Australian Journal of Zoology, 2000, **48**, 661–667

Novel exocrine glands in the hindleg tarsi of the ant *Nothomyrmecia macrops*

Johan Billen[A], *Fuminori Ito*[B] *and Christian Peeters*[C]

[A]Zoological Institute, University of Leuven, Naamsestraat 59, B–3000 Leuven, Belgium. Email; Johan.Billen@bio.kuleuven.ac.be
[B]Biological Laboratory, Faculty of Agriculture, Kagawa University, Takamatsu 760, Japan.
[C]Laboratoire d'Ecologie, Université Pierre et Marie Curie, CNRS UMR 7625, 7 Quai Saint Bernard, F–75252 Paris 05, France.

Abstract

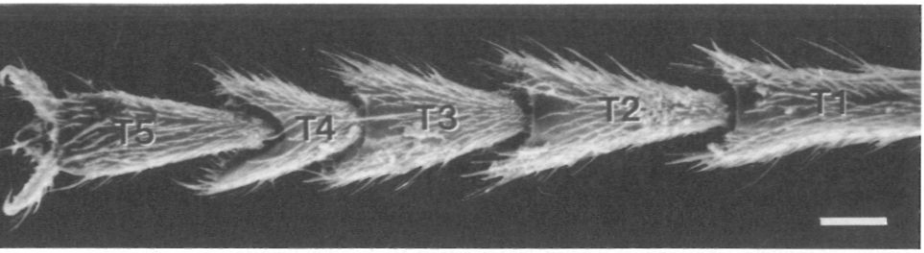
The third tarsomere of the hindlegs of both workers and queens of *Nothomyrmecia macrops* is almost entirely filled with a hitherto unknown exocrine gland (which does not occur in the closely related *Myrmecia*). Each of the approximately 30 secretory cells is connected to the outside via a duct cell. These open individually via large cuticular pores at the mesoventral side of the anterior part of the tarsomere. The diameter of the duct cells is narrow near the secretory cell, but gradually increases towards their opening site. The rounded secretory cells show a well developed Golgi apparatus and numerous clear vesicles. The function of this gland is not yet known, although its opening site may be indicative of the deposition of marking substances. At the mediodistal side of tarsomeres 2, 3 and 4 in the three pairs of legs, a glandular thickening of the epidermal epithelium occurs; this represents another novel exocrine structure in ants. This epithelial gland occurs in both *Nothomyrmecia* and *Myrmecia*.

Introduction

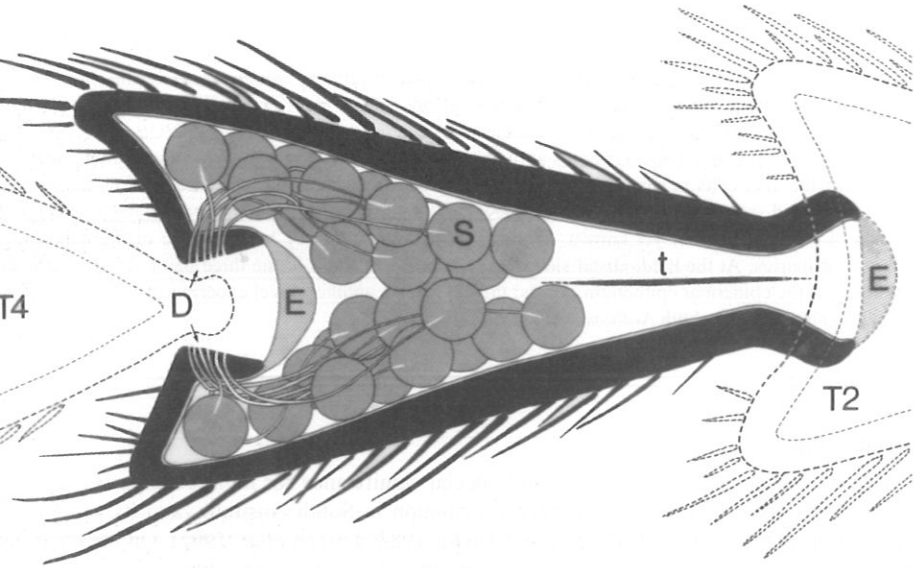
The ant *Nothomyrmecia macrops* is of special significance for myrmecology because of its ancestral position, its limited geographic distribution in South Australia, and its simple social organisation (Taylor 1978; Hölldobler and Taylor 1983; Jaisson *et al.* 1992). On the other hand, its apparent similarity to the bulldog ants of the genus *Myrmecia* has led to comparative morphological (Kugler 1980; Billen 1990) and chemical studies (Billen *et al.* 1988*a*, 1988*b*; Jackson *et al.* 1990) from which a closer relationship between both Australian ant genera may be concluded. During our study of the exocrine system of *N. macrops*, we discovered a conspicuous gland in the third tarsomere of the hindlegs of both workers and queens, as well as an epithelial gland at the mediodistal end of the tarsomeres in the three pairs of legs. We here describe both hitherto unknown exocrine glands in ants.

Material and Methods

Workers and queens of *Nothomyrmecia macrops* Clark, 1934 were collected in Poochera, South Australia. Workers of *Myrmecia pyriformis* F. Smith, 1858 were examined for comparison. The tarsi of the three pairs of legs were fixed in 2% cold glutaraldehyde buffered at pH 7.3 with sodium cacodylate and postfixed in 2% cold osmium tetroxide in the same buffer. After dehydration in a graded acetone series, they were embedded in araldite and sectioned with a Reichert Ultracut E microtome using a diamond knife. Semithin sections for light microscopy were stained with methylene blue and thionin;



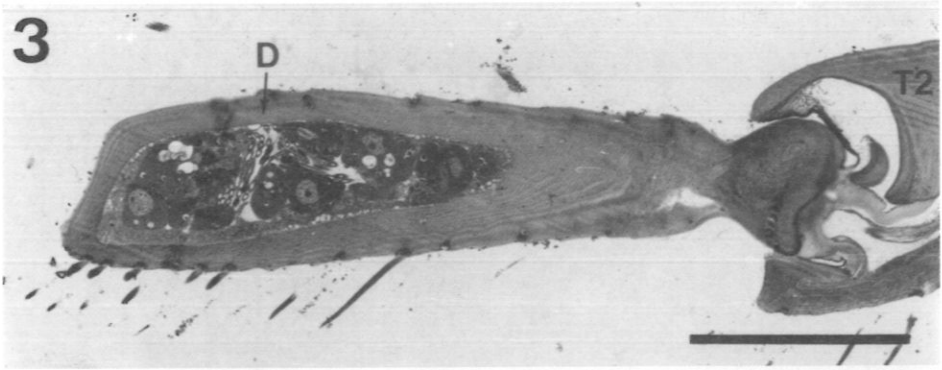
g. 1. SEM photograph of hindleg tarsus of *N. macrops* worker (T1 to T5 correspond with tarsomere mbering). The third tarsomere shows no special external features. Scale bar, 100 μm.



g. 2. Semi-schematical view from above of the third tarsomere of the hindleg with glandular uctures, based on reconstruction of serial sections. D, duct cells; E, glandular epithelium; t, hindleg don; S, secretory cells; T2–4, tarsomeres 2–4.

sults

The tarsomeres of *Nothomyrmecia macrops* are arrow-shaped with two laterodistal processes g. 1). The third tarsomere of the hindlegs of both workers and queens is for the greater part ed with an exocrine gland hitherto unknown in ants. Examination of serial sections showed t approximately 30 rounded secretory cells occur, each cell being connected to the outside via associated duct cell (Figs 2–6). The duct cells run distally, where they divide into left and nt bundles of approximately 15 ducts each. These bend downward in the distal portion of the somere (Figs 2–6), where they curve towards the mesoventral side of the tarsal processes,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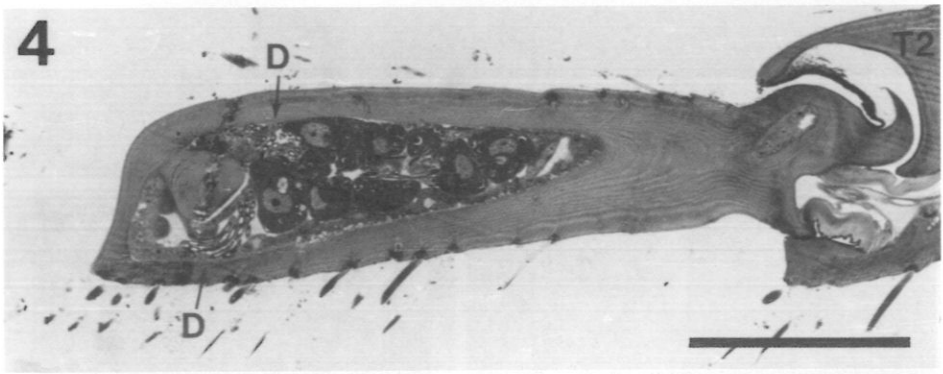
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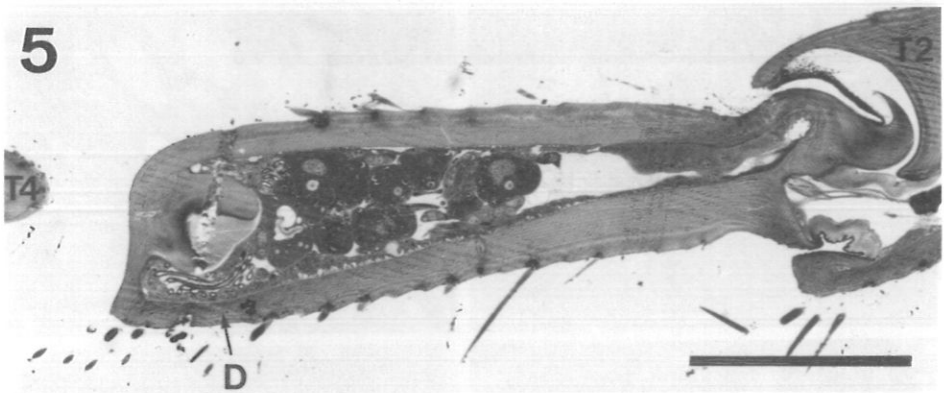
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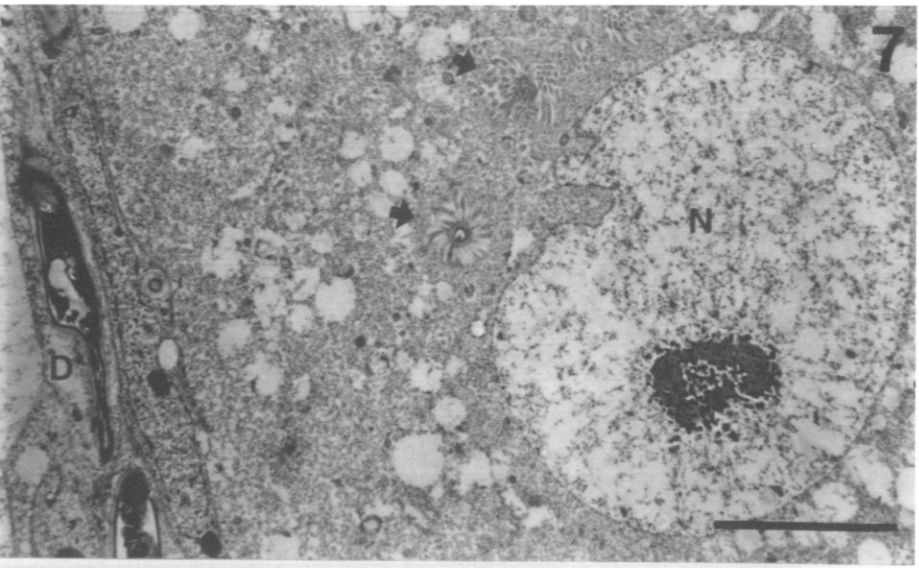
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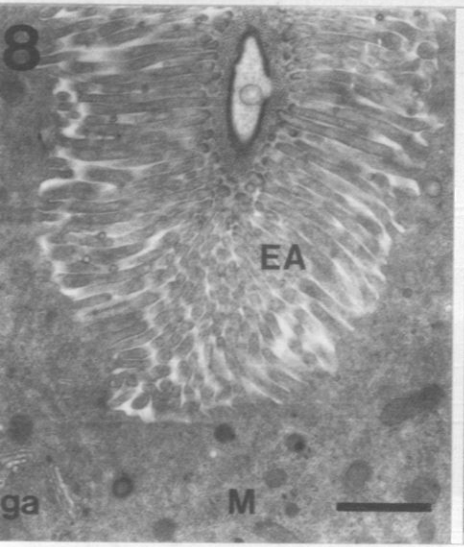
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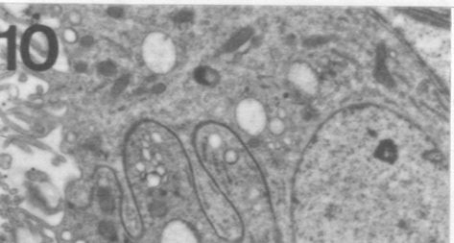
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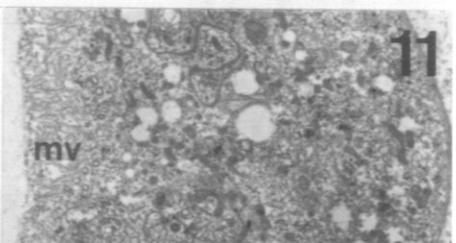
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tarsomere. For comparison, we also sectioned the third tarsomere of the hindlegs of *Myrmecia pyriformis* workers, but could find no similar exocrine gland.

The secretory cells are approximately 25 μm in diameter with a centrally located rounded nucleus, which is about 10 μm in diameter (Fig. 7). Their cytoplasm appears dense with numerous clear vesicles and a well developed Golgi apparatus (Fig. 8). Mitochondria are rather sparse. Some scattered ribosomes are present, although there is no evidence for the presence of either smooth or granular endoplasmic reticulum. A conspicuous end apparatus displays a tortuous course in the secretory cell, as can be seen from its repeated occurrence on the same plane of a histological section (Fig. 7). Its microvillar sheath generally shows a regular arrangement, while the central cuticular ductule shows an electron-dense epicuticle with local fenestrations, and a more fibrillar procuticle (Fig. 8). Its lumen has an internal diameter of approximately 0.4 μm. Small secretory droplets may be distinguished in the lumen of the ductule (Fig. 8). In the broader portion of the duct cells, lamellar inclusions can be seen in the lumen (Fig. 9). The gradually increasing diameter of the duct cell is very characteristic. It ranges from 0.4 μm near its junction with the secretory cell up to 1.5 μm at the site where it opens to the outside (Fig. 9).

In addition to the conspicuous cluster of glandular cells that fill the distal portion of the tarsomere of the fourth hindleg, we also found a hitherto unknown glandular epithelium underneath the mediodistal tegument of tarsomeres 1–4. This glandular epithelium occurs in the three pairs of legs in both queens and workers (Figs 2, 6), reaching a thickness of approximately 8 μm in workers (Fig. 10) and 12 μm in queens (Fig. 11). It is characterised by an apical microvillar border and sinuous contacts between neighbouring cells in the apical region. The cells have a rounded nucleus with a diameter of approximately 3 μm. Their cytoplasm contains numerous electron-dense rounded vesicles, scattered mitochondria, and a moderately developed granular endoplasmic reticulum (Fig. 10). These epithelial glands also occur in the mediodistal part of the various tarsomeres of *Myrmecia pyriformis*.

Discussion

Social insects, and ants in particular, can be thought of as walking glandular batteries (Hölldobler and Wilson 1990) as they are endowed with an impressive variety of exocrine glands all over their bodies (Billen and Morgan 1998). Several previously unknown glands have been found on the various leg segments in ants (e.g. Leuthold 1968; Schoeters and Billen 1993; Hölldobler *et al.* 1996; Billen *et al.* 2000), as well as in bees (Cruz Landim *et al.* 1998) and termites (Bacchus 1979; Costa Leonardo 1994). The tarsae in ants have been reported to contain glands in the basitarsus (Hölldobler and Palmer 1989*b*; Hölldobler *et al.* 1992; Ito and Billen 1998) and in the pretarsus (Hölldobler and Palmer 1989*a*), but no glandular structures have so far been described for the intermediate tarsomeres. The conspicuous exocrine gland described here in the distal part of the tarsomere of the third hindleg in both workers and queens of *Nothomyrmecia macrops* therefore represents another novel gland.

The gland is formed by Type III secretory cells, according to the classification of Noirot and Quennedey (1974), but shows the unusual characteristic of duct cells with a gradually increasing diameter in their course from the secretory cell towards their opening to the outside. The cytoplasmic organisation gives no clear indication as to the possible chemical nature of the secretory products, although the presence of lamellar material inside the duct lumen may be

Figs 7–11. Electron micrographs of glandular structures in third tarsomere of the hindleg. D, ducts;

ıked with glandular activity (Billen and Morgan 1998). The function of this novel tarsal gland *N. macrops* still remains unknown. The opening site of the ducts at the mesoventral side of e tarsomere may conveniently allow deposition of the secretory products onto the substrate ıth each foot step. It has been suggested that workers of *N. macrops* mark their nest entrances ıth colony-specific scent signals, although the glandular origin of these has not been tablished (Hölldobler and Taylor 1983). The eventual involvement of the tarsal glands in this haviour, however, needs to be investigated with behavioural experiments. In this regard, it is markable that this tarsal gland occurs in both the worker and queen caste.

Furthermore, the epithelial gland underneath the mediodistal part of tarsomeres 1–4 in all ree legs of both *N. macrops* and *Myrmecia pyriformis* has not been described before. It is rmed by Type I secretory cells, according to the classification of Noirot and Quennedey 974), that discharge their secretion directly through the overlaying cuticle. The function of this and also remains unknown at present, although the repeated occurrence in the various tarsal gments, as well as its location at the articulation between tarsomeres may be indicative of a mple lubricant function.

The present report of two previously unknown glands provides yet another example of the erwhelming variety of the exocrine system in the Formicidae. The presence of the nspicuous gland in the third tarsomere of the hindlegs of *Nothomyrmecia*, and its absence in *yrmecia pyriformis* provides a remarkable difference between these genera (although more ecies of *Myrmecia* should be examined for confirmation), and as such supports their separate assification. On the other hand, both genera also display a number of obvious similarities that ggest a close relationship. Among these are also glandular characters, such as the common esence of the mediadistal tarsomere gland as described here (although this gland may also be esent in other ant genera) and the presence of the sting-bulb gland, which appears to be a ique glandular structure that does not occur in ants other than *Nothomyrmecia* and *Myrmecia* illen 1990).

cknowledgments

We are very grateful to Dirk Corstjens, Koen Collart, Eric Schoeters and Julien Cillis for eir help in preparation of specimens for microscopy. This work was supported through grant o. G.0254.96 from the Flemish Fund for Scientific Research. We thank the Australian Nature onservation Agency for permit to export the ants, and Mike Schwarz for tactical support.

eferences

cchus, S. (1979). New exocrine gland on the legs of some Rhinotermitidae (Isoptera). *International Journal of Insect Morphology and Embryology* **8**, 135–142.

len, J. (1990). The sting bulb gland in *Myrmecia* and *Nothomyrmecia* (Hymenoptera : Formicidae): a new exocrine gland in ants. *International Journal of Insect Morphology and Embryology* **19**, 133–139.

len, J., and Morgan, E. D. (1998). Pheromone communication in social insects – sources and secretions. In 'Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites'. (Eds R. K. Vander Meer, M. D. Breed, K. E. Espelie and M. L. Winston.) pp. 3–33. (Westview Press: Boulder.)

len, J. P. J., Jackson, B. D., and Morgan, E. D. (1988*a*). Secretion of the Dufour gland of the ant *Nothomyrmecia macrops* (Hymenoptera : Formicidae). *Experientia* **44**, 715–719.

len, J. P. J., Jackson, B. D., and Morgan, E. D. (1988*b*). The contents of the pygidial gland of the primitive ant *Nothomyrmecia macrops* (Hymenoptera : Formicidae). *Experientia* **44**, 794–797.

Hölldobler, B., and Palmer, J. M. (1989*a*). Footprint glands in *Amblyopone australis* (Formicidae, Ponerinae). *Psyche* **96**, 111–121.

Hölldobler, B., and Palmer, J. M. (1989*b*). A new tarsal gland in ants and the possible function in chemical communication. *Naturwissenschaften* **76**, 385–386.

Hölldobler, B., and Taylor, R. W. (1983). A behavioral study of the primitive ant *Nothomyrmecia macrops* Clark. *Insectes Sociaux* **30**, 384–401.

Hölldobler, B., and Wilson, E. O. (1990). 'The Ants.' (Harvard University Press: Cambridge, MS.)

Hölldobler, B., Obermayer, M., and Wilson, E. O. (1992). Communication in the primitive cryptobiotic ant *Prionopelta amabilis* (Hymenoptera : Formicidae). *Journal of Comparative Physiology* **170A**, 9–16.

Hölldobler, B., Obermayer, M., and Peeters, C. (1996). Comparative study of the metatibial gland in ants (Hymenoptera, Formicidae). *Zoomorphology* **116**, 157–167.

Ito, F., and Billen, J. (1998). Larval hemolymph feeding and oophagy: behavior of queen and workers in the primitive ponerine ant *Prionopelta kraepelini* (Hymenoptera, Formicidae). *Belgian Journal of Zoology* **128**, 201–209.

Jackson, B. D., Morgan, E. D., and Billen, J. P. J. (1990). A note on the pygidial glands of primitive Australian ants: a new source of odorous chemicals. In 'Chromatography and Isolation of Insect Hormones and Pheromones'. (Eds A. R. McCaffery and I. D. Wilson.) pp. 335–341. (Plenum Press: New York.)

Jaisson, P., Fresneau, D., Taylor, R. W., and Lenoir, A. (1992). Social organisation in some primitive Australian ants. I. *Nothomyrmecia macrops* Clark. *Insectes Sociaux* **39**, 425–438.

Kugler, C. (1980). The sting apparatus in the primitive ants *Nothomyrmecia* and *Myrmecia*. *Journal of the Australian Entomological Society* **19**, 263–267.

Leuthold, R. H. (1968). A tibial gland scent-trail and trail-laying behavior in the ant *Crematogaster ashmeadi* Mayr. *Psyche* **75**, 233–248.

Noirot, C., and Quennedey, A. (1974). Fine structure of insect epidermal glands. *Annual Review of Entomology* **19**, 61–80.

Schoeters, E., and Billen, J. (1993). Glandes coxales de *Pachycondyla obscuricornis* (Formicidae, Ponerinae). *Actes des Colloques Insectes Sociaux* **8**, 183–186.

Taylor, R. W. (1978). *Nothomyrmecia macrops*: a living-fossil ant rediscovered. *Science* **201**, 979–985.

Manuscript received 19 May 2000; accepted 20 September 2000