

# Fine structure of the postpygidial gland in *Aenictus* army ants

Johan Billen<sup>1</sup>, Bruno Gobin<sup>1</sup> and Fuminori Ito<sup>2</sup>

<sup>1</sup> Zoological Institute University of Leuven  
Naamsestraat 59 B-3000 Leuven, Belgium  
<sup>2</sup> Biological Laboratory Faculty of  
Agriculture Kagawa University Takamatsu  
760, Japan

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## Abstract

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Army ants of the genus *Aenictus* are characterized by the presence of a conspicuous postpygidial gland, which is the source of the trail pheromone. The paired gland at each side consists of a reservoir sac into which the secretory cells open through their accompanying duct cells. The secretory cells are characterized by a well developed Golgi apparatus, numerous mitochondria and strands of smooth endoplasmic reticulum. The reservoir opens near the abdomen tip, which facilitates deposition of the secretory products onto the substrate. The large reservoir of the postpygidial gland may enable the incessant trail laying of at least one of the investigated species.

Professor Johan Billen, Zoological Institute, Naamsestraat 59, B-3000 Leuven, Belgium. E-mail: Johan.Billen@bio.kuleuven.ac.be

## Introduction

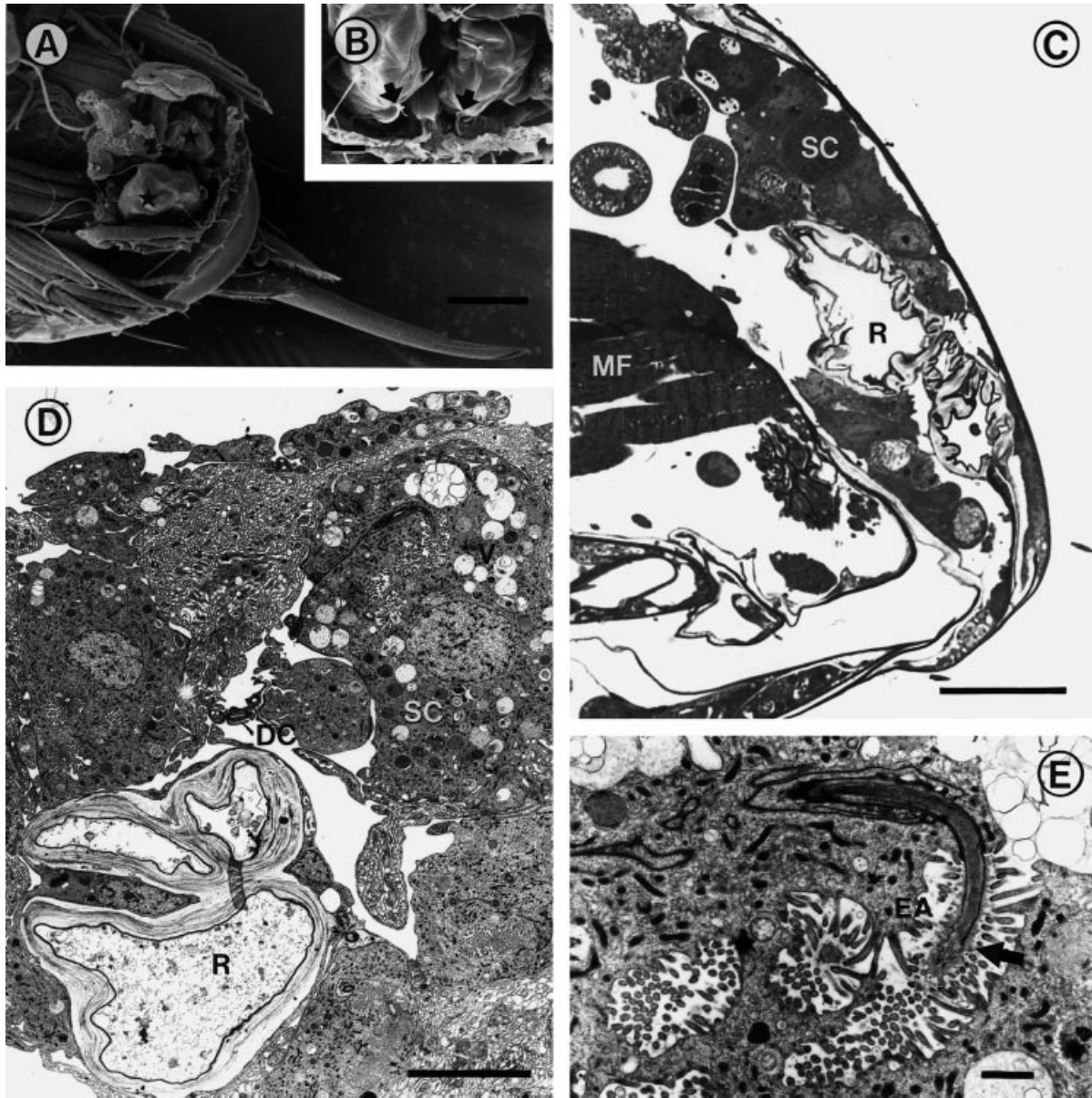
Social organization in ants relies for a very considerable part on the pheromonal secretions that find their origin in an overwhelming variety of exocrine glands (Hölldobler and Wilson 1990; Billen and Morgan 1998). Pheromonal communication is especially important among the army ants, which have the largest known societies in ants. Colonies of the Old World species *Dorylus wilverthi* and *Aenictus laeviceps* contain up to 22 million and 110 000 individuals, respectively (Raignier and van Boven 1955; Schneirla and Reyes 1966), while 2 million are reported for the New World species *Eciton burchelli* (Rettenmeyer 1963). Army ants are especially known for their impressive raiding columns (Gotwald 1995), the organization of which no doubt has a pheromonal basis. Thus far, the existence of a trail pheromone could be demonstrated in only three species. The Neotropical *Eciton burchelli* follows trails from a specialized glandular epithelium associated with the 7th abdominal sternite (Billen 1992). The venom gland is the source of the trail substance in *Dorylus molestus* (Billen and Gobin 1996), while in *Aenictus* sp. near *laeviceps*, the trail pheromone originates from the paired postpygidial glands, and was found to comprise an initiating and an orientating component, which could be

chemically identified as 2-methyl-nicotinate and 2-methyl-anthranilate, respectively (Oldham *et al.* 1994). The postpygidial glands are found underneath the left and right posterior margins of the 7th tergite, and are exceptionally large in representatives of the Aenictinae. In contrast, the postpygidial gland is very small in all other species (of Dorylinae, Ecitoninae, Nothomyrmecinae, Myrmecinae, Ponerinae and Pseudomyrmecinae), in which they occur (Hölldobler and Engel 1978), containing only few secretory cells and most often no reservoir.

We here report on the morphology and ultrastructure of the well developed postpygidial gland in *Aenictus*, which at the same time represents the first description of the fine structural organization of this gland in ants.

## Materials and Methods

Raiding workers were collected of *Aenictus alticola* Wheeler, 1930, *A. dentatus* (Forel, 1911), *A. laeviceps* (F. Smith, 1857) (all three from Kuala Lumpur, Malaysia), *A. javanus* Emery, 1896 (from Bogor, Indonesia), *A. rotundatus* Mayr, 1901 (from Nairobi, Kenya) and *A.* sp. near *laeviceps* (from Hong Kong). Dissected postpygidial glands as well as abdominal tips were fixed in cold 2% glu-



**Fig. 1**—**A**, Scanning micrograph of the abdominal tip with extruded sting and removed tergites of *Aenictus rotundatus* showing the paired postpygidial gland. Scale bar: 50  $\mu\text{m}$ . —**B**, Scanning micrograph detail of postpygidial glands in *A. rotundatus*. Arrows indicate duct cells. Scale bar: 10  $\mu\text{m}$ . —**C**, Longitudinal semithin section through the abdominal tip of *A. javanus* MF muscle fibres, R reservoir, SC secretory cells. Scale bar: 50  $\mu\text{m}$ . —**D**, Survey of

postpygidial gland secretory cells (SC), duct cells (DC) and reservoir (R). Note electron-lucid and granular vesicles (V) in secretory cells (electron micrograph *A. sp.* near *laeviceps*). Scale bar: 10  $\mu\text{m}$ . —**E**, Junction between duct cell and secretory cell with formation of the end apparatus (EA) in *A. sp.* near *laeviceps*. Note transition of continuous cuticle in duct cell to fenestrated appearance in end apparatus (arrow). Scale bar: 1  $\mu\text{m}$ .

taraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose, and post-fixed in cold 2% osmium tetroxide in the same buffer. Tissues were dehydrated through a graded acetone series and embedded in Araldite. Semithin sections with a thickness of 1  $\mu\text{m}$  were stained with methylene blue and thionin, and were

used for light microscopy. Thin sections were double-stained in an LKB 2168 Ultrastainer and viewed in a Zeiss EM 900 electron microscope. Following fixation, material for scanning microscopy was dehydrated through an ethanol series, critical point dried and examined in a Philips SEM 515 scanning microscope.

## Results

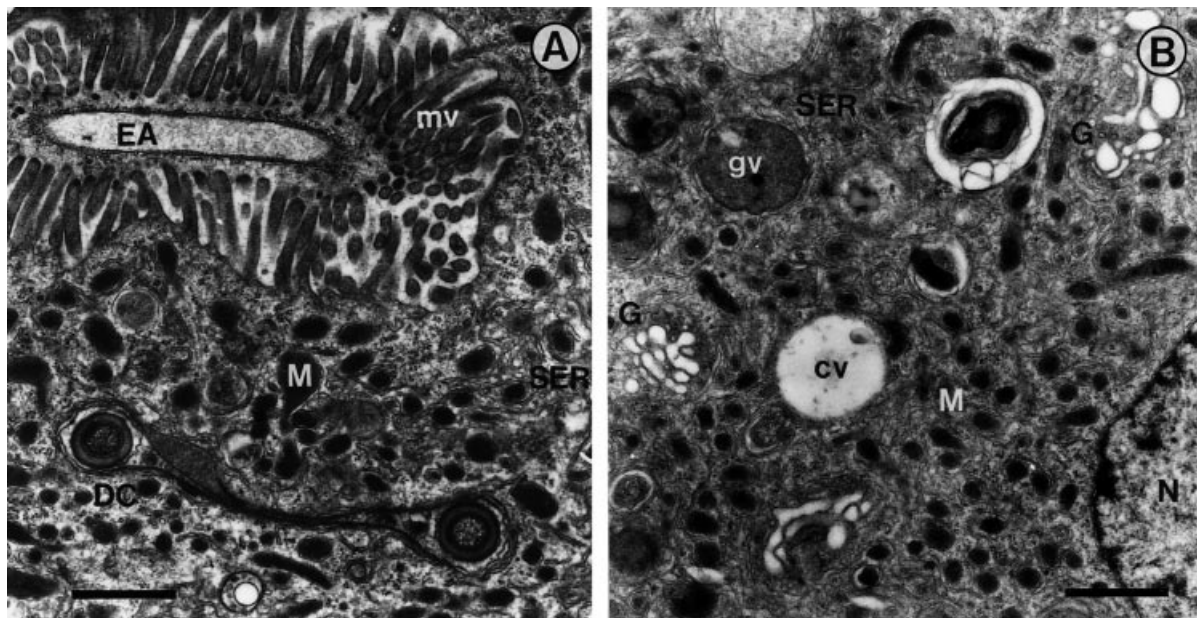
Workers of all *Aenictus* species examined have a pair of well developed postpygidial glands (Fig. 1 A–C). In *A. javanus* and *A. rotundatus*, the glands have a bright red colour that makes them even visible through the pale cuticle of live workers as two conspicuous red spots. Each gland is formed by 15–20 secretory cells which open into a reservoir via their accompanying duct cells. The reservoir has a diameter that varies from 70 to 100  $\mu\text{m}$ , and is lined with a cuticular intima with a thickness around 2  $\mu\text{m}$  and a flattened epithelium of hardly 0.5  $\mu\text{m}$  (Fig. 1D). Slender duct cells form the connection between the reservoir and each secretory cell (Fig. 1E). They contain a duct with a diameter of 0.35  $\mu\text{m}$  and a continuous cuticular lining with a thickness of approx. 0.1  $\mu\text{m}$  (Figs 1E, 2A). The rounded to polygonal secretory cells have a diameter between 20 and 25  $\mu\text{m}$  and occur both on top and underneath the reservoir (Fig. 1C–D). At the junction between the duct cell and the secretory cell, the duct loses its continuous intima and continues as a fenestrated cuticular lining that together with the surrounding microvilli forms the sinuous end apparatus (Fig. 1E). The microvilli are tightly arranged or may be separated by considerable extracellular spaces (Figs 1E, 2A). The secretory cells are characterized by a rounded nucleus, a well developed Golgi apparatus, an abundance of small electron-dense mitochondria and scattered strands of smooth endoplasmic reticulum (Fig. 2 A, B). The

cytoplasm further reveals the occurrence of many rounded electron-lucid and few granular vesicles with a diameter of up to 1  $\mu\text{m}$  (Figs 1D, 2B). The reservoirs of the postpygidial gland open into the dorsal part of the cloacal chamber.

## Discussion

The cytoplasmic organization of the large secretory cells of the postpygidial gland of *Aenictus* corresponds with that of other pheromone producing glands (Billen and Morgan 1998; Quennedey 1998). The occurrence of a well-developed Golgi apparatus and smooth endoplasmic reticulum is in line with the production of the low weight substances that were identified in *Aenictus* sp. near *laeviceps* and *A. rotundatus* (Oldham et al. 1994). These secretory compounds are probably contained in the numerous electron-lucid vesicles in the cytoplasm. They are secreted into the glands' reservoir which itself opens dorsally near the abdomen tip.

All investigated species of *Aenictus* have well elaborated postpygidial glands with a conspicuous reservoir. This is in sharp contrast to the few glandular cells and the absence of a reservoir in the majority of species that have a postpygidial gland (Hölldobler and Engel 1978). Although we do not know the function of this gland in the other Aenictinae, the occurrence of a large reservoir allows it to serve as the source of the trail pheromone in *Aenictus* sp. near *laeviceps*.



**Fig. 2**—**A**, Detail of end apparatus (EA) and section through duct cells (DC) in *Aenictus* sp. near *laeviceps*. Note strands of smooth endoplasmic reticulum (SER) and abundance of electron-dense mitochondria (M). mv microvilli. Scale bar: 1  $\mu\text{m}$ . —**B**, Detail of

cytoplasm with well developed Golgi apparatus (G), numerous mitochondria (M) and strands of smooth endoplasmic reticulum (SER). cv clear vesicle, gv granular vesicle, N nucleus (*A. sp.* near *laeviceps*). Scale bar: 1  $\mu\text{m}$ .

The majority of trail pheromones in ants originates from abdominal glands that are either associated with the sternites or the sting, thus allowing easy deposition of the active compounds onto the substrate (Billen and Morgan 1998). The use of tergal glands as source of trail substances therefore at first appears functionally less appropriate. The postpygidial gland opening, however, is situated dorsally in the cloacal chamber, thus bringing the secretory products conveniently to the abdominal tip, from where they can be released via the sting. Also in some dolichoderines, a dorsally situated source of the trail pheromone may be found, such as the pygidial gland in *Tapinoma simrothi* (Simon and Hefetz 1991). The presence of a large reservoir in the postpygidial gland of *Aenictus*, combined with the efficient mechanism for depositing secretion onto the substrate probably reflects its functional significance, as trail following in army ants represents a very important part of their behavioural repertoire. Thanks to these morphological characteristics the very high trail laying capacity of these insects can be realized.

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