

Femoral and tibial glands in the ant genus *Strumigenys* (Hymenoptera, Formicidae)

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ABSTRACT. The distal part of the femur and tibia of the six legs of workers, queens and males of five of the six *Strumigenys* species examined is characterized by a dorsally occurring epithelial gland. In *S. rogeri*, we could find this gland only on the midleg femur and on the hindleg femur and tibia of queens, whereas it appears absent in workers. The glandular area is externally visible as an apparently smooth oval patch with numerous minute pores with a diameter around 100 nm. Both the femoral and tibial glands are formed by a considerable thickening of the tegumental epidermis in a region where the overlaying cuticle is reduced to approximately half the thickness it has elsewhere. The glandular epithelium is formed by secretory cells with basally located rounded nuclei and with an irregular microvillar differentiation of the apical cell membrane. Their cytoplasm contains numerous secretory vesicles of variable size and electron density, and scattered free ribosomes. The function of these hitherto unknown leg glands so far remains unknown.

KEY WORDS: morphology, ultrastructure, exocrine glands, femoral glands, tibial glands, *Strumigenys*, Formicidae.

INTRODUCTION

Ants are known for the overwhelming variety of glands that make up their exocrine system (HÖLLDOBLER & WILSON, 1990; BILLEN & MORGAN, 1998). Although most attention has been given to the glands in the head, thorax and abdomen, also the legs were recently found as a possible location for exocrine glands. Among these leg glands, the pretarsal glands represent a common exocrine structure for the six legs in the various castes of all ant species (BILLEN, 1993), while other glands are only found in ants of a particular phyletic group, caste and/or leg pair, like the metatibial gland, that is characteristic for the hindlegs of workers in the doryline section (BOLTON, 1990; HÖLLDOBLER et al., 1996). The glands that may occur in a particular leg segment moreover do not need to be homologous. The tibial glands in *Crematogaster* ants, for instance, are formed by an internalized epithelium surrounding a central reservoir (LEUTHOLD, 1968; BILLEN, 1984), while a tibial gland formed by bicellular secretory units has been reported in the army ant *Dorylus molestus*

Gerstaecker, 1858 (BILLEN, 1997). Still another tibial gland is the metatibial gland in ants of the doryline section, that represents a glandular modification of the ventral tegumental epidermis (HÖLLDOBLER et al., 1996). In this paper, reference is made to a personal communication by B. Bolton that also the myrmicine *Strumigenys* displays a tibial gland, although its dorsal position makes it not homologous with the ventrally occurring metatibial gland in the doryline section. The present contribution deals with the morphology and ultrastructure of this tibial gland and of a structurally similar but hitherto unknown femoral gland in *Strumigenys*.

MATERIAL AND METHODS

Strumigenys lewisi Cameron, 1886 was collected in Takamatsu, Japan. Colonies of the other species for this study were collected in Indonesia and brought to Belgium for further morphological work. Colonies of *S. koningsbergeri* Forel, 1905 were collected at Mt. Sarak, Bogor, *S. nanzanensis* Lin & Wu, 1996 in Ujung Kulon, and *S. rogeri* Emery, 1890, *S. signae* Forel, 1905 and *Strumigenys* sp. (colony code FI97-586, belonging to the *S. lyroessa* group) in Kebun Raya. Voucher specimens of

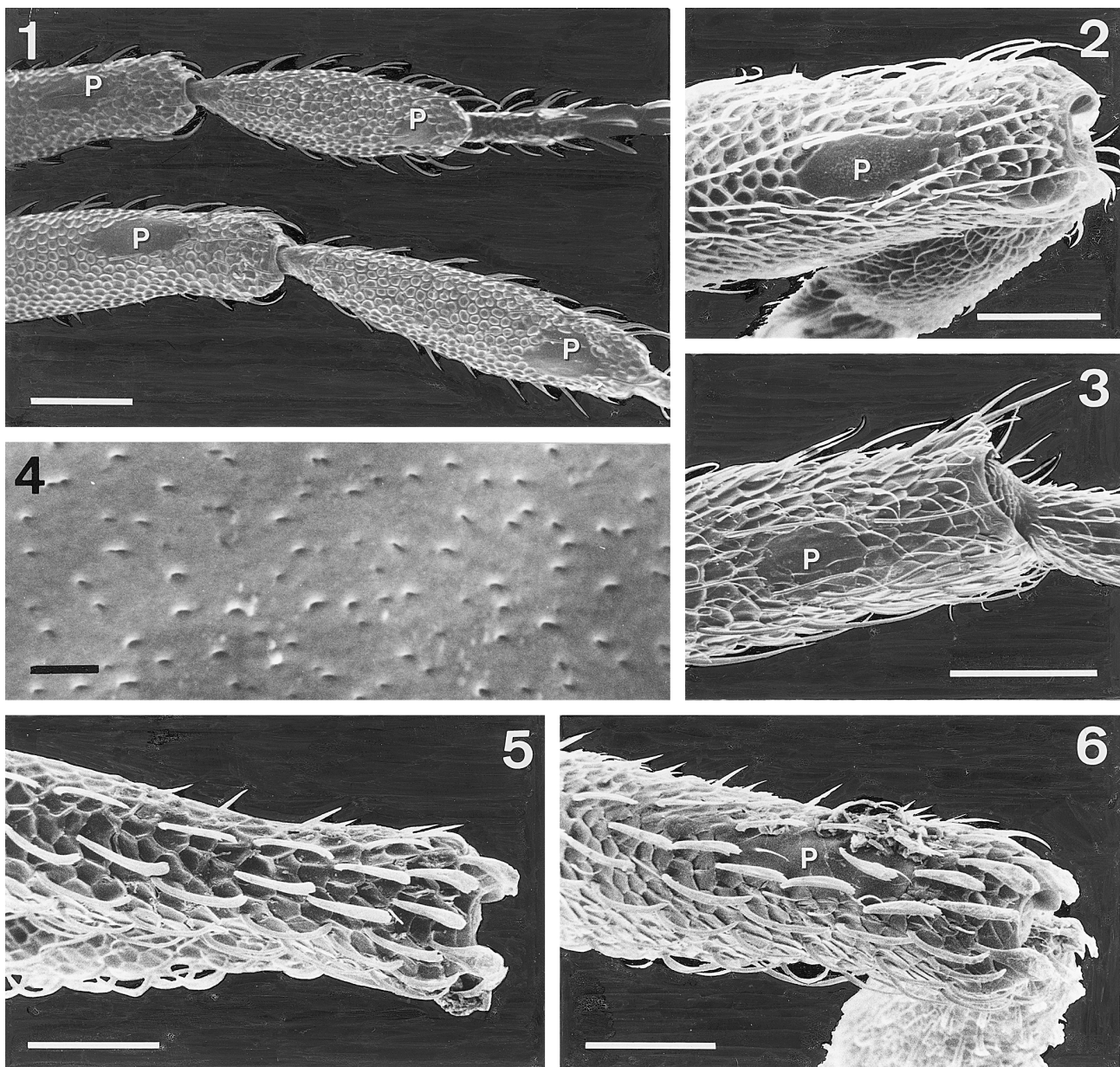
the Indonesian species have been deposited in the Bogor Zoological Museum and in the London Natural History Museum.

Leg parts were fixed in 2% cold glutaraldehyde buffered with 50 mM Na-cacodylate and 150 mM saccharose and postfixed in 2% osmium tetroxide. After dehydration in a graded acetone series and embedding in Araldite, they were sectioned with a Reichert Ultracut E microtome. Semi-thin sections for light microscopy were stained with methylene blue and thionin, double stained thin sections were viewed in a Zeiss EM900 electron

microscope. Air-dried specimens for scanning microscopy were coated with gold and observed with a Philips SEM 515 microscope.

RESULTS

Near the distal part of both the femur and tibia of the six legs of the workers, queens and males of the *Strumigenys* species studied, we found an apparently smooth dorsal patch amidst the hairy leg sculpturation (Figs 1-3). Only in *S. rogeri*, the presence of this structure is considerably



Figs 1-6. – Scanning electron micrographs illustrating the position of the dorsally occurring smooth cuticular patches (P) overlying the epithelial glands in femur and tibia. – 1. Mid- and hindleg femora and tibiae of a queen of *Strumigenys* sp. FI97-586, scale bar 100 μ m. – 2. Foreleg femur of *S. lewisi* queen, scale bar 50 μ m. – 3. Hindleg tibia of *S. lewisi* male, scale bar 50 μ m. – 4. Detail of cuticular pores in smooth patch of midleg femur of *S. sp.* FI97-586 worker, scale bar 1 μ m. – 5. Distal part of hindleg tibia in worker of *S. rogeri* showing absence of smooth patch, scale bar 50 μ m. – 6. Same region of hindleg tibia in queen of *S. rogeri* showing presence of smooth patch, scale bar 50 μ m.

more restricted as we only found it on the midleg femur and on the hindleg femur and tibia of the queen. It could not be found on the legs of *S. rogeri* workers (Figs. 5, 6 - males of this species were not available for examination).

On the femur, the patch occurs at approx. 50 μm from the articulation with the tibia, and measures approx. 25 x 15 μm in the worker and 50 x 25 μm in the queen. The hindleg femoral patch is larger, measuring 35 x 17 μm in the worker and 80 x 25 μm in the queen. On the tibia, it occurs approx. 20 to 30 μm from the articulation with the basitarsus, and for the three leg pairs measures approx. 25 x 15 μm in the worker and 30 x 15 μm in the queen. At high magnification, these patches show very numerous pores with a diameter of approx. 100 nm and a pore density between 1.5 and 2.5 pores/ μm^2 for both the femoral and tibial patches (Fig. 4).

The glandular epithelium in both the femur and tibia reaches a thickness of 7 to 10 μm , whereas the non-glandular epidermal lining of the leg tegument has a thickness of less than 1 μm . In the region of the glandular epithelium, the overlying cuticle is reduced to a thickness of 3 to 5 μm , which is approximately half the thickness it has elsewhere in the leg (Figs 7-10). In this part overlaying the gland, numerous irregular channels traverse the cuticle and open at the exterior surface through minute pores (Figs 4, 9, 11). Due to the small size and high number of these pores, the cuticular surface that corresponds with each secretory cell shows approx. 100 such pores. The secretory cells have basally located rounded nuclei with a diameter of 1.5 to 3 μm , and display an irregular microvillar differentiation of their apical cell membrane (Figs 9, 11). Their cytoplasm is characterized by numerous rounded secretory vesicles with a diameter ranging from 0.1 to 2 μm . Vesicles of variable electron density occur, and become confluent with the intermicrovillar spaces apically (Fig. 9). Numerous scattered free ribosomes and few mitochondria occur (Fig. 12), while endoplasmic reticulum and other cytoplasmic organelles are scarce.

DISCUSSION

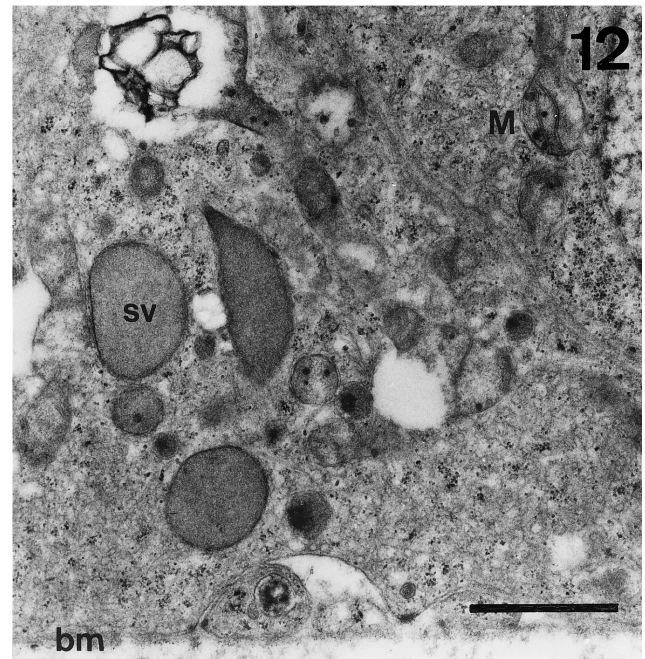
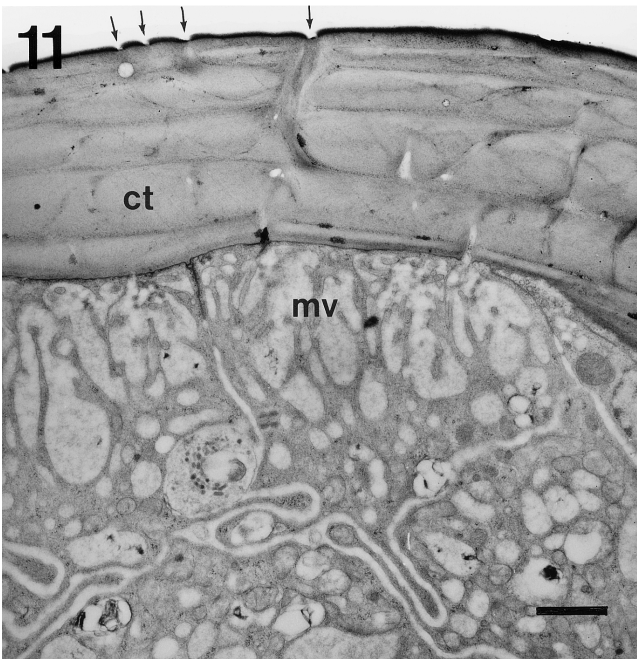
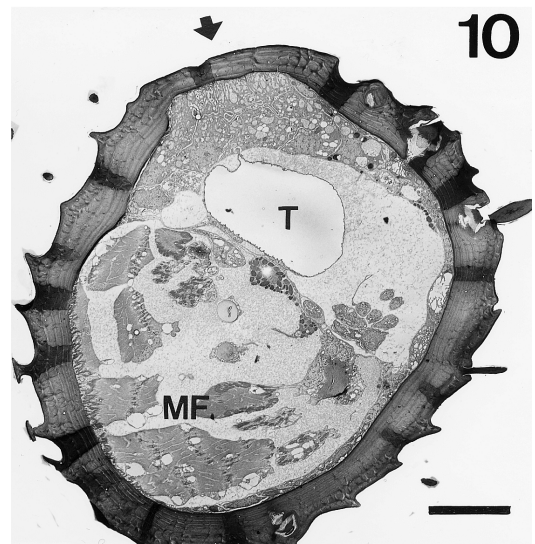
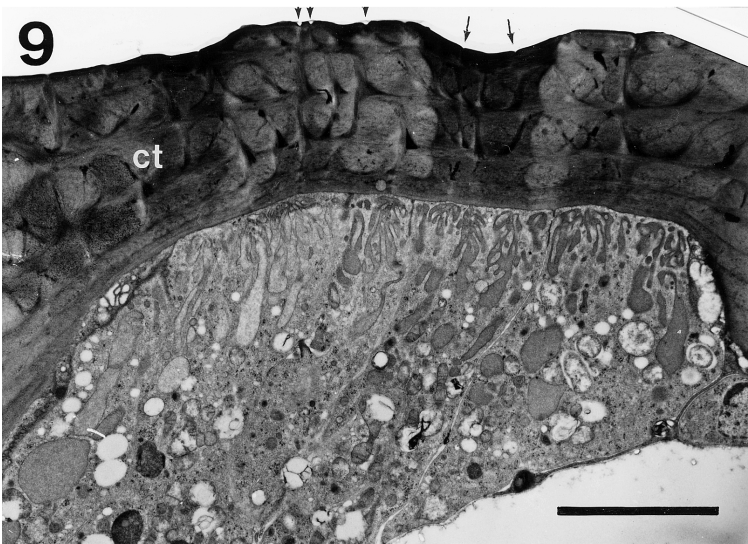
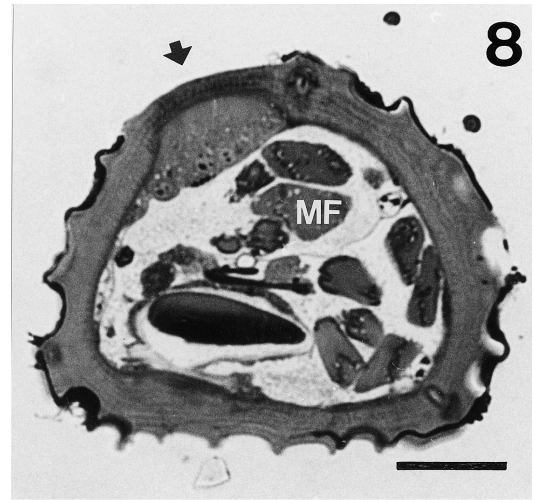
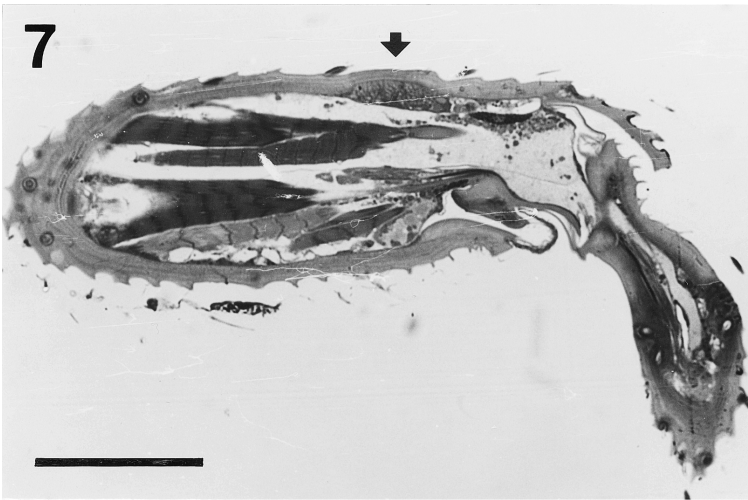
The presence of exocrine glands in the tibia is known for a number of ants, and even comprises various glands with different structural organization and/or location within the tibia. The epithelial gland that is here described on the distal part of the tibia in several *Strumigenys* species, however, is not homologous to any of the other known tibial glands in ants. It is structurally most similar to the metatibial gland that is characteristic for the hindlegs ants of the doryline section (HÖLDOBLER et al., 1996), although the gland in the myrmicine *Strumigenys* species occurs in a dorsal instead of a ventral position. It moreover occurs in the three leg pairs and can be found in workers, queens and males. Tibial glands with externally visible pore plates have also been reported for the six legs of some rhinotermitid termites (BACCHUS, 1979), but these larger and less numerous pores each represent the

opening of an individual duct cell and associated secretory cell. The much smaller pores of *Strumigenys*, however, represent cuticular channels, and therefore do not have any relationship with the number of secretory cells.

The femoral glands of *Strumigenys* appear as the structural homologues of the tibial glands with a similar occurrence in the femur of all legs and in all castes. They also represent the first example of an exocrine gland in the femur of ants. The occurrence of glands in the femur was recently reported for some meliponine bees, although these are some scattered unicellular glands occurring in the three castes as well as an internal sac-like gland that is only found in queens (CRUZ LANDIM et al., 1998).

The presence of these femoral and tibial glands is a phylogenetically useful feature, as it represents a synapomorphy of all the genera in the *Strumigenys*-group (subtribe Strumigenyiti), while the glands are absent from all other groups of Dacetoniini. In workers of some *rogeri*-group species the gland is obvious but in others, including *S. rogeri* itself and some other African members of the group, it is vestigial or even absent. Despite this, the presence of the glands is an apomorphy of the strumigenyiform clade of genera (BOLTON, 1999). Subsequent reduction or loss of the gland has occurred in a number of individual species and a few entire species-groups, both in *Strumigenys* and its sister genus, *Pyramica*.

The structural organization of both the tibial and femoral glands is a clear example of the epithelial type (class 1 secretory cells according to the classification of NOIROT & QUENNEDEY, 1974). Compared to other epithelial glands, these leg glands in *Strumigenys* are fairly small, both in terms of external surface and gland volume. Cell height hardly reaches 10 μm , whereas the metatibial gland epithelium e.g. may have a thickness of up to 80 μm (HÖLDOBLER et al., 1996). The apparently poor occurrence of endoplasmic reticulum and other cytoplasmic organelles make it difficult to judge about the metabolic potential of these leg glands, although the obvious presence of vesicles and their structural association with the apical microvilli should be indicative for the secretory activity. Also the presence of hundreds of transcuticular channels that open as minute pores at the surface is in agreement with an effective mechanism for release of the secretory products to the outside. The chemical composition of the secretion and its function so far remain highly speculative. The general presence of the gland on the six legs in the three castes may make a pheromonal function less probable. Also a function as lubricant, that is often postulated for glands with unknown function, seems not very likely for these tibial and femoral glands considering their position that is not directly associated with any articulation where such smearing function may be more meaningful. The high number of ribosomes scattered in the cytoplasm may indicate the secretion has a protein fraction, although further research will be needed to find out about the function of these peculiar glands in *Strumigenys* ants.



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REFERENCES

- BACCHUS, S. (1979). New exocrine gland on the legs of some Rhinotermitidae (Isoptera). *Int. J. Insect Morphol. & Embryol.*, 8: 135-142.
- BILLEN, J.P.J. (1984). Morphology of the tibial gland in the ant *Crematogaster scutellaris*. *Naturwissenschaften*, 71: 324-325.
- BILLEN, J. (1993). Morphology of the exocrine system in ants. In: Ed. V.E. KIPYATKOV, *Proc. Coll. Social Insects*, St. Petersburg: 1-15.
- 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. & E.D. MORGAN (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, M.L. WINSTON & K.E. ESPELIE, Westview Press, Boulder, Oxford: 3-33.
- BOLTON, B. (1990). Army ants reassessed: the phylogeny and classification of the doryline section (Hymenoptera, Formicidae). *J. Nat. Hist.*, 24: 1339-1364.
- BOLTON, B. (1999). Ant genera of the tribe Dacetoniini. *J. Nat. Hist.*, 33: 1639-1689.
- CRUZ LANDIM, C., R.L.M.S. DE MORAES, H.C. SALLES & R.D. REGINATO (1998). Note on glands present in Meliponinae (Hymenoptera, Apidae) bees legs. *Revta bras. Zool.*, 15: 159-165.
- HÖLLDOBLER, B. & E.O. WILSON (1990). *The Ants*. Harvard University Press, Cambridge, Mass. (pp. 732)
- HÖLLDOBLER, B., M. OBERMAYER & C. PEETERS (1996). Comparative study of the metatibial gland in ants (Hymenoptera, Formicidae). *Zoomorphology*, 116: 157-167.
- 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. & A. QUENNEDEY (1974). Fine structure of insect epidermal glands. *Annu. Rev. Entomol.*, 19: 61-80.

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Legends to the figures (see opposite page)

Fig. 7. – Longitudinal semithin section through midleg tibia and basitarsus of queen of *Strumigenys nanzanensis*, showing dorsal tibial gland epithelium (arrow). Scale bar 50 µm.

Fig. 8. – Transverse semithin section through distal part of hindleg femur of *S. koningsbergi* worker with dorsal femoral gland (arrow). MF = muscle fibres, scale bar 10 µm.

Fig. 9. – Electron micrograph of epithelial gland in hindleg femur of *S. sp.* FI97-586 worker. Note irregular vertically orientated transcuticular channels and their opening at surface via small pores (arrows). ct = cuticle, scale bar 5 µm.

Fig. 10. – Electron micrograph showing transverse section through distal part of hindleg tibia of *S. nanzanensis* queen with occurrence of epithelial gland (arrow). MF = muscle fibres, T = trachea, scale bar 10 µm.

Fig. 11. – Detail of cuticle (ct) and apical cytoplasm with irregular microvilli (mv) in epithelial gland of hindleg tibia of *S. nanzanensis* queen. Note pores opening on cuticular exterior side (arrows), scale bar 1 µm.

Fig. 12. – Detail of cytoplasm in basal region of glandular epithelium in midleg femur of *S. sp.* FI97-586 worker. bm = basement membrane, M = mitochondria, sv = secretory vesicle, scale bar 1 µm.