New characteristics of the male reproductive system in the Meliponini bee, *Friesella schrottkyi* (Hymenoptera: Apidae): histological and physiological development during sexual maturation*

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**Abstract** – Despite the biological importance of Meliponini bees, there are still many doubts about their reproductive physiology and phylogeny. When compared with other bee species studied up to now, the reproductive system of *Friesella schrottkyi* (Friese) has very different characteristics during sexual maturation, which includes: (1) the release of individualized testicular spermatozoa, rather than spermatozoa in bundles, (2) spermatozoa reaching the seminal vesicles only nine days after emersion of the adult bees, (3) holocrine secretion carried out by the seminal vesicle epithelium, (4) big lipid droplets abundant in the seminal vesicle epithelium and (5) membranous inclusions found in the seminal vesicle epithelium during the period of sexual maturation. These characteristics may represent important physiological characteristics as well as evolutionary differences in relation to other Meliponini studied and may be useful in studies of this bee group, as soon as similar descriptions are obtained for other closely related species.

**Friesella schrottkyi**/ seminal vesicle / morphology / holocrine secretion / Hymenoptera / reproductive system

**1. INTRODUCTION**

The Meliponini tribe (Apidae), which includes *Friesella schrottkyi* (Friese), is distributed in all tropical and subtropical regions of the Southern Hemisphere (Michener, 2000; Silveira et al., 2002). Besides the use of these species to enhance agricultural production, the interest in studying and preserving these species derives from their importance as pollinators of native vegetation. In order to protect a species, it is important to understand its reproductive behavior, as well as its systematics. Despite the importance of the stingless bees, there are many unresolved points related to their reproduction, taxonomy and phylogeny (Rasnitsyn, 1988; Dowton and Austin, 1994; Dowton et al., 1997; Ronquist et al., 1999).

Secretions from accessory glands of the male reproductive system have been associated with reproductive success in many species. Some functions of these secretions are: spermatozoon nutrition, helping spermatozoon displacement along the ducts and inducing post-copula behavior in females (Gillott, 2003). In species that form spermatophores, sperm are packed by secretory
materials from the glands before to their transfer to the female (Chen, 1984; Sturm, 2003). Therefore, it is noteworthy that Meliponini bees do not have such glands (Kerr, 1948; Ferreira et al., 2004).

Measuring almost 3 mm in length, *F. schrottkyi* is a small bee that is distributed throughout the Brazilian southeast (Silveira et al., 2002). These bees are not aggressive; their colonies are small with approximately 300 individuals and irregular combs are found inside the colonies. Males of *F. schrottkyi* are produced throughout the year from queen or workers’ unfertilized eggs (Velthuis et al., 2005).

*Friesella* is a monotypic genus (Camargo and Pedro, 2008) that is closely related to another Meliponini genus: *Plebeia* (Michener, 1990, 2000).

This study describes morphophysiological aspects of the male reproductive system of *F. schrottkyi* during sexual maturation, and aspects related to sperm release from the testes, with the intention of contributing to the study of reproductive physiology.

## 2. MATERIAL AND METHODS

To obtain adult males of known ages, from 0 (just emerged) to 14 days, combs were taken from colonies of *F. schrottkyi*, maintained in the Central Apiary of the Universidade Federal de Viçosa (Viçosa, MG, Brazil), and conditioned in Petri dishes. These were maintained in BOD at 28.7 °C. They were inspected daily and the males were marked with a nontoxic paint for later age identification. As each adult emerged, the individuals were transferred to a “colony” previously mounted with workers, also maintained in BOD, and fed daily with a sucrose solution and pollen taken from colonies at the apiary.

### 2.1. Histology

Reproductive systems were dissected in cacodylate buffer (pH 7.2), fixed in Stefanini liquid (Stefanini et al., 1967), dehydrated in an alcoholic series and embedded in historesin. Histological sections were submitted to: (1) PAS (Junqueira and Junqueira, 1983) for neutral carbohydrate detection; (2) Alcian Blue pH 2.5 and pH 0.5 (Bancroft and Stevens, 1996) for acid glycoconjugates and (3) Xyloidine Ponceau (Mello and Vidal, 1980) for protein detection. They were also submitted to acid and alkaline phosphatase detection (Bancroft and Stevens, 1996).

To identify the nucleus, some histological slides were stained for 15 min in 0.2 μg/mL 4,6-diamino-2-phenylindole (DAPI) in phosphate buffer. They were examined with an epifluorescence microscope (Olympus, BX60), equipped with a BP 360–370 nm excitation filter.

### 2.3. Transmission Electron Microscopy (TEM)

Reproductive systems were fixed in a solution containing 2.5% glutaraldehyde, 0.2% picric acid, 3% sucrose and 5 mM CaCl₂ in 0.1 M cacodylate buffer, pH 7.2. The material was post-fixed in 1% osmium-tetroxide in the same buffer for 2 h. Dehydration was carried out in acetone and embedding of the organs in epon. Ultrathin sections were stained with 2% uranyl acetate and 0.2% lead citrate and observed with the Zeiss Leo 906 transmission electron microscope, at 60 kV.

## 3. RESULTS

### 3.1. Reproductive system

The reproductive system of *F. schrottkyi* males consists of two testes, each made up of four follicles. From each follicle originates an efferent duct, and the four tubular structures with Entellan (Merck), examined and photographed with a photomicroscope, Olympus CX31.

For lipid removal, seminal vesicles were fixed for 12 h in 2.5% glutaraldehyde, dehydrated to absolute alcohol and xylene, re-hydrated and post-fixed in osmium tetroxide. Thin sections were stained with toluidine blue.

### 2.2. Histochemistry

Reproductive systems were dissected in cacodylate buffer (pH 7.2), fixed in Stefanini liquid (Stefanini et al., 1967), dehydrated in an alcoholic series and embedded in historesin. Histological sections were submitted to: (1) PAS (Junqueira and Junqueira, 1983) for neutral carbohydrate detection; (2) Alcian Blue pH 2.5 and pH 0.5 (Bancroft and Stevens, 1996) for acid glycoconjugates and (3) Xyloidine Ponceau (Mello and Vidal, 1980) for protein detection. They were also submitted to acid and alkaline phosphatase detection (Bancroft and Stevens, 1996).

To identify the nucleus, some histological slides were stained for 15 min in 0.2 μg/mL 4,6-diamino-2-phenylindole (DAPI) in phosphate buffered saline. They were examined with an epifluorescence microscope (Olympus, BX60), equipped with a BP 360–370 nm excitation filter.