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Morphological and Histological Structure of Adexinal Glands of some Solitary Bee Species (Hymenoptera – Apoidea)

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Abstract

Solitary bees are diverse and very important for plant and crop pollination. They are extensively studied taxonomically, but little is known about their anatomy and physiology compared to honey bees. Dufour's gland is important for many physiological functions in social and solitary bees. The present study addresses the morphological and histological structure of Dufour's gland in ten bee species representing bee families Andrenidae, Colletidae, Halictidae, Melittidae, Megachilidae, and Apidae. Results indicated that the shape and size of the glands tend to differ from one species to the other. However, on histological bases, the intern seems to be the same among the secretion cell types. The gland varied in length and size in the studied species, probably due to nesting behavior differences: ground and cavity nesting. Further studies are needed to clarify the different secretions produced by Dufour's gland and their functions in each species.

Introduction

In female Apocrita (Hymenoptera), the two glands connected to the sting apparatus are the Dufour's and poison glands. The Dufour's gland (often referred to as "alkaline gland" or "basic gland" in the older literature) was first described in the 19th century (Dufour, 1841). It is an ectodermal abdominal gland located near the proximal end of the abdomen (Mitra, 2013).

Different shapes and functions of Dufour's gland have been discovered. It secretes chemicals, similar to all other exocrine glands, which vary in nature and function among taxa. These secretions are part of materials used in larval food, nest building, and other communicative functions like nest and nestmate recognition, host marking, fertility signaling, and as a trail and sex pheromone. These functions are essential for the biology of solitary and social bee species (Mitra, 2013). The variation in the morphological structure of the sting apparatus and the glands associated with bees is also important for understanding bee systematic and phylogenetics (Packer, 2003). In nonsocial bees, although many features of the Dufour's glands belonging to many tribes from major genera have been reported, the characteristics of glands of the 16,000 or more species of solitary bees in the world remain unknown (Michener, 2007). The histological and anatomical aspects of the adnexal glands of the sting apparatus of 48 species of Andrenidae Colletidae, Megachilidae, Halictidae, Melittidae, and Anthophoridae were compared (Lello, 1971a, b, c, d; Lello, 1976). However, no further studies were reported ever since except the structure and position of the Dufour's gland in the sting apparatus of solitary cavity-nesting bees, *Megachile rotundata* (F.) and *Osmia lignaria* Say, (Pitts-Singer et al., 2012).

There are plenty of studies on the Hymenopteran species Dufour's gland in some geographical regions. Therefore, conducting more studies is mandatory to explore more information about solitary bee's physiology. This study is the first in Egypt and the Middle East on Dufour's gland

in solitary bees. The study addresses the morphological and histological structure of Dufour's gland in ten bee species representing bee families Andrenidae, Colletidae, Halictidae, Melittidae, Megachilidae, and Apidae.

Material and methods

Species collection

Bee species were collected from different sites of the Suez Canal region during 2022 and 2023. Bees were transported live to the laboratory for identification. Ten species were chosen and subjected to the study, which includes the following: Andenidae (*Andrena aegyptiaca* Friese, 1899; *A. savignyi* Spinola, 1838 and *A. flavipes* Panzer, 1799), Colletidae (*Colletes lacunatus* Dours, 1872), Halictidae (*Lasioglossium vagans* Smith, 1857), Melittidae (*Dasypoda sinuata* Pérez, 1895), Apidae (*Xylocopa pubscens* Spinola, 1838) and Megachiliade (*Chalicodoma nigripes* Spinola, 1838, *C. siculum* Rossi, 1792 and *Osmia latreillei* Spinola, 1806).

Microscopic examination

Dufour's glands were dissected from collected adult female bees after being decapitated, and its abdomen was cut open lengthwise. The glands were directly fixed in aquas bouin's solution for at least 24 hours. After fixation, glands were washed and photographed with a stereoscopic camera (Velap). Dufour's glands of 3-5 females were measured using micrometer microscope eyepieces for biological microscope lens, WF10X/18mm.

Histological studies

Fixed glands were dehydrated in an ethyl alcoholic series ranging between 70% – 90%, then embedded in paraffin wax and serial sectioned 5/x thick. These glands were impeded in paraffin and stained with Hematoxylin and Eosin (Lello, 1971a). A stained tissue photomicrograph was taken using the same previously reported camera.

Table 1. The variation of Dufour's gland length between the studied species.

Bee species	Dufour's gland length (mm)
Andrena egyptiaca Friese, 1899	5 – 6.8
Andrena flavipes Spinola, 1838	11 – 17
Chalicodoma nigripes Spinola, 1838	1.5 – 2
Chalicodoma siculum Rossi, 1792	3 – 4
Colletes lacunatus Dours, 1872	11 – 15
Lasioglossium vagans Smith, 1857	5 – 6.5 (2 – 3 for branch)
Osmia latreillei Spinola, 1806	2.5 – 3.5
Xylocopa pubescens Spinola, 1838	12.5
Dasypoda sinuata Pérez, 1895	2 – 2.5

Results

Dufour's gland morphological structure

Dufour's gland position was found between the rectum and vagina, which is somewhat different from well-developed to vestigial. Dufour's glands were much larger in the examined bee species of Apidae, Halictidae, Colletidae, and Andrenidae. In contrast, reports of other studied species of Megachilidae and Melittidae indicated that the gland length was much smaller (Table 1).

The Dufour's gland in *Colletes lacunatus* was large (ranging from 11 to 15 cm). It has an inverted "U" shape with the concavity turned caudally, taking up the majority of the abdominal cavity. The distal portion hung freely in the abdominal cavity and was thinner than the remaining gland. The exterior surface was milk-white and featured irregular transversal furrows (Fig 1C).

In all Andrenidae studied species, the gland appeared as a large, horseshoe-shaped, milk-white sac. The smooth, semitransparent external membrane allowed the observation of the internal folds of the wall (Fig 1A; B)

In *Lasioglossium vagans* Smith 1857 (Halictidae), the gland had a "U" shape as in *C. lacunatus*. The gland occupies a significant part of the abdominal cavity. It was white and showed transversal ridges. A secondary sac emerged in its proximal segment and was set within the larger segment concavity. Dufour's glands in Halictidae species are not as large as those of Colletidae and Andrenidae species but are larger than the similar glands in Megachilidae and Melittidae studied species (Fig 1D). In all studied species, the glands were single, usually sack-shaped, except in Halictidae, which was branched.

In *Xylocopa pubescens* Spinola 1838 (Apidae), the gland was a ribbon-like structure, with a long tubular occupying much of the abdomen, which is opened into the sting chamber (Fig 1F).

The glands in *Chalicadoma nigripes*, *Osmia latreillei* (Megachilidae), and *Dasypoda sinuata* (Melittidae) were very similar in their tubular sac-like appearance, arrangement, and structure. However, they were much smaller than other studied species (Fig 1G; H; I), while in *Chalicadoma siculum,* the gland was larger (Fig 1E).

Dufour's gland histological structure

The Dufour's gland lumen was lined by cuticle and subcuticle layers. The secretory cells were class 1 because the apical surface of each one reached a cuticular lining, which was reported to be secreted by the cells characterized as epithelial glandular cells lined with cuticles in the secretory pole. Dufour's gland presented secretory cells individually provided with an intracellular canal, which took the intercellular secretion to the lumen, perforating the intima. The intima was constituted by a second layer of flat cells that lined the glandular epithelium and produced the cuticle that involved the lumen.

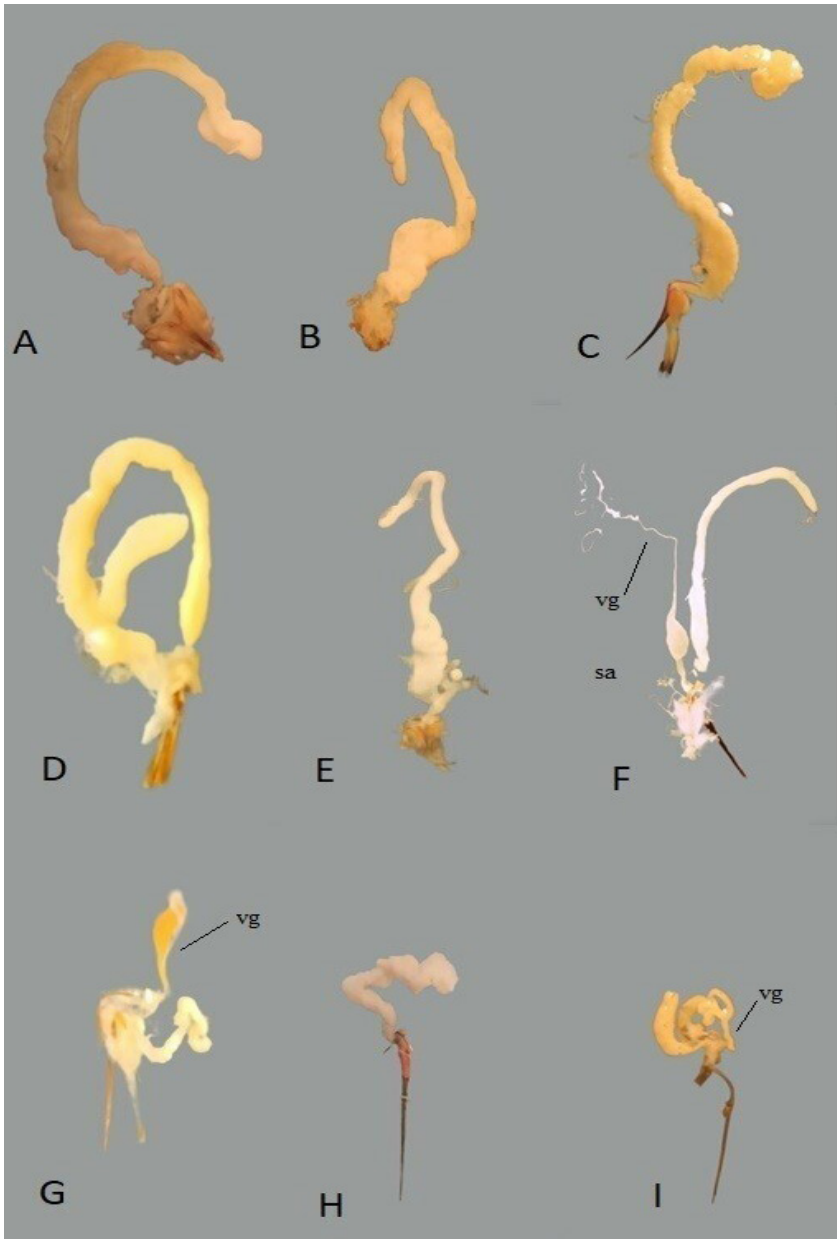

Fig 1. Morphological shapes of Dufour's gland of the studied species. A: *Andrena aegyptiaca;* B: *Andrena flavipes;* C: *Colletes lacunatus;* D: *Lasioglossium vagans;* E: *Chalicodoma siculum;* F: *Xylocopa pubscens;* G: *Osmia latreillei;* H: *Chalicodoma nigripes;* I: *Dasypoda sinuate.* (sa: sting apparatus – vg: venom gland).

The epithelial lining folds to the lumen and the folds corresponded to the furrows observed externally in Colletidae and Andrenidae studied species (Fig 2 D; F), showing an irregular pattern to the inner aspect of the gland.

The basal and luminal surfaces of the gland were characterized by very deep and numerous invaginations, imparting an undulant appearance to both surfaces. The gland cells were mainly tall, simple columnar epithelium, but a cuboidal or trapezoidal cell was occasionally shown. Each cell has a single nucleus with multiple nucleoli. The nucleus may be located apically or basally (Fig 2 A-F).

The cuticle was found covering the entire luminal surface of the gland. The deeply invaginated surface has caused this cuticular material to be disposed of over extensive lateral cell areas and on their apical surfaces. Round nuclei with sharp nuclear membranes, dense chromatin, and nucleoli characterize secretory cells starting in the proximal third. Their cytoplasm was pink on Hematoxylin and Eosin and homogeneously granular vacuoles were also observed.

Discussion

In female Apocrita (Hymenoptera), the two glands connected to the sting apparatus are the poison gland and Dufour's gland. (Lello, 1971a, b, c, d). Studying Dufour's gland in bees would deepen our knowledge and appreciation

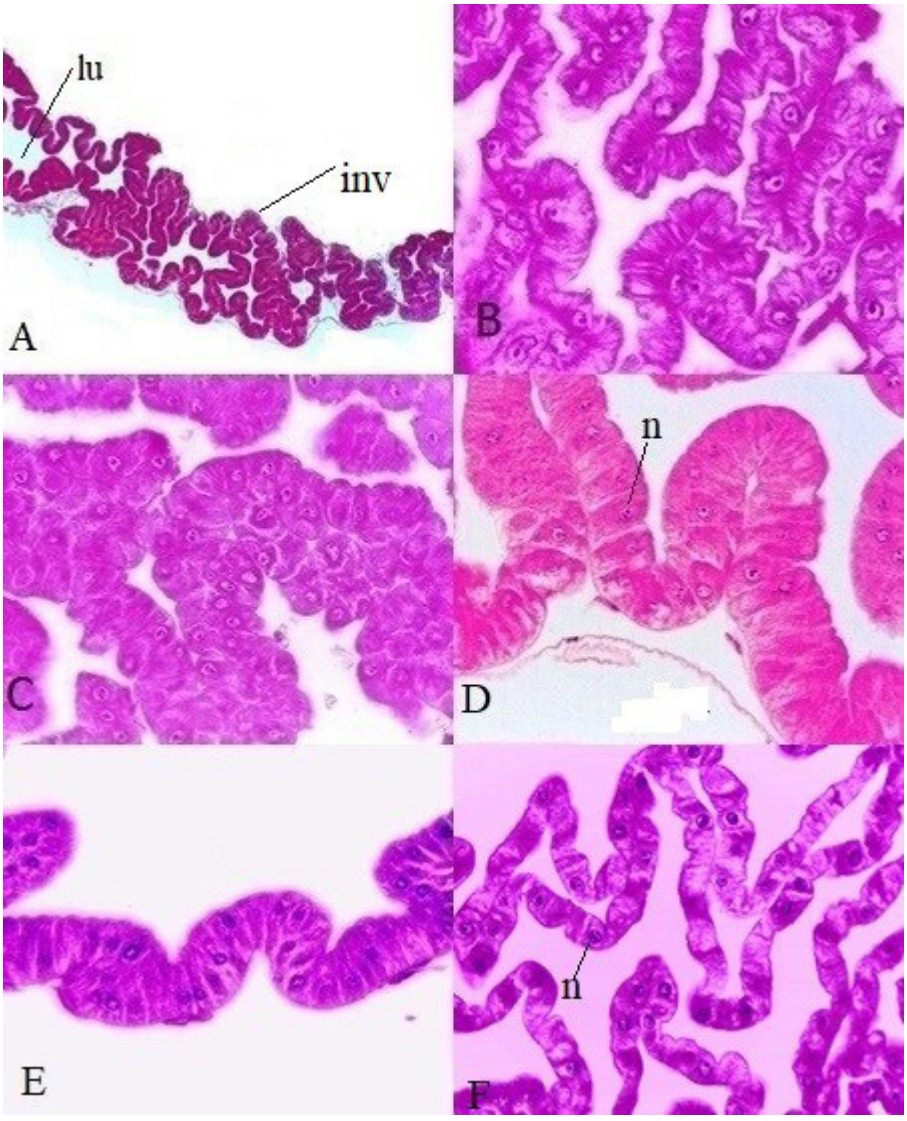

Fig 2. Histological structure of Dufour's gland of studied species. A & B: *Xylocopa pubscens;* C: *Chalicodoma siculum;* D: *Colletes lacunatus;* E: *Lasioglossium vagans;* F: *Andrena savignyi.* (n: nucleus, with multiple nucleoli – inv: invaginations – lu: lumen).

of how different living styles have influenced and how different organs have been adapted for various purposes in different lineages (Mittra, 2013). Many functions have been described for Dufour's gland in bees. In non-social bees, the gland produces hydrophobic lining and cementing substances in the nest (Mitra & Gadagkar, 2014; Cane & Brooks, 1983; Cane, 1981; Batra, 1964, 1966, 1970; Lello, 1971a, b, c, d), sexual attractants (Smith et al., 1985), recognition or nest marking (Bergström & Tengö, 1974; Shimron et al.,1985), or even trail pheromones (Vinson et al., 1978). Its secretion can serve as larva1 food (Norden et al., 1980) and pheromones for food source-marking (Franckie & Vinson, 1977).

Dufour's glands of 10 species belonging to 5 families of solitary bees were collected, and their morphology and histology were studied. The results showed that the glands differed in size and shape. In Colletidae, Andrenidae, and Halictidae species, the glands were larger than those of Megachillidae and Melittidae species. In *X. pubscens* and *C. siculum*, the glands were medium size. Only in *L. vagans* the gland was branched. In the histological study, the Dufour's gland lumen is lined with cuticle and subcuticle, and the secretory cells are class 1 (Noirot & Quennedey, 1974). These results were in line with some previous studies (Lello, 1971a, b, c, d; Lello, 1976) on Apidae, Anthophoridae, Andrenidae, Colletidae, Halictidae, Melittidae and Megachilidae. Differences between studied families and species in shape and size might be attributed to differences in the gland function and the nature of the species nesting.

The glandular epithelium of Dufour's gland in Hymenoptera was constituted by a single cell layer, whose luminal surface was covered by a thick and undifferentiated cuticle secreted by the epithelial cells (Abdalla, 1999). Dufour's gland secretion was more closely associated with earthen-nested bees. Since these have not been living

underground, they did not need progeny protection from excess moisture, and probably, for that reason, they have relatively small glands (Cane, 1983; Batra, 1984).

Dufour's gland secretions were likely presented in the lining of nest cells of other burrowing bees. There was a close infrageneric chemical similarity between the lining of nest cell and the secretions of Dufour's gland previously reported for species of *Colletes* by Duffield et al. (1980), *Lasioglossum* (Duffield et al., 1981) and *Andrena* (Bergstrom & Tengo, 1974; Tengo & Bergstrom, 1978). Dufour's gland of Colletidae bees, which includes ground-nesting species, produces a unique cellophane-like membrane (Almeida, 2008). Several Halictidae are known to line the walls of their cells with a protective membrane. *Lasioglossum zephyrum* lined the cell walls of smooth earth with a water-repellent, bright membrane of salivary origin (Batra, 1964). In the case of species of *Xylocopa* and cavity-nesting bees of Megachilidae (Ali et al., 2017; Shebl et al., 2018; Kamel et al., 2019) the Dufour's gland plays another role in nest recognition cue as found in *Osmia lignaria* and *Megachile rotundata* (Pitts-Singer et al., 2012).

Because they lack the glands necessary to produce sterile, hydrophobic secretions to line brood cells, leafcutter bees layer cells and shield larvae and pupae from pathogens using plant materials like resins and leaves (Mitra, 2013).

In the case of the Melittidae species, they built their nests on the grounds without cell lining (Rozen, 1987; Michener, 2007). However, since the secretions of the gland were also utilized as pheromones by certain bees, it is unclear if all oil-collecting bees that employed floral oil for cell lining had a smaller Dufour's gland or larval food (Cane, 1983; Norden et al., 1980).

More research is needed on solitary bee glands to understand their sociobiology and nesting behavior better.

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Authors' Contribution

K.M.: conceptualization, investigation, writing: original draft, writing: review and editing.
M.S.: conceptualization, investigation, writing: original draft, writing: review and editing.

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