

## COMPARATIVE STUDY OF WAX GLANDS IN FOUR MELIPONINI BEES (HYMENOPTERA, APIDAE) PRODUCING DIFFERENT QUANTITIES OF WAX

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### ABSTRACT

The developmental degree of the wax glands was compared in four Meliponini bees, that produce different quantities of wax. The histological data and height average of the wax epithelium during the time in which the maximum production of wax is expected, are in accordance with the rates of wax produced by the species. In *Lestrimelitta limao* (Smith, 1863) a species which has cleptobiotic habits, and frequently rob wax from the attacked colonies, the height of wax epithelium was the lowest among the studied species. The cells seem to show an abnormal vacuolated cytoplasm, in the phase in which they would be producing wax.

KEYWORDS. Stingless bees, wax glands, development, wax production.

### INTRODUCTION

The eusocial bees of Apidae family build their nests with wax, to which they add different kinds of exogenous materials (HEPBURN & KURSTJENS, 1988). In *Apis mellifera* L., Apini, the wax is produced by epithelial abdominal glands (SNODGRASS, 1956), constituted by cells of class I of NOIROT & QUENNEDEY (1974), which are located ventrally from the third to sixth sternites (DREYLING, 1903). In meliponines the glands are also present from the third to the sixth abdominal segments but, dorsally, in the tergites (DRORY, 1873; CRUZ-LANDIM, 1967). In both classes of bees the wax glands have a developmental and functional dynamics that are related to worker division of labor in the colony (RÖSCH, 1927, 1930; SNODGRASS, 1956). In newly emerged workers the region where the wax glands will developed, appear as an ordinary epidermis, but a few days after the adult emergency the epidermal cells turn columnar and active in wax production. Later, in old aged forager workers, the cells height decrease and the region become a simple and flat layer of epidermal epithelium again.

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In *Apis mellifera* the wax glands are active approximately between 6 and 18 days of adult life (RÖSCH, 1930). Both meliponines and *A. mellifera* produce wax at approximately the same age, which coincide with the phase when workers are nursing (HEBLING *et al.*, 1964). The amount of wax used in the nest construction and the type of material added to it, vary from one species to another, according to the habits (MILBORROW *et al.*, 1987) and in the same species according to the nest location and the environmental resources.

The aim was to search for morphological and developmental differences in the wax glands, related to wax production in four species of Meliponini, according to their habits or behavioral characteristics, which interfere in wax production.

### MATERIAL AND METHODS

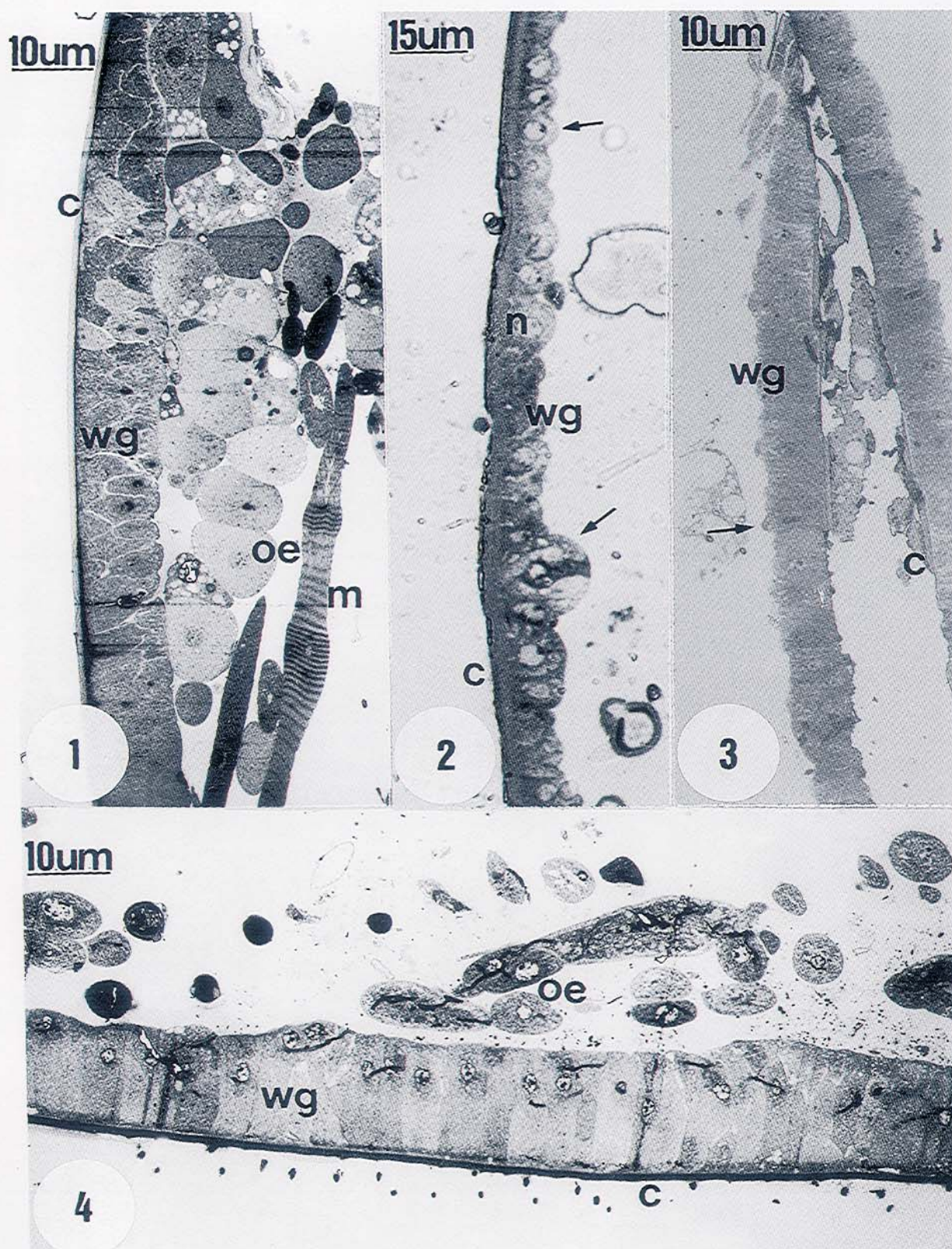
The species used in this study were *Trigona recursa* Smith, 1863, *T. hypogea* Silvestri, 1902, *Cephalotrigona capitata* (Smith, 1854) and *Lestrimelitta limao* (Smith, 1863). The workers were middle aged, which could be observed by the evaluation of the tegument color due to the esclerotization of the tegument. Some abdomens were separated from the body and fixed in Bouin fixative, embedded in historesin, and sectioned. The sections 6µm thick were stained with hematoxylin and eosin, for histological study. Other abdomens were dehydrated, dried at critical point dryer for analysis under scanning electron microscope (SEM). Vaucher specimens are deposited in the collection of the Centro de Estudos de Insetos Sociais, and the histological slides and SEM samples in the Departamento de Biologia both of Instituto de Biociências de Rio Claro, Universidade Estadual Paulista, Rio Claro, SP, Brazil.

The height of the epithelium was measured in the glands of five workers of each species. Ten measurements were made in the tergites chosen of each bee, the average height of the epithelium was calculated for each species.

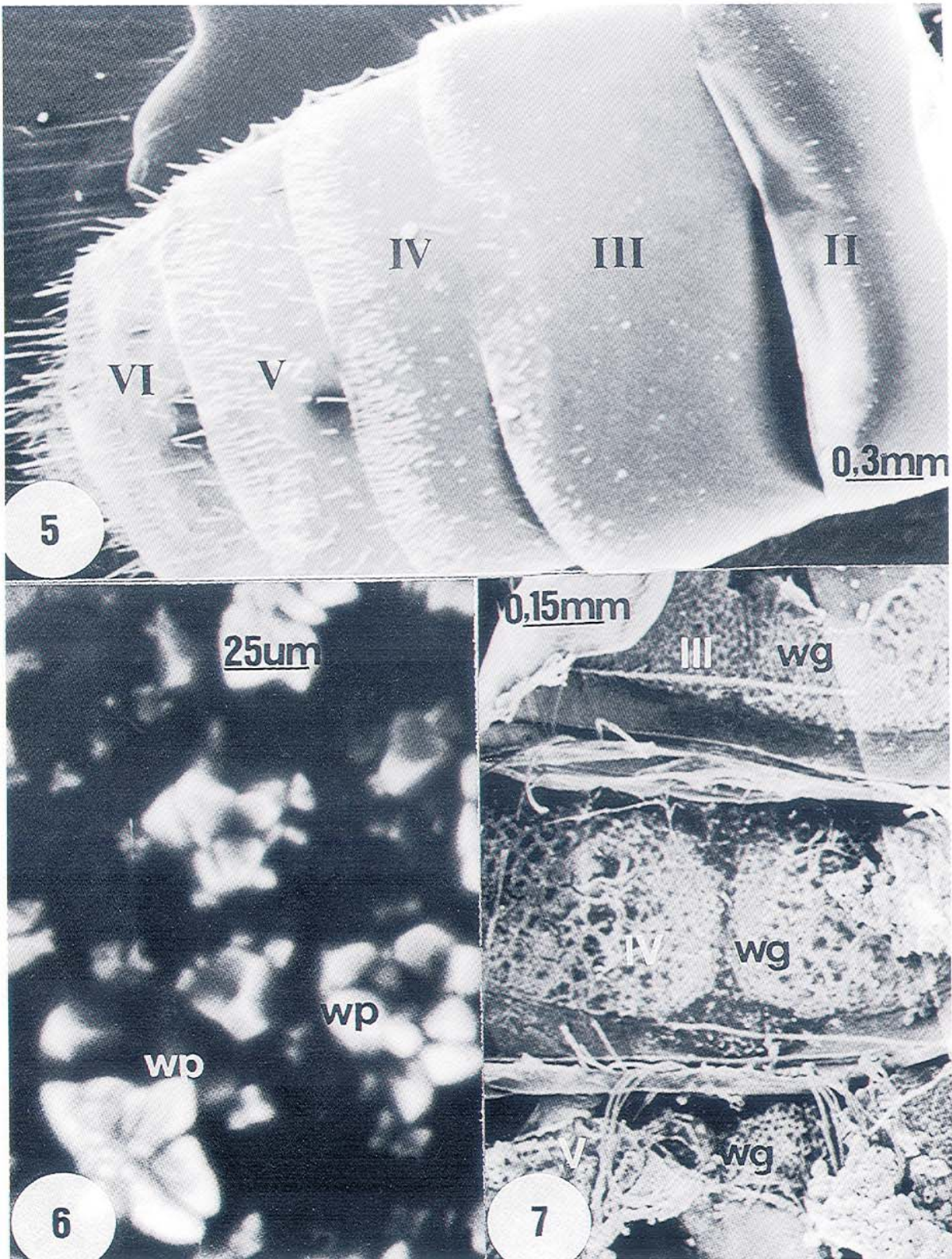
### RESULTS AND DISCUSSION

The wax glands has the maximum development when the workers are working inside of the colony, mainly in the brood area, building and provisioning brood cells. These tasks are done, in most of the known species of Meliponini, by workers between 5 and 20 days of adult life (KERR & SANTOS NETO, 1956; HEBLING *et al.*, 1964; GIANINNI, 1997).

The epidermis of the region of the wax gland appeared developed in all middle aged workers of the species studied (figs. 1-4) although with different degrees among them. Some histological differences were, also, detected. In *Lestrimelitta limao*, a cleptobiotic bee, that apparently uses stoled wax for nest construction, the basal surface of the cell, in contact with hemolymph, appeared vacuolated and bulging into the cavity of the body (fig. 2). In this species the cells are cubical and the average height of the epithelium is 4µm. In *Trigona hypogea*, a necrophagous bee, that usually use mainly resin to build its semi-subterraneous nest, the glandular epithelium has an average height of 8µm and columnar cells. Some of the cells also have basal protuberances toward the hemolymph (fig. 3). *Trigona recursa* and *Cephalotrigona capitata* have wax glands with usual morphological features. These species build theirs nest in tree trunks and produce a great amount of wax, that they mix to resin in order to build the nests. Their glandular cells are columnar, with basal nuclei, enlarged intercellular spaces and perpendicularly striated cytoplasm (figs. 1, 4). The height average of the epithelium in *Trigona recursa* is 10µm while in *Cephalotrigona capitata* reaches 16µm. The greatest height found in *Cephalotrigona capitata* is in accordance with its reported increased wax productivity (ROUBIK 1979, 1989).



Figs. 1 - 4. Wax glands (wg) of nurse workers: 1, *Trigona recursa*; 2, *Lestrimellita limao*; 3, *Trigona hypogea*; 4, *Cephalotrigona capitata*. The arrows point to the basal bulges of the glandular cells (c, cuticle; m, muscle; n, nucleus; oe, oenocytes).



Figs. 5 - 7. 5, Dorsal outer view of *Trigona hypogea* abdomen (II-VI tergites); 6, outer view of tergites of *Cephalotrigona capitata* showing the wax pores (wp); 7, inner view of *Trigona recursa*, (III-V tergites) showing the wax gland (wg).

Therefore the histological features and epithelial heights size data seem to confirm the expected condition from the observed behavior related to wax production by the species, that is, species that produce great amounts of wax have more developed wax glands than those species that requires less wax in their nests as *Trigona hypogea*, or use wax robbed from other bees as *Lestrimelitta limao*.

Concerning, the features of the outer surface of the sternites a SEM dorsal view of the abdomen of *Trigona hypogea* shows a smooth surface without any differentiation related to the presence of the wax gland (fig. 5), while in *Cephalotrigona capitata* were observed pores full of wax crystals (fig. 6). The tergites of *Trigona recursa*, by the internal surface, allowed to detect that developed wax epithelium (fig. 7), does not occupy all the surface of the tergite, but forms two blocks medially separated by a glandular free region between them.

The present results indicate that although the epidermis of the segments where in the proper time a wax gland will develop, undergo changes in all species studied, the histological features of the epithelium there differentiated and the height achieved by the cells is different in each one. The results, show, also, that the developmental degree reached by the wax epithelium is compatible with the amount of wax produced by the species. *Lestrimelitta limao* and *Trigona hypogea* besides have lower wax epithelium have histological features that suggest that the wax producing cells present some abnormality in the phase in which the wax must being produced, characterized by basal vacuolation of the cytoplasm, which causes a bulging toward the body cavity.

The results indicate that the wax glands development is adapted to the amount of wax used in the nest construction (*Trigona hypogea*, *T. recursa* and *Cephalotrigona capitata*) and to the way the wax is obtained (*Lestrimelitta limao*).

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