Novel thoracic glands in the ant *Myopias hollandi*

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**ABSTRACT**

Besides the common labial and metapleural glands, four novel exocrine glands are described in the thorax of both workers and queens of the ponerine ant *Myopias hollandi*. From anterior to posterior, these glands were designated as the propleural pit gland, the posterolateral pronotal gland, the anterolateral propodeal gland and the metasternal process gland. They all correspond with class-3 glands, that are made up of bicellular units that each comprise a secretory cell and a duct cell. In the propleural pit gland, the ducts are characterized by a gradually widening diameter, while in the three other glands the ducts show a portion which displays a balloon-like expansion, that on semithin sections stains very dark. For none of these novel glands the function is known as yet, although ultrastructural examination indicates that they produce a non-proteinaceous and therefore possibly pheromonal secretion.

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**1. Introduction**

Ants are well known for their astonishing variety of exocrine glands, the total number of which in 2009 was set at 75 (Billen, 2009a). This impressive number in the meantime has grown to 85, as several novel glands have since been reported in *Protanilla wallacei* (Billen et al., 2013a), in the genus *Myopias* (Billen et al., 2013b), and in *Tatuidris tatusia* (Billen and Delsinne, 2014) and *Brachyponera semnaaensis* (Billen and Al-Khalifa, 2016). The classification of glands goes back to the pioneer paper of Noirot and Quennedey (1974), that distinguishes between class-1 epithelial glands and class-3 glands (the glandular cells that were initially indicated as class-2 were later considered as homologous with oenocytes: Noirot and Quennedey, 1991; Billen and Sobotnik, 2015). Class-3 glands are made up by bicellular units, each unit comprising a secretory cell and its accompanying duct cell. The junction between both cells is formed by the secretion-collecting end apparatus, that continues into the duct cell as a narrow cuticle-lined duct. The ducts have an almost universal internal diameter around 0.5–1 μm among insect species, and eventually open into a reservoir or through the external tegument as a small pore with the same diameter size (Billen et al., 2013b). A remarkable exception of class-3 glands with such constant duct diameter has been reported for *Myopias* ants, in which all examined class-3 glands show ducts with gradually increasing diameter (Billen et al., 2013b). In our attempt to check for duct diameter in other class-3 glands of *Myopias* ants, we discovered the existence of four hitherto unknown class-3 glands in the thorax of *M. hollandi*, which we here describe. *M. hollandi* is the same species as the *M. maligna* studied by Billen et al. (2013b), the name change resulting from a recent revision of the genus *Myopias* (Probst et al., 2015).

**2. Material and methods**

Workers and queens of *Myopias hollandi* were collected from Padang, Indonesia. Voucher specimens are deposited in the Bogor Zoological Museum, Indonesia. The anterior as well as posterior part of the thorax was fixed in cold 2% glutaraldehyde in a 50 mM cacodylate buffer at pH 7.3 with 150 mM saccharose added. After postfixation in 2% osmium tetroxide in the same buffer and dehydration in a graded acetone series, tissues were embedded in Araldite. Serial sections with a thickness of 1 μm were made with a Leica EM UC6 ultramicrotome, stained with methylene blue and thionin and viewed in an Olympus BX-51 microscope. Material for scanning microscopy was critical point dried in a Balzers CPD 030 instrument and examined in a JEOL JSM-6360 scanning microscope.
3. Results

Our examination of serial semithin sections of the thorax of both workers and queens for gland structures revealed the presence of 6 exocrine glands, of which four are so far unknown exocrine structures. After a brief description of the common labial and metapleural glands, we here describe the four novel glands from anterior towards posterior.

3.1. Labial gland

With its opening site at the labium, the labial gland strictly spoken is a cephalic gland, though we include it here as its major secretory part is situated in the prothorax. As in other ponerine ants, the labial gland is of the acinar type (Fig. 1). The spherical acini have a diameter around 80 μm, with a large central cell that is full of dark secretory granules and that is surrounded by pale peripheral cells (Fig. 2A). The acini are connected by labial gland ducts to 4 enlarged reservoir sacs. These have a diameter around 100 μm, and have a thin epithelial lining surrounding a large subcuticular space. The cuticle that lines the reservoir lumen shows taenidial reinforcements, that resemble the tracheal cuticle (Fig. 2A).

3.2. Metapleural gland

The metapleural gland consists of a compact cluster of pear-shaped class-3 secretory cells of 70 × 25 μm that open through their accompanying duct cells into the ventral part of a heavily sclerotized atrium at either side (Figs. 1 and 2B). The ducts show a slightly enlarging internal diameter from approx. 0.5 μm near their junction with the secretory cell to nearly 2 μm at their opening site into the atrium (Fig. 2B). The atrium has a diameter around 150 μm, and opens to the outside through a round opening near the implantation of the hind coxa (Fig. 7D).

3.3. Propleural pit gland

In the anterior part of the prothorax, close to the propleural plate cuticle, we found in both queens and workers a left and right cluster of approx. 10 class-3 cells at each side (Fig. 1). The polygonal gland cells have a diameter around 20 μm with a rounded nucleus of 10 μm (Fig. 3A and B). From each cell, a duct cell leads anteriorly to join a bundle formed by ducts from other gland cells. Along their long trajectory of approx. 100 μm, the internal diameter of the ducts gradually widens from 0.5 to almost 10 μm. In addition, the duct lumen fills up with a dark staining secretion (Fig. 3A,D,E). The ducts of both cell clusters eventually open to the exterior through the nostril-like pit that has a diameter of almost 50 μm and that occurs at the anterior side of each propleural plate (Fig. 4A and B). We unfortunately were not able to analyse the gland with electron microscopy.

3.4. Posterolateral pronotal gland

A short distance before the posterolateral margin of the pronotum at either side in both workers and queens we find a cluster of 15–20 round class-3 secretory cells with a diameter around 30 μm (Figs. 1 and 5). Each secretory cell is connected to a duct cell, in which the cuticular ductule has an internal diameter around 0.5 μm near its junction with the secretory cell (Fig. 6A). The ducts are characterized by a conspicuous bulbous widening that reaches a diameter of up to 20 μm. The lumen of both the narrow duct and the inflated part is filled with a dark staining secretion (Fig. 5A–H). More distally, the ducts become narrow again and move posteriorly into a thin space that is formed by a back-folded portion of the lateral pronotal cuticle (Figs. 1 and 5). The ducts open through the inner side of this back-folded cuticle (Fig. 1), and therefore are not externally visible, although serial semithin sections clearly illustrate the course of the ducts (Fig. 2C–H).

Ultrastructural observation reveals rounded nuclei with a diameter around 10 μm (Fig. 6A). The cytoplasm is characterized by a prominent Golgi apparatus (Fig. 6B) and abundant large mitochondria (Fig. 6B–E). Also numerous spherical vesicular inclusions with a diameter between 1 and 2 μm are found (Fig. 6A,C,D). These show a foam-like heterogeneous content, and are mainly found near the end apparatus (Fig. 6A,D), which is an indication they probably represent secretion. There is no granular endoplasmic reticulum. The cuticular lining of the end apparatus has an internal diameter of 0.3—0.5 μm, and is surrounded by short microvilli with a length of approx. 1 μm (Fig. 6C–E).

3.5. Anterolateral propodeal gland

Underneath the anterolateral region of the propodeum we find a cluster of approx. 20 ovoid class-3 secretory cells at either side (Figs. 1 and 7A). The cells measure around 40 × 20 μm, and are each connected to a duct cell that contains a narrow cuticular canal with a diameter of less than 1 μm over a length of 30 μm. The ducts then

Fig. 1. Schematic top view of the thorax, drawn to real scale, showing the various exocrine glands in the thorax. The insets give a close-up of the 4 novel glands (class-1 glands green, class-3 glands red). ALPG: anterolateral propodeal gland, LG: labial gland, mp: metasternal process, MPG: metapleural gland, MSPG: Metasternal process gland, PLPG: Posterolateral pronotal gland, pp: propleural pit, PPG: propleural pit gland.
suddenly widen to continue as a balloon-shaped inflated part with a length around 40 \( \mu m \) and a diameter around 15 \( \mu m \) that is filled with dark staining secretion. When approaching the propodeal cuticle, the majority of the inflated ducts becomes narrow again to reach a diameter around 1 \( \mu m \) and runs through the cuticle as a thin canal of this size (Fig. 7B); some ducts still keep their inflated appearance when they enter the cuticle, and only display a sudden diameter reduction while already crossing the cuticle (Fig. 7C). All ducts eventually cross the cuticle as narrow canals that open to the outside as small scattered pores with a diameter around 1 \( \mu m \) (Fig. 7D and E). The anterolateral propodeal gland occurs in both queens and workers. The morphological characteristics with widening ducts as described here is found in queens, while workers appear to have narrow ducts without an inflated portion. Our study material unfortunately did not allow to analyse the gland with electron microscopy.

3.6. Metasternal process gland

The posterior region of the metasternite in both queens and workers is characterized by the presence of two metasternal processes, which occur as a left and right cone-shaped dome (Fig. 8A). These processes have a height of around 100 \( \mu m \) with a distance of around 150 \( \mu m \) between their apices. Histological examination reveals that each process is filled with 15–20 polygonal class-3 secretory cells with a diameter around 20 \( \mu m \) (Fig. 8C). Each secretory cell is connected to a duct cell that is oriented towards the interior of the thorax. The ducts initially have an internal diameter of 0.5 \( \mu m \) (Fig. 8G), but then abruptly inflate to form a sac-like portion with a diameter around 20 \( \mu m \) and length around 30 \( \mu m \), in which dark staining material is accumulated (Fig. 8C,G). The ducts at either side then become narrow again to reach a diameter of 0.5–1 \( \mu m \), and run posteriorly as two bundles (Fig. 8D–F) that will eventually open through a left and right side sieve-plate. These are located approx. 80 \( \mu m \) posterior to the base of the ipsilateral process (Fig. 8A,B,F), close to the inner side of the hindleg implantation. Each sieve-plate appears as a region with 15–20 pores with a diameter of 1 \( \mu m \) that is partly covered by irregularly shaped cuticular scales (Fig. 8B). Ultrastructural observation shows the presence of numerous mitochondria (Fig. 8H). Other organelles are less apparent, granular endoplasmic reticulum could not be found.

4. Discussion

Leaving aside the wide variety of over 20 exocrine glands in the legs (Billen, 2009b; Billen et al., 2013a), the thorax in ants is usually not a very glandular body part, as it only contains the labial glands in the prothorax and the metapleural glands in the metathorax. Our finding of 4 additional hitherto unknown paired glands in the thorax of *Myopias hollandi*, therefore, is exceptional and brings the currently known exocrine repertoire of ants to a total number of 89 glands. It also illustrates the particular wealth of exocrine glands in *Myopias* ants, as also a survey of their abdominal glands revealed the occurrence of two glands that had not been found in any other ant genus previously (Billen et al., 2013b). Ponerine ants in general are known for their well-developed exocrine system (Jessen and Maschwitz, 1983; Jessen et al., 1979), which in turn can be linked to their heavily sclerotized cuticle (Peeters et al., 2017) that needs a significant production and use of lubricating substances.

We consider the propleural pit gland a novel exocrine structure in ants. More than a century ago, Janet already described a paired ‘prothoracic gland’ in queens of *Lasius niger*, with ducts also running anteriorly but opening through the articulation membrane at the anterior side of the sternum (Janet, 1907). The gland we here describe in *Myopias hollandi*, however, has its ducts arranged in bundles that run anteriorly to open through the nostril-like anterior pit of the propleural plate. Although *L. niger* queens do also have these propleural pits, the different opening site as described by Janet (1907) indicates that both glands are different structures. The function of the novel propleural pit gland remains unknown, and requires further behavioural observations. Another gland that has been reported in this anterior part of the prothorax is the propleural gland of fungus-growing ants. This gland also consists of class-3 secretory cells, but the ducts open directly through the underlying propleural gland, where they provide mutualistic bacteria with nutrients (Currie et al., 2006).

The posterolateral pronotal gland is situated anterior to the lateral junction between the pronotum and the upper part of the mesopleuron. The duct cells following a peculiar course before opening at the inner side of the back-folded cuticular portion of the posterior pronotal wall, which makes the glandular pores invisible from the exterior. As no gland has ever been reported at this location in any ant species, this represents another novel exocrine structure. Bolton (1999), without providing any
Fig. 3. A. Longitudinal section through the propleural pit gland of a queen (anterior side to the left). B-E. Transverse serial sections of the propleural pit gland (black arrows) of a worker, B being the most posterior section at the level of the secretory cells, and E the most anterior section near the external opening. Note conspicuous widening of ducts with dark contents anteriorly. DC: duct cells, LD: labial gland duct, MF: muscle fibres, N: nerve, PP: propleural plate, SC: secretory cells.
Fig. 4. Scanning micrographs of the anterior thorax of a worker in lateral (A) and ventral view (B), showing the opening of the propleural pit gland (arrow); the location of the gland, drawn to real scale, is indicated in white dashed lining.

Fig. 5. Semithin sections of posterolateral pronotal gland (A-B worker, C-H queen). A. Cross section through posterior prothorax, showing location of posterolateral pronotal gland with inflated ducts (white arrows; fc: foreleg coxae, LG: labial gland, mn: mesonotum, pn: pronotum). B. Detail of secretory cell (SC) and duct cell (DC), that enlarges to form inflated duct (ID). C–H. Successive cross section views of right side wall of posterior prothorax (C most anteriorly, H most posteriorly; D-H all shown at same magnification). Note how initially narrow ducts (black arrows in C) considerably inflate (white arrows in C–F) before they become narrow again (black arrows in E–H) and eventually open at inner side of posterior pronotal margin in H.
illustration, mentions about a ‘mesopleural gland’ in species of the dacetonine genus *Strumigenys*, that should be associated with a large circular excavation, which in these ants occurs anteriorly in the mesopleuron near its junction with the posterolateral pronotal margin. Our histological examination of this region in several *Strumigenys* species did not reveal any glandular tissue, however, which confirms the posterolateral pronotal gland that we here describe in *Myopias hollandi* is the only known exocrine structure in this part of the thorax. The function of this novel gland as yet remains unknown. The absence of granular endoplasmic reticulum in the secretory cells indicates that a non-proteinaceous secretion is produced. This may either be linked with a function in lubricating the lateral articulation between the pro- and mesothorax, or with a pheromonal role, which will require studying any peculiar behaviour that may be associated with this part of the thorax.
The anteroventral region of the propodeum, adjacent to the tentorial pit that marks the externally visible division between the upper and lower metapleuron, at either side shows a region with approx. 20 scattered pores with a diameter of 1 μm. These represent the opening of the anterolateral propodeal gland, which has not been found before in any ant species. The presence of individual subepithelial glands that open at the tegumental surface through a small pore is a common feature in ants (Gobin et al., 2003), though it differs from this novel propodeal gland in which the secretory cells form a prominent cluster. Another difference is the inflation of the ducts, at least in the queens (see below). The opening site, which is not in the near vicinity of any articulating sclerites, makes a lubricant function unlikely. Although we could not examine the ultrastructural features of the secretory cells, a pheromonal function seems plausible, and will require behavioural observations.

Many ants possess two metasternal processes that appear as a left and right cuticular projection of their posteroventral alitrunk. Our finding in M. hollandi, that each cone-shaped process is filled with a cluster of gland cells that release their secretion more posteriorly through a sieve-plate near the inner margin of the hindleg coxae, represents the first description of this novel metasternal process gland. The peculiar ventral opening site leaves it to speculation what the function of the gland may be. We do not think that a merely lubricating role for the hindleg coxae is likely, as this

Fig. 7. Anterolateral propodeal gland in queen A. Cross semithin section through anterior propodeal region, showing location of paired anterolateral propodeal gland (encircled). B.C. Details of right side gland with secretory cells (SC), and ducts with narrow (black arrows) and inflated part (ID) penetrating the propodeal cuticle. D. Scanning micrograph of posterior thorax, showing gland region in framed part. E. Enlargement of framed part in D, showing scattered pores where ducts open. ID: inflated duct, FC: fat cells, MF: muscle fibres, MPG: metapleural gland opening, MsG: mesothoracic ganglion, ps: propodeal spiracle, tp: tentorial pit.
Fig. 8. Metasternal process gland (A,B,G,H worker, C-F queen). A. Ventral view scanning micrograph of the two metasternal processes (mp) and ventral articulation membrane that connects to petiole (vap). Black arrows indicate region where duct cells open (framed part is enlarged in B). C–F. Successive cross section ventral views of posterior metathorax, all at same magnification (C at level of metasternal processes filled with secretory cells (SC), F at level of duct openings), showing the course of ducts (white arrows indicate inflated ducts, black arrows indicate narrow ducts). G. Electron micrograph of secretory cell with end apparatus (EA) and rounded nucleus (Ns) and of narrow and expanded ducts (black and white arrows, resp.). H. Detail of cytoplasm showing end apparatus (EA) and mitochondria (M). Nd: nucleus duct cell.
would rather suppose a gland that opens through the articulation membrane. In addition, there is no reason why only the hindlegs would be equipped with such specialized gland. The absence of granular endoplasmic reticulum in the gland cells indicates that the secretion is non-proteinaceous, and hence may possibly have a pheromonal function. Behavioural and chemical examination may help to shed light on this.

The labial and metapleural glands form part of the standard exocrine equipment of ants. The labial (= salivary) gland belongs to the acinar type, which among ants is common for Ponerinae, and which represents a modification of class-1 glands as reflected by a series of complex cellular differentiations that occur during the pupal stages (Lommele, et al., 2003). The metapleural gland, that is best known for its production of antibiotics, shows the same general morphology as in other ants (Hölldobler and Engel-Siegel, 1984). Together with the 4 other novel glands that we here describe, it belongs to the group of class-3 glands, which are formed by bicellular units that each comprise a secretory cell and a duct cell (Noirot and Quennedey, 1974).

The internal diameter of the duct cell canal in insects in general, as well as of the pores through which they open, is commonly constant and measures between 0.5 and 1 μm, regardless the size of the animal. We hypothesize that this remarkable feature is linked to physiochemical properties such as capillarity and optimal transportation of secretion. Myopius ants in this regard are a peculiar exception, as the ducts of their class-3 glands start with a diameter of 0.5 μm near their junction with the secretory cell, but then gradually widen up to 5 μm near their opening site (Billen et al., 2013b). We here also report on such gradually widening ducts in the metapleural gland, with a gentle increase from 0.5 to 2 μm, while it is very pronounced in the novel prepleural pit gland where duct diameter increases from 0.5 to almost 10 μm. The functional meaning of such widening ducts remains unclear.

The three other novel glands that we here describe (posterolateral pronotal gland, anterolateral propodeal gland and meta-thermal process gland) show another peculiarity, as their ducts start narrow, then considerably inflate to a balloon-like central portion, and then become narrow again before opening to the exterior as a pore with a diameter around 1 μm. To the best of our knowledge, such inflated ducts have been reported only twice in ants so far, first for a sternal abdominal gland in workers of Cylindromyrmex whymperi (Gobin et al., 2001), and more recently for tergal abdominal glands in queens of Eciton hamatum (Hölldobler, 2016). A bulbous ampulla, although with a heavily sclerotized lining, that forms part of the conducting canal has also been described in firebugs, with the additional peculiarity that such ampulla is formed by a separate cell that is intercalated between the secretory cell and the proper duct cell (Farine, 1987; Quennedey, 1998). Also the inflated part of the duct in Cylindromyrmex whymperi is formed by one or even more intercalated duct cells (Gobin et al., 2001). As in Cylindromyrmex, we believe that the inflated portion of the ducts in Myopius hollandi functions as a kind of reservoir chamber for the secretory cell. As the volume of the balloon-like extension can be similar to that of the secretory cell, the amount of stored secretion appears considerable. As the there is no direct muscular supply associated with either the inflated ducts or the secretory cells, we assume that release of secretion is regulated by the indirect effect of general body musculature that causes changes in hemolymph pressure.

The function of these novel glands remains unknown. A rather ordinary function such as lubricant production is not likely, as the glands are not located at an articulation site (except for the posterolateral pronotal gland). Their peculiar morphology rather makes them more suitable for a specialized, possibly pheromonal function, which at least fits with the ultrastructural absence of granular endoplasmic reticulum. Future behavioural observations can hopefully shed more light on the function of these glands and of the social organization of these fascinating ants.

Acknowledgements

We are grateful to An Vandoren and Alex Vrijdaghs for their great help in section preparation and scanning microscopy. Roberto Keller is acknowledged for giving valuable anatomical advice and Benoît Guénard for the identification of the ants studied. This study was supported through a Grant-in-Aid for Scientific Research from JSPS (B, Nr. 24405010, 16H05769).

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