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Dehiscent organs used for defensive behavior of kamikaze termites of the genus *Ruptitermes* (Termitidae, Apicotermitinae) are not glands

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ABSTRACT

During Isoptera evolution, the caste of soldiers disappeared in some Apicotermitinae termites as in the Neotropical *Ruptitermes*. Paired dorsolateral structures located between the metathorax and abdomen of foraging workers of *Ruptitermes* were previously denominated dehiscent glands, and are responsible for releasing an adhesive secretion that immobilizes enemies, causing their death. In this study, we investigated the morphology of dehiscent organs of workers of *Ruptitermes reconditus*, *Ruptitermes xanthochiton*, and *Ruptitermes pitan* and also second instar larvae of *R. reconditus* using light, laser scanning confocal, and transmission electron microscopy. Additionally, we performed a preliminary protein analysis using SDS-PAGE to further characterize the secretion of *Ruptitermes* dehiscent organs. Our results showed that the dehiscent organs do not exhibit the typical characteristics of the exocrine glandular cells class I, II or III of insects, suggesting that they constitute a new type of defensive organ. Thus, the denomination dehiscent gland was not used but dehiscent organ. Dehiscent organs in larvae are formed by fat body cells. In workers, dehiscent organs are composed by compact masses of cells that accumulate a defensive secretion and are poor in organelles related to the production of secretion. Since the dehiscent organs are not glands, we hypothesize that the dehiscent organs originate from larval fat body. The defensive secretion may have been produced at younger developmental stages of worker or the defensive compounds were absorbed from food and accumulated in the worker fat body. Histochemical techniques and SDS-PAGE revealed that the secretion of *Ruptitermes* dehiscent organs is constituted mainly by a protein of high molecular weight (200 kDa). In conclusion, the dehiscent organs are extremely different from the exocrine glands of termites and other insects described until now. In fact, they seem to be a specialized fat body that is peculiar and exclusive of *Ruptitermes* termites.

1. Introduction

Termite colonies contain apterous and alate castes. The apterous caste is composed by workers and soldiers envolved with colony labor and defense, respectively, while the alate caste is responsible for colony reproduction. The latter caste includes the king and one or more queens, responsible for egg production and maintenance of the population. All species are eusocial and have both worker and soldier castes, or only workers (Boomsma, 2009).

The occurrence of the soldier caste has an important impact in the defense system of the colony since soldiers are equipped with specific morphological adaptations in the head and powerful mandibles very useful in defense activities (Deligne et al., 1981), defensive behavioral strategies (Pasteels and Bordereau, 1998), and exocrine glands that perform chemical defense. Among exocrine glands related to defense are the salivary glands (Maschwitz et al., 1972; Šobotník et al., 2010a), frontal glands (Costa-Leonardo and Kitayama, 1991), and labral glands (Šobotník et al., 2010a).

Soldierless Apicotermitinae is a taxonomic group of termites that have been received less attention in comparison with other termite taxonomic groups. In terms of species diversity and abundance, the Apicotermitinae dominate African and Neotropical rainforests, where they generally feed on soil organic fractions (Bourguignon et al., 2015a). Particularly, the genus *Ruptitermes* Mathews, 1977 is endemic to South America and is relatively abundant in the Cerrado ecoregion of central Brazil. Acioli and Constantino (2015) recently published a taxonomic review of this dominant Neotropical taxon in which they described nine new species of *Ruptitermes*, including one of the species studied in the present research.

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The soldier caste has been lost in some termite species, like all Neotropical Apicotermitinae (Sands, 1972) and some Termitinae (Ahmad, 1976; Miller, 1984). Therefore, during the process of evolution, worker caste has also developed and specialized in a variety of defensive strategies. The "kamikaze termites" are termite workers that have been named as such because they "explode" during a fight with their enemies. In fact, what really occurs is a rupture of the abdominal body wall of these individuals with release or not of a viscous-like secretion, a phenomenon known as abdominal dehiscence (Sands, 1982). This secretion rapidly turns opaque and thick when it comes into contact with air (Mill, 1984), and acts by immobilizing termite and enemy, which usually both die. When the source of the viscous secretion is glandular the altruistic suicide of termites is named autothysis (Bordereau et al., 1997).

The salivary glands were thought to be the structures responsible for, and involved in *Ruptitermes* bursting (Fontes, 1992; Mathews, 1977). However, Costa-Leonardo (2004), using light microscopy, verified that other organs different from salivary glands were functioning as defensive structures in some *Ruptitermes* species, and named them "dehiscent glands" since the secretion is released through abdominal dehiscence. This author also showed that dehiscent glands are paired structures located between the metathorax and the first abdominal segment of workers, which produce a viscous-like secretion. Later, Šobotník et al. (2012) identified a new exocrine gland responsible for producing copper-rich protein crystals with a molecular weight of ~76 kDa, in workers of *Neocapritermes taracua*. These crystals react with secretions from the salivary glands, causing rupture of the body wall of these workers.

Exocrine gland cells of insects can be classified into three categories (classes I, II and III) based on their cellular structure and the way the secretion is released through the cuticle (Noirot and Quennedey, 1974, 1991; Quennedey, 1975). Additionally, it is known that class I epidermal cells are the predominant type in the adhesive glands of insects (Dettner et al., 1985). Nevertheless, the ultrastructure of dehiscent organs was never studied. Light microscopy about dehiscent organs (Costa-Leonardo, 2004) not elucidated to which class (I, II or III) the glandular cells belong. Similarly, little is known about their morphological structure. For these reasons, throughout this research, the term "dehiscent gland" (Costa-Leonardo, 2004) was not used but "dehiscent organ".

The present study investigated the morphology of dehiscent organs in termite workers belonging to three species: *Ruptitermes reconditus* (Silvestri, 1901), *Ruptitermes xanthochiton* (Mathews, 1977), and *Ruptitermes pitan* (Acioli and Constantino, 2015), using light, electron, and laser confocal microscopy. A preliminary protein analysis using SDS-PAGE was also performed in order to further characterize the secretion of the dehiscent organs. Our aim was to elucidate the morphology and functioning of the dehiscent organs used for the defense of Neotropical *Ruptitermes*, and, hence, contribute to the knowledge of the defense system of soldierless Apicotermitinae species.

2. Material and methods

2.1. Insects

Worker termites of *R. reconditus*, *R. xanthochiton*, and *R. pitan* were collected during foraging activities and second instar larvae of *R. reconditus* were collected from shallow galleries under the exit of the subterranean nests located at the campus of the Universidade Estadual Paulista (UNESP), Rio Claro, Brazil.

2.2. Light microscopy

2.2.1. Total preparations.

The dehiscent organs of four workers of *R. reconditus* were dissected in physiological solution for insects, and stained with 0.5% methylene blue. After another addition of physiological solution, the material was covered by a glass coverslip and observed under a light microscope.

2.2.2. Histology and histochemistry

For light microscopy, thorax and abdomen of ten workers of *R. reconditus*, *R. xanthochiton,* and *R. pitan* were isolated in buffered saline solution for insects, and fixed in 4% formaldehyde for 2 h. Afterwards, the samples were dehydrated, embedded in Leica historesin, and placed in the same resin containing a catalyst. After resin polymerization, the blocks were cut into 4–6 μm slices, mounted on histological slides, and stained with hematoxylin-eosin (HE) for comparative morphology, and bromophenol blue (BB) and xylidine Ponceau (XP) for protein detection. Five whole second instar larvae of *R. reconditus* were included for histology following the same procedures described above and the histological slices were stained with hematoxylin-eosin (HE).

2.3. Laser scanning confocal microscopy

Immunocytochemical techniques were performed for the detection of:

2.3.1. Plasma membrane (red) and nucleus (green)

The dehiscent organs of ten workers of *R. reconditus* and *R. xanthochiton* were dissected in 3.5% formaldehyde, incubated first with Alexa 633, and after with SYTO 11. Between each step, the organs were washed 2 times with phosphate-buffered saline (PBS) solution.

2.3.2. Microtubules (blue) and nucleus (green)

The dehiscent organs of five *R. reconditus* workers were dissected in 3.5% formaldehyde, immersed in 0.1% Triton X100, and incubated overnight in monoclonal anti-tubulin α/β (Sigma–Aldrich) diluted 1:100, inside a humid chamber. Afterwards, the organs were incubated first with Alexa 405, and finally with SYTO 11. Between each step, the organs were washed 2 times with PBS.

2.3.3. Actin (green) and nucleus (red)

The dehiscent organs of five workers of *R. reconditus* and *R. pitan* were dissected in 3.5% formaldehyde, immersed in 0.1% Triton X100, treated with RNAse (10 mg/ml), incubated with propidium iodide in the dark, and stained with Phalloidin-FITC (Sigma–Aldrich; 7.5 mM). Between each step, the organs were washed 2 times with PBS.

2.3.4. Endomembrane (red) e nucleus (green)

The dehiscent organs of five workers of *R. reconditus* were dissected in PBS, immersed in endomembrane marker, and incubated with SYTO 11.

All total preparations of dehiscent organs were analyzed under a Leica TCS SP5 II confocal laser scanning microscope. The LAS AF software (Leica) was used for image analysis.

2.4. Transmission electron microscopy (TEM)

The dehiscent organs of ten workers of *R. reconditus* and *R. pitan* were dissected and fixed for 48 h in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). In previous assays, we fixed the organs for 2, 4, and 24 h and, in all cases, the material was found