Netherlands Journal of Zoology
52 (1): 69-75 (2002)

NOVEL EXOCRINE GLANDS IN THE LEGS OF THE PONERINE ANT *AMBLYOPONE RECLINATA* (HYMENOPTERA, FORMICIDAE)

by

MARJOLEIN TIJSKENS [1], FUMINORI ITO [2] and JOHAN BILLEN [1,*]

([1] *Zoological Institute, University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*; [2] *Faculty of Agriculture, Kagawa University, Ikenobe, Miki, 761-0795, Japan*)

ABSTRACT

The legs of workers of the ponerine ant *Amblyopone reclinata* harbour at least five different exocrine glands. The pretarsal arolium gland is a common structure in the legs of all Hymenoptera, the pretarsal footprint gland is also found in the hindlegs of other *Amblyopone* species, while the epithelial metatibial gland is found in ants of the doryline section. Two novel glands are added to this repertoire: an epithelial gland surrounding a central reservoir space occurs in the tibia of the front- and hindlegs, and a cluster of three secretory cells was found in the distal part of the hindleg tibia adjacent to the metatibial gland, their accompanying duct cells open through the articulation membrane that connects the hindleg tibia and metatarsus. A possibly glandular epithelium was found in the tibial spur of the hindlegs.

KEY WORDS: *Amblyopone reclinata*, exocrine glands, morphology, Ponerinae, ultrastructure.

INTRODUCTION

The large variety of exocrine glands represents a well-known characteristic of social insects, with a correspondingly vast number of secretory products that form the cornerstone of the social organization and communication system of these insect societies (HÖLLDOBLER & WILSON, 1990). The number of known exocrine glands among the Formicidae has steadily increased in the past few decades, with the discovery of several novel glands made possible because of the use of plastic embedding and new sectioning techniques (BILLEN & MORGAN, 1998). This has also resulted in the finding of several previously unknown glands in the appendages such as the antennae (BILLEN, 2000; ISIDORO *et al.*, 2000) and especially the legs.

We here report on at least two novel glands that occur in the legs of workers of the ponerine ant *Amblyopone reclinata*.

* Corresponding author; e-mail: Johan.Billen@bio.kuleuven.ac.be

MATERIAL AND METHODS

Amblyopone reclinata Mayr, 1879 colonies were collected in the Bogor Botanical Gardens, Indonesia, and kept in artificial plaster nest boxes. The various leg parts of worker individuals were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 0.050 M Na-cacodylate and 0.150 M saccharose, and postfixed in 2% osmium tetroxide in the same buffer. Dehydration was carried out in a graded acetone series and preceded embedding in Araldite. Semithin 1 μm sections were stained with methylene blue and thionin and viewed in a Zeiss Axioskop microscope, double stained 70 nm thin sections were examined in a Zeiss EM900 electron microscope.

RESULTS

The legs of *Amblyopone reclinata* workers contain an impressive variety of exocrine glands (fig. 1). The pretarsus is characterised by the presence of the ventrally opening epithelial arolium gland in all six legs that is a common feature for all Hymenoptera, as well as a dorsally situated and

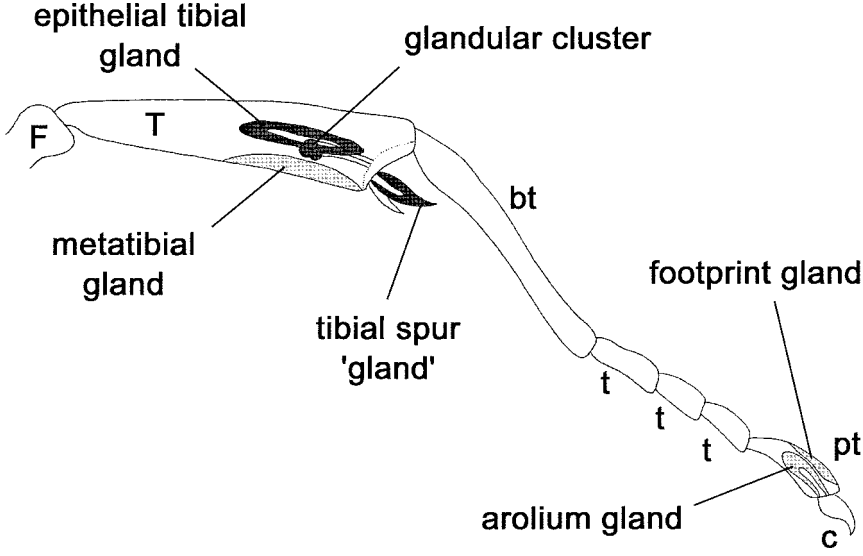


Fig. 1. Schematical survey of the hindleg of an *A. reclinata* worker with indication of the various exocrine glands. The novel structures described here are shown in black. bt = basitarsus, c = claw, F = femur, pt = pretarsus, T = tibia, t = tarsomere.

equally epithelial 'footprint gland' in the hindlegs only (occurrence shared with the congeneric *A. australis*, see HÖLLDOBLER & PALMER, 1989a). The distal part of the hindleg tibia displays at its ventral side a conspicuous epithelial metatibial gland that is shared with other species belonging to the doryline section (HÖLLDOBLER *et al.*, 1996). In contrast to the smooth external cuticle lining this gland in other species, *A. reclinata* shows conspicuous hairs in this region (figs 4, 5). In addition to these glands that were already known for other ponerine species, we found some other glandular structures.

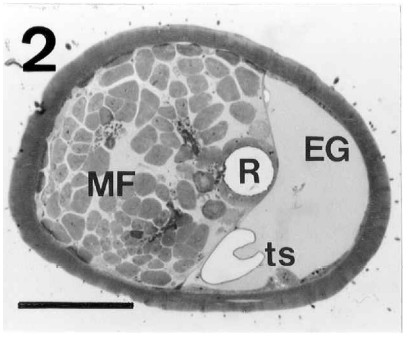
Centrally located in the distal part of the tibia of the hindleg, and to a lesser extent also in the foreleg, is a glandular epithelium that surrounds a central reservoir with a lumen diameter around 40 μm (figs 1 and 2). The epithelium has a thickness between 7 and 10 μm and is formed by cubic cells that display a microvillar apical cell membrane (fig. 3). The cytoplasm contains many mitochondria and large lipid droplets. The cells are covered by a cuticular intima with a thickness around 1 μm that is characterised by the presence of pore canals (fig. 3). Histological sections indicate a connection between this gland and the tibial tendon, although their precise relationship could not be determined due to technical problems with the serial sections in this region.

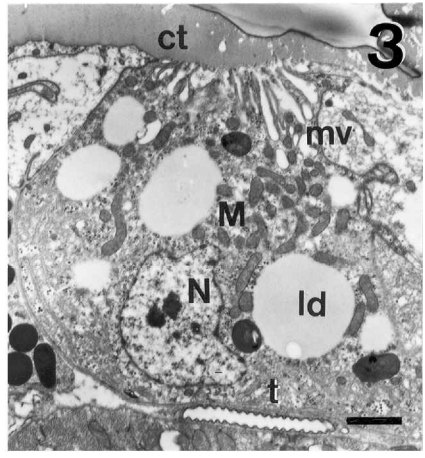
Adjacent to the metatibial gland in the hindlegs we found a cluster of three rounded glandular cells with a diameter of approx. 20 μm (figs 4 and 5). Each cell has an end apparatus that continues in a slender duct cell, that opens into the articulation space between the tibia and the basitarsus. The glandular cells contain numerous lipoidic inclusions with a diameter around 1 μm (fig. 6).

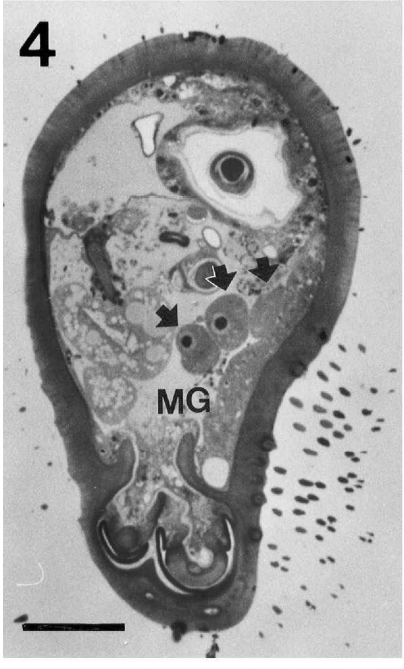
In addition to these glandular structures inside the hindleg tibia, the epidermis of the hindleg tibial spur appears as a fairly thick epithelial layer with a thickness of approx. 10 μm, which may represent another novel glandular tissue (fig. 7). Ultrastructural examination revealed the presence of a well developed granular endoplasmic reticulum. The cuticle shows small pore canals to the outside.

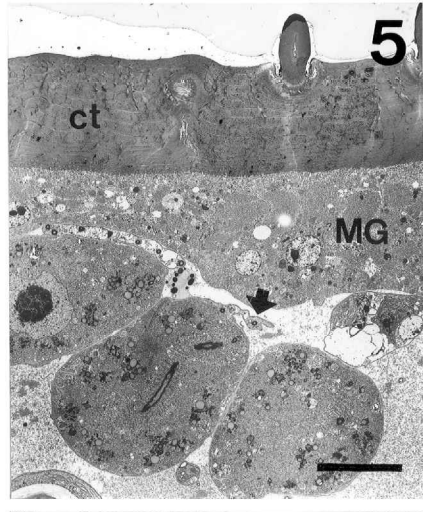
DISCUSSION

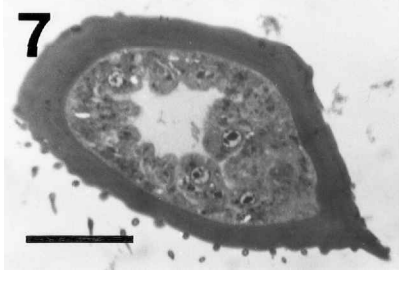
The high number of exocrine structures found in the legs of *Amblyopone reclinata* is an obvious example of the wealth of exocrine glands that occur in ants in general, and in ponerine species in particular (*e.g.* see JESSEN & MASCHWITZ (1983) and JESSEN *et al.* (1979) for an illustration of the variety of abdominal glands). The occurrence of exocrine glands in ant legs has been well documented in the past 10 years with glands found

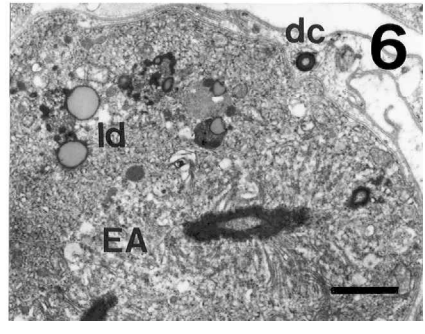

2
MF
R
EG
ts


ct
3
mv
M
N
ld
t


4
MG


5
ct
MG


7


dc
6
ld
EA

in the various leg parts: coxa (SCHOETERS & BILLEN, 1993), femur (BILLEN *et al.*, 2000a), various glands in the tibia (LEUTHOLD, 1968; BILLEN, 1984; HÖLLDOBLER *et al.*, 1996; BILLEN, 1997; BILLEN *et al.*, 2000a), basitarsus (HÖLLDOBLER & PALMER, 1989b; HÖLLDOBLER *et al.*, 1992), the tarsomeres (BILLEN *et al.*, 2000b), and the pretarsus (HÖLLDOBLER & PALMER, 1989a; BILLEN & MORGAN, 1998).

The novel glands described in the present article have never been found before in ponerine ants, although they show similarities to glandular structures encountered in other subfamilies. The tibial epithelial gland surrounding a reservoir and somehow associated with the tibial tendon may be similar to the tibial gland that so far has only been found in the hindleg of species of the myrmicine genus *Crematogaster* (LEUTHOLD, 1968). Also the ultrastructural features show obvious similarities, such as the well developed microvillar border, the occurrence of lipid droplets, and the presence of cuticular pore canals (BILLEN, 1984). In *Crematogaster* ants, this tibial gland is the source of trail pheromones, that find their way to the outside at the leg's distal extremity via the hindleg tendon (PASTEELS *et al.*, 1970). In *Amblyopone*, however, the apparently unique footprint gland in the hindleg pretarsus is involved in the elaboration of recruitment substances. This was shown for *A. australis* by HÖLLDOBLER & PALMER (1989a), and confirmed by own observations on *A. reclinata* using extracts of the hindleg pretarsi. Extracts of the hindleg tibia, containing the here described tibial gland, however, did not elicit any trail following activity of the kind shown in *Crematogaster* ants (LEUTHOLD, 1968), which makes the function of the tibial epithelial gland in *A. reclinata* still questionable.

The cluster comprising three glandular cells in the distal part of the hindleg tibia occurs in a similar position as a gland cluster with five to

---

Fig. 2. Semithin section through hindleg tibia, showing the epithelial gland (EG) surrounding a central reservoir. Scale bar: 100 μm.

Fig. 3. Electron micrograph of epithelial gland in hindleg tibia. Note pore channels in cuticle. Scale bar: 1 μm.

Fig. 4. Semithin section through distal part of hindleg tibia near articulation with basitarsus, showing epithelial metatibial gland and adjacent cluster of three secretory cells (arrows). Scale bar: 50 μm.

Fig. 5. Electron micrograph showing epithelial metatibial gland and cluster of three glandular cells. Arrow indicates section through duct cell. Scale bar: 10 μm.

Fig. 6. Ultrastructural detail of secretory cell with end apparatus (EA) and duct cells (dc) in the hindleg metatibia. Scale bar: 2 μm.

Fig. 7. Semithin section through hindleg tibial spur with apparently glandular epithelium inside. Scale bar: 20 μm.

ct = cuticle, ld = lipid droplet, M = mitochondria, MF = muscle fibres, MG = metatibial gland, mv = microvilli, N = nucleus, R = reservoir, t = tracheole, ts = tracheal sac.

ten cells found in the hindleg tibia of workers of the army ant *Dorylus molestus* (BILLEN, 1997). Its function remains unknown, although the opening of the ducts at the articulation between tibia and basitarsus, together with the presence of lipoid inclusions in the secretory cells, may indicate a smearing function. Such function could be well understood in ponerine ants that are known to have a heavily sclerotised tegument. The presence of a conspicuous epithelium inside the hindleg tibial spur may represent yet another novel exocrine structure in ant legs, although further studies on its occurrence and function will be needed.

ACKNOWLEDGEMENTS

We greatly acknowledge the help of Koen Collart and Julien Cillis in tissue preparation. We also thank LIPI for permission to work and collect material in Indonesia. This work was supported through grant No. G.0254.96 from the Flemish Fund for Scientific Research and grant No. 08041141 for Overseas Research from the Japanese Ministry of Education, Science and Culture.

REFERENCES

BILLEN, J.P.J., 1984. Morphology of the tibial gland in the ant *Crematogaster scutellaris*. Naturwissenschaften **71**: 324-325.

BILLEN, J., 1997. Morphology and ultrastructure of the metatibial gland in the army ant *Dorylus molestus* (Hymenoptera, Formicidae). Belg. J. Zool. **127**: 179-186.

BILLEN J., 2000. A novel exocrine gland in the antennal scape of the army ant *Eciton burchelli*. Belg. J. Zool. **130**: 159-160.

BILLEN, J. & E.D. MORGAN, 1998. Pheromone communication in social insects — sources and secretions. In: R.K. Vander Meer, M.D. Breed, M.L. Winston & K.E. Espelie (Eds): *Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites*: 3-33. Westview Press, Boulder: Oxford.

BILLEN, J., F. ITO & B. BOLTON, 2000a. Femoral and tibial glands in the ant genus *Strumigenys* (Hymenoptera, Formicidae). Belg. J. Zool. **130**: 111-115.

BILLEN, J., F. ITO & C. PEETERS, 2000b. Novel exocrine glands in the hindleg tarsi of the ant *Nothomyrmecia macrops*. Aust. J. Zool. **48**: 661-667.

HÖLLDOBLER, B. & J.M. PALMER, 1989a. Footprint glands in *Amblyopone australis* (Formicidae, Ponerinae). Psyche **96**: 111-121.

HÖLLDOBLER, B. & J.M. PALMER, 1989b. A new tarsal gland in ants and the possible function in chemical communication. Naturwissenschaften **76**: 385-386.

HÖLLDOBLER, B. & E.O. WILSON, 1990. *The Ants*. Cambridge, Massachusetts: Harvard University Press, pp. 732.

HÖLLDOBLER, B., M. OBERMAYER & E.O. WILSON, 1992. Communication in the primitive cryptobiotic ant *Prionopelta amabilis* (Hymenoptera: Formicidae). J. Comp. Physiol. **170A**: 9-16.

HÖLLDOBLER, B., M. OBERMAYER & C. PEETERS, 1996. Comparative study of the metatibial gland in ants (Hymenoptera, Formicidae). Zoomorphology **116**: 157-167.

ISIDORO, N., R. ROMANI, D. VELASQUEZ, R. RENTHAL, F. BIN & S.B. VINSON, 2000. Antennal glands in queen and worker of the fire ant, *Solenopsis invicta* Buren: first report in female social Aculeata (Hymenoptera, Formicidae). Insectes Soc. **47**: 236-240.

JESSEN, K. & U. MASCHWITZ, 1983. Abdominaldrüsen bei *Pachycondyla tridentata* (Smith): Formicidae, Ponerinae. Insectes Soc. **30**: 123-133.

JESSEN, K., U. MASCHWITZ & M. HAHN, 1979. Neue Abdominaldrüsen bei Ameisen. I. Ponerini (Formicidae, Ponerinae). Zoomorphologie **94**: 49-66.

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

PASTEELS, J.M., R.M. CREWE & M.S. BLUM, 1970. Etude histologique et examen au microscope électronique à balayage de la glande sécrétant la phéromone de piste chez deux *Crematogaster* nord-américains (Formicidae, Myrmicinae). C.R. Acad. Sc. Paris **271D**: 835-838.

SCHOETERS, E. & J. BILLEN, 1993. Glandes coxales de *Pachycondyla obscuricornis* (Formicidae, Ponerinae). Actes Coll. Insectes Soc. **8**: 183-186.