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ORIGINAL PAPER

New exocrine glands in ants: the hypostomal gland and basitarsal gland in the genus *Melissotarsus* (Hymenoptera: Formicidae)

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Abstract Fisher and Robertson (Insect Soc 46: 78–83, 1999) discovered the production of silk-like secretions emerging from slit-shaped openings along the anterior margin of the ventral hypostoma of *Melissotarsus* ant workers. The current histological study describes a hitherto unknown hypostomal gland from which this silk-like substance originates. In addition, this study describes a new basitarsal gland in the three pairs of legs of *Melissotarsus* workers.

Keywords *Melissotarsus* · Exocrine glands · Hypostoma · Basitarsus · Silk production · *Rhopalomastix*

Introduction

The bodies of ants are crowded with exocrine glands. More than 40 anatomically distinct exocrine glands have been found in all ant species taken together, and the total continues to climb (Billen 2009, 2011; Hölldobler and Wilson 2009). In most cases investigated, the glands manufacture chemical compounds used as communication signals or in defense against predators and microorganisms (Billen and Morgan 1998; Hölldobler and Wilson 1990, 2009).

In 1999, Fisher and Robertson (1999) reported the discovery of silk production in hitherto unknown exocrine glands in the heads of workers of the myrmicine species *Melissotarsus emeryi*. Their observation suggested that the silk-producing glands are located in cuticular depressions on the ventral portion of the anterior margin of the hypostoma and that silk was applied in construction with the aid of modified protarsi in the form of "silk brushes" (Fisher and Robertson 1999). Silk production by exocrine glands in adult ant workers is a novel function in ants, and such "hypostomal glands" have never been described before. We therefore conducted a histological study of putative, hitherto unknown exocrine glands in *M. emeryi*. In addition, we conducted comparative studies with the three other known *Melissotarsus* species, i.e., *Melissotarsus beccarii*, *Melissotarsus insularis*, and *Melissotarsus weissi*, and compared these findings with those found in the sister genus *Rhopalomastix.*

In addition, *Melissotarsus* workers seem to be endowed with a special basitarsal gland in the three pairs of legs. This was inferred from the presence of particular cuticular structures at the distal outer portion of the basitarsi (Delage-Darchen 1972; Billen 2009). The current study provides the first histological description of this unique glandular structure.

Material and methods

The genus *Melissotarsus* is known from Saudi Arabia (Collingwoord 1985), South Africa (Bolton 1973, 1982), and Madagascar (Fisher et al. 1998). Live specimens of *M. emeryi* used in this study were collected from nests in *Leucospermum praemorsum* (Proteaceae) in South Africa, Western Cape Province, fixed in alcoholic Bouin's solution, and stored in 75 % ethanol. For histological investigations, the

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head, alitrunk, gaster, front, middle, and hind legs were imbedded in Spurr's low-viscosity medium (Spurr 1969) and serially sectioned at 1.5–3.5 μm using a Reichert-Jung microtome model 2050 with a histo-diamond knife. The sections were attached to egg albumin-coated slides and stained on a hot plate with Mallory's solution (1 % methylene blue, 1 % azure II in 1 % sodium borate) or Burns and Bretschneider (1981) toluidine blue/basic fuchsin; the latter staining technique required the removal of the plastic.

All histological preparations and some of the scanning electron microscope (SEM) work were conducted at the laboratory of B. Hölldobler at the University of Würzburg. Further analysis of the histological sections and additional SEM work were carried out at the School of Life Science at Arizona State University in 2013. For comparative morphological studies with *M. beccarii* (Cameroon), *M. insularis* (Madagascar), *M. weissi* (Cameroon), and *Rhopalomastix sp.* (Malaysia), we used specimens preserved in 70 % ethanol.

Results

The hypostomal gland

Workers of all *Melissotarsus* species are very small, but they have, relative to their size, large heads and sturdy-looking legs (Fig. 1). Our SEM studies confirm the findings by Fisher and Robertson (1999). The rim of the buccal cavity is lined with slit-shaped openings and setae. Threads resembling silk can be seen emerging from some of the cuticle slits (Fig. 2). Transverse sections of the head of *M. emeryi* workers revealed two large clusters of glandular cells, each cell sending a long duct to the V-shaped margin of the buccal cavity between the base of the mandibles and maxillary labial complex (Fig. 2). Each gland cell measures approximately 30–35 μm; we counted a total of 135–140 cells. The mass of glandular cells fills almost the entire ventral region of the head case, the inside of which bulges outward more than in most other ant species.

We found several identical-looking glandular cells inside the massive mandibles but were unable to unequivocally determine where the ducts of these cells connect with the cuticle. In addition, we noticed a striking structure inside the mandibles which appears to be a chordotonal organ, but the light microscopic images did not allow a more detailed analysis (Fig. 3).

Fig. 1 *Melissotarsus weissi* worker. *Arrows* point to the basitarsi of the fore, middle, and hind legs (photograph courtesy Alex Wild)

Following the terminology proposed by Noirot and Quennedey (1974) and elaborated by Billen (2009, 2011), these cells of the hypostomal gland are class 3 cells, as each secretory cell is associated with a duct cell. This silk-producing glandular structure is not derived from one of the known exocrine glands in the head of ants. We confirmed the presence of a relatively small mandibular gland, propharyngeal gland, maxillary gland, postpharyngeal gland, and labial gland (terminology according to Emmert 1968).

Dissections of the specimens preserved in ethanol of the other three known species of *Melissotarsus* (*M. beccarii*, *M. insularis*, and *M. weissi*) confirmed the presence of a large hypostomal gland. For *Rhopalomastix sp.*, a sister genus of *Melissotarsus*, we were unable to identify a hypostomal gland by dissecting ethanol-preserved specimens; however, SEM images clearly indicate that this genus is also endowed with a hypostomal gland (Fig. 4). The rim of the buccal cavity is lined with grooves which appear to be glandular openings, because emerging secretions can be seen in some of the grooves. The consistency of the secretions seems to be different to those of *Melissotarsus*, although in one case, we observed a fine silk-like thread coming out of the glandular opening.

Basitarsal gland

A remarkable feature of the *Melissotarsus* workers is the "swollen" basitarsus of the forelegs, but the basitarsus of the middle and hind legs also bulges out more than in most ant species (Figs. 1 and 5a, b). Delage-Darchen (1972) noticed that the distal, outer portion of the basitarsus in the three pairs of legs has cuticle pits with pores inside and suggested that these might be glandular openings. Recently, Billen (2009) published a SEM photograph of such a cuticle pit at the basitarsus of the middle leg, showing pores oozing with a "toothpaste-like secretory material." We found the same kind of structure in *M. emeryi* (Fig. 5b, c), and our histological studies of the basitarsi of front, middle, and hind legs revealed large glandular cells (35–45 μm in diameter), replete with large vacuoles and long ducts that lead to a pore plate (~25–30 μm in diameter) (Fig. 6). These secretory cells also are class 3 cells. SEM studies of the basitarsi of the three pairs of legs did not indicate a presence of this kind of basitarsal gland in *Rhopalomastix*.

The basitarsus of the front leg, in contrast to that of the middle and hind legs, also houses a large cleaning gland (sometimes called antenna-cleaning gland), consisting of a glandular epithelium approximately 25 μm thick and 120 μm long (Fig. 6a).

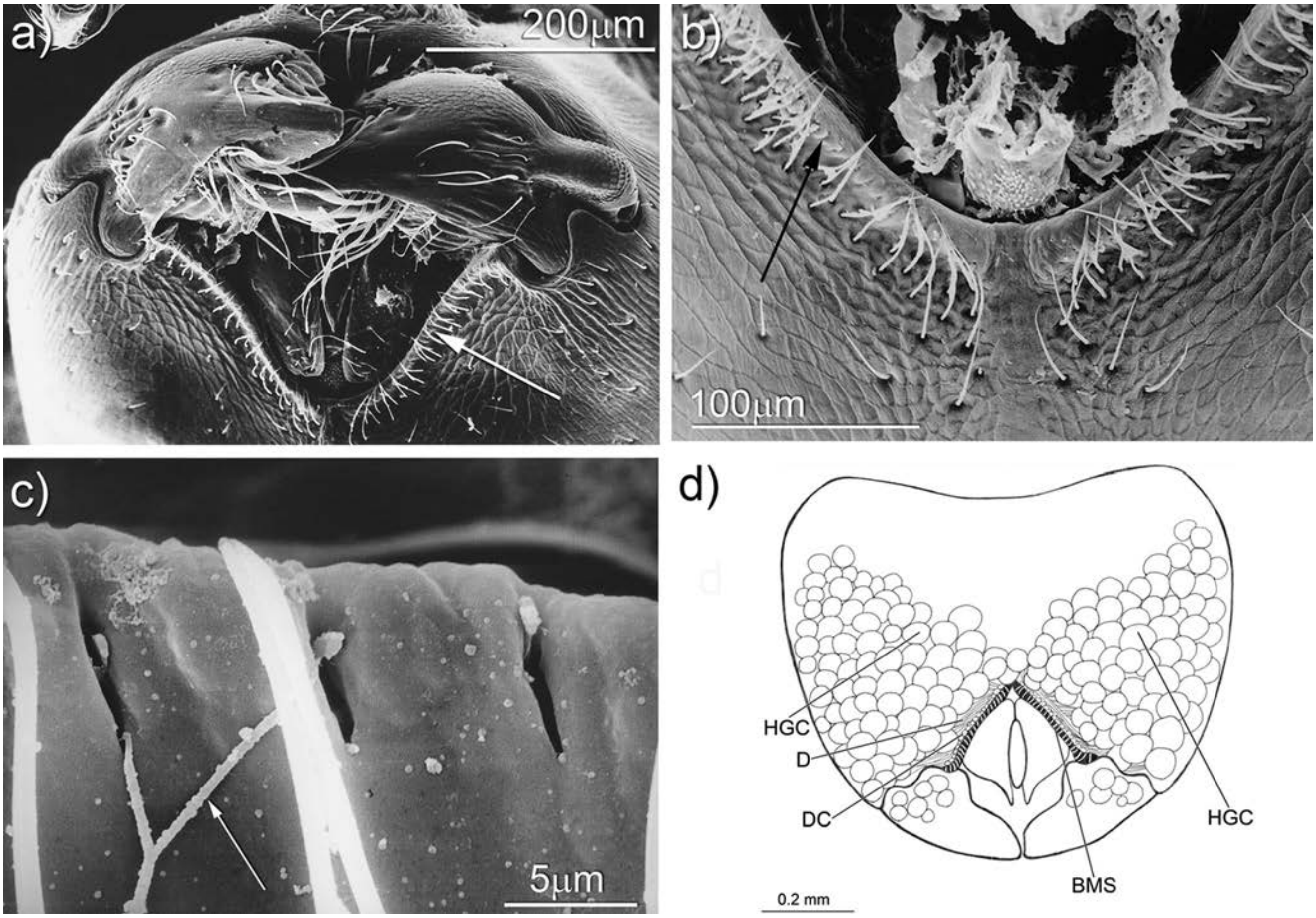

Fig. 2 Scanning electron microscope images of the margin of the buccal cavity which is lined with slit-shaped openings and setae of *Melissotarsus emeryi*; (*arrows* in images **a** and **b**). **c** Close-up of the slit-shaped openings with silk-like threads coming out of two openings and merge into one thread (see *arrow*). **d** Schematic illustration of the hypostomal gland, located below the brain and pharynx in the ventral portion of the head case; *HGC* hypostomal gland cell, *D* glandular cell duct, *DC* glandular duct cell, *BMS* buccal margin slit openings

This gland has been previously described in several ant species (Schönitzer and Lawitzky 1987; Schönitzer et al. 1996; Hölldobler et al. 1992; Billen 2009). The secretions of the glandular cells seep through densely spaced cuticular pores on the base of the glandular epithelium (Fig. 7; see also Billen 2009). The presence of two major glands in the foreleg basitarsus of *Melissotarsus* explains the strikingly swollen shape of this tarsal segment.

Discussion

Melissotarsus workers produce silk, although the chemical nature of this silk-like secretion is not yet known. This is currently the only known case of silk production by adult worker ants (Fisher and Robertson 1999). Except for the fact that silk threads were seen extruding from the slit-like openings along the buccal rim or the ventral part of the anterior margin of the hypostoma, nothing was known about the structure and size of this putative gland. In fact, it was entirely feasible that the silk gland of *Melissotarsus* is a modification of a known head gland, such as the propharyngeal or maxillary gland.

Our histological studies clearly demonstrate that this silk gland is a hitherto unknown gland, consisting of over 100 single glandular cells, with each cell draining its secretion through cellular ducts that lead to the slit-shaped cuticular openings along the buccal margin. We call the gland the hypostomal gland. It is by far the largest exocrine gland entirely located in the head of *Melissotarsus* workers. The labial gland of *Melissotarsus* is also quite large, but the major secretory section of this gland is located in the alitrunk.

Mony et al. 2007 consider the production of silk by adult workers of *Melissotarsus* a unique adaptation to living in a network of chambers and tunnels excavated with their massive, powerful mandibles into the bark and wood of live trees. Older workers frequently exhibit heavily worn mandibles (Delage-Darchen 1972; Mony et al. 2007). Although it is not exactly known how and to what degree silk is employed in nest construction, Fisher and Robertson (1999) suggest that

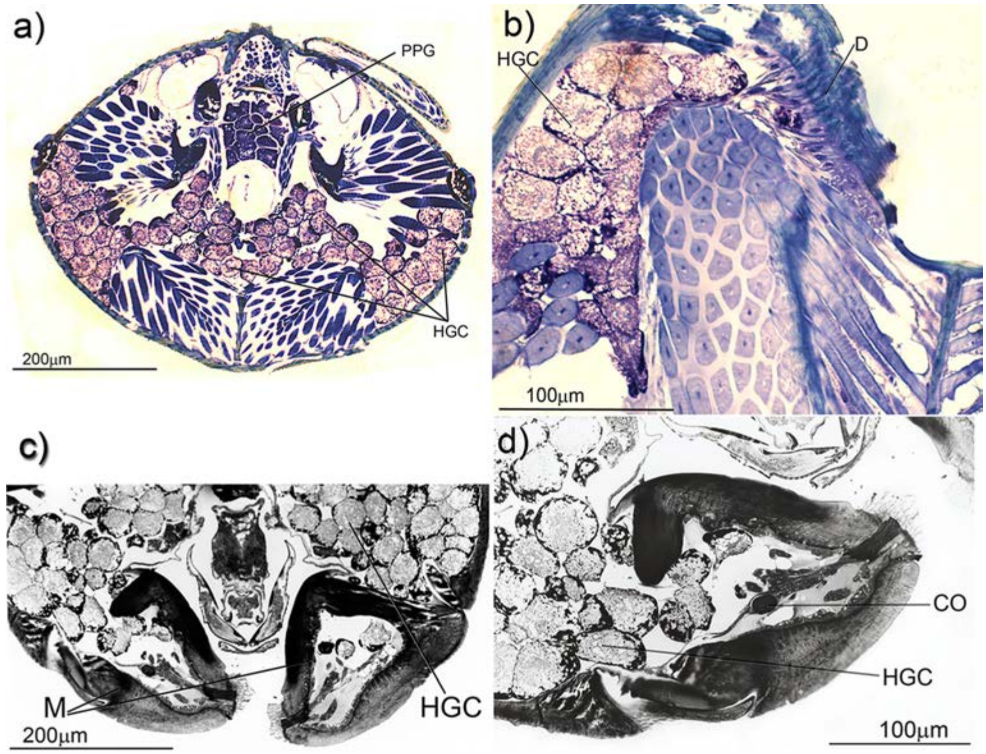

Fig. 3 Transverse sections through the head of *Melissotarsus emeryi.* **a** Section at the level of the eyes. **b** left side of parts of the hypostomal gland depicting glandular cells (HPC) and glandular cell ducts (D) penetrating the cuticle of the buccal margin. **c** Section through mandibles (M) with hypostomal gland cells (HGC). **d** Close-up image of the section through one mandible with hypostomal gland cells and cordotonal organ (CO). *PPG* propharyngeal gland, *HGC* hypostomal gland

silk might be used to seal the tunnel walls or to serve in colony defense.

Melissotarsus workers have never been seen to forage outside their nest galleries. Several authors have reported that the ants cultivate phloem sap-sucking coccids of the family Diaspididae, not for honeydew, but most likely for meat (Ben-Dov 1978, 2010; see Schneider et al. 2013 for relevant literature). Apparently, *Melissotarsus* workers spend their entire life inside the nest galleries, stretching over large portion of a major tree. In certain areas of managed forests in Madagascar

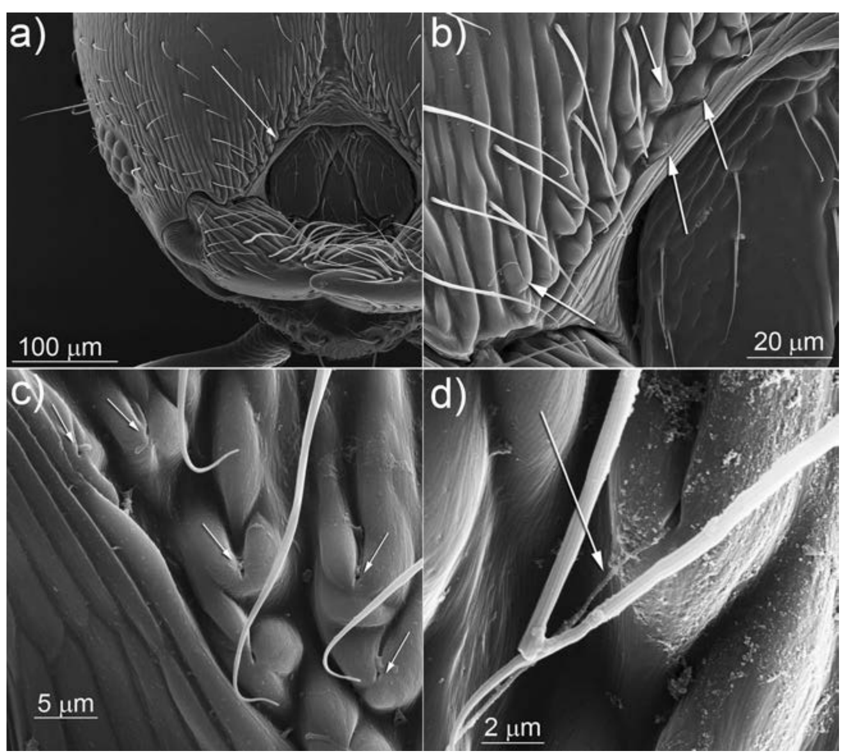

Fig. 4 Scanning electron microscopic images of the margin of the buccal cavity of *Rhopalomastix* sp. which is lined with groove-shaped openings (see *arrows* in images **a** and **b**). **c**, **d** Close-up image of the groove-shaped openings with secretions coming out of the openings (see *arrows*)

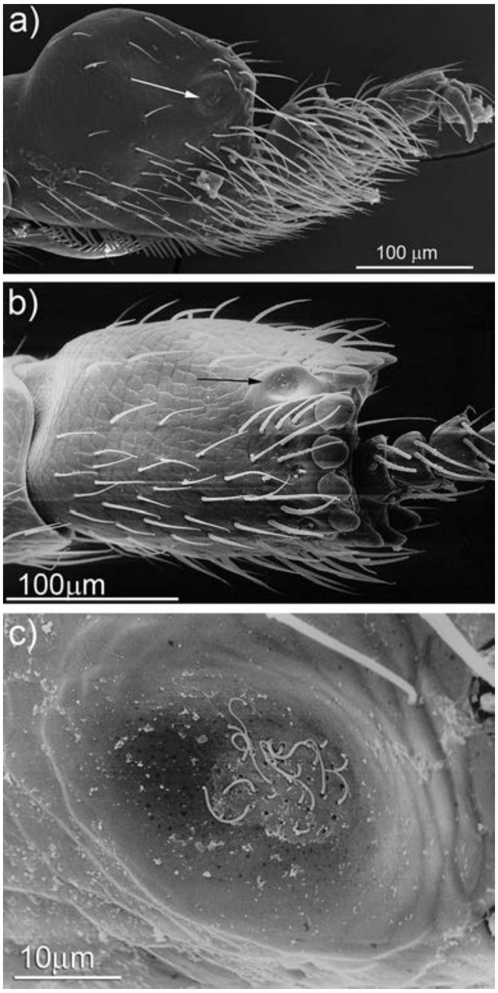

Fig. 5 Basitarsal gland in *Melissotarsus emeryi*. **a** Electron microscopic image (SEM) of the "swollen" front leg basitarsus. *Arrow* indicates the opening of the basitarsus gland. **b** SEM image of the basitarsus of the middle leg with basitarsus gland opening (*arrow*). **c** Close-up SEM image of the opening of basitarsal gland of the middle leg; the pores and secretions oozing from some of the pores are clearly visible

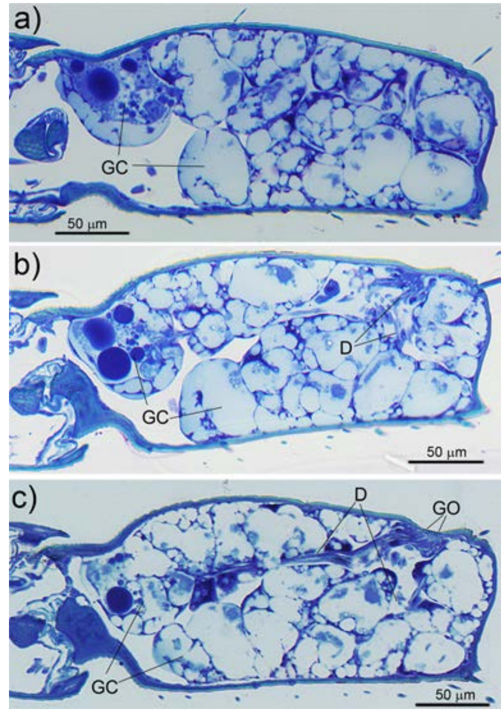

Fig. 6 Longitudinal sections through the basitarsus of the front leg, large glandular cells (GC) replete with vacuoles and the glandular cell ducts (D) penetrating the cuticle through the pores in the glandular opening (GO) are clearly visible

and Africa, *Melissotarsus* can become a real pest problem (Ben-Dov 1978; Prins et al. 1990; Fisher and Robertson 1999; Mony et al. 2002, 2013).

When walking inside their nest tunnels, *Melissotarsus* workers move with the middle legs held upward so that they can touch the tunnel roof (Delage-Darchen 1972). The function of this unusual behavior is not clear yet, but it has been suggested that the ants walk in this manner to chemically mark the galleries. In fact, as already mentioned, Delage-Darchen (1972) first noticed crater-shaped pits with tiny pores on the outer dorsolateral distal part of the basitarsus of each of the three pairs of legs. Our histological studies demonstrated that these pits are indeed the exit area for secretions produced by massive basitarsal glands consisting of large glandular cells richly endowed with vacuoles and ducts that communicate between glandular cells and cuticular pores. In fact, these gland cells appear to be special vesicles replete with secretions that are drained through ducts. In some sections, some ducts appear to merge into a single duct (see Fig. 6b), but the light microscopic images did not allow a more detailed analysis. In addition, the basitarsus of the front legs is endowed with a large cleaning gland which occurs in many ant species, although the size of this gland varies among species.

In a splendid review of all the exocrine glands found in the legs of ants, Johan Billen lists 20 different glands discovered to date (Billen 2009). From the very different structures of the

Fig. 7 Longitudinal section through the basitarsus of the front leg of *Melissotarsus emeryi* showing the cleaning gland epithelium (CG). On the base of the epithelium, densely spaced cuticular pores embedded between layers of fine hairs through which the glandular secretions seep through the cuticle

basitarsal glands in other ant species, e.g., the amblyoponine species *Onychomyrmex* (Hölldobler and Palmer 1989) and *Prionopelta amabilis* (Hölldobler et al. 1992), we conclude that the basitarsal gland in *Melissotarsus* is an independent development, as is the remarkable hypostomal gland. SEM studies suggest that worker ants of the phylogenetically most closely related genus *Rhopalomastix* posess the hypostomal gland but not the basitarsal gland.

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References

Ben-Dov Y (1978) *Andaspis formicarum* n. sp. (Homoptera, Diaspididae) associated with a species of *Melissotarsus* (Hymenoptera: Formicidae) in South Africa. Insect Soc 25:315–321. doi:10.1007/BF02224296

Ben-Dov Y (2010) On new taxa and some described armoured scale insects (Hemiptera: Diaspididae) living in the galleries of the ant *Melissotarsus insularis* Santschi (Hymenoptera: Formicidae) in Madagascar. Zootaxa 2368:49–58

Billen J (2009) Occurrence and structural organization of the exocrine glands in the legs of ants. Arthropod Struct Dev 38:2–15. doi:10.1016/j.asd.2008.08.002

Billen J (2011) Exocrine glands and their function in the communication system of social insects. Formos Entomol 31:75–84

Billen J, Morgan ED (1998) Pheromone communication in social insects-sources and secretions. In: Vander Meer RK, Breed MD, Winston ML, Espelie KE (eds) Pheromone communication in social insects: ants, wasps, bees and termites. Westview Press, Boulder, pp 3–33

Bolton B (1973) The ant genera of West Africa: a synonymic synopsis with keys (Hymenoptera: Formicidae). Bull Br Mus Nat Hist Entomol 27:319–368

Bolton B (1982) Afrotropical species of the myrmicine ant genera *Cardiocondyla*, *Leptothorax*, *Melissotarsus*, *Messor* and *Catalaucus* (Formicidae). Bull Br Mus Nat Hist Entomol 45:307–370

Burns MS, Bretschneider A (1981) Thin is in. American Society of Clinical Pathologists. Educational Product Division, Chicago

Collingwoord CA (1985) Hymenoptera: Fam. Formicidae of Saudi Arabia. In: Buttiker W, Krupp F (eds) Fauna of Saudi Arabia vol 7. Pro Entomologia. Natural History Museum, Basel, pp 230–302

Delage-Darchen B (1972) Une fourmi de Cote-d'Ivoire: *Melissotarsus titubans* Del., n. sp. Insect Soc 19:213–226. doi:10.1007/BF02226627

Emmert W (1968) Die Postembryonalenwicklung sekretorischer Kopfdrüsen von *Formica pratensis* Retz. und *Apis mellifica* L. (Ins. Hym.). Z Morphol Tiere 63:1–62

Fisher BL, Robertson HG (1999) Silk production by adult workers of the ant *Melissotarsus emeryi* (Hymenoptera, Formicidae) in South African fynbos. Insect Soc 46:78–83. doi:10.1007/s000400050116

Fisher BL, Ratsirarson H, Razafimandimby S (1998) The ant fauna (Hymenoptera: Formicidae) in the littoral forest of Station Forestière de Tampolo, Madagascar. In: Goodman SM, Ratsirarson J (eds) Inventaire biologique de forestière de tampolo, serié sciences biologiques. Centre d'Information et de Documentation Scientifique et Technique, Antananarivo, pp 107–129

Hölldobler B, Palmer JM (1989) A new tarsal gland in ants and the possible role in chemical communication. Naturwissenschaften 76:385–386. doi:10.1007/BF00366215

Hölldobler B, Wilson EO (1990) The ants. Belknap Press, Cambridge

Hölldobler B, Wilson EO (2009) The superorganism. W.W. Norton and Comp, New York

Hölldobler B, Obermayer M, Wilson EO (1992) Communication in the primitive cryptobiotic ant *Prionopelta amabilis* (Hymenoptera: Formicidae). J Comp Physiol A 170:9–16

Mony R, Kenne M, Orivel J, Dejean A (2002) Biology and ecology of pest ants of the genus *Melissotarsus* (Hymenoptera: Formicinae), with special reference to tropical fruit tree attacks. Sociobiology 40:645–654

Mony R, Fisher BL, Kenne M, Tindo M, Dejean A (2007) Behavioral ecology of bark digging ants of the genus *Melissotarsus*. Funct Ecosyst Comm 1:121–128

Mony R, Dejean A, Bilong CFB, Kenne M, Rouland-Lefèvre C (2013) *Melissotarsus* ants are likely able to digest plant polysaccharides. Comptes Rendus Biol 336:500–504

Noirot C, Quennedey A (1974) Fine structure of insect epidermal glands. Ann Rev Entomol 19:61–80

Prins AJ, Robertson HG, Prins A (1990) Pest ants in urban and agricultural areas of South Africa. In: Vander Meer RK, Jaffe K, Cedeno A (eds) Applied myrmecology: a world perspective. Westview Press, Boulder, pp 25–33

Schneider SA, Giliomee JH, Dooley JW, Normark BB (2013) Mutualism between armoured scale insects and ants: new species and observations on a unique trophobiosis (Hemiptera: Diaspididae; Hymenoptera: Formicidae: *Melissotarsus emeryi*). Syst Entomol 38:805–817. doi:10.1111/syen.12033

Schönitzer K, Lawitzky G (1987) A phylogenetic study of the antenna cleaner in Formicidae, Mutillidae and Tiphiidae (Insecta, Hymenoptera). Zoomorphology 107:271–285

Schönitzer K, Dott H, Melzer RR (1996) The antenna cleaner gland in *Messor rufitarsis* (Hymenoptera, Formicidae). Tissue Cell 28:107–113. doi:10.1016/S0040-8166(96)80049-8

Spurr AR (1969) A low viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43