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The dorsoproximal intramandibular gland in *Leptanilla clypeata*, a novel exocrine gland in ants

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ABSTRACT. Workers of *Leptanilla clypeata* have a conspicuous epithelial gland underneath the dorsoproximal part of the mandibles that is different from all other intramandibular glands that are so far found in ants. Because of its unique location, we suggest to name this novel structure as the 'dorsoproximal intramandibular gland'. The gland occurs in an excavation of the mandibular cuticle and is made up by columnar cells with apical microvilli and basal invaginations. The presence of smooth endoplasmic reticulum indicates the elaboration of a non-proteinaceous secretion, although the function of the novel gland remains unknown.

Keywords Leptanillinae, Formicidae, morphology, ultrastructure
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INTRODUCTION

Species of the subfamily Leptanillinae are among the most elusive ants because of their mainly hypogaeic lifestyle, the small size of their colonies, and the extremely small individual size that hardly exceeds 2 mm in workers. In species of the type genus *Leptanilla*, the workers are predators of geophilomorph centipedes, while the queens are characterized on a diet of larval hemolymph (Masuko 1990; Ito & Yamane 2021). Because of their cryptic lifestyle and small size, information on the internal anatomy of leptanilline ants is very limited, and mainly goes back to studies of the exocrine glands in *Leptanilla japonica* Baroni Urbani, 1977, and *L. escheri* (Kutter 1948; Hölldobler et al. 1989) and of larval structures that are involved in larval hemolymph feeding in *L. japonica* (Masuko 1989, 2008). Morphological information on *Protanilla* is given in a report on the exocrine glands in *P. wallacei* Taylor 2013 nec Hölldobler & Wilson 1990 (Billen et al. 2013) and in a study of the internal head anatomy in *P. lini* Terayama, 2009 (Richter et al. 2021b). The survey of the exocrine system in *P. wallacei* describes the impressive variety of 26 glands, one of which is a novel structure referred to as the "intramandibular epithelial gland", that lines the ventroproximal wall of the mandibles (Billen et al. 2013). While performing a survey study of the exocrine system of *Leptanilla clypeata* Yamane & Ito, 2001, however, we did not find any trace of such ventroproximal intramandibular gland. Instead, we found a conspicuous dorsally located epithelial gland inside the mandibles, that we here describe.

MATERIAL AND METHODS

A queenless colony fragment of *Leptanilla clypeata* with approx. 50 workers and 50 larvae was collected in the Bogor Botanical Gardens, West Java, Indonesia, in March 1997 (colony code FI97-556). For histological examination of the mandibles, the anterior part of the heads was cut off and fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Tissues were postfixed in 2% osmium tetroxide in the same buffer, dehydrated in a graded acetone series, and embedded in Araldite. Serial semithin sections with a thickness of 1 µm were made with a Leica EM UC6 ultramicrotome, stained with methylene blue and thionin, and viewed under an Olympus BX-51 microscope. Thin sections of 70 nm thickness were viewed under a Zeiss EM900 electron microscope. Heads for scanning microscopy were attached to aluminium stubs with double-adhesive tape, sputtered with gold, and examined under a JEOL JSM-6360 scanning microscope.

RESULTS

Histological sections revealed the presence of a conspicuous glandular thickening of the tegumental epithelium in the upper proximal part of the mandibles. Both on transverse (Fig. 1A) and longitudinal sections (Fig. 1B) the glandular epithelium appears more or less pentagonal. It is made up by tall columnar cells with a height of 15 µm and is located in a prominent excavation of the mandibular cuticle. The heavily sclerotized lining of the mandibles has a thickness around 10-15 µm along the mandibular surface, but is abruptly reduced to only 2 µm where it covers

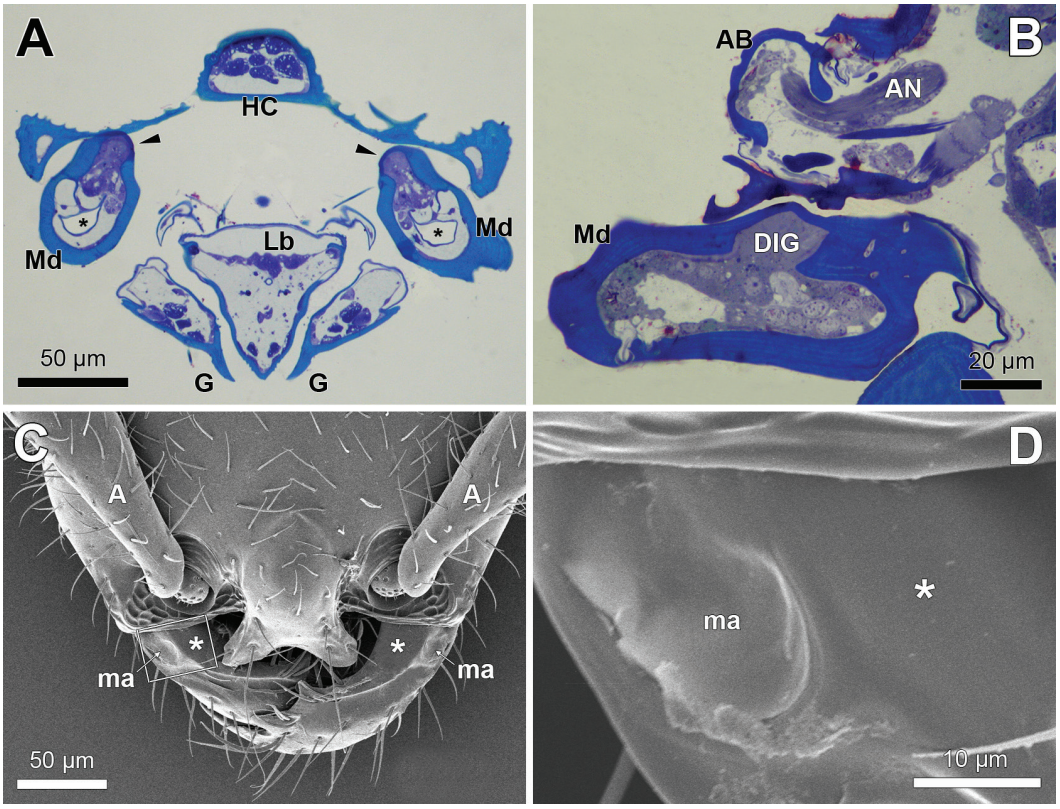

Fig. 1. A. Transverse semithin section through the mouthparts (G: galea, HC: head capsule, Lb: labium, Md: mandible), arrowheads indicate dorsoproximal intramandibular gland, asterisks show mandibular gland duct. **B**. Longitudinal semithin section through anterior head part and mandible, note conspicuous dorsoproximal intramandibular gland (DIG). **C**. Scanning micrograph of anterior head part (A: antenna, ma: mandalus, white asterisks: smooth upper proximal margin of mandible). **D**. Enlargement of framed area in C.

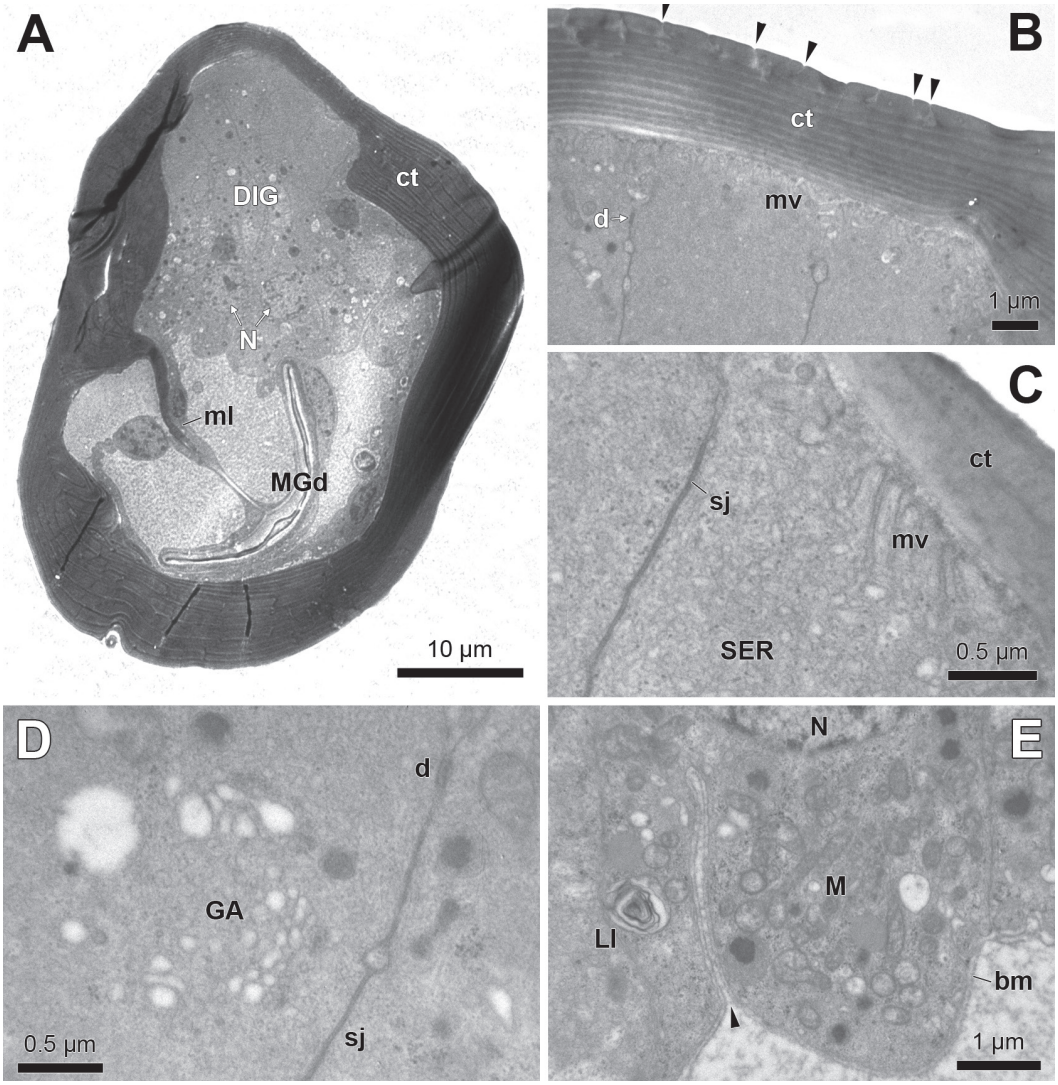

Fig. 2. Electron micrographs of the dorsoproximal intramandibular gland (DIG). **A**. Transverse section through proximal part of mandible (ct: cuticle, MGd: mandibular gland duct, ml: mandalus lamella, N: nucleus). **B**,**C**. Apical cytoplasm with microvilli (mv), smooth endoplasmic reticulum (SER), desmosome (d) and septate junction (sj). Arrowheads indicate minute depressions on cuticular surface. **D**. Central cytoplasm with Golgi apparatus (GA) and septate junction. **E**. Basal cytoplasm with numerous mitochondria (M), lamellar inclusion (LI) and deep basal invaginations (arrowhead). bm: basement membrane.

the epithelium (Fig. 1A,B). Under the scanning electron microscope, the upper side of each mandible shows a large circular mandalus (which represents the site where the mandibular gland duct opens: Gotwald 1969; Richter et al. 2021a) at the outer side, and a smooth surface at the mesal side (Fig. 1C). This smooth area corresponds with the region of the thickened epithelium, and appears entirely smooth at high magnification without any sculpturing or hairs (Fig. 1D).

Ultrastructural observation further illustrates the glandular characteristics of the epithelium with columnar cells that have basally located round nuclei (Fig. 2A). The cuticle overlaying the epithelium shows minute pores of hardly 100 nm at its surface (Fig. 2B). The apical cell membrane is differentiated into a microvillar border (Fig. 2B,C), while the lateral cell junctions are reinforced with an apical desmosome, followed by an extended septate junction zone (Fig. 2C,D).

The cytoplasmic organelles include an extensive smooth endoplasmic reticulum, Golgi apparatus, some lamellar inclusions and numerous mitochondria (Fig. 2C-E). The basal portion of the glandular cells shows an irregular outline with numerous deep basal invaginations (Fig. 2E).

DISCUSSION

The thickened epithelium that occurs underneath the dorsal proximal portion of the mandibles in *Leptanilla clypeata* workers represents the characteristics of an active exocrine gland. The presence of a well-developed smooth endoplasmic reticulum indicates the elaboration of a non-proteinaceous secretion. The apical microvilli and basal invaginations represent modifications of the cell membrane that provide a significant surface increase that allows an efficient uptake of precursor molecules and discharge of secretory products (Noirot & Quennedey 1974; Billen & Morgan 1998). Minute depressions on the cuticular surface overlaying the glandular epithelium probably correspond with the site where secretion is released. Although these depressions were clearly noticeable with TEM, we could not see them with SEM, which may be because of their very small size and/or observation at an inappropriate angle.

The location underneath the dorsoproximal part of the mandible and its peculiar fit within the surrounding dorsal cuticle make the glandular epithelium of *L. clypeata* workers different from all other intramandibular glands that have so far been described in ants. The bowl-shaped basimandibular gland in *Strumigenys* ants also occurs in a kind of cuticular excavation, but has a clearly ventroproximal position and also differs in its ultrastructural features (Wang et al. 2021). Other epithelial glands inside ant mandibles have been found near the distal tip in *Strumigenys* (formerly *Pyramica*) *membranifera* Emery, 1869 (Billen & Espadaler 2002), lining the ventral cuticle in *Protanilla wallacei* (Billen et al. 2013) and several Ponerini (Martins & Serrão 2011), and underneath the mandibular pit in *Brachyponera sennaarensis* (Mayr 1862) (Billen & Al-Khalifa 2016). In addition to these epithelial glands (that belong to class-1 following the standard classification of Noirot & Quennedey 1974), also a number of class-3 intramandibular glands can be commonly found, that were initially simply termed "intramandibular glands" (Schoeters & Billen 1994). As so many glands can thus be found inside ant mandibles, they should best be precisely named. We therefore suggest to designate the novel gland we here describe for *L. clypeata* as the "dorsoproximal intramandibular gland". The function of the gland remains unknown and will require further examination. As the ultrastructural features are indicative for the production of a non-proteinaceous secretion, a pheromonal role cannot be excluded, but can only be studied when live material of these elusive ants will become available again.

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