NOTES ON THE MORPHOLOGY OF *Tetrapedia diversipes* KLUG 1810 (TETRAPEDIINI, APIDAE), AN OIL-COLLECTING BEE

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ABSTRACT

Some groups of bees collect oil from flowers and use this product to feed the larvae and to line the nests and brood cells, as is the case for bees of the Neotropical genus *Tetrapedia* (Tetrapediini, Apidae). They are solitary and construct their nests on pre-existing cavities in wood. Aiming to bring a better understanding of the oil collecting structures of Neotropical oil bees, in this study we examined the foreleg morphology of female of *Tetrapedia diversipes* Klug, showing on SEM the adaptations of forebasitarsus for collecting oil from flowers. The metasoma of female bees was measured and dissected using stereomicroscope and the size and shape of the Dufour's gland were estimated. *T. diversipes* hold a curved comb on the basitarsus of the front leg to collect oil and a mixture of slender and branched hairs on the scopa of the hind leg to transport it. These structures are very similar on other examined *Tetrapedia* species. The Dufour's gland of *T. diversipes* is reduced, occupying about 2.2% of the metasoma. Further investigation of the chemical composition of the Dufour's gland secretion, of the cell lining and of the collected floral oil might clarify the role of these components on *T. diversipes'* life.

Key words: Dufour's gland, floral resource, elaiophore, nesting material, Neotropical bee

INTRODUCTION

The main floral resources collected by bees are pollen and nectar. But some groups of bees, additionally, collect oil from the flowers [4,25,30]. This is the case for some members of the Mellitinae, Ctenoplectrini, Centridini, Tapinostapidini and Tetrapediini. The last three tribes are exclusively found in the western hemisphere and are specially diverse and abundant in the Neotropical region [17]. Oil-collecing behavior presumably evolved independently in five groups of bees.

Oil-producing flowers and oil-collecting bees were first described by Vogel [29]. Currently, around 1800 species of plants belonging to the following 8 botanical families are believed to secrete fatty oils instead of nectar as a reward [4,14,26,31]: Cucurbitaceae, Iridaceae, Krameriaceae, Malpighiaceae, Orchidaceae, Primulaceae, Scrophulariaceae and Solanaceae. The oil flowers are found most abundantly in Neotropical savannas and forests [4]. The plant tissues specialized in producing oils are called elaiophores, and they can be trichomatic or epithelial [30].

For the activity of oil sampling, the oil bees are equipped with elaborate modified hairs: setal combs and pads [10]. These structures are usually located on the legs, fore- and/or midtarsus (most groups) or on the metasomal sterna (*Tapinotaspoides*) [10,15,21,22,30]. With the combs and pads, the females scrape the region of the oil glands and then transfer the oil to the scopa on the hind legs for the transportation. The morphology and position of the oil-sampling apparatus of the bees are correlated mainly with the different types of floral elaiophores [19,30].

It is assumed that these bees collect oil for two reasons. First, the collected oils might replace the sugars in the nectar as an energy source for the larvae. For this reason, the females mix oil with pollen in the provision. Second, the females line their brood cells in the nest with the floral oil, presumably to waterproof them [9]. Also, the use of the floral oil, as an adhesive substance for the transport of soil and sand grains was described for *Tetrapedia diversipes* Klug

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[2] and Centridini [12,27]: when the females land in the ground the oil causes soil grains to stick to the scopal hairs, facilitating their transport.

The tribe Tetrapediini is composed of two genera: *Tetrapedia* and *Coelioxoides.* In Brazil there are 18 described species of *Tetrapedia* [18,24] all of which are apparently oil-collecting bee. *Coelioxoides* are cleptoparasites of *Tetrapedia* [22].

The nest architecture and the nesting biology of *Tetrapedia diversipes* have been studied by Alves dos Santos *et al*. [2]. This species is bivoltine, with the first generation being active in the spring, while the second being active in the summer. Females nest in wood and usually use pre-existing cavities made by beetles in tree trunks for their nests. The nests are linear and the cell walls are lined with a mixture of sand sediment and oil. We assume that these oils are a replacement for the Dufour's gland secretion used by other bees. With this assumption in mind, we have studied and report here, the morphology of the oil-collecting apparatus and the Dufour's gland of females of this species.

MATERIAL AND METHODS

The bees

Since *T. diversipes* use pre-existing cavities, they are relatively common in wood trap nests. Females were obtained from active nests maintained on the Campus of the University of São Paulo, in the City of São Paulo. Details of the nest architecture and nesting biology are given in Alves-dos-Santos *et al.* [2]. All the *Tetrapedia* species in the entomological collections of the University of São Paulo and the University of Kansas (USA) were examined for comparison of the oil collecting structures.

Scanning electron microscopy

The scanning electron microscope images of the female legs were obtained at the Microscopy Laboratory of the Institute of Biosciences of the University of São Paulo. The legs of the specimens were dissected, dried and placed directly on the stubs with double-face adhesive tape. They were then coated with gold palladium (Balzer SCD 050) and examined with a Zeiss DSM940 scanning electron microscope operated at 10 kV.

Internal morphology

Freshly killed females were dissected in cold insect saline solution. The Dufour's gland and the state of development of the ovaries were examined for each female. Only females with developed ovaries, i.e., containing 2-3 mature eggs were considered (n=5). The last metasomal segments, with the sting, the reproductive organs and the Dufour's gland and the acid gland were separated. The length and width of the Dufour's glands were measured using an ocular micrometer adapted for a binocular microscope. The width at three equidistant points along the length of the gland (anterior, medium and posterior) were used to calculate the mean width of the gland. The length was measured by summing the distances between the most anterior and the medium points and between the medium and the most posterior points. The size of the gland was inferred by multiplying the mean width by the length. The glands were photographed under a stereomicroscope and preserved in 4% paraformaldehyde. The other parts of the specimens were also preserved in 4% paraformaldehyde.

External metasomal measurements

The relationship between the size of the metasoma and of the Dufour's gland was assessed by measuring the length and width of the metasomas and multiplying these two values to obtain a rough estimate of the size of the Dufour glands of our *T. diversipes* specimens.

All the material is deposited at the Bee Laboratory at the IB/University of São Paulo in São Paulo.

RESULTS

Females of *Tetrapedia diversipes* exhibit peculiar morphological adaptations on the basitarsi of the forelegs and on the hind legs respectively to collect, manipulate and transport the floral oil to the nest (Figs. 1 A-D and 2 A-D). The oil-collecting organ is a strong and elaborate setal comb situated on the basitarsi of the front legs (Fig. 1A). This structure is present in females and males. The comb is curved in the proximal portion and concave dorsally; the distal portion is straight along the outer margin of the basitarsus (Fig. 1B). The comb is composed of several rows of simple hairs arranged densely and a row of flattened setae. The setae are longer on the basal portion (about twice the length of the distal setae) and gradually become smaller distally (Fig. 2A-C). Long, simple and short, branched hairs are mingled ventrally to the comb where we suppose the oil first accumulates (Fig. 2A).

The oil-manipulating and carrying structures also include on the hind legs of the female, a densely pectinated tibial spur and the scopa on the hind tibia and basitarsus. The scopa consists of a mixture of slender, branched hairs along the ventral part of the basitarsus and in the distal portion of the tibia, and of stout, long, simple and relative inflexible hairs emerging either above the plumose hairs (Fig. 2D) or

Figure 1. Oil collecting structures of *Tetrapedia* bees. **A -** Front view of a *Tetrapedia* female showing the location of the combs on the forelegs (indicated by the **arrows**). **B -** Right forebasitarsus with the modified setae forming a curved comb that is used to scrape the floral oil. **C-D -** Scope on the hind legs of the female with oil among the setae (**C**) and full of pollen mixed with oil (**D**) after foraging activities. Note the shiny pollen mass.

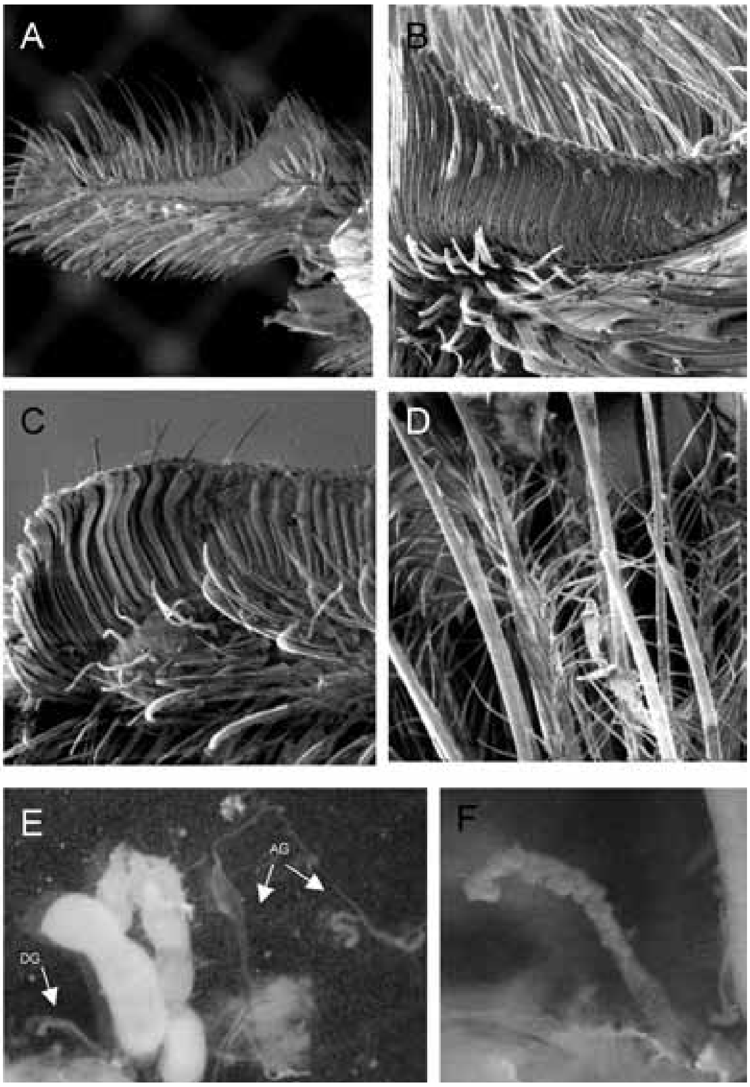

Figure 2. Scanning electron micrographs of the oil collecting structures of *Tetrapedia diversipes.* **A -** Forebasitarsus of *T. diversipes* female showing the structure involved and adapted for oil sampling. **B-C -** The comb is curved and composed of unbranched, flattened, apically curved setae. **D -** A combination of stiff, simple hairs and slender, branched hairs for holding oil and pollen on the scope of hind tibia. **E -** Developed ovaries with mature eggs, the Dufour's gland (**DG**), acid glands (**AC**) and the sting of *T. diversipes*. **F -** The narrow and convoluted Dufour's gland.

all over the dorsal tibia and basitarsus. The oil usually accumulates in the dense wooly layer formed by the plumose hairs; and the pollen (usually mixed with oil) is settled between the simple and the branched hairs (Fig. 1C,D). The structures related to oil collection and transportation are very similar among all of the *Tetrapedia* species examined in museum collections, with the same basic pattern, varying a little in the size and the color of the setae. Thus, for example, the comb is small and shallow in *T. clypeata* Friese, while in *T. rugulosa* Friese it is larger and deeper and the long setae are dark golden distally.

The Dufour's gland of *T. diversipes* has a long, narrow and convoluted shape, as we can see in Figure 2F. In average the length of the Dufour gland is 1.95 ± 0.56 mm (n=5). The width of the gland varies along its length, with the narrowest portion measuring 0.056 mm and the largest measuring 0.12 mm, the mean width being 0.091 ± 0.009 mm. The mean total size of the gland (obtained multiplying the mean length by the mean width) is 0.178 ±0.058 mm² (Table 1). The mean length and width of the metasoma of *T. diversipes* is 3.42 ± 0.153 mm and 2.34 ± 0.106 mm, respectively, that resulted in a mean size of 8.018 ± 0.268 mm² (Table 2). The ratio between the size of the Dufour's gland and the size of the metasoma (DG/Ab) indicated that the gland occupies about 2.2% of the metasoma what apparently seems a quite reduced portion. Figure 2E shows a Dufour's gland, along with the ovary and the sting.

DISCUSSION

In contrast to other oil-collecting species of the tribes Centridini and Tapinotaspidini, the comb used to scrape the oil is turned distally in *Tetrapedia*, which certainly implies different movements while foraging for the oil on the flowers. Neff & Simpson [19], who described the oil sampling structures in all the groups, observed that *Tetrapedia* females hang upside-down beneath *Banisteriopsis* (Malpighiaceae) flowers while grasping the petal with the midlegs and working on the oil glands with the forelegs. According to these authors it seems that this inverted position for collecting oil places the comb in a "right side up" orientation. But even in this upside down position, the females still have to scrape the glands in a distal direction, following the curved shape and arrangement of the comb. It is still unclear how the female handle the oil that is collected. Additional observations on flowers other than those of the Malpighiaceae may help us to understand how *Tetrapedia* performs the sampling.

Although the comb is turned to a distal position in *T. diversipes*, the structure of its oil collecting apparatus presents many similarities with the combs from the other oil bees, such as the usually flattened setae forming a broad and salient surface (like a spade) on the basitarsus. In *Centris* the same structure is also present on the midlegs, thereby enabling the females to collect oil from four elaiophores at the same time. The association of *Centris* with Malpighiaceae flowers is well known; the female grasps one petal of the flower and use the fore- and midlegs to sample oil from the elaiophores [4,23,28]. According to Vogel [30] the females perform synchronic forward and backwards movements with the legs over the epithelial oil glands. The oil is then transferred to the scopa of the hind legs.

To transport the oil to the nest, the same morphological adaptations found in *Tetrapedia* females occurs in most groups of oil bees, that is, the combination of plumose and simple hairs. According to Roberts and Vallespir [21] the woolly layer on the scope has a marked capacity of attract the oil by capillary action, and the long and stout hairs protect the integrity of the wooly hairs. Without the reinforcement provided by the rigid setae, the woolly hairs would collapse due to the surface tension caused by the oil.

The use of floral oil in nest cell linings and pollen provision was first demonstrated and reported by Cane *et al.* [6] for *Macropis nuda* (Melittidae), an oil bee from the United States specialized to *Lysimachia* flowers. These authors described the foraging behavior of this species and showed that the cell lining of *M. nuda* is composed of lipids acquired from *Lysimachia* oil glands. In contrast, the lipids secreted by the tiny, linear, and poorly tracheated Dufour's gland of *M. nuda*, the other possible source of the lining material, have a different chemical composition [6]. The related *Melitta* females, which presumably secrete waxy nest cell linings, possesses an enormous Dufour's gland, twice bifurcately branched and heavily tracheated (Cane 1982 cited in [6]). Some chemical components of the Dufour's gland secretion of *Melitta* are remarkably similar to those of the *Lysimachia* elaiophores [5]. This may be a functional convergence of floral oil and bee gland secretion for similar physical characteristics.

Table 1. Dufour's gland parameters of *Tetrapedia diversipes* females (in mm).

Specimens	Length	Width 1	Width 2	Width 3	Mean width	Size (mm²)
T. diversipes 1	1.222	0.093	0.111	0.093	0.099± 0.011	0.121
T. diversipes 2	1.852	0.102	0.074	0.056	0.077± 0.023	0.143
T. diversipes 3	2.630	0.102	0.093	0.083	0.092± 0.009	0.244
T. diversipes 4	1.667	0.093	0.120	0.056	0.089± 0.033	0.149
T. diversipes 5	2.370	0.111	0.074	0.111	0.098± 0.021	0.243

Table 2. Abdominal measurements of *T. diversipes* females (in mm).

Specimens	Length	Width	Size (mm²)	DG/Ab
T. diversipes 1	3.643	2.214	8.066	0.015
T. diversipes 2	3.429	2.357	8.082	0.018
T. diversipes 3	3.214	2.286	7.347	0.030
T. diversipes 4	3.429	2.500	8.572	0.020
T. diversipes 5	3.389	2.367	8.022	0.027

Ab- abdomen, DG- Dufour's gland.

The chemistry of the Dufour's gland extracts have been studied in several groups of bees: Colletidae (*Hylaeus, Colletes*), Andrenidae, Halictidae (*Nomia*), *Melitta* in many non-corbiculated Apidae, and also corbiculate Apidae, including Meliponini [1,11,20]. The glandular secretions in most groups of solitary bees are dominated by a homologous series of saturated and unsaturated macrocyclic lactones, isopentenyl esters and fatty acids. In Meliponini the mixtures are very complex and species-specific. In the majority of the studied species, the Dufour's gland secretions appear to generate the wax-like, waterproof lining of the brood cell [16].

This is the reason why hypertrophied Dufour's glands are frequent in ground-nesting bees that typically apply the glandular waxy or oily secretion to the inner surface of their nest cell walls [3,5,6,7,13] and also in the Megachilidae bees that build their cells with pieces of petals and leaves, "or other material brought in from the outside" [9,17].

We assumed that *T. diversipes* would have a relatively small Dufour's gland, because they build their nests in small holes in tree trunks, and line the cells with oil [2]. This supposition was based on the assumption that the floral oil would replace the secretion of the Dufour's gland. Indeed, our data show that the Dufour's gland occupies only 2.2% of the metasomal size, what apparently seems a quite reduced portion, when compared to the Dufour's gland of *Colletes validus* and *C. inaequalis* that occupy the "larger portion of the abdominal cavity" [13]. Supporting such a view we propose to study the Dufour's gland of *Tetrapedia* with more details employing more species for further comparisons.

Preliminary chemical analysis of the oil composition of the nests of oil bees has shown that the substances present in the cell walls are more complex than those found in the pollen mass (Reis *et al.* in prep) suggesting the addition of secretions made by the bees. Future studies of *T. diversipes* should include investigation of the chemical composition of the Dufour's gland secretion, of the nest cell lining and of the components used in cell provisioning. In addition, the volatile compounds of Dufour's gland extracts should be tested in behavioral studies.

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