golgi complexes are generally composed of a stack of 4 or 5 cisternae that are dilated peripherically. The cisternae are associated with vesicles (diameter: 0.05–0.4 μm). In the nucleus region, both rough endoplasmic reticulum (ER) and numerous cisternae of smooth endoplasmic reticulum (sER) are found, rough ER is rare, however, in the apical part of the cells. Proximally, transitions of smooth and rough ER can be seen. In addition, elongated mitochondria often orientated apicobasally are present.

Distal to the nuclei is a zone with numerous lysosomal bodies of different shape and size. Among these bodies, large secondary lysosomes are present that contain...
inhomogenous electron-dense material as well as vesicular and granular inclusions (Fig. 2d). They are most frequent in the middle region of the cells, and can hardly be seen in the basal and apical regions. More distally, secondary lysosomes with a smooth spherical outline and a rather homogeneous matrix are found. Their diameter is about 0.4–0.5 µm. Often these lysosomes contain coiled membranes (multilamellar bodies). In addition, primary lysosomes (Fig. 2b) are present solely in the apical plasma.

Near the cuticle, the gland cells possess cup-shaped invaginations forming deep extracellular spaces. These cups extend about 6–8 µm into the gland cells. Protruding from the gland cells into the extracellular space there are numerous microvilli (Fig. 2b). They are generally found in the lateral part of the cups, while the proximal part is smooth and lacks microvilli. Near the apical cell membrane, many cisternae of sER and vesicles of low electron density are found throughout. In addition, stalked membrane loops are visible indicative of endo- or exocytotic events. Distal to the cup-shaped extracellular spaces a band of spongiform material is found. This material is directly connected to the fibrillar cuticle, and also fills the inner section of the pore canals that pass across the whole cuticle (Fig. 2a). The canals have a diameter of approximately 0.2 µm. Their inner part projecting across the endocuticle is slightly broader than the outer part that passes the exocuticle.

The ‘bande poreuse’ of other ants
In addition to Messor rufitarsis, the antenna cleaner has been examined with the SEM in Myrmica laevinodis, Tetramorium guineense, Atta sexdens (Myrmicinae), Paraponera clavata, Ectatomma tuberculatum (Ponerinae), and in Myrmecia gulosa (Myrmecinae). In all these species a pore region is present indicating that
also the gland cells can be found (Fig. 1). Apart from this, pores and fissures exhibit a species-specific shape and arrangement. While numerous deep fissures are found in *Messor* (Fig. 1a,d), *Myrmica* (Fig. 1c,e) and *Myrmecia* (Fig. 1f), they are less developed or absent in *Atta, Ectatomma, Tetramorium* (Fig. 1b), and especially *Paraponera* (Fig. 1g), where only pores of a round shape are present.

**Discussion**

Fine structural characteristics of the cell cluster beyond the 'bande poreuse' (Fig. 3) clearly indicate that the cells function as an epidermal gland in *Messor rufitarsis*. Basal labyrinth, highly active nucleus region, distal microvilli, and large pores within the cuticle are generally seen as typical gland characters (Noirot and Quennedey, 1974). Since only one cell type takes part in the formation of the gland, and since specialized canal cells are lacking, the antenna cleaner gland of *Messor rufitarsis* resembles class 1 according to the most common classification of Noirot and Quennedey (1974, 1991) or type B according to Billen (1987). In addition, the basic outfit of these cells is similar to that of ion transporting epithelia as in the anal organ housefly larvae (Schwantes, 1989). In the antenna cleaner gland, however, the massive cuticle and the shape of the pores indicate that it is rather a secretory organ than an organ taking up substances from the environment. In addition, the large amount of smooth ER is conspicuous, this is generally regarded as a requisite of glands with a non-proteinous secretion (Noirot and Quennedey, 1974) and especially of pheromone producing glands (Billen, 1991). A further feature of the antenna cleaner gland is the large amount and variability of lysosomal bodies. This indicates a very large turnover of membranes. Another peculiarity of the gland is the presence of relatively large pores passing through the cuticle. Cicuticular pores are not found in the majority of epithelial glands. Exceptions are the tibial glands in *Creptagaster* and Pavan's gland in Dolichoderinae (Billen, 1991). The same situation is found in pheromone producing glands in a moth (Sreng and Sreng, 1988). Possibly the thick and robust cuticle of the leg does not permit passage of larger molecules.

As can be seen from previous light microscopic investigations (Janet, 1894; Whelden, 1960; Fanfani and Dazzini Valcurone, 1990), there is also a gland in the basitarsus of *Myrmica laevinodis* (Myrmicinae), *Creptagaster striatula* (Myrmicinae) and *Rhytidoponera metallica* (Ponerinae). In these species pores were also described which pass the cuticle above the glandular cells. As also inferred by Hölldobler et al. (1992), who examined the phylogenetically primitive ponerine species *Prinopelta amabilis*, our findings on altogether six previously unexamined species indicate that an antenna cleaner gland might be present throughout the Formicidae (see also Schönitzer and Lawitzky, 1987). Therefore, it might represent another exocrine gland of the ant's legs in addition to the basitarsal glands (Hölldobler et al., 1992), footprint glands in the hind leg tarsi (Hölldobler and Palmer, 1989) and the tarsal glands which generally provide adhesion on smooth surfaces and have been found in all Hymenoptera previously studied (e.g. Billen, 1986, 1990; Lensky et al., 1985).

Though its position justifies to name the gland described here the 'antenna cleaner gland' a direct impact on grooming is as yet not clear. Since a gland appears to lack in the grooming apparatus of other Hymenoptera (e.g. in Mutillidae, Tiphidae and Apoidea, Schönitzer and Renner, 1980; Schönitzer, 1986; Schönitzer and Lawitzky, 1989), its presence must not necessarily be coupled with the primary function of the antenna cleaner, viz., the cleaner might serve for cleaning, and for the distribution of the gland's secretion as suggested for fly pheromone glands (Dillwith and Blomquist, 1982). Hölldobler et al. (1992) have therefore suggested that the gland might cooperate with the basitarsal gland of the hindlegs in trail and recruitment behaviour. Alternatively, the gland might also distribute secretion over the antennae, and hence take part in intraspecific communication.

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**REFERENCES**


