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Morphology and ultrastructure of the Dufour gland of *Myzinum* sp. (Tiphiidae)

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Abstract

The Dufour gland of two *Myzinum* females was studied with light and electron microscopy, and is formed by a large sac lined with a monolayered secretory epithelium. The epithelium displays a crenellate appearance, which is the result of the peculiar shape of the secretory cells, that have a cupola-like central portion and a more flattened appearance in the contact region with other cells. The ultrastructural organization is indicative for the elaboration of a non-proteinaceous secretion. The gland opens ventrally to the sting base, but does not open through the sting, as does the venom gland duct. The sting itself is dorsally curved, which may be a functional adaptation to facilitate stinging large beetle larvae from above, as these are the common hosts for tiphiid wasps.

Keywords

Morphology, ultrastructure, Dufour gland, curved sting, *Myzinum*, Tiphiidae

Introduction

Social insects no doubt are the champions of having the most elaborate exocrine system among the hexapods, with 149 known glands (Billen and Šobotník 2015). As only 20 of these occur in termites, it is clear that this impressive gland variety is especially prominent in the social Hymenoptera, with 84 glands in ants, 53 in bees and bumblebees,

and 49 in wasps (Billen and Šobotník 2015). Much less studied is the exocrine system of solitary Hymenoptera, with almost nothing known of the glands in the Tiphiidae. Tiphiidae comprises seven subfamilies, of which the Myzininae are distributed around the world and are one of the most diverse groups of Tiphiidae (Kimsey 1991). The genus *Myzinum* Latreille is still largely unknown regarding its biology and taxonomy (Kimsey 2009, Spence and Hodges 2010). The species in this genus present a strong sexual dimorphism (Kimsey 1991), with fully winged males that are easy to recognize by their last hook-like metasomal segment. Females can be winged, brachypterous, or wingless (Pate 1947, Kimsey 1991). They are stronger than the males and their antennae are curved (see Fig. 1D). Tiphiidae are parasitoid wasps of beetle larvae in general and Myzininae species probably parasitize Scarabaeidae and Cicindelidae (Pate 1947, Brown 1985, Kimsey 1991). Males are pollinators and are commonly collected flying in groups. However, females are harder to collect because they are found alone, usually on flowers (Kimsey 2009). Thus, the limited knowledge of internal body structure in this parasitoid wasp family is also due to the low chance of finding females alive, as live material is needed for proper tissue fixation for microscopical analysis. In addition, proper species identification of *Myzinum* wasps is difficult because males and females are very rarely collected together. Kimsey (2009) proposed a key for *Myzinum* in North America, but as this key comments that the North American fauna is different from South America, we do not have tools to identify the species used in the present work. All those characteristics make it difficult to study these wasps and literature is very scarce.

As a modification of the accessory glands of the reproductive system, the Dufour gland is one of the standard glands of female Hymenoptera. Both in solitary and social species, several functions have been attributed to the gland, ranging from provisioning of larval food and nesting material to several pheromonal functions (reviewed in Hefetz 1987, Abdalla and Cruz-Landim 2001a, Mitra 2013). Because of the very scarce information about *Myzinum* biology, we can only speculate about the function of their Dufour gland. Literature shows that its secretion can be associated with host marking in solitary wasps (Mitra 2013). Parasitoid wasps mark their hosts to avoid superparasitism and guarantee the fitness of their offspring. Odor marks may be used in host discrimination (Sheehan et al. 1993) and could be associated with Dufour gland secretion. An analysis of the Dufour gland morphology and chemistry in four species of bethylid and pteromalid ectoparasitoid wasps confirms its opening into the oviduct and supports its involvement in the elaboration of a lipidic secretion (Howard and Baker 2003).

Several studies have been done on the anatomy and ultrastructure of the Dufour gland, mainly in the social Hymenoptera (Billen 1986 for ants, Abdalla et al. 1999 for bumblebees, Abdalla and Cruz-Landim 2001b for bees, Billen 2006 and Fortunato and Turillazzi 2012 for wasps). In ants, the appearance of the epithelial gland cells shows subfamily-specific characteristics (Billen 1986). In Tiphiidae, however, no information on this gland is available until now (Robertson 1968, Mitra 2013). As we had the luck to collect two live *Myzinum* females, we here report on the morphology and ultrastructure of their Dufour gland, which brings the first information on this gland in tiphiid wasps.

Material and methods

We collected two live *Myzinum* females at the Estação Ecológica do Noroeste Paulista (EENP), São José do Rio Preto, SP, Brazil (20°50'43.6"S 49°26'05.5"W). Because of the complex taxonomy in this genus, we refer to them as *Myzinum* sp.1 and sp.2, of which sp.1 is characterized by a black and yellow thorax, whereas sp.2 has an almost entirely black thorax (Fig. 1). *Myzinum* sp.1 is similar in its color pattern to *M. maculata*, which is also a common species in insect collections. Reference specimens of both species can be found in the entomological collection at the Departamento de Zoologia e Botânica at the UNESP-campus in São José do Rio Preto, SP (Brasil). From the sp.1 female, the Dufour gland was carefully dissected, so that optimal fixation quality for microscopy could be obtained. From the sp.2 female, we fixed the posterior abdomen part to have material of Dufour's gland in situ with its surrounding tissues. Both the dissected gland and the abdomen part were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Tissues were postfixed in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, they were embedded in Araldite and sectioned with a Leica EM UC6 microtome. Semithin 1 µm sections of both females were stained with methylene blue and thionin and viewed in an Olympus BX-51 microscope, double stained 70 nm thin sections of the gland of the sp.1 female were examined in a Zeiss EM900 electron microscope. To illustrate the sting shape, two pinned collection specimens of *Myzinum* sp. 1 were treated with 10% KOH during 1.5 hours, and then further dissected under insect Ringer solution.

Results

The Dufour gland is a wide elongated sac that opens ventrally of the sting base. The main portion of the gland is formed by a monolayered epithelium with a thickness of approximately 10 µm (Fig. 2A). At its lumen side, it is lined with a cuticular intima around 1 µm, while isolated muscle strands occur at the basal side (Fig. 2B). The epithelium has a crenellate appearance, which is the result of the peculiar shape of the secretory cells. Each cell has a cupola-like central portion, in which the round nucleus with a diameter of 5 µm is apically situated (Fig. 2B). More laterally, cell height is considerably less, and reaches a thickness of hardly 2 µm. The resulting cell shape is schematically illustrated in Figure 2C.

Electron microscopy shows how intercellular junctions occur at the thin lateral cell parts (Fig. 3A, C). The apical cell membrane is differentiated into microvilli with a length around 1 µm (Fig. 3B), although they are less developed and shorter in the lateral portions of the cell (Fig. 3C–E). The cytoplasm contains scattered mitochondria, and probably a diffuse network of smooth endoplasmic reticulum, of which tubular extensions can be found inside the apical microvilli (Fig. 3C). The basal cell membrane shows some deep basal invaginations that penetrate up to 3 µm into the gland cell

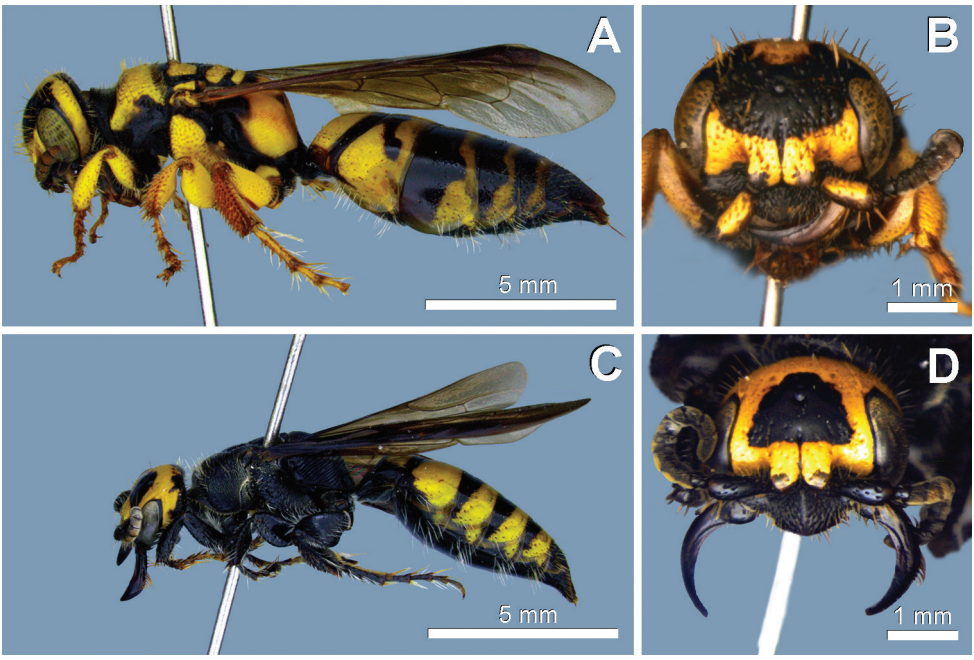

Figure 1. A, B Profile and frontal view of female of *Myzinum* sp.1. **C, D** Profile and frontal view of female of *Myzinum* sp.2.

(Fig. 3C). The lateral cell contacts show a pattern with folded and interdigitating cell membranes, with desmosomes in the apical region, followed by septate junctions, and undifferentiated parallel membranes in the basal part (Fig. 3D, E). Intercellular spaces may be wedged in between the two neighbouring cell membranes (Fig. 3D). On one occasion, we observed an intercellular bridge, that appeared as a barrel-shaped tube between the two cells. The connecting structure has a smooth electron-dense lining and has a diameter of 0.4 µm and a length of 0.6 µm (Fig. 3E).

A remarkable observation in our *Myzinum* females was the dorsally curved sting (Fig. 4A–C), that results in the sting shaft being parallel with the posterior tergites, rather than having a more 'horizontal' orientation parallel with the posterior sternites, as is the usual case. In its posterior part, the reservoir sac abruptly narrows into a thin slit-like duct that opens ventrally of the sting base (see black arrows in Fig. 5A,B), whereas the venom gland duct enters the sting, and thus will discharge its secretion through the sting (see white arrows in Fig. 5A, B). The slit-like duct has a length of almost 200 µm and a width around 80 µm, and is provided with a massive muscular supply at both the dorsal and ventral side (Fig. 5A–C). The muscle fibres cannot attach directly onto the rigid duct cuticle, as the gland epithelium forms an intermediate layer (Fig. 5D). The pulling forces of the myofilaments are transmitted to parallel bundles of microtubules that occur in the epithelial cells. These microtubules run perpendicularly to the inner cuticular surface, to which they adhere. The contact region between myofilaments and microtubules is characterized by a tortuous shape and numerous hemidesmosomes (Fig. 5D, E).

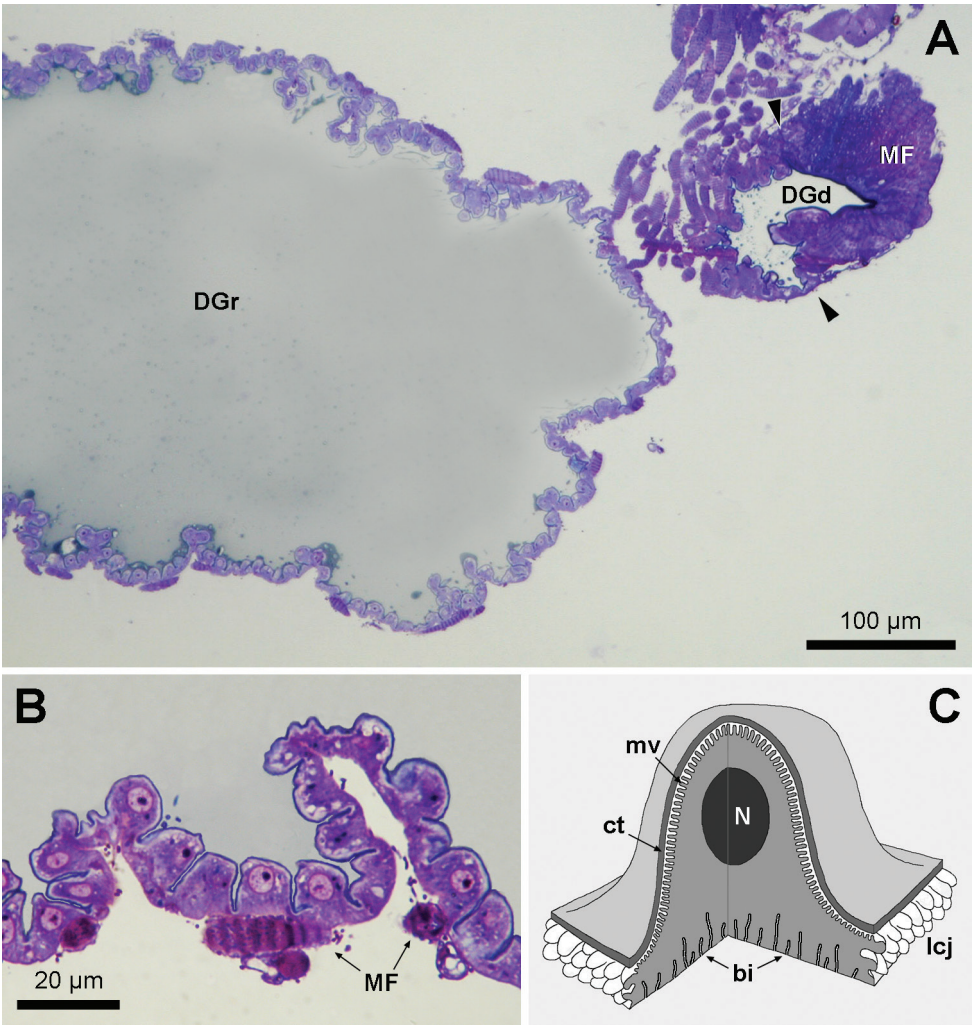

Figure 2. A Semithin section through the Dufour gland reservoir sac (DGr) and duct region (DGd) in *Myzinum* sp.1. The arrowheads indicate the transition between the reservoir and duct region **B** Detail of crenellate epithelial lining of reservoir sac with strands of surrounding muscle fibres (MF) **C** Schematical view of cupola-like shape of gland cell. bi: basal invaginations, ct: cuticle, lcj: lateral cell junction, mv: microvilli, N: nucleus.

Discussion

Our observations revealed that the epithelial gland cells have a peculiar shape with a cupola-like central part. This cell shape does not correspond to any of the 8 types that have been described in ants (Billen 1986), but does show some resemblance to the epithelial appearance in vespid wasps (see figure 2A in Billen 2006). The presence of smooth endoplasmic reticulum, including its extension into the apical microvilli,

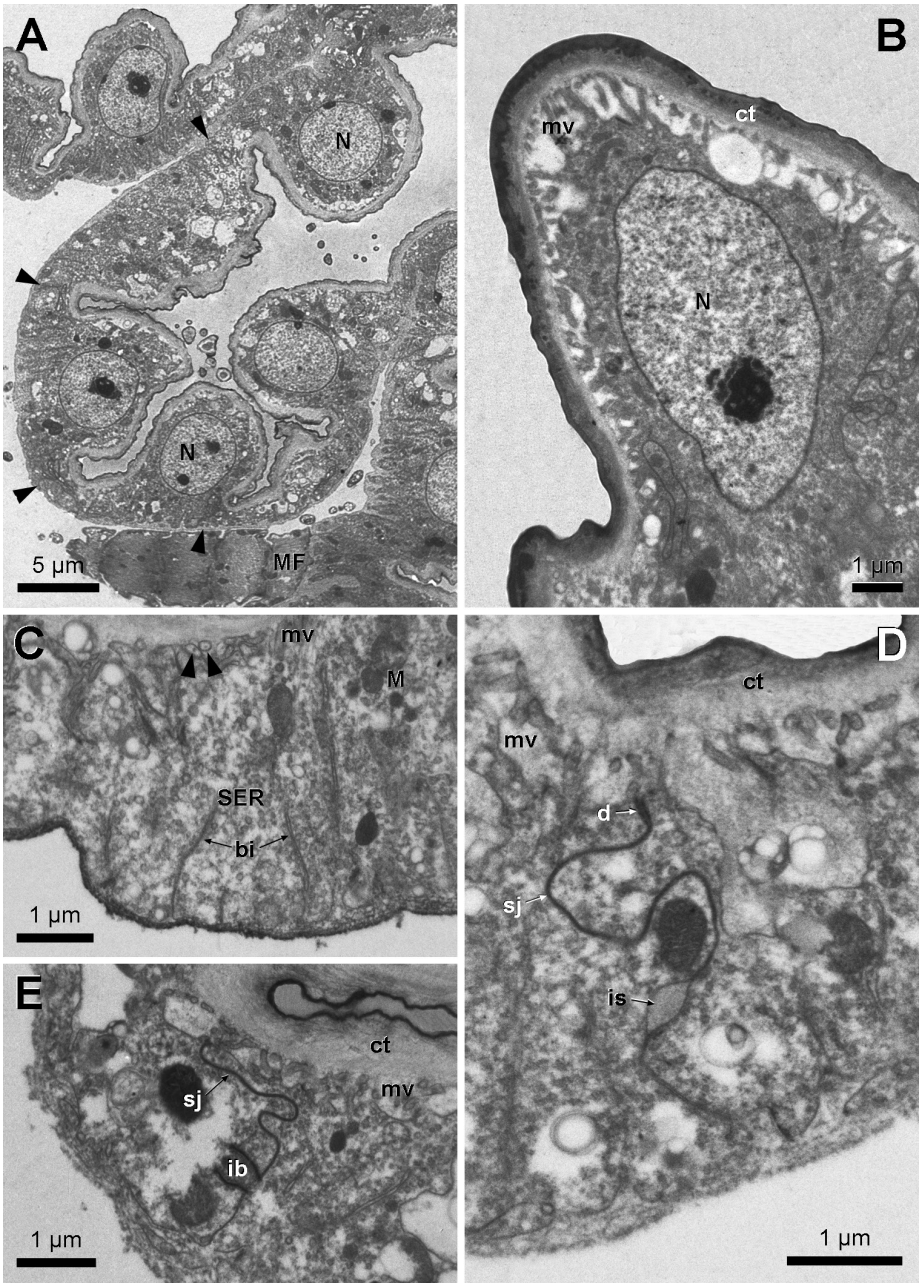

Figure 3. Electron micrographs of Dufour gland secretory cells in *Myzinum* sp.1. **A** Low magnification survey of crenellate epithelium, arrowheads indicate cell junctions **B** Cupola-like apical part of gland cell with nucleus (N) **C** Low lateral part of gland cell with apical microvilli (mv) and basal invaginations (bi). Arrowheads indicate extensions of smooth endoplasmic reticulum (SER) into microvilli **D** Detail of intercellular junction with apical desmosome (d), followed by septate junction (sj). Note intercellular space (is) wedged in between neighbouring cell walls. **E**. Occurrence of intercellular bridge (ib). ct: cuticle, M: mitochondria.

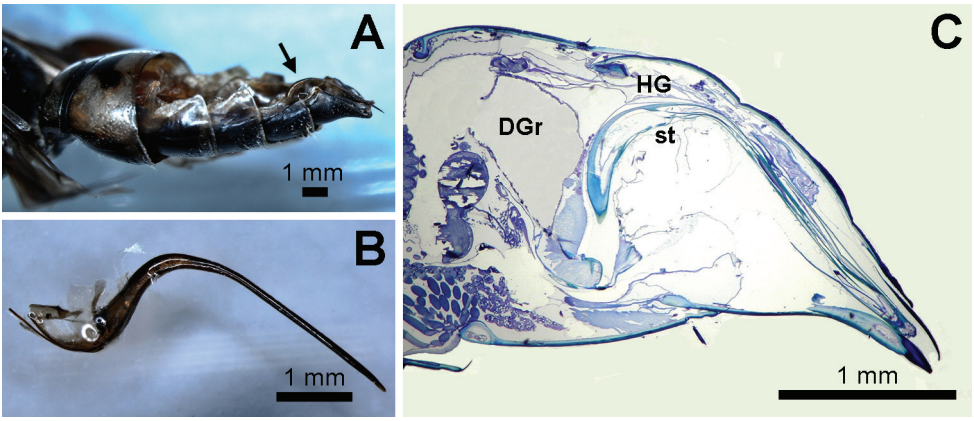

Figure 4. A Partially dissected gaster of a collection specimen of *Myzinum* sp.1, showing the dorsally curved sting (arrow) **B** Overview of sting (*Myzinum* sp.1) **C** Longitudinal semithin section through the posterior abdomen part of *Myzinum* sp.2 female, showing upward curved sting (st). DGr: Dufour gland reservoir, HG: hindgut.

is in line with the general ultrastructural appearance of Dufour's gland in other Hymenoptera (Billen 1986, 2006, Abdalla and Cruz-Landim 2001a). This cytoplasmic characteristic supports the elaboration of a non-proteinaceous secretion, which therefore can also be postulated to be the case in Tiphiidae. This is also in agreement with the lipidic secretion that has been found in Dufour's gland of four bethylid and pteromalid ectoparasitoid wasps (Howard and Baker 2003). The occurrence of apical microvilli and basal invaginations represent an increase of the cell surface, and can be understood by giving the epithelial cells an efficient uptake of precursor molecules from the hemolymph basally, and an efficient discharge of secretory products apically. An interesting observation was the occurrence of an intercellular bridge, as such structures usually do not occur in somatic tissues of adult insects. Intercellular bridges, on the other hand, are common in the germarium of ovarioles, where they occur as 1.2 µm wide connecting channels between neighbouring cystocytes, that allow transport of large amounts of material to the future oocyte (Billen 1985).

At the general anatomical level, the Dufour gland reservoir is surrounded by a loose network of muscle fibres, that upon contraction push the secretion towards the duct region. In this duct region, a conspicuous muscular supply with dorsal and ventral muscles attaching onto the slit-like duct is very prominent. This muscular arrangement is similar to that described in ants (Billen 1986) and wasps (Billen 2006), and will result in active opening of the duct by muscular contraction, while passive closing is the result of the thickened cuticle returning to its rest position when muscles stop contracting. At the cell level, the muscular forces onto the rigid duct cuticle have to be transmitted through the epithelium layer. This is achieved by the occurrence of bundles of parallel microtubules in the epidermal cells and hemidesmosomes that form a strong mechanical system ensuring efficient transmission of pulling forces. This set-up corresponds to the typical myo-epidermal junction, that was first described in Diptera

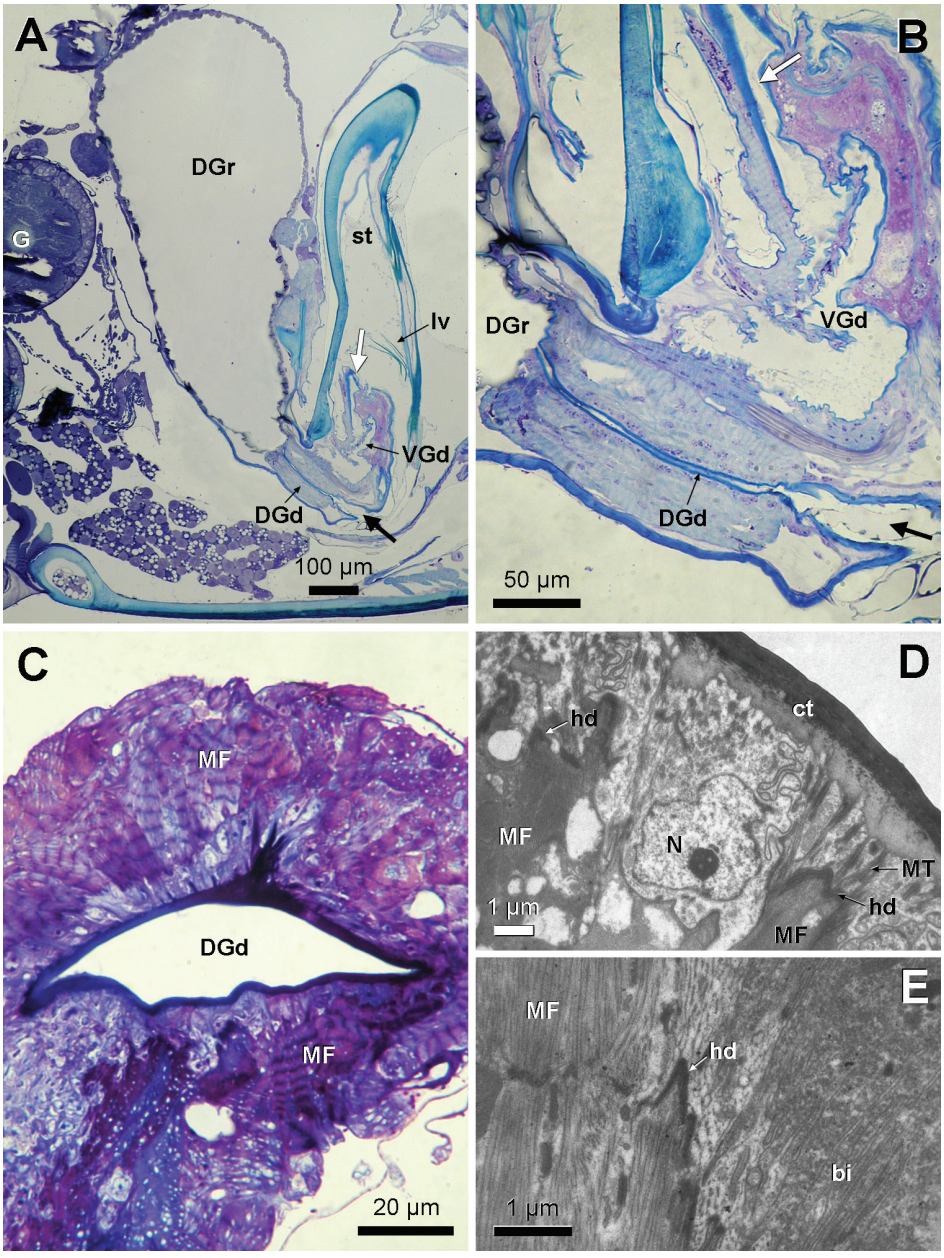

Figure 5. A Longitudinal semithin section through sting base region in *Myzinum* sp.2, showing Dufour gland opening ventrally of the sting base (black arrow), whereas the venom gland duct opens through the sting (white arrow) **B** Enlargement from A showing sting base region **C** Cross semithin section through Dufour gland duct in *Myzinum* sp.1, showing slit-like duct with attachment of dorsal and ventral muscle fibres (MF) **D** and **E** Electron micrographs of muscular attachments onto Dufour gland duct of *Myzinum* sp.1. Myofilaments of muscle fibres (MF) transmit their pulling force onto bundles of microtubules (MT) in the duct cells via hemidesmosomes (hd). bi: basal invaginations, DGd: Dufour gland duct, DGr: Dufour gland reservoir sac. G: ganglion, lv: lancet valves, N: nucleus, st: sting, VGd: venom gland duct.

(Auber 1963), and that is also found in the duct region of Dufour's gland (Billen 1982, 1986, 2006).

As in mutillids (Hermann 1968), the sting in *Myzinum* shows a dorsally curved sting, which so far has never been reported for tiphiid wasps. Both Mutillidae and Tiphiidae are parasitoids of larvae of other insects. Mutillidae are commonly described to be parasitoids of social insects, however, Brothers et al. (2000) provided new records for Mutillidae hosts, including beetle larvae that are generally described as hosts for Tiphiidae. These beetle larvae can be quite big in comparison to the size of their parasitoids, thus their curved sting shape may be related with the possibility to sting the host larva from above. The Dufour gland duct of *Myzinum* approaches the sting base, but unlike the venom gland duct does not open through the sting. In this regard, it is different from the situation in ants, where both the venom and Dufour gland ducts open through the sting, and thus release their secretory products via the sting shaft (Billen 1986, 1987). Dufour's gland opening at the base of the sting shaft as is the case in *Myzinum* is similar to the situation in wasps and bees (Billen 1987, Fortunato and Turillazzi 2012, Martin et al. 2005), and corresponds with an opening in the dorsal wall of the oviduct.

Even though the South American species taxonomy of tiphiid wasps is controversial and mostly unknown, we believe that all information on this group of wasps is very welcome and important.

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