Acta Zoologica (Stockholm) **89**: 201–204 (July 2008) doi: 10.1111/j.1463-6395.2008.00323.x

A novel exocrine gland in the trochanter of ant legs

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Keywords:
morphology, ultrastructure, trochanter, exocrine glands, ants, Formicidae

Accepted for publication:
18 July 2007

Abstract

Billen, J. 2008. A novel exocrine gland in the trochanter of ant legs. — *Acta Zoologica* (Stockholm) **89**: 201–204

We found a hitherto unknown gland in the trochanter of several ant species. The gland occurs at the proximal ventral part of the trochanter in all legs. It consists of a thickening of the tegumental epithelium, the lining cuticle of which is characterized by narrow vertical pores that lead the secretion to the outside. Its function is probably that of producing lubricant substances to allow optimal manoeuvrability of the articulation between the trochanter and the coxa.

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Introduction

The spectacular variety of the exocrine system in ants is well illustrated in the number of glands that can be found in their legs. Exocrine glands have indeed been found in the coxa, femur, tibia, basitarsus, the tarsomeres and the pretarsus [see Billen and Ito (2006), and references therein]. This list comprises all leg segments, except for the trochanter, in which so far no glands have been reported among ants. This, together with the existence of trochanter glands in bees (Cruz-Landim *et al.* 1998; Cruz-Landim 2002), prompted us to have a closer look for exocrine glands in the trochanter of ants. We found a clear epithelial gland in the several species investigated and describe this novel exocrine structure for ant legs.

Materials and Methods

The species studied in this work are listed in Table 1. Their trochanters were prepared by cutting transversely through the coxa and femur of the fore-, mid- and hindlegs, and fixing these leg fragments in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose. This was followed by postfixation in 2% osmium tetroxide in the same buffer. Tissues were then dehydrated in a graded acetone series and embedded in Araldite. Semithin 1-μm sections were stained with methylene blue and thionin and viewed with an Olympus BX51 microscope. Double-stained 70-nm thin sections were examined in a Zeiss EM900 electron microscope.

Results

We found a conspicuous ventral glandular epithelium in the proximal part of the trochanter in all species investigated. The gland occurs in the three leg pairs, and exists in workers as well as queens. The epithelium has a thickness of approximately 15 μm (ranging from 10 μm in the smaller species like *Probolomyrmex* sp. and *Proceratium itoi*, up to 17 μm in

Table 1 List of species examined with collection locality

Subfamily	Tribe	Species	Locality	Caste
Formicinae	Camponotini	*Camponotus rufipes* Fabr., 1775	Viçosa, MG, Brazil	w
	Oecophyllini	*Oecophylla longinoda* (Latreille, 1802)	Miri, Malaysia	w
Myrmicinae	Attini	*Atta laevigata* (F. Smith, 1858)	Caucagua, Venezuela	w
Ponerinae	Platythyreini	*Platythyrea* sp.	Kebun Raya Bogor, Indonesia	w
	Ponerini	*Diacamma* sp.	Nakijin, Okinawa, Japan	w
		Leptogenys diminuta (F. Smith, 1857)	Kebun Raya Bogor, Indonesia	Q
		Odontomachus rixosus (F. Smith, 1857)	Kebun Raya Bogor, Indonesia	Q
		Odontoponera transversa (F. Smith, 1857)	Pangandaran, W. Java, Indonesia	w
Proceratiinae	Probolomyrmecini	*Probolomyrmex* sp.	Padang, Indonesia	w
	Proceratiini	*Proceratium itoi* (Forel, 1918)	Cape Manazuru, Japan	w

In the right column, Q (queen) and w (worker) indicate the caste of the corresponding specimens examined.

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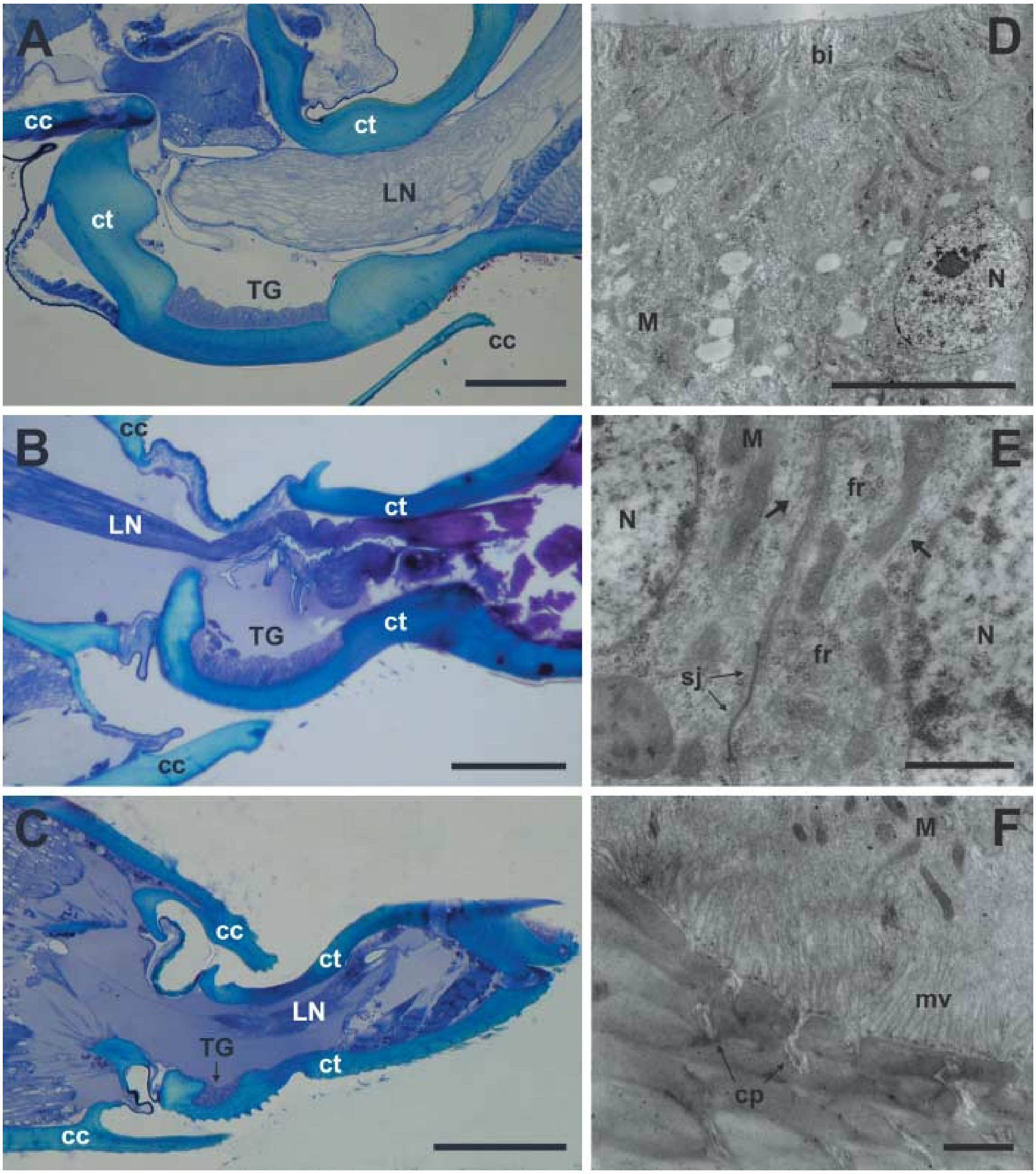

Fig. 1—**A**. Longitudinal semithin section through proximal part of foreleg trochanter of *Camponotus rufipes* worker, indicating position of trochanter gland epithelium (TG). cc = cuticle of coxa, ct = cuticle of trochanter, LN = leg nerve (scale bar 50 µm). —**B**. Longitudinal section through proximal part of midleg trochanter of *Leptogenys diminuta* queen (scale bar 50 µm). —**C**. Longitudinal section through trochanter of hindleg of *Probolomyrmex* sp. worker (scale bar 50 µm). —**D**. Electron micrograph of basal part of trochanter gland epithelium in midleg of *Atta laevigata* worker, showing basal invaginations (bi). N = nucleus (scale bar 5 µm). —**E**. Electron micrograph detail of central cytoplasm of trochanter gland in midleg of *Diacamma* sp. worker, showing numerous mitochondria (M), free ribosomes (fr) and microtubules (arrows). sj = septate junction (scale bar 1 µm). —**F**. Electron micrograph of apical part of trochanter gland epithelium in midleg of *Diacamma* sp. worker, showing apical microvilli (mv) and cuticular pores (cp) (scale bar 1 µm).

the larger ones like *Diacamma* sp., *Leptogenys diminuta* and *Odontoponera transversa*). The glandular epithelium is lined by a cuticle of approximately the same thickness as the epithelium itself. In the more proximal as well as more distal regions, the cuticle is twice as thick, which makes the glandular epithelium 'fit' in a well-defined cuticular depression at the trochanter's inside (Fig. 1A–C). The outer surface of the cuticular area where the gland occurs is opposite the

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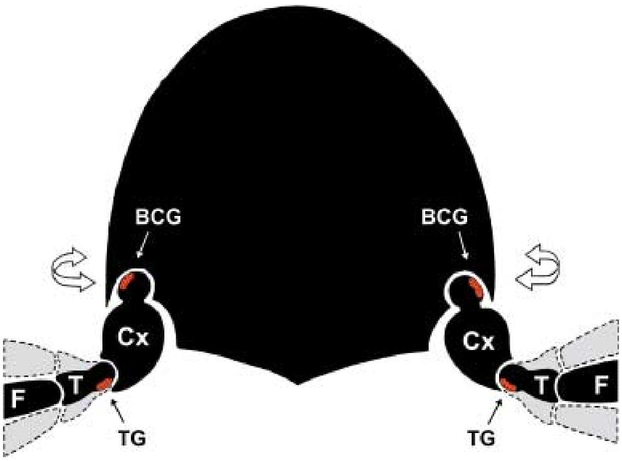

Fig. 2—Schematic cross-section through the thorax, with indication of the location of the trochanter glands (TG) and basicoxal glands (BCG). The trochanter glands occur at a position where up-and-down movement against the coxal basis is maximal, while the position of the basicoxal glands is where the ball-and-socket-like proximal part of the coxa shows maximal manoeuvrability with the thorax. Cx, coxa; F, femur; T, trochanter.

ventral distal margin of the coxa. The cuticle overlaying the glandular epithelium displays a different texture compared to its appearance elsewhere, because it contains a fine striation perpendicular to the cuticular surface. In low-magnification light micrographs (Fig. 1A,B), this is only visible as a slightly darker area, though the striated pattern becomes very clear in the electron micrographs (Fig. 1F).

At the ultrastructural level, the epithelial cells are characterized by basal invaginations (Fig. 1D) and apical microvilli (Fig. 1F). The cytoplasm contains many mitochondria, microtubules and scattered free ribosomes (Fig. 1E), while other organelles are not obvious. Intercellular contacts reveal the presence of septate junctions in the apical region (Fig. 1E). Nuclei are rounded and occupy a central position in the cells (Fig. 1D,E). The cuticle shows many pore canals with a diameter of 0.2 μm that run perpendicular to the cuticular surface (Fig. 1F), that correspond with the striations observed in the light microscope.

Discussion

The discovery of a glandular epithelium in the trochanter brings another illustration of the great variety of exocrine glands in ants. Its presence in all the species examined and its occurrence in the three leg pairs and in both workers and queens indicate that it is probably a common structure with a general function. Its appearance closely resembles that of the basicoxal gland (Billen and Ito 2006) and of the glandular epithelium in the proximal part of the basitarsus (see figure 11 in Billen 1997). The position of these glands in all three cases is in the articulation region with the next leg segment or thorax, which is suggestive for a function of producing lubricant substances (Fig. 2). Such function finds additional support in the precise anatomical position, as the trochanter gland occurs ventrally, which is where the up-and-down movement between the coxa and the trochanter is maximal, whereas the basicoxal gland occurs dorsally (which likewise is where the mainly anteroposterior coxal movements against the corresponding thoracic cavity are maximal). The basicoxal gland is absent in the forelegs, which can be explained by the limited movements between the thorax and the foreleg coxae (Billen and Ito 2006). The manoeuvrability between the coxa and trochanter, however, is high in the three leg pairs, and helps explain the presence of the trochanter gland in all legs.

Details of the trochanter gland at the ultrastructural level give further support for the suggested elaboration of lubricant substances. As an epithelial structure, the trochanter gland corresponds to class 1 glands following the classification of Noirot and Quennedey (1974). The presence of basal invaginations and apical microvilli provides an increased surface area, which is important for the uptake of precursor molecules from the haemolymph into the cytoplasm, and later for the release of the secretory products (Billen and Morgan 1998). The further transportation of the secretion to the outside is facilitated by the presence of transcuticular pores. The cytoplasmic composition with many mitochondria and scattered free ribosomes, but lacking granular endoplasmic reticulum, is in agreement with the elaboration of a non-proteinaceous secretion. Such composition may well correspond to the oily products that have been mentioned as lubricants to reduce the friction between cuticular parts in the intersegmental glands of heavily sclerotized ponerine ants (Attygalle *et al.* 1996). Similar to the basicoxal gland cells, the trochanter gland cells also contain an abundance of microtubules in their cytoplasm; these may provide structural strength for a tissue that can be exposed to considerable mechanical forces because of its position at an articulation point between cuticular structures (Billen and Ito 2006).

Acknowledgements

My sincere thanks go to An Vandoren for her skilful help in section preparation for microscopy. This work was funded through grant OT/2001/24 from the K.U.Leuven Research Fund.

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