

# The basicoxal gland, a new exocrine structure in poneromorph ants (Hymenoptera, Formicidae)

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## Abstract

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The tegumental epithelium of the outer dorsolateral region in the proximal part of the coxae in the mid- and hindlegs of both workers and queens of the ants *Odontomachus rixosus* and *O. simillimus* is differentiated into a conspicuous and hitherto unknown exocrine gland. The glandular cells display a clear microvillar differentiation of their apical cell membrane, and are lined with the tegumental cuticle, which in this part contains crack-like channels perpendicular to its surface, that carry the glandular secretions to the outside. Apical microvilli support the transport of substances, and contain an extension of tubular smooth endoplasmic reticulum in their centre. The function of the gland may be that of providing lubricant substances to the articulation region of the generally heavily sclerotized ponerine ant species. The gland is also found in several other ponerine and amblyoponine species, but not in the ectatommine species studied. The foreleg coxae lack a basicoxal gland in all species examined, which may be explained by the more limited articulation between the thorax and the coxae in the forelegs compared to the mid- and hindlegs.

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## Introduction

Social insects, and ants in particular, can be considered as flying or walking glandular batteries (Hölldobler and Wilson 1990). They are endowed with an extensive variety of exocrine glands that occur all over their bodies. This not only applies to the head, thorax and abdomen but also the ants' legs have been reported as the possible location for a number of glands. Among the various leg parts, glands have been found in the coxae (Schoeters and Billen 1993), femur (Billen *et al.* 2000a), tibia (Leuthold 1968; Billen 1984, 1997; Hölldobler *et al.* 1996), basitarsus (Hölldobler and Palmer 1989a; Hölldobler *et al.* 1992; Schönitzer *et al.* 1996), the tarsomeres (Billen *et al.* 2000b) and the pretarsus (Hölldobler and Palmer 1989b; Billen 1993; Billen *et al.* 2005). The distribution of these glands may be very common or specific for particular taxa, it may vary according to the ant caste considered, and it may also vary between the three leg pairs. In addition, some of the glands belong to the epithelial type, whereas others are formed by bicellular units, each of these

consisting of a secretory cell and a duct cell. The latter gland type is encountered in the coxal glands of the ponerine ant *Pachycondyla obscuricornis* and a few other species (Schoeters and Billen 1993), where the secretory cells occur near the articulation membranes between the coxa and thorax, and between coxa and femur. We here report on our finding of another hitherto unknown exocrine gland in the coxae of ponerine ants, but which corresponds to the epithelial type. We describe its general histology and ultrastructure in *Odontomachus* species, and discuss its occurrence among other poneromorph species (*sensu* Bolton 2003). Because of its occurrence in the proximal part of the coxae, and to distinguish it from the previously described coxal gland with bicellular units (Schoeters and Billen 1993), we propose that this newly described gland be designated the basicoxal gland.

## Materials and Methods

Table 1 presents a survey of the ant species examined for this study, together with their locality of collection. Voucher

**Table 1** List of ant species examined with collection locality, and indication of presence (+) or absence (–) of the basicoxal gland epithelium in the three leg pairs

Subfamily	Tribe	Species	Locality	Foreleg	Midleg	Hindleg
Amblyoponinae	Amblyoponini	<i>Amblyopone reclinata</i> Mayr, 1879	Kebun Raya Bogor, Indonesia	–	+	+
		<i>Prionopelta kraepelini</i> Forel, 1905	Kebun Raya Bogor, Indonesia	–	+	+
Ectatomminae	Ectatommini	<i>Ectatomma quadridens</i> (Fabricius, 1793)	Manaus, Brazil	–	–	–
Ponerinae	Platythyreini	<i>Gnamptogenys striatula</i> Mayr, 1884	Santa Rosa National Park, Costa Rica	–	–	–
		<i>Platythyrea</i> sp.	Kebun Raya Bogor, Indonesia	–	–	–
	Ponerini	<i>Leptogenys diminuta</i> (F. Smith, 1857)	Kebun Raya Bogor, Indonesia	–	+	+
		<i>Leptogenys kraepelini</i> Forel, 1905	Mt. Gede, W. Java, Indonesia	–	+	+
		<i>Leptogenys myops</i> (Emery, 1887)	Kebun Raya Bogor, Indonesia	–	+	+
		<i>Myopias emeryi</i> (Forel, 1913)	Ulu Gombak, Malaysia	–	+	+
		<i>Odontomachus rixosus</i> F. Smith, 1857	Kebun Raya Bogor, Indonesia	–	+	+
		<i>Odontomachus simillimus</i> F. Smith, 1858	Kebun Raya Bogor, Indonesia	–	+	+
		<i>Odontoponera transversa</i> F. Smith, 1857	Pangandaran, W. Java, Indonesia	–	+	+
		<i>Probolomyrmex dammermani</i> Wheeler, 1928	Kebun Raya Bogor, Indonesia	–	+	+
Proceratiinae	Proceratiini	<i>Proceratium</i> sp.	Padang, W. Sumatra	–	+	+
Myrmicinae	Crematogastrini	<i>Crematogaster inflata</i> F. Smith, 1857	Ulu Gombak, Malaysia	–	–	–

specimens of all Indonesian species are deposited in The Bogor Zoological Museum, West Java.

Anterior (with the forecoxae) and posterior thorax halves (with the mid- and hindcoxae attached) were fixed in 2% cold glutaraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose, and post-fixed in 2% cold osmium tetroxide in the same buffer. Dehydration was performed through a graded acetone series and was followed by embedding in araldite. Semi-thin sections at 1 µm for light microscopy were stained with methylene blue and thionin, thin sections (at 70 nm for electron microscopy) were double stained with lead citrate and uranyl acetate, and examined in a Zeiss EM900 electron microscope.

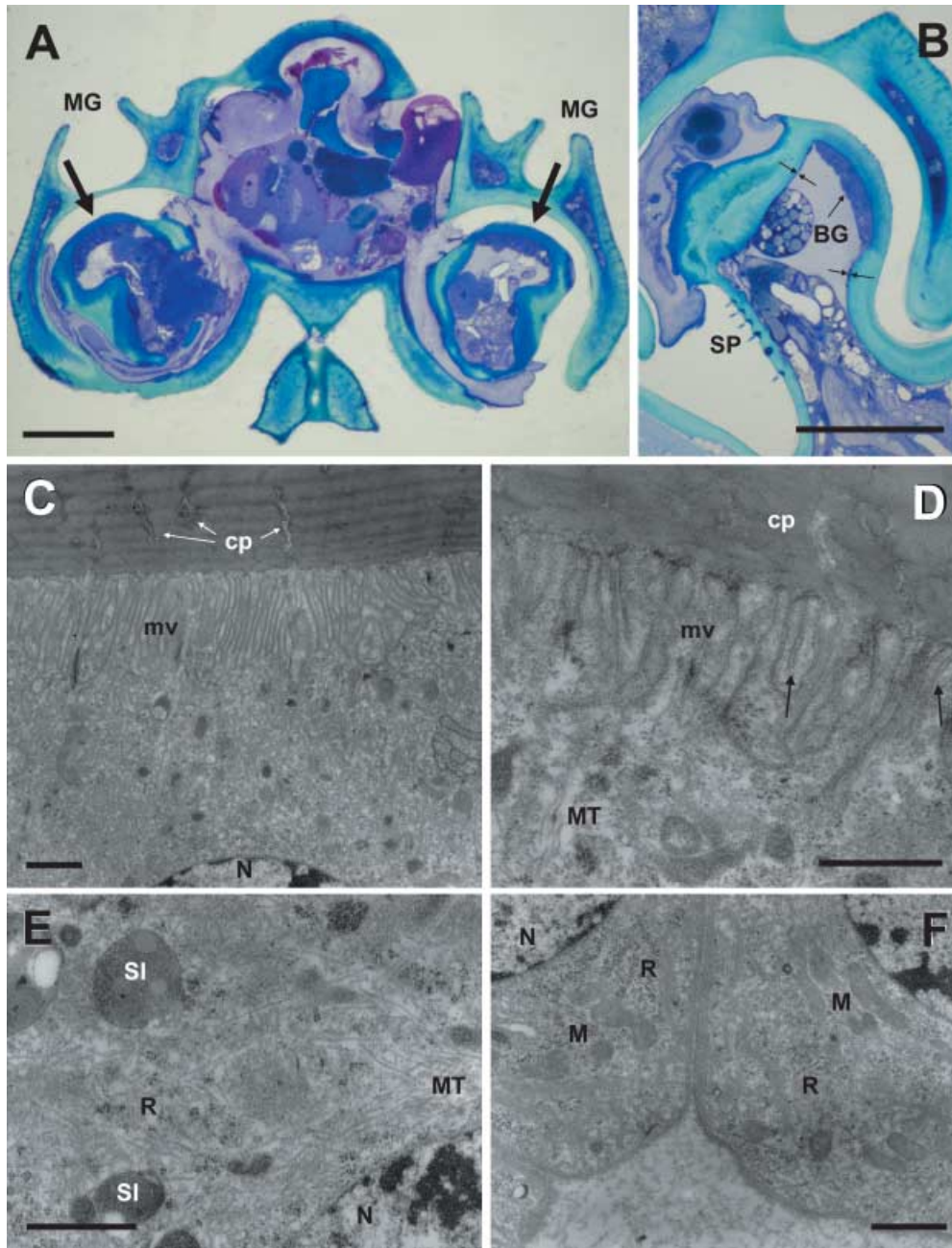
## Results

The coxae are the most proximal part of the insects' legs and articulate with the thorax. In ants, the coxae are more or less bottle-shaped, with the bottleneck part articulating with the thorax. For the mid- and hindlegs, these proximal coxal parts fit within a ventral cavity of the thorax to allow optimal manoeuvrability as a ball-and-socket joint. Cross-sections through the thorax of both workers and queens of *Odontomachus rixosus* and *O. simillimus* show the presence of a glandular differentiation of the tegumental epithelium at the outer-dorsal side of this rounded proximal part of the coxae of the mid- and hindlegs (Fig. 1A,B). The epithelium of this basicoxal gland reaches a thickness of 12–15 µm, and is covered with

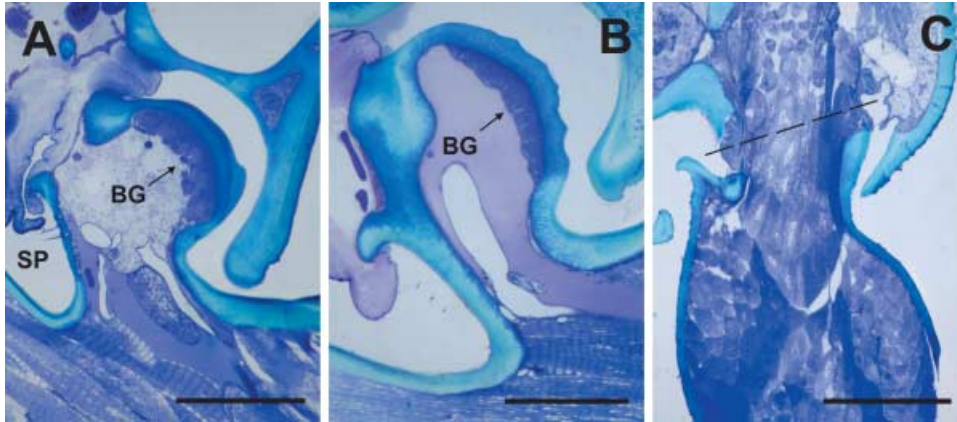
a cuticular layer of approx. 10 µm thickness. Elsewhere, the coxal tegumental epidermis is reduced to flattened cells with a thickness of hardly 1 µm (Fig. 1B). In the region overlying the thick glandular epithelium, and only there, the cuticle displays numerous irregular crack-like channels with a diameter of about 0.2 µm; these run perpendicular to the cuticular surface, where they apparently open (Fig. 1C).

Underneath the cuticle, the epithelial cells show a clear microvillar differentiation of their apical cell membrane (Fig. 1C,D). The cells have rounded nuclei with a diameter of 5 µm, and are situated in the basal part. Their cytoplasm contains many scattered free ribosomes and mitochondria. Granular endoplasmic reticulum or Golgi apparatus do not occur, while tubular strands of smooth endoplasmic reticulum extend into the microvillar fingers (Fig. 1D). A very obvious characteristic in the cytoplasm is the abundance of microtubules, which occur in a disorderly manner (Fig. 1D,E). Rounded secretory inclusions have electron-dense granular contents (Fig. 1E). The basal cell membrane shows a simple topography without invaginations (Fig. 1F).

A comparative survey of other poneromorph species revealed the presence of a similar basicoxal gland also in the mid- and hindlegs of several other species of the Ponerinae (except in *Platythyrea* sp.) (Fig. 2A,B) and in *Amblyopone reclinata* (Amblyoponinae). In the two species of the Ectatomminae that we examined, however, no basicoxal glands could be found (Table 1). In the single non-poneromorph



**Fig. 1**—**A**. Semi-thin cross-section through the posterior tip of the metathorax of an *Odontomachus simillimus* worker showing the implantation of the hindlegs and location of the basicoxal gland (arrows). The lateral grooves are the most posterior part of the metapleural gland orifices (MG) (scale bar 100  $\mu$ m). —**B**. Semi-thin cross-section through proximal part of the midleg coxa in an *O. rixosus* queen showing basicoxal gland epithelium (BG). The different texture of the cuticle overlaying the glandular epithelium is the result of the presence of cuticular pores in this region. Note very flat tegumental epithelium in non-glandular regions (between double arrows), and sensory pegs (SP) at inner side of coxa (scale bar 100  $\mu$ m). —**C**. Electron micrograph of apical region of basicoxal gland epithelium, showing cuticular pores (cp) and apical microvilli (mv) (hindleg *O. simillimus* worker, scale bar 1  $\mu$ m). —**D**. Detail of apical microvilli (mv) with extensions of smooth endoplasmic reticulum (arrows) and proximal part of cuticular pore (cp), MT = microtubules (hindleg *O. rixosus* queen, scale bar 1  $\mu$ m). —**E**. Cytoplasm of gland cell with abundance of microtubules (MT), numerous free ribosomes (R) and secretory inclusions (SI) (hindleg *O. rixosus* queen, scale bar 1  $\mu$ m). —**F**. Basal part of epithelial cells, showing few mitochondria (M) and free ribosomes. Note simple topography of basal plasmalemma (hindleg *O. simillimus* worker, scale bar 1  $\mu$ m). M = mitochondria, N = nucleus.



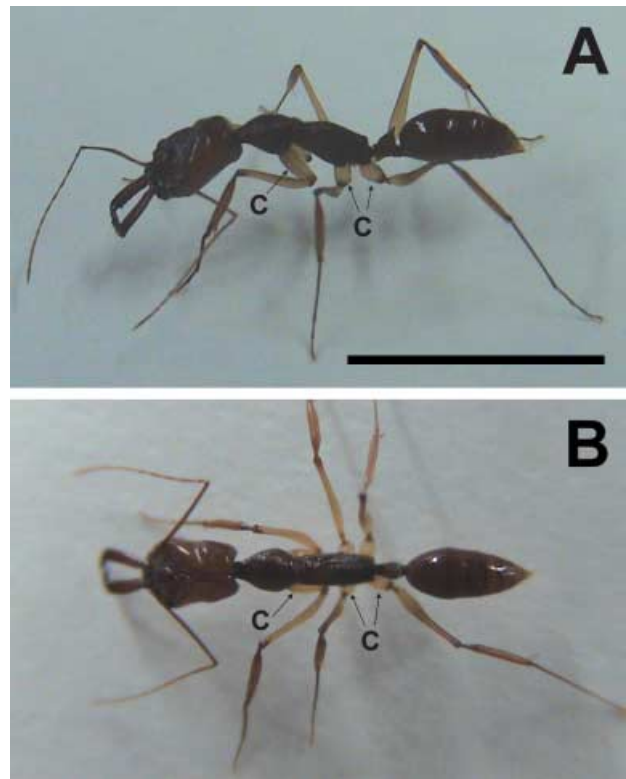
**Fig. 2**—Semi-thin cross-sections through the rounded proximal part of the coxae with basicoxal gland (BG) of —**A.** *Leptogenys diminuta* queen (hindleg) and —**B.** *Odontoponera transversa* worker (hindleg). —**C.** Cross-section through foreleg coxa of *Odontomachus simillimus* worker, showing the straight and broad connection with the prothorax without presence of a basicoxal gland (the dashed line indicates the separation between the prothorax, above the line, and the foreleg coxa below). Scale bars 100  $\mu$ m. BG = basicoxal gland, SP = sensory pegs.

species we examined (*Crematogaster inflata*, subfamily Myrmicinae) basicoxal glands were also absent (Table 1).

The forelegs in all species studied were characterized by the absence of a basicoxal gland (Table 1). Although the foreleg coxae also have the general bottle-shaped appearance, their proximal part does not display the typically rounded ball-and-socket articulation with the thorax as we observed for the mid- and hindleg coxae. Instead, the lateral walls of the proximal foreleg coxae form a straight and rather broad tube-like connection with the prothorax, with a uniformly thin epithelial lining underneath the tegumental cuticle (Fig. 2C). Observation of live ants supports the more limited movements of the foreleg coxae. Whereas the front coxae can move a little bit in the vertical plane (Fig. 3A), they show no lateral movement: regardless of the position of the foreleg, the front coxae remain in the same position along the main body axis (Fig. 3B). This is in sharp contrast to, for example, the hindleg coxae, which can turn through almost 90° according to the position of the leg (compare the position of the coxae of the left and right hindlegs in Fig. 3B).

## Discussion

The discovery of the basicoxal gland provides yet another example of the variety of exocrine structures in ants, and brings it close to an overall diversity of 70 different glands in this insect family. Glands in the coxae of ant legs were first reported by Schoeters and Billen (1993) in *Pachycondyla obscuricornis* and some other species, although these coxal glands correspond to the class-3 exocrine glands in the standard classification of Noirot and Quennedey (1974), in which functional units comprising a secretory cell and an accompanying duct cell occur. Similar class-3 gland cells have also been reported in the coxae of some stingless bee



**Fig. 3**—**A, B.** Lateral and top views, respectively, of *Odontomachus rixosus* worker, illustrating the natural position of the coxae (C) and their articulation with the remainder of the legs. —**B.** The top view clearly illustrates how the foreleg coxae remain underneath the prothorax, regardless of whether the foreleg is held forward (right foreleg) or to the side (left foreleg). The hindleg coxae, in contrast, turn considerably sideward according to the leg's position (compare sideward pointing coxa of right hindleg with backward pointing coxa of left hindleg).

species (Cruz-Landim *et al.* 1998; Cruz-Landim 2002), but otherwise we do not know of the description of coxal glands in social Hymenoptera. A survey of the leg exocrine glands in termites also does not mention coxal glands in this group (Soares and Costa-Leonardo 2002), although there is a brief report of ‘epidermal glands’ in the coxae of neotenic reproductives of *Prorethra simplex* termites (Šobotník *et al.* 2003). In contrast to the known Hymenopteran coxal glands, however, the novel basicoxal gland described here is formed by simple epithelial cells and thus corresponds to the class-1 glands according to Noirot and Quennedey (1974). Coxal glands are also known among the chelicerate arthropods, where they are involved in water balance and osmoregulation, although these are structurally totally different and complicated glandular formations (Filimonova 2004; references therein).

The structural characteristics of the basicoxal glands reveal an epithelium that discharges its secretion into the articulation space surrounding the coxal implantation of the mid- and hindlegs. The presence of pores allows transport of the secretory products through the cuticle, with this porous region exactly coinciding with the region of the glandular epithelium. Microvillar differentiation of the apical cell membrane provides an increase of the surface area, which enables more efficient transport of secretion. The latter is also facilitated by the occurrence of tubular extensions of smooth endoplasmic reticulum into the microvillar fingers. Such an association between smooth endoplasmic reticulum and microvilli also occurs in the Dufour gland of social wasps (Billen 2006), which is known for the discharge of hydrocarbons. The elaboration of a similar kind of secretion may also be the case for the basicoxal glands. Free ribosomes are fairly numerous in their cytoplasm but as no granular endoplasmic reticulum was found, a highly proteinaceous secretion seems not very likely. The abundance of microtubules in the cytoplasm may represent a structural reinforcement, as the position of the basicoxal glands at an articulation point can expose them to considerable mechanical forces. This anatomical position, together with their cytoplasmic characteristics, makes us hypothesize that the basicoxal glands may have a lubricant function. The presence of the gland in both workers and queens is in line with this idea. A similar lubricant function has also been suggested for other intersegmental glands in ponerine ants, of which the oily secretions reduce the friction between sclerotized cuticular parts (Attygalle *et al.* 1996).

The obvious absence of a basicoxal gland in the forelegs (Table 1) can also be understood through the suggested function of the gland as a source of lubricant substances. While the proximal part of the coxae in the mid- and hindlegs has a rounded shape that fits within a socket-like articulation cavity of the thorax, the implantation of the foreleg coxae occurs through a more tubular proximal part, which does not seem to allow the manoeuvrability provided by the ball-and-socket joints of the mid- and hindlegs. The need for lubricant

substances at the junction between the thorax and the highly manoeuvrable coxae of the mid- and hindlegs therefore is much higher than at the junction with the less flexible foreleg coxae.

Although we found basicoxal glands in the mid- and hindlegs of most species for which we had material available, some species do not possess the glands (see Table 1). All Ponerini species studied contain the glands, which may be linked with their heavy sclerotization and therefore even higher need for lubricants. Among the species without basicoxal glands, the ponerine *Platythyrea* sp. and the myrmicine *Crematogaster inflata* are less sclerotized, although the two ectatommine species studied are fairly sclerotized but also lack the basicoxal glands. Whether this reflects eventual phylogenetic differences will need the examination of more species. Besides the degree of sclerotization, species may also differ in their precise walking mechanism, but this will require a careful comparative biomechanical analysis of leg movements in various species, which was beyond the scope of the present study.

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