

## The exocrine system of *Aneuretus simoni* (Formicidae, Aneuretinae)

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**ABSTRACT.** This paper reviews the morphology of the 11 exocrine glands that we found in *Aneuretus simoni* workers. In comparison with their stingless sister group Dolichoderinae, the stinging *A. simoni* has a well-developed venom gland with long and slender secretory filaments. They also share the occurrence of Pavan's gland as source of the trail pheromone, although this gland has a bilobed appearance in *Aneuretus*, while it only has one lobe in dolichoderine species. The intramandibular gland is well-developed, while the metapleural gland has the lowest number of secretory cells known in ant workers. All glands described occur in both minor and major workers, with sometimes clear differences between both worker castes. Major workers have larger propharyngeal glands, which can be understood by their presumed role in food storage in the colony, and also have metapleural glands with twice as many secretory cells than minor workers. Minor workers in turn have a more developed venom gland, which is in line with their higher activity in nest defence and prey capture.

**Keywords:** *Aneuretus simoni*, Aneuretinae, exocrine glands, morphology, histology, ultrastructure

### INTRODUCTION

*Aneuretus simoni* Emery, 1893 is the sole living representative of the subfamily Aneuretinae, which is the sister group to the Dolichoderinae. This phylogenetic position is well-supported and based on morphological, fossil and molecular data (Ward et al. 2010; Ward 2014, and references therein). The species is endemic to Sri Lanka, where it lives in pieces of rotting wood on the ground in forests of the wet and intermediate zones in the southeastern parts of the country (Wilson et al. 1956; Dias et al. 2011, 2013; Dias & Ruchirani 2014; Dias & Udayakantha 2016). This very limited geographical distribution together with its unique phylogenetic position

make *A. simoni* a vulnerable ant species, which has led to its inclusion in the IUCN Red List as 'endangered' (Fellowes & Brühl 2009).

Information on the biology of *A. simoni* has mainly been provided by the studies of Wilson et al. (1956), Traniello and Jayasuriya (1981a,b, 1985) and Jayasuriya and Traniello (1985). From these papers we know that the small colonies are polydomous and contain one or sometimes more dealate queens, an average of 65 minor workers and 2 (Wilson et al. 1956) or 3 (Jayasuriya & Traniello 1985) major workers (but note that the colony fragment we had available for the present study contained 4 major workers !). The worker caste is clearly dimorphic with no intermediate individuals between the minor and major workers

(Wilson et al. 1956; based on this paper, we here also refer to the large workers as ‘majors’ rather than ‘soldiers’). Morphometric data on minor and major workers as well as queens are given in Dias (2014), and include head width measurements of 0.44–0.51 mm for minor workers, 0.61–0.86 mm for major workers, and 0.82 mm for queens.

The major workers do not participate in brood care or colony defence, but show a high activity during nest moving, when they lay trails and transport brood and workers. Majors also show a high rate of trophallaxis with both adult and callow minor workers, and therefore may play a role in food storage (Traniello & Jayasuriya 1985). Mature minor workers, on the other hand, are mainly in charge of nest defence, while callow minors in such situations retreat with brood towards the nest interior (Traniello & Jayasuriya 1985). The callow workers are also involved in queen-related acts, brood care and foraging. This early foraging is explained in the species’ food habits, in which insect prey is directly offered to the larvae (Wilson et al. 1956), thus making the otherwise unrelated tasks of foraging and larval provisioning part of the same behavioural sequence (Traniello & Jayasuriya 1985). It is noteworthy that callow minor workers are “unusually active in foraging-related tasks” (Traniello & Jayasuriya 1985). During prey capture, the ants use their well-developed sting. They are not exclusive predators, however, as they also collect carbohydrates from rotting fruit (Jayasuriya & Traniello 1985). The presence of a sting clearly distinguishes *Aneuretus* from the Dolichoderinae, although the detailed anatomy of the sclerites of the venom apparatus with the furcula fused to the anterior sting base in both groups supports the very close relationship between Aneuretinae and Dolichoderinae (Blum & Hermann 1978).

Studies on the exocrine system of *A. simoni* have been given in brief anatomical descriptions of the pygidial and Pavan’s gland, together with a documentation of their respective functions in alarm communication and trail laying (Traniello & Jayasuriya 1981). We added more detailed reports on the intramandibular gland (Billen & Verbesselt 2016a), Pavan’s gland (Billen & Verbesselt 2016b) and the metapleural gland (Billen 2017). In the present paper, we bring a review on all previously studied exocrine

glands of *A. simoni*, together with novel histological and ultrastructural data on the other glands, that had not yet been studied before.

## MATERIAL AND METHODS

During a short visit to Gilimale Forest in southern Sri Lanka in December 1986, we found a nest fragment of *Aneuretus simoni* in a decomposing fallen twig. This sample contained 16 minor and 4 major workers, of which 13 minors and 2 majors were prepared for sectioning (light and electron microscopy), the remaining 3 minors and 2 majors were prepared for scanning microscopy. The latter were mounted on stubs and coated with a thin layer of gold before examination in a JEOL JSM-6360 scanning microscope. For sectioning, workers were separated into head, thorax and abdomen, these tissues were then fixed in 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose, and post-fixed in 2% osmium tetroxide. After dehydration in a graded acetone series, tissues were 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 examined with an Olympus BX-51 light microscope. For ultrastructural analysis, we studied thin sections of 70 nm thickness, double stained with uranyl acetate and lead citrate, under a Zeiss EM900 electron microscope.

## RESULTS AND DISCUSSION

The following survey includes a description of the 11 exocrine glands that we found during the examination of our serial sections (Fig. 1). According to the commonly used classification of insect exocrine glands by Noirot and Quennedey (1974), glands are either formed by simple epithelial cells (class-1) or are formed by structurally more complex bicellular units (class-3), of which each unit comprises a secretory cell and a duct cell. The connection between both cells is known as the end apparatus, that serves as a draining device to guide the secretion from the secretory cells via the duct cells to the exterior. The various glands will be shown in the order from head

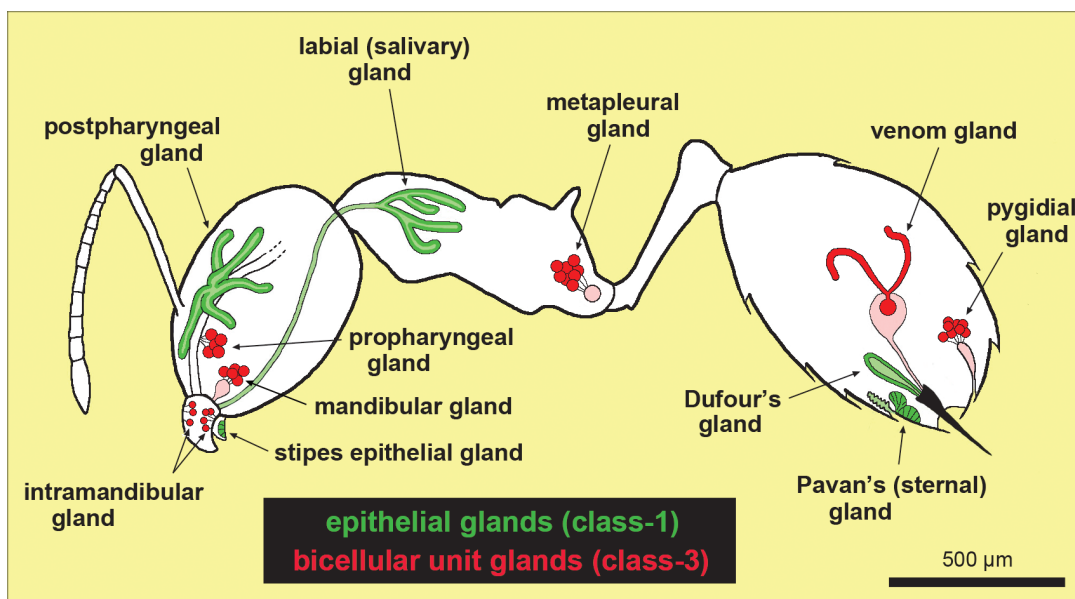


Fig. 1. Profile drawing of a minor worker with indication of the various exocrine glands (drawn to scale).

to abdomen. All longitudinal section figures are shown with the anterior side to the left.

### 1. Intramandibular gland

Both minor and major workers show a clear intramandibular gland with class-3 cells (Fig. 2A,B). In both castes, approx. 8 cells open through the proximal upper surface of each mandible, while approx. 12 cells open through the proximal lower surface. The round to polygonal cells have a diameter of  $16.6 \pm 2.7 \mu\text{m}$  in minor and  $15.6 \pm 2.7 \mu\text{m}$  in major workers. Ultrastructural examination of the secretory cells reveals the presence of a well-developed granular endoplasmic reticulum and Golgi apparatus, which is indicative for the elaboration of a proteinaceous, and hence non-pheromonal secretion. More details can be found in Billen and Verbesselt (2016a).

Class-3 intramandibular glands are commonly found in ants, and were first described by Schoeters and Billen (1994). The secretory cells discharge their contents mainly through tiny pores onto the upper mandibular surface via their corresponding duct cells, as has also been found in *Polyergus rufescens* (Grasso et al. 2004) and in *Atta laevigata* (Martins et al. 2015). In *Oecophylla longinoda*, the gland cells open both at the upper and lower mandibular surface, and have a

function in substrate marking and nestmate recruitment (Roux et al. 2010), which is the only case in which the function of the intramandibular gland is known. A special kind of class-3 intramandibular gland has been found in *Tatuidris tatusia*, as the ducts of all secretory units cluster together in one main bundle, that opens through a single sieveplate at the ventral proximal side of each mandible (Billen & Delsinne 2014). The structural complexity of intramandibular glands is further illustrated by the existence of class-1 glands as well. Epithelial intramandibular glands underneath the ventral mandibular surface have been found in some *Pachycondyla* species (Martins & Serrão 2011) and *Protanilla wallacei* (Billen et al. 2013). Still another epithelial intramandibular gland has recently been described in *Brachyponera sennaarensis*, where it is associated with the peculiar mandibular pit that is a diagnostic characteristic for this genus (Billen & Al-Khalifa 2016).

The intramandibular gland in *A. simoni* is special as its class-3 cells open through both mandibular surfaces, and even more through the lower surface. In other ants, class-3 intramandibular glands mainly open through the upper surface (Schoeters & Billen 1994; Roux et al. 2010), which may indicate a more prominent function of

the gland in *Aneuretus*. Minor and major workers show no difference, which suggests that the intra-mandibular gland function is not caste-specific. A second remarkable characteristic is that the secretory cells in *A. simoni* contain a well-developed granular endoplasmic reticulum, whereas in other ant species the intramandibular gland usually has smooth endoplasmic reticulum. The secretion in *Aneuretus* therefore has a proteinaceous nature, in contrast to the non-proteinaceous and possibly pheromonal secretion of most other ants (Billen & Verbesselt 2016a).

## 2. Stipes epithelial gland

The inner proximal part of each stipes shows an obvious epithelial gland with a thickness of  $24.0 \pm 3.7 \mu\text{m}$  in minor (Fig. 2B) and  $17.0 \pm 3.4 \mu\text{m}$  in major workers (Fig. 2C). The basally located round nuclei have a diameter around  $3 \mu\text{m}$ .

The existence of an epithelial gland inside the maxillary stipes has so far only been reported in workers as well as queens of *Protanilla wallacei* (Billen et al. 2013). We noticed its presence also in workers of other phylogenetically distant ant genera such as *Proceratium* and *Solenopsis* (own unpubl. obs.), which may indicate this gland is more common than currently known. As the left and right side glands are directed towards the labium (see Figs 2B,C) and hence come in contact with food, they may have a digestive function.

## 3. Mandibular gland

Although all ants have a pair of class-3 mandibular glands, it was difficult to find them in our *Aneuretus* sections, as we estimate there are only around 7 secretory cells per gland while the reservoir appears collapsed (Fig. 3A). The cells moreover are quite small with a diameter of  $16.2 \pm 3.0 \mu\text{m}$  in minor workers; the head sections of the 2 majors we had available did not allow any proper count or measurement. Ultrastructural observation reveals a well-developed smooth endoplasmic reticulum in the secretory cells (Fig. 3B).

The presence of smooth endoplasmic reticulum is indicative for the elaboration of a non-proteinaceous and hence possibly pheromonal secretion, which is often the case for mandibular glands (Billen & Morgan 1998). One of the

most commonly reported functions for the mandibular gland in ants is that of producing alarm pheromones (Maschwitz 1964). Behavioural experiments on *Aneuretus simoni* using glandular extracts, however, showed that the mandibular gland is not involved in any form of alarm communication, but that this role is attributed to the pygidial gland (Traniello & Jayasuriya 1981b). Although the ultrastructural characteristics of the mandibular gland in *A. simoni* make a pheromonal function likely, further behavioural observation and testing will be necessary to verify this.

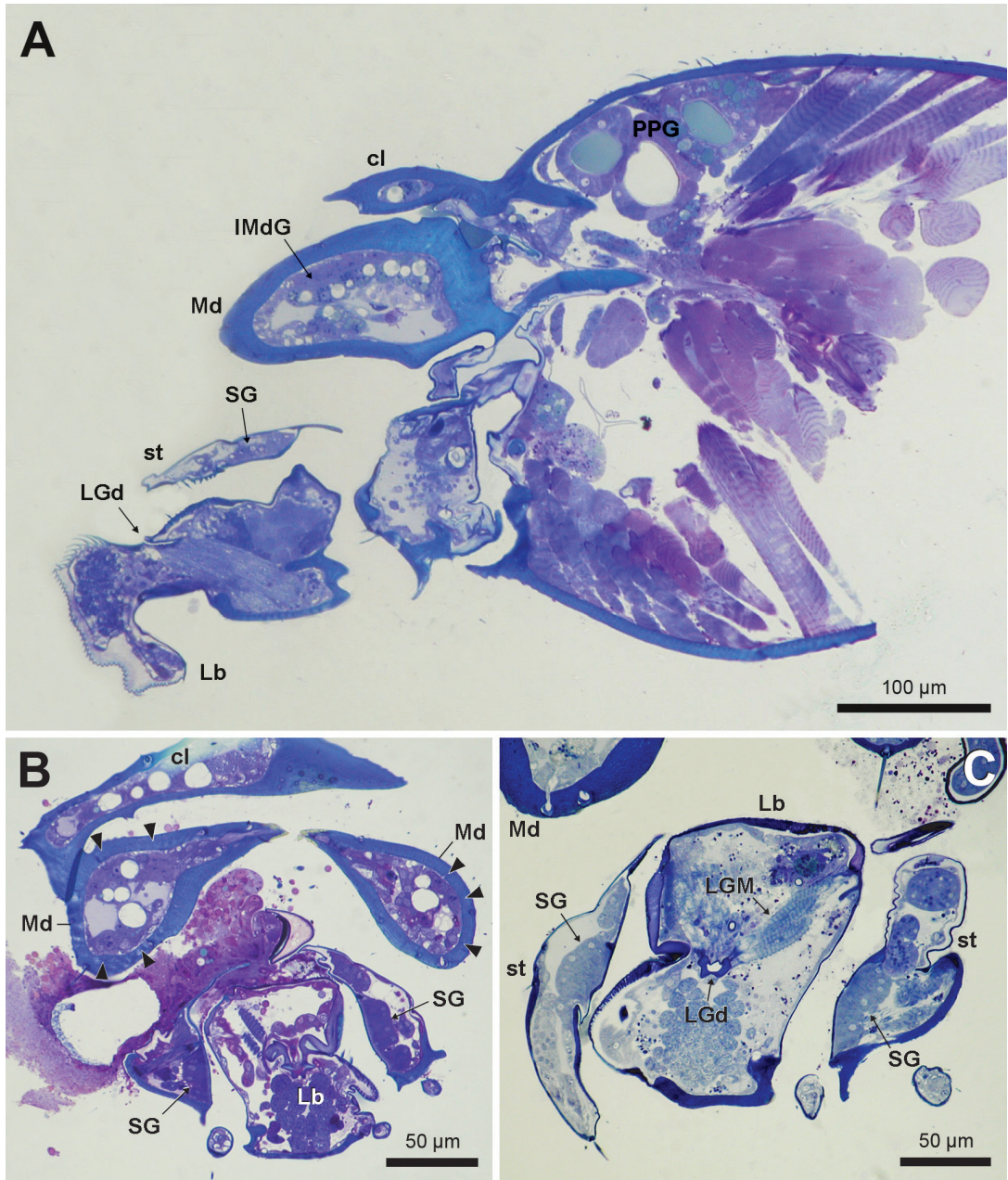
## 4. Propharyngeal gland

The propharyngeal gland is formed by a left and right cluster of class-3 secretory cells that open through their accompanying ducts in the anterior lateral region of the pharynx (Fig. 3C), and forms part of the standard exocrine system that occurs in all ants. In *A. simoni*, we count approx. 12 cells per side, with an average diameter of  $36.2 \pm 4.7 \mu\text{m}$  in minor and  $48.4 \pm 6.8 \mu\text{m}$  in major workers. The ducts have an internal diameter of  $0.5 \mu\text{m}$  and at each side form a bundle that opens into the lateral pharynx (Fig. 3D). The secretory cells contain an abundance of granular endoplasmic reticulum, which illustrates the clearly proteinaceous nature of the secretory products (Fig. 3E). The secretion appears as round electron-dense droplets with a diameter up to  $1 \mu\text{m}$ , that accumulate around the end apparatus (Fig. 3F), from where they are drained into the ducts.

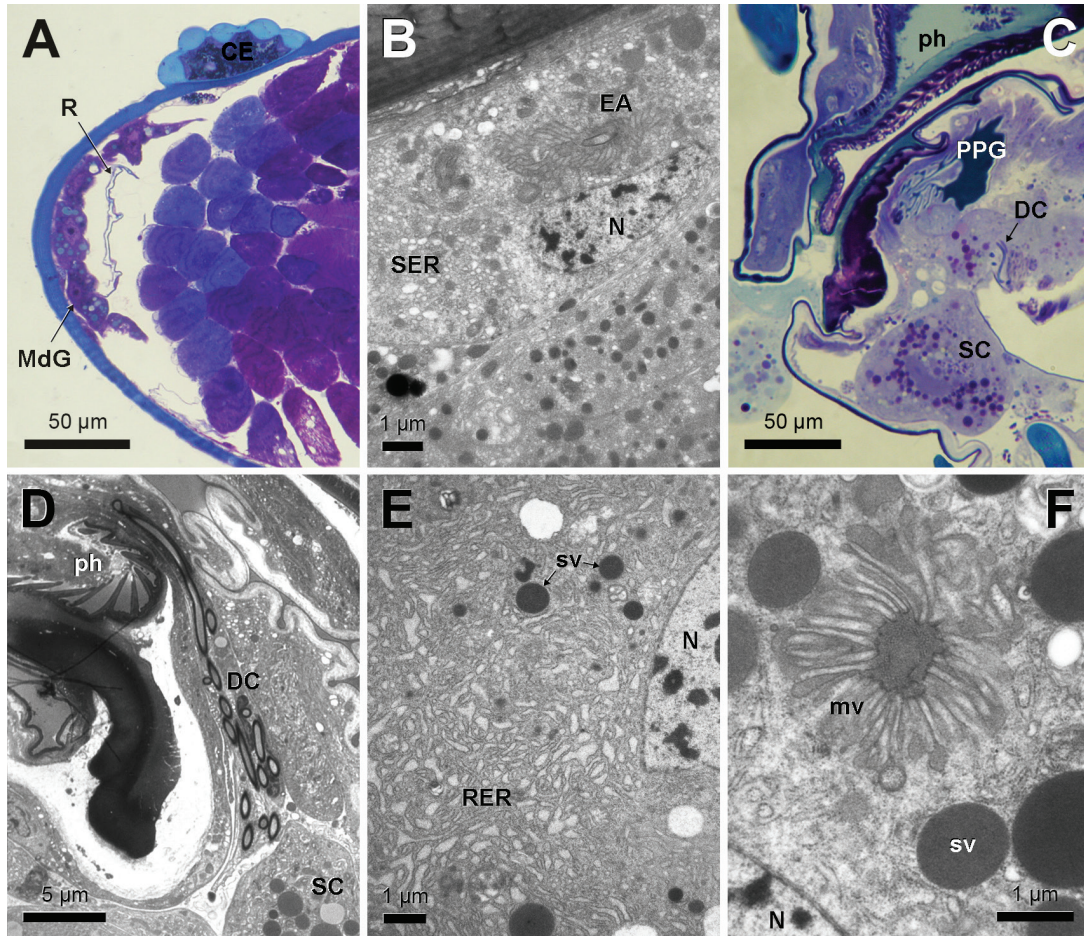
The general anatomy as well as the ultrastructural organization of the propharyngeal gland in *Aneuretus* is similar with that in other ants, and corresponds with the commonly accepted function of producing digestive enzymes (Boonen & Billen 2016, and references therein).

## 5. Postpharyngeal gland

The postpharyngeal gland is a unique exocrine structure that among social insects is only found in ants (as well as in crabronid digger wasps: Strohm et al. 2007, 2010). It is formed by multiple tubules or lobes that surround the branched central lumen, with epithelial class-1 cells forming the wall. In *A. simoni*, it is glove-shaped with 4 tubular extensions, that open in the posterior part of the pharynx. Although no muscles are



**Fig. 2.** – **A.** Longitudinal section through the head of a minor worker with indication of the intramandibular gland (IMdG), stipes epithelial gland (SG) and postpharyngeal gland (PPG). **B.** and **C.** Cross sections through the mouthparts of a minor (**B**) and major (**C**) worker showing the intramandibular gland cells both underneath the upper and lower mandibular surface (arrowheads) and the stipes epithelial gland (SG). cl: clypeus, Lb: labium, LGd: labial gland duct, LGM: labial gland duct muscles, Md: mandible, st: stipes.

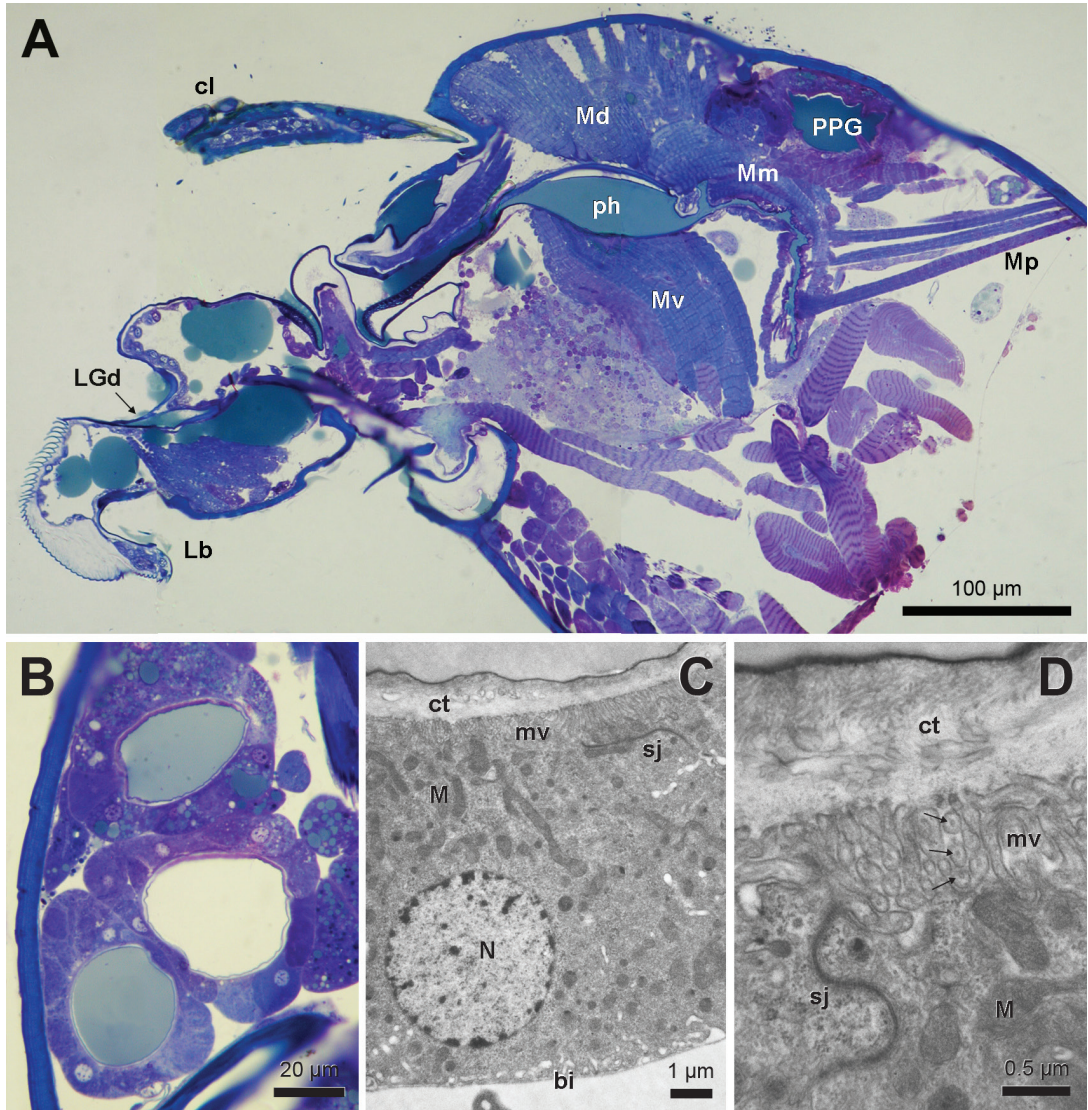


**Fig. 3.** – **A.** Cross section through head showing mandibular gland secretory cells (MdG) and reservoir (R). **B.** Electron micrograph of secretory cell of mandibular gland with end apparatus (EA) and smooth endoplasmic reticulum (SER). **C.** Longitudinal section through anterior pharynx (ph), postpharyngeal gland tubule (PPG) as well as duct cells (DC) and secretory cell (SC) of propharyngeal gland. **D-F.** Electron micrographs of propharyngeal gland showing duct cells (**D**), general view of secretory cell with secretory vesicles (sv) and abundant granular endoplasmic reticulum (RER) (**E**) and detail of end apparatus with microvilli (mv) surrounded by secretory vesicles (**F**). CE: compound eye, N: nucleus. All images in this figure are from minor workers.

directly associated with the gland, it is affected by various muscle groups that control the pharyngeal movements (dorsal and ventral anterior muscles, as well as muscles in the middle and posterior region: Fig. 4A). The more or less cubic epithelial cells have a height of  $11.8 \pm 1.6 \mu\text{m}$  in minor workers and approx.  $13 \mu\text{m}$  in majors. The gland tubules have a large lumen of 25-30  $\mu\text{m}$  (Fig. 4B). The epithelial cells have a round nucleus of 4-5  $\mu\text{m}$  and are apically lined with a cuticle of approx. 1  $\mu\text{m}$  thick. The apical cell membrane is differentiated into a clear microvil-

lar border, while the basal cell membrane shows numerous basal invaginations (Fig. 4C). The cytoplasm contains numerous mitochondria and smooth endoplasmic reticulum, of which extensions continue into the microvilli (Fig. 4D). The lateral cell contacts show septate junctions in their upper part (Fig. 4D).

The cytoplasmic organization of the secretory cells is in line with the general appearance of the postpharyngeal glands in other ants, and thus corresponds with a tissue involved in lipid metabolism (Eelen et al. 2006). Besides a num-



**Fig. 4.** – Postpharyngeal gland images of minor workers. **A.** Longitudinal section through anterior part of head showing pharynx (ph) and associated pharyngeal musculature (Md: dorsal muscle, Mm: Muscle middle region, Mp: posterior muscle, Mv: ventral muscle). **B.** Close-up of postpharyngeal gland tubules. **C.** Electron micrograph of postpharyngeal gland epithelium with apical microvilli (mv) and basal invaginations (bi). **D.** Ultrastructural detail of apical cytoplasm and microvillar border of postpharyngeal gland epithelium. Note extensions of smooth endoplasmic reticulum in core of microvilli (small arrows). cl: clypeus, ct: cuticle, Lb: labium, LGd: labial gland duct, M: mitochondria, N: nucleus, PPG: postpharyngeal gland, sj: septate junction.

ber of possible secondary and mainly digestive functions, the main function of the postpharyngeal gland in ants is associated with their system of nestmate recognition as the gland contains the same hydrocarbons as those that appear on the epicuticle (Bagnères & Morgan 1991; Eelen et al.

2006 and references therein). Although no direct experimental data on the chemical composition of the postpharyngeal gland are available for *A. simoni*, it is likely that the gland is also involved in nestmate recognition in this species, as Jayasuriya and Traniello (1985) suggested that nestmate

recognition and perhaps intraspecific territoriality is well developed. The presence of smooth endoplasmic reticulum at least is compatible with the elaboration of hydrocarbons, as is known for other ant species (Bagnères & Blomquist 2010).

### 6. Labial (salivary) gland

The labial gland opens at the tip of the labium (Figs 2C,4A), from where an unpaired duct runs towards the thorax. After crossing the neck, the duct branches into several secondary ducts, that continue as secretory tubules that are made up of class-1 secretory cells (Fig. 5A). The tubules have a diameter of  $18.1 \pm 3.0 \mu\text{m}$  in minor and  $22.8 \pm 4.7 \mu\text{m}$  in major workers. The ducts are lined with epithelial cells with a thickness around  $5\text{--}7 \mu\text{m}$  that are loaded with mitochondria, and with slender microvilli underneath the cuticle (Fig. 5B). The electron-dense cuticle lining the internal lumen has a thickness of  $0.1 \mu\text{m}$ , and is characterized by the occurrence of paler zones, that very much resemble the taenidial reinforcements in the cuticular lining of tracheal tubes (Fig. 5C). The cells forming the secretory tubules contain a well-developed granular endoplasmic reticulum and an abundance of spherical electron-dense secretory vesicles with a diameter of  $0.5\text{--}1.2 \mu\text{m}$  (Figs 5D,E).

While the designation labial gland is linked to its anatomical opening, the gland is also known as the salivary gland following its major function. This function is clearly reflected in the cytoplasmic organization of the secretory cells, as the secretory vesicles most likely represent the digestive enzymes that have been assembled in the granular endoplasmic reticulum (Billen et al. 2013). The long ducts show the characteristics of a transport epithelium with well-developed microvilli and large amounts of mitochondria. The taenidia-like differentiation of the duct cuticle probably represents a mechanical reinforcement to prevent collapsing of the lumen. The tubular appearance of the secretory part is common for all non-ponerine ants, while Ponerinae are characterized by labial glands with an acinar appearance. A rather doubtful report by Gama and Cruz Landim (1982) mentions that the dolichoderine *Conomyrma brunnea* has an acinar labial gland (as in ponerines), but without providing supporting evidence.

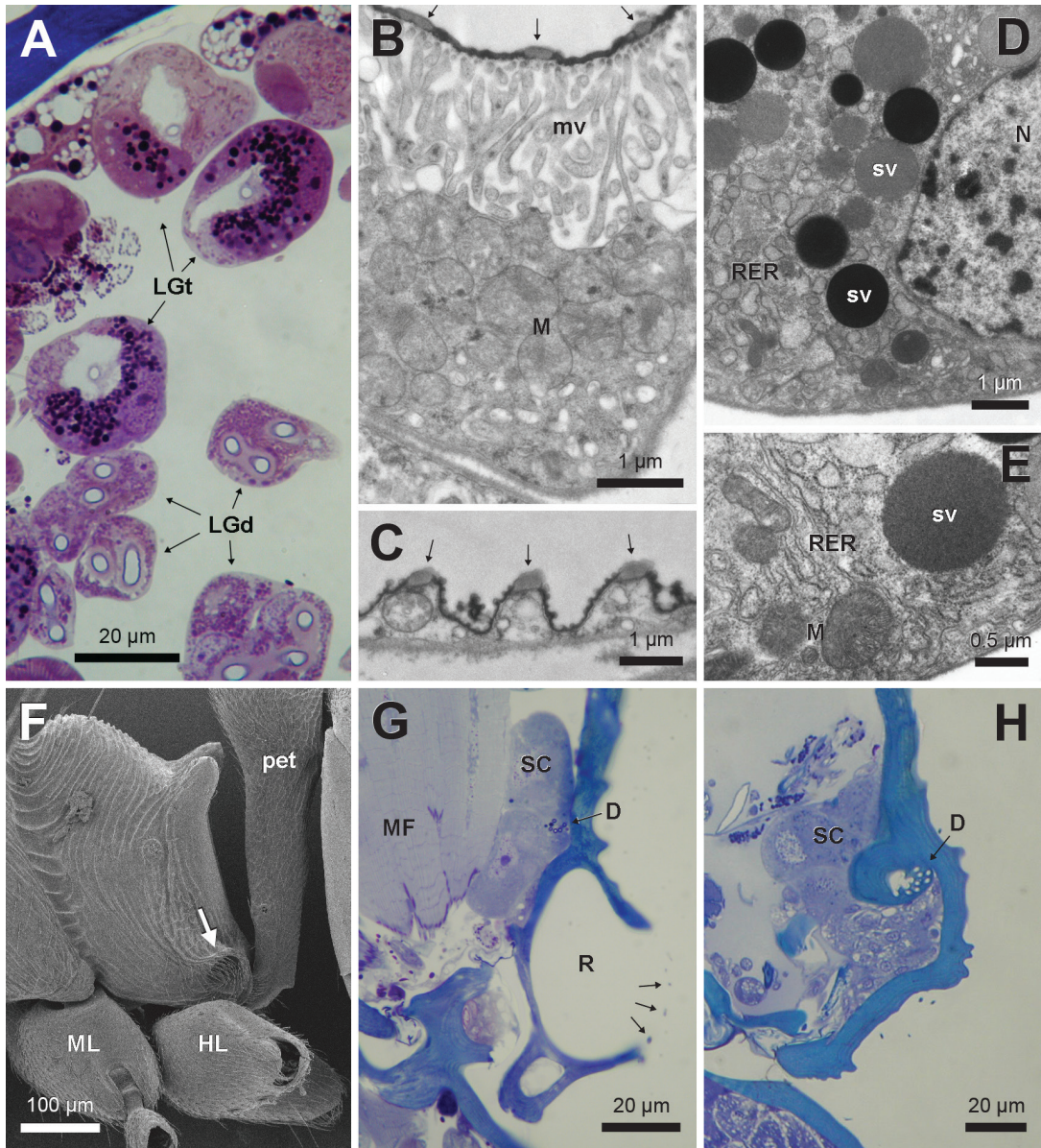
### 7. Metapleural gland

The paired metapleural gland is situated in the posterior part of the thorax, where each gland opens to the outside near the articulation with the hind-leg coxa through a large round opening with a diameter around  $40 \mu\text{m}$  (Fig. 5F). The external orifice is connected to a round and heavily sclerotized reservoir chamber from which a few tens of stiff bristle hairs protrude (Fig. 5F,G). The spherical class-3 secretory cells are found dorsally to the reservoir, into which their ducts open through a clear sieveplate (Fig. 5H). Minor workers have 6 secretory cells at each side with a diameter of  $32.6 \pm 4.7 \mu\text{m}$ , majors have 12 cells per side with a diameter of  $36.4 \pm 2.7 \mu\text{m}$ . More information, including the ultrastructural characteristics that reveal a prominent smooth endoplasmic reticulum, are given in Billen (2017).

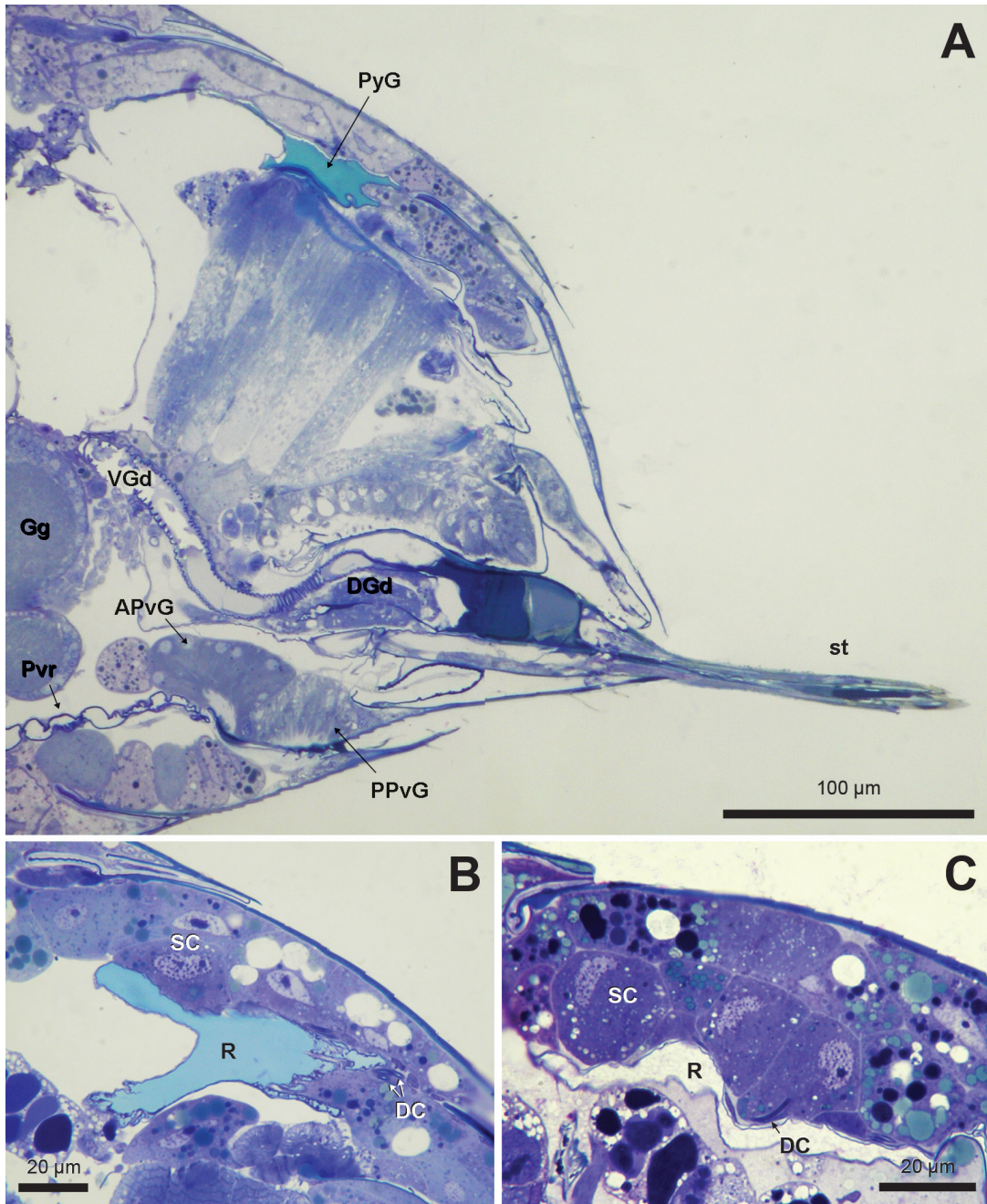
Together with the postpharyngeal gland, the metapleural gland represents a unique neofunction that is characteristic for the Formicidae. This gland mainly acts in the production of antibiotic substances, although other functions may also be possible (reviewed by Yek & Mueller 2011 and Tragust 2016). It is striking that major *A. simoni* workers have a metapleural gland with twice as many cells than minors, which may be linked to activities inside the nest (Jayasuriya & Traniello 1985). The absolute number of secretory cells, however, is the lowest of all worker ant species studied so far (Hölldobler & Engel-Siegel 1985; Billen 2017; Pech & Billen 2017). Although the ultrastructural appearance of the metapleural gland in *A. simoni* fits with the commonly known function of antibiotics production, a functional examination of the gland's secretion is needed to confirm whether the low number of gland cells can indeed suppress the growth of microorganisms in their nests, or whether they serve another role.

### 8. Pygidial gland

We found approx. 8 large round class-3 secretory cells with a diameter of  $28.4 \pm 2.0 \mu\text{m}$  in minor workers (we were not able to make proper estimates of cell number and cell size in major workers). The ducts open through the deeply invaginated articulation membrane between the 6<sup>th</sup> and 7<sup>th</sup> abdominal tergite, that acts as an enlarged reservoir (Fig. 6A-C).



**Fig. 5.** – A. Semithin section through prothorax of major worker with labial gland tubules (LGt) and ducts (LGd). B. Electron micrograph of labial gland duct cell of major worker showing abundant mitochondria (M) and irregularly arranged microvilli (mv). Note taenidia-like reinforcements of duct cuticle (small arrows). C. Detail of thin trachea wall to show real taenidia (small arrows). D,E. Electron micrographs of labial gland secretory cell (minor worker) with secretory vesicles (sv) and well-developed granular endoplasmic reticulum (RER). F. Scanning micrograph of posterior thorax of major worker showing metapleural gland orifice (white arrow). G,H. Cross semithin sections through posterior thorax of minor (G) and major worker (H), showing secretory cells (SC), ducts (D) and reservoir (R) of metapleural gland. Small arrows indicate dispenser hairs. HL: hind-leg coxa, MF: muscle fibres, ML: mid-leg coxa, N: nucleus, pet: petiole.



**Fig. 6.** – Semithin longitudinal sections of minor workers. **A.** Section through abdomen tip, showing pygidial gland (PyG), venom gland duct (VGd) and Dufour's gland duct (DGd) that both open through sting base, and Pavan's gland that consists of anterior part (APvG), posterior part (PPvG) and reservoir sac (Pvr). **B,C.** Images of pygidial gland with secretory cells (SC), duct cells (DC) and reservoir (R). Gg: ganglion, st: sting.

Well-developed pygidial glands with a considerably enlarged reservoir have been described as a characteristic for the Dolichoderinae, in which the large size has long resulted in their designation as ‘anal glands’ (Pavan & Ronchetti 1955; Billen 1986b). They function as part of the alarm-defence system by the production and release of a repellent secretion, also known as the ‘*Tapinoma*-odour’, that resembles rancid butter (Wilson et al. 1956). Although we agree with Traniello and Jayasuriya (1981b) that *A. simoni* has a pygidial gland that is ‘morphologically similar to Dolichoderinae’ with large glandular cells, we feel that the gland is not as large and has fewer cells than in most dolichoderines. Interestingly, Wilson et al. (1956) already noticed that defending *A. simoni* workers never produced anal droplets as do dolichoderines, nor that they have the ‘*Tapinoma*-odour’. In their study of chemical communication in *A. simoni*, Traniello and Jayasuriya (1981b) found that the pygidial gland elicits alarm communication, in which mature minor workers aggregate at the nest entrance where they show excited behaviour, whereas young workers hide with the brood in the deeper nest regions. These authors consider the pygidial gland as part of the glandular anatomy of the ancestral aneuretines, but that the repellent function of the secretory products evolved in the Dolichoderinae only.

### 9. Venom gland

As in the majority of ants, the venom gland of *A. simoni* is formed by two slender blind-ending secretory filaments, that open and join into the convoluted gland portion, that is situated inside the venom reservoir (Fig. 7D). The venom duct connects the reservoir to the sting base, so that the venom can be discharged through the sting shaft (Fig. 7A,B). The two filaments have a length of at least 200  $\mu\text{m}$ , and a diameter of  $15.9 \pm 3.1 \mu\text{m}$  in minor and  $12.4 \pm 4.3 \mu\text{m}$  in major workers. Each filament is made up by class-3 secretory cells that are arranged in a pseudo-epithelial way around the thin central cuticular duct, into which the ducts discharge the secretion. At the ultrastructural level, the secretory cells contain numerous ribosomes and an end apparatus to drain the secretion towards the duct cells (Fig. 7E,F). Both filaments merge just before entering the venom reservoir, where they continue into the convoluted

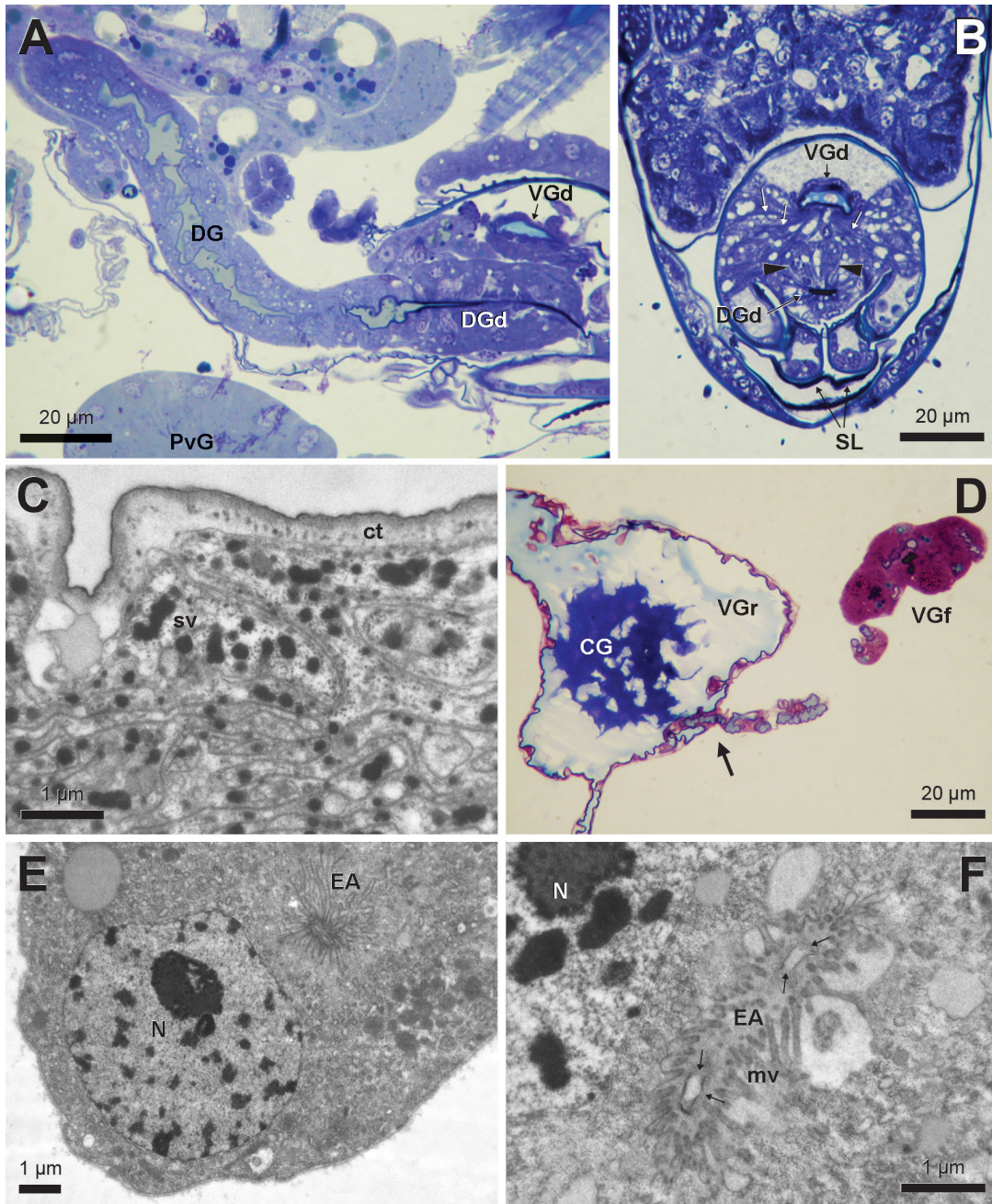
gland. In this proximal part, the filaments do not contain secretory cells, but are mainly formed by their central duct only (Fig. 7D). The convoluted gland contains secretory tissue, although we could not get a more detailed view. The reservoir sac has a diameter around 100  $\mu\text{m}$ , its wall is formed by a squamous and cuticle-lined epithelium of hardly 1  $\mu\text{m}$  (Fig. 7D). The venom duct enters dorsally into the sting bulb, where it gets a slit-like shape. Bundles of muscle fibres attach onto the sclerotized ventral wall of the duct, and upon contraction cause a dilatation and hence opening of the duct (Fig. 7B).

The well-developed appearance of the venom gland of *A. simoni* can be explained by the presence of their sclerotized and exsertile sting, which distinguishes them from the Dolichoderinae. Wilson et al. (1956) already described the presence of a well-developed sting as a primitive character. Also the long and slender venom gland filaments of *A. simoni* differ from the usually globose shape they have in the majority of the Dolichoderinae (Blum & Hermann 1978; Billen 1986b), although some dolichoderines may also have long venom gland filaments (Billen & Taylor 1993). Behavioural observations revealed that *A. simoni* indeed uses its sting to paralyze prey, and that conspecific workers from different nests upon contact readily start long-lasting combats during which they bite and sting each other (Jayasuriya & Traniello 1985).

### 10. Dufour’s gland

The tubiform Dufour’s gland is made up of an epithelium of class-1 secretory cells, that line the central lumen (Fig. 7A). The epithelium has a thickness of  $12.2 \pm 1.5 \mu\text{m}$  in minor and  $11.4 \pm 0.8 \mu\text{m}$  in major workers. It is apically lined with a cuticle of approx. 0.5  $\mu\text{m}$ , and at the ultrastructural level shows highly folded intercellular membranes and numerous small and dark secretory vesicles. Apical microvilli could not be recognized (Fig. 7C). The gland opens into the sting bulb, ventrally of the venom gland duct (Fig. 7A). The Dufour gland duct equally appears slit-like, with muscles attaching at both the upper and lower side, in order to open the duct upon contraction (Fig. 7B).

The function of Dufour’s gland in ants, in spite of its simple anatomy, still remains poor-



**Fig. 7.** – **A,B.** Semithin longitudinal (**A**) and transverse section (**B**) through sting base region of minor worker. Both Dufour's gland duct (DGd) and venom gland duct (VGd) are slit-shaped and open through sting base (in **B**, black arrowheads indicate muscles of Dufour's gland duct, small white arrows indicate muscles of venom gland duct). **C.** Electron micrograph of apical part of Dufour's gland epithelium of major worker, showing cuticle (ct), irregular cell membranes and dark secretory vesicles (sv). **D.** General view of venom gland of minor worker. Arrow indicates how secretory filaments (VGf) penetrate into reservoir sac (VGr) to make contact with convoluted gland (CG). **E,F.** Electron micrographs of venom gland secretory filaments in minor (**E**) and major worker (**F**). Small black arrows show discontinuous cuticle of end apparatus (EA). DG: Dufour's gland, mv: microvilli, N: nucleus, PvG: Pavan's gland, SL: sting lancets.

ly understood. In some species, it produces trail pheromones, but this is not the case in *A. simoni* (and the Dolichoderinae), where Pavan's gland is in charge of this. In a comparative ultrastructural study of the Dufour gland epithelium among ants from 8 subfamilies, Billen (1986a) described subfamily-specific types. The few data on *A. simoni* unfortunately do not allow to describe an 'aneuretine type', nor to make a meaningful comparison with the other types. The anatomical organization of how the duct opens into the sting base is similar to that in stinging ants, and allows an active opening of the gland duct upon contraction of the dorsal and ventral muscle groups (Billen 1986a).

### 11. Pavan's (sternal) gland

Underneath the central anterior part of the 7<sup>th</sup> abdominal sternite in both minor and major workers, a conspicuous glandular epithelium with tall cylindrical cells is found, that is associated with a wrinkled reservoir sac, that is formed by an unpaired invagination of the intersegmental membrane between the 6<sup>th</sup> and 7<sup>th</sup> sternite (Fig. 6A). In both worker castes, the epithelium is bilobed with a mushroom-shaped anterior and a semi-spherical posterior portion. The most prominent ultrastructural feature is the well-developed smooth endoplasmic reticulum. A more detailed description of Pavan's gland in *A. simoni* can be found in Billen and Verbesselt (2016b).

The presence of Pavan's gland is a unique exocrine character that is only shared by *Aneuretus* and the Dolichoderinae, although the latter do not show a bilobed appearance of the glandular epithelium. In both groups, the gland produces trail pheromones (Wilson & Pavan 1959 for Dolichoderinae; Traniello & Jayasuriya 1981a,b for *A. simoni*). This pheromonal function is in agreement with the presence of a well-developed smooth endoplasmic reticulum (Billen 1986b for Dolichoderinae; Billen & Verbesselt 2016b for *A. simoni*).

## CONCLUSION

Our survey study of the exocrine system of *Aneuretus simoni* revealed the presence of 11 glands, distributed over head (5), thorax (2) and abdomen

(4). The overall number probably will be higher than this, which on the one hand will be because we only had a total of 13 minor and 2 majors available for this study. On the other hand, when we collected these ants back in 1985, we did not specifically prepare the legs, as almost nothing at that time was known about the presence of leg glands (the presence and high diversity of 20 glands in the legs of ants only became apparent in our review study more than 2 decades later; see Billen 2009). The rather primitive fixation conditions in the field without the availability of a dissection microscope moreover did not allow such specific tissue preparation.

The 11 glands described here occur in both minor and major workers. In spite of the low numbers of workers we had available for microscopy, especially the major workers, some peculiar differences between both worker castes became apparent. Major workers have clearly larger secretory cells in their propharyngeal gland, which indicates that they are more active in food digestion. This may possibly be linked with the behavioural observations by Traniello and Jayasuriya (1985) that majors play a role in food storage in the colony, although food storage as such does not necessarily include a digestive function. Food storage moreover is more likely to happen in digestive organs such as the crop, as the propharyngeal gland lacks a reservoir and therefore is not structurally suitable for any storage function. Glandular secretions may possibly be added to regurgitated food, although this remains pure speculation without data on food exchange in this species. Majors also have a more developed metapleural gland with twice as much secretory cells than minor workers, although the overall number of metapleural gland cells is the lowest known among ants (Billen 2017). It therefore remains unknown whether this gland does also produce antibiotic substances as it does in most ant species. The difference between minor and major workers, however, is apparent and calls for a more thorough analysis to determine the exact function of this gland. Among the abdominal glands, the venom gland appears more developed in minor workers, which can be related to their pronounced activity in nest defence and prey capture (Traniello & Jayasuriya 1985). Pavan's gland, that produces the trail pheromone, is

well-developed in both minor and major workers (Billen & Verbesselt 2016b), which can be understood by minor workers using trails especially during foraging, while majors lay trails during nest emigration (Traniello & Jayasuriya 1985).

In comparison with their sister group the Dolichoderinae, the presence of Pavan's gland in *Aneuretus* is the most striking exocrine character, as no other ants possess this gland. It is remarkable, however, that this gland is bilobed in *Aneuretus*, while it only has one single lobe in the Dolichoderinae (Billen & Verbesselt 2016b). The most conspicuous difference between Dolichoderinae and *Aneuretus* is the presence of a well-developed and sclerotized sting in the latter (Wilson et al. 1956). This is also reflected in the presence of a well-developed venom gland with long and slender secretory filaments in *Aneuretus*, whereas dolichoderines usually have short globose filaments (Billen 1986b). The lack of sufficient material for electron microscopy unfortunately did not allow to thoroughly study Dufour's gland in *Aneuretus*, as this gland was found to show a subfamily-specific ultrastructural appearance (Billen 1986a).

Although the present study adds to our knowledge of the exocrine system of *Aneuretus simoni*, the chemical composition of the glands as well as the precise function of several glands remains unknown, and calls for further study of the social organization and communication system of this ant species.

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