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The metapleural gland of *Aneuretus simoni* (Formicidae, Aneuretinae)

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ABSTRACT. *Aneuretus simoni* workers have a paired metapleural gland that in minor workers contains six secretory cells on each side, while majors have 12 cells per side. The heavily sclerotized reservoir opens above the articulation of the hind-legs through a large round orifice with a diameter around 40 µm. Stiff dispenser hairs emerge from the orifice, and assist in guiding the secretory products to the exterior. The rounded to ovoid cells display a well-developed smooth endoplasmatic reticulum, which is a common characteristic for the metapleural gland in ants. This cytoplasmic organization is in agreement with the production of antibiotic substances, which is the most common function of this gland. The difference in gland size between minor and major workers may either indicate that majors are more efficient in producing antibiotics, or that the gland may serve another function, as the cell numbers in *A. simoni* are the lowest known of all ant species studied so far.

Keywords: *Aneuretus simoni*, Aneuretinae, metapleural gland, morphology, ultrastructure

INTRODUCTION

Ants can be considered as walking glandular batteries (Hölldobler & Wilson 1990), with an impressive exocrine repertoire in which a total of 85 glands can be distinguished (Billen & Šobotník 2015; Billen & Al-Khalifa 2016). Neoformations that are only known in ants are the postpharyngeal gland in the head, the metapleural gland in the thorax, and the pygidial gland in the abdomen. Of these, the paired metapleural gland is of particular interest as its major function is the production of antibiotics that protect the ants and their nests against microorganisms (Maschwitz et al. 1970; Maschwitz 1974; Beattie et al. 1986; Yek & Mueller 2011). Especially in fungus-growing ants, the gland has been well studied as these ants have well-developed metapleural glands in response to the strong pressure from parasites they experience (Poulsen et al. 2003; Fernández-Marín et al. 2006; Vieira et al. 2012). In a comparative histological study of the metapleural gland in 53 ant species representing all major subfamilies, Hölldobler and Engel-Siegel (1984) list its absence only in workers of 8 species belonging to the formicine genera *Camponotus*, *Dendromyrmex*, *Oecophylla*, *Polyergus* and *Polyrhachis*. In all other 45 species, the gland exists, with cell numbers ranging between 473 in *Atta sexdens* and 14 in *Aneuretus simoni*. This cell number indication is the only available information in literature on the metapleural gland of *A. simoni*, as Hölldobler and Engel-Siegel (1984) do not provide any histological images for this species, nor any information on eventual caste differences between minor and major workers. As we had embedded material available of both minor and major workers of *A. simoni*, we studied the metapleural gland in both castes, and here present our findings.

MATERIAL AND METHODS

We were able to collect a nest fragment of *A. simoni* in a decomposing fallen twig in Gilimale Forest in southern Sri Lanka in December 1986, containing 16 minor and 4 major workers. Of these, 3 minor and 2 major workers after ethanol preservation were mounted on stubs, coated with a thin gold layer and examined in a JEOL JSM-6360 scanning microscope. For the remaining workers, the thorax was transversely cut so that the posterior thorax portions could be fixed between 5 and 24 hours in 2% glutaraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose. Tissues were then post-fixed in 2% osmium tetroxide in the same buffer, dehydrated in a graded acetone series and embedded in Araldite. Serial semithin sections of 1 µm thickness were made with a Leica EM UC6 ultramicrotome, stained with methylene blue and thionin, and viewed with an Olympus BX-51 microscope. Thin sections of 70 nm thickness were double stained with uranyl acetate and lead citrate and examined with a Zeiss EM900 electron microscope.

RESULTS

Externally, the metapleural gland orifice at each side appears as a round opening with a diameter around 40 µm, that is situated in the posterior part of the thorax, just above the insertion of the hind-leg coxae (Fig. 1. A). The appearance is similar in major and minor workers (Fig. 1. B, C), and shows a few tens of stiff bristle hairs that emerge from the gland orifice, and that form a kind of basket-like structure over the orifice (Fig. 1.). Sections through the posterior thorax in both worker castes clearly show the implantation of these bristles in the dorsal wall of the heavily sclerotized reservoir chamber (Fig. 2. A,B). Dorsally to this reservoir chamber are the rounded to ovoid secretory cells of the metapleural gland, that have a diameter of 32.6 ± 4.7 µm in minor workers and 36.4 ± 2.7 µm in major workers. Each secretory cell is connected to a duct cell of approx. 0.5-1 µm internal diameter, the ducts forming a bundle that opens near the implantation of the bristle hairs (Fig. 2. A,B). The number of ducts, and hence also the number of secretory cells, can be clearly seen on cross sections, and reveals a clear difference between the two worker castes, as minors have six cells on each side, whereas majors have 12 cells per side (Fig. 2. C, D). There are no muscle fibres directly associated with the gland cells or the reservoir chamber, although the thoracic leg muscles touch the secretory cells (Fig. 2. A, B, 3B), and may thus have some indirect effect by creating pressure onto the cells.

Electron microscopy shows a cytoplasm with a clear end apparatus with an internal diameter of 0.3-0.5 µm, of which the epicuticular lining is discontinuous (Fig. 3. A). Numerous secretory vesicles occur (Fig. 3. A-D), of which some have a lamellar appearance (Fig. 3. D). The most prominent cell organelles are smooth endoplasmic reticulum and Golgi apparatus (Fig. 3. C,D). Nuclei have a diameter around 10 µm and can have an irregular outline (Fig. 3. A,D).

DISCUSSION

Aneuretus simoni Emery, 1893 is endemic to Sri Lanka, where it occurs in the southeastern part (Dias & Udayakantha 2016). It is the sole living representative of the subfamily Aneuretinae, which is the sister group to the Dolichoderinae (Brown 1954; Wilson et al. 1956; Taylor 1978; Brady et al. 2006; Ward 2007; Moreau & Bell 2013). Information on its exocrine system is limited to a brief description of the pygidial and sternal (= Pavan's) glands (Traniello & Jayasuriya 1981) and two recent papers on the intramandibular (Billen & Verbesselt 2016a) and Pavan's gland (Billen & Verbesselt 1916b). For the metapleural gland, the only available information is that *A. simoni* has 14 secretory cells, which is the lowest number of all ants studied (Hölldobler & Engel-Siegel 1984). It was not specified, however, whether this number is per side or for both sides together, nor whether this is for minor or major workers.

The cytoplasmic appearance of the secretory cells with an abundant smooth endoplasmic reticulum and a well-developed Golgi apparatus is in agreement with the elaboration of a non-proteinaceous secretion, which in turn fits with the most common function of the metapleural gland

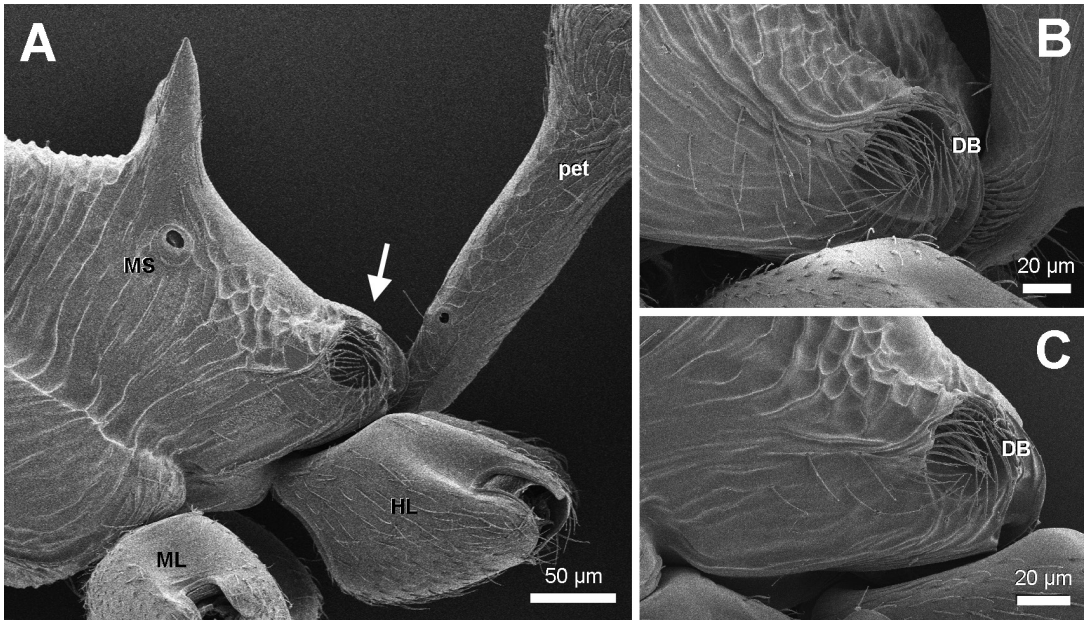

Fig. 1. A. Scanning micrograph showing the circular exterior opening (arrow) of the metapleural gland in minor worker. **B**. Detail of gland opening in major worker. **C**. Detail of gland opening in minor worker. DB: dispenser bristles, HL: hind-leg coxa, ML: mid-leg coxa, MS: metathoracic spiracle, pet: petiole.

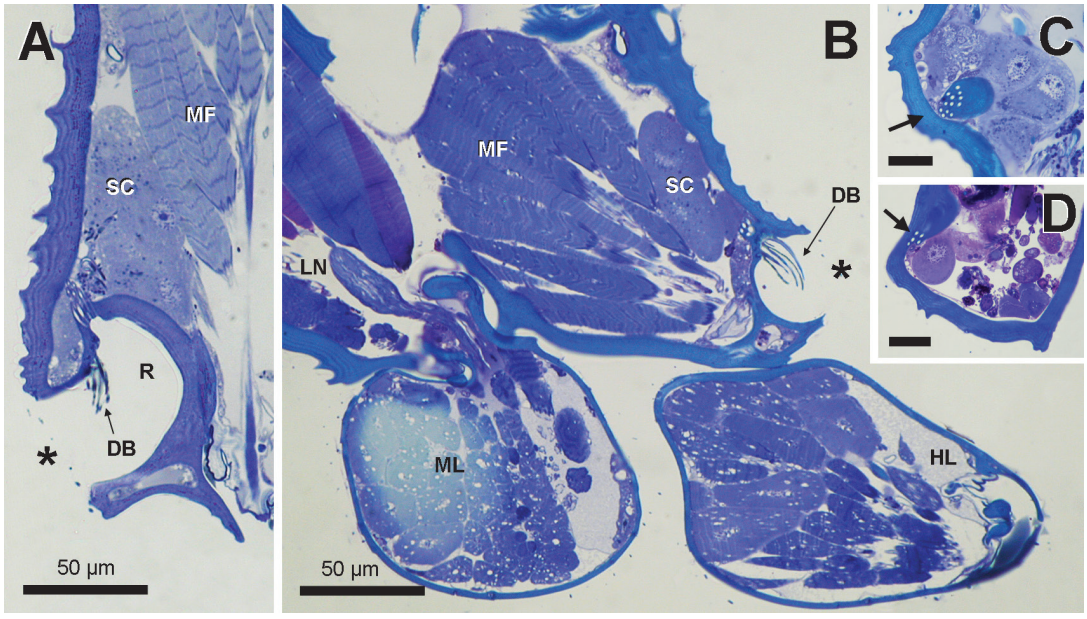

Fig. 2. Semithin sections through metapleural gland. **A**. Transverse section of major worker. **B**. Longitudinal section of minor worker. Note thoracic muscle fibres (MF) adjacent to secretory cells (SC). Cross sections in region where ducts (arrows) approach reservoir chamber are shown in **C** (major worker) and **D** (minor worker). DB: dispenser bristles, HL: hind-leg coxa, ML: mid-leg coxa, MS: metathoracic spiracle, R: reservoir chamber. Asterisks indicate external opening of gland. Scale bar in C and D is 20 µm.

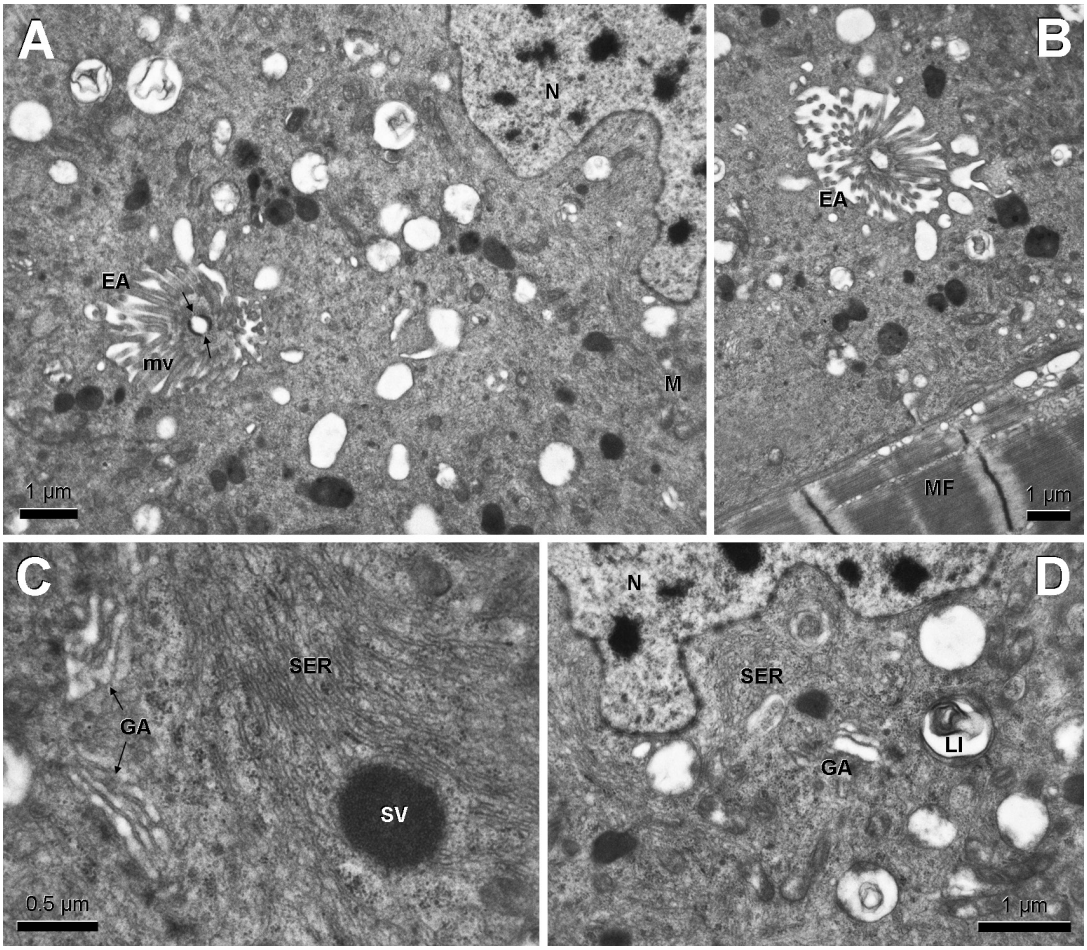

Fig. 3. Electron micrographs of secretory cells (minor workers). **A**. General view of cytoplasm with nucleus (N) and end apparatus (EA). Note discontinuous inner epicuticular lining of end apparatus (arrows). **B**. Detail of end apparatus. **C**. Detail of cytoplasm with abundant smooth endoplasmic reticulum (SER), Golgi apparatus (GA) and secretory vesicle (SV). **D**. View of cytoplasm with smooth endoplasmic reticulum, Golgi apparatus and lamellar inclusions (LI). M: mitochondria, MF: muscle fibre, mv: microvilli of end apparatus.

as a source of antibiotics. The bristle hairs that protrude from the reservoir chamber are thought to act as dispenser structures to guide the secretion to the exterior (Hölldobler & Engel-Siegel 1984). Although the large orifice allows secretion to flow out easily, it has been reported that ants can adjust the production of secretion according to the degree of microorganism infection they encounter (Fernández-Marín et al. 2006). Our results reveal a conspicuous difference between the two worker castes, as majors have 12 secretory cells per side whereas minors have only six. This may reflect a higher individual contribution of the majors towards nest antisepsis. It has been reported already that major workers are not involved in defence and foraging (Jayasuriya & Traniello 1985), which confirms that their function is more related to inside-nest activities. The very low number of secretory cells, on the other hand, may raise the question whether *A. simoni* can efficiently suppress microorganisms in its nests, or whether their metapleural gland serves other functions, as can be the case in a number of ant species (Yek & Mueller 2011).

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REFERENCES

Beattie AJ, Turnbull CL, Hough T and Knox RB, 1986. Antibiotic production: a possible function for the metapleural glands of ants (Hymenoptera: Formicidae). *Annals of the Entomological Society of America* 79: 448 – 450.

Billen J and Šobotník J, 2015. Insect exocrine glands. *Arthropod Structure and Development* 44: 399 – 400.

Billen J and Al-Khalifa M, 2016. A novel intramandibular gland in the ant *Brachyponera sennaarensis*. *Insectes Sociaux* 63: 321 – 326.

Billen J and Verbesselt S, 2016a. The intramandibular gland of *Aneuretus simoni* (Formicidae, Aneuretinae). *Asian Myrmecology* 8: 95 – 99.

Billen J and Verbesselt S, 2016b. Morphology and ultrastructure of Pavan's gland of *Aneuretus simoni* (Formicidae, Aneuretinae). *Asian Myrmecology* 8: 101 – 106.

Brady SG, Fisher BL, Schultz TR and Ward PS, 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences U.S.A.* 103: 18172 – 18177.

Brown WL Jr, 1954. Remarks on the internal phylogeny and subfamily classification of the family Formicidae. *Insectes Sociaux* 1: 21 – 31.

Dias RKS and Udayakantha WS, 2016. Discovery of the Sri Lankan Relict Ant, *Aneuretus simoni* Emery (Formicidae, Aneuretinae) and the nest density of the species in a selected region of Meethirigala Forest Reserve, Sri Lanka. *Asian Myrmecology* 8: 49 – 56.

Fernández-Marín H, Zimmerman JK, Rehner SA and Wcislo WT, 2006. Active use of the metapleural glands by ants in controlling fungal infection. *Proceedings of the Royal Society B: Biological Sciences* 273: 1689 – 1695.

Hölldobler B and Engel-Siegel H, 1984. On the metapleural gland of ants. *Psyche* 91: 201 – 224.

Hölldobler B and Wilson EO, 1990. The Ants. *Cambridge, Mass.: Harvard University Press,* pp. 732.

Jayasuriya AK and Traniello JFA, 1985. The biology of the primitive ant *Aneuretus simoni* (Emery) (Formicidae: Aneuretinae). I. Distribution, abundance, colony structure, and foraging ecology. *Insectes Sociaux* 32: 363 – 374.

Maschwitz U, 1974. Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. *Oecologia* 16: 303 – 310.

Maschwitz U, Koob K and Schildknecht H, 1970. Ein Beitrag zur Funktion der Metapleuraldrüse der Ameisen. *Journal of Insect Physiology* 16: 387 – 404.

Moreau CS and Bell CD, 2013. Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution* 67: 2240 – 2257

Poulsen M, Bot ANM and Boomsma JJ, 2003. The effect of metapleural gland secretion on the growth of a mutualistic bacterium on the cuticle of leaf-cutting ants. *Naturwissenschaften* 90: 406 – 409.

Taylor RW, 1978. *Nothomyrmecia macrops*: a living-fossil ant rediscovered. *Science* 201: 979 – 985

Traniello JFA and Jayasuriya A, 1981. Chemical communication in the primitive ant *Aneuretus simoni*: the role of the sternal and pygidial glands. *Journal of Chemical Ecology* 7: 1023 – 1033.

Vieira AS, Bueno OC and Camargo-Mathias MI, 2012. Morphophysiological differences between the metapleural glands of fungus-growing and non-fungus-growing ants (Hymenoptera, Formicidae). *PloS ONE* 8: e43570.

Ward PS, 2007. Phylogeny, classification, and species-level taxonomy of ants (Hymenoptera: Formicidae). *Zootaxa* 1668: 549 – 563.

Wilson EO, Eisner T, Wheeler GC and Wheeler J, 1956. *Aneuretus simoni* Emery, a major link in ant evolution. *Bulletin of the Museum of Comparative Zoology at Harvard College* 115: 81 – 99.

Yek SH and Mueller UG, 2011. The metapleural gland of ants. *Biological Reviews* 86: 774 – 791.