Comparative morphology and histochemistry of the colleterial glands in different-aged females of *Coptotermes gestroi* (Blattaria, Isoptera, Rhinotermitidae)

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Introduction

- Colleterial glands, also referred as female accessory glands, are associated with the female reproductive system;

- These structures are not omnipresent among insect taxa and may display several functions (Gillott, 2002);

- Within the monophyletic Dyctioptera, these glands are laterally disposed in cockroaches and responsible for secrete compounds that are mixed and result in a hardened casing, the ootheca (Stay and Roth, 1962; Courrent et al., 2008);

- However, their morphology and function are not well-understood within Isoptera (termites). In these insects, the glands are disposed as anterior and posterior, opening into the genital chamber (Soltani-Mazouni e Bordereau, 1987).

Coptotermes gestroi

- Subterranean termite species of particular economic interest (Grace, 2014);
- Similar to most termite species, *C. gestroi* queens lay eggs individually, but the morphophysiological features of the colleterial glands in non-egg-laying and egg-laying queens are unknown.
- Aiming to contribute to the knowledge of the reproduction in this termite species, we conducted morphological, morphometrical, and histochemical analyses of the colleterial glands in different-aged females of *C. gestroi.*

Alate females

***C. gestroi* colony**

Material and methods

Termites

- Alate females and males were collected during the swarming flight, sexed, and randomly paired in 9 cm Petri dishes containing moistened and decayed pine sawdust;

- Non-egg-laying females were obtained from swarming flights (alate females) and from 2-old-colonies after pairing;

- Egg-laying queens were obtained from 80-d- and 4-yr-old colonies after pairing.

General morphology of the colleterial glands

- Colleterial glands were isolated from ten queens from 2-d- and 80-d-old colonies (five of each group), and photographed using a stereomicroscope for posterior description.

Material and methods

- **Histology, Histochemistry, and Morphometry of the colleterial glands**
- Abdomens from at least three alate females and queens from 4-yr-old colonies were fixed in FAA or alcoholic Bouin, dehydrated in an etanol serie, and included in historesin

- Sections with a 3µm thickness were obtained using a Leica RM 2245 microtome. After, they were stained with Hematoxyilin-eosin, xylidine-Ponceau (detection of total proteins), and PAS (detection of polysaccharides).

- Aiming to evaluate morphometrical changes in the colleterial glands, we randomly selected nine cross-sections of each gland in two different females from each group (non- and egg-laying females). The epithelium height was measured using the “straight tool” of the software (https://imagej.nih.gov/ij), and posteriorly compared using the software R (RStudio Team, 2020).

Results

- Colleterial glands in *C. gestroi* are composed of anterior and posterior glands, which are distally ramified but join a common basal trunk. This trunk, in turn, opens into the genital chamber;

- The anterior gland was always longer than the posterior one, although the latter was thicker than the former.

Results

- Histological sections showed that the anterior and posterior colleterial glands were very similar in alate females, although they were well-developed and easily distinguishable in queens from 4-yr-old colonies.

- The secretion present in the lumen of the posterior gland distal tubules was weakly stained with xylidine-Ponceau in queens from 4-yr-old colonies. However, the same secretion was strongly stained with PAS. Despite differences in staining intensity were observed, histochemical tests suggest the occurrence of glycoproteins in the lumen of the posterior gland. The lumen of the distal tubules of the anterior gland, in turn, did not react to any histochemical test in both females, similar to that of posterior gland in alate females

- The morphometrical evaluation showed that epithelium height of both glands increased in egg-laying-queens from 4-yr-old colonies ($P < 0.0001$, t test).

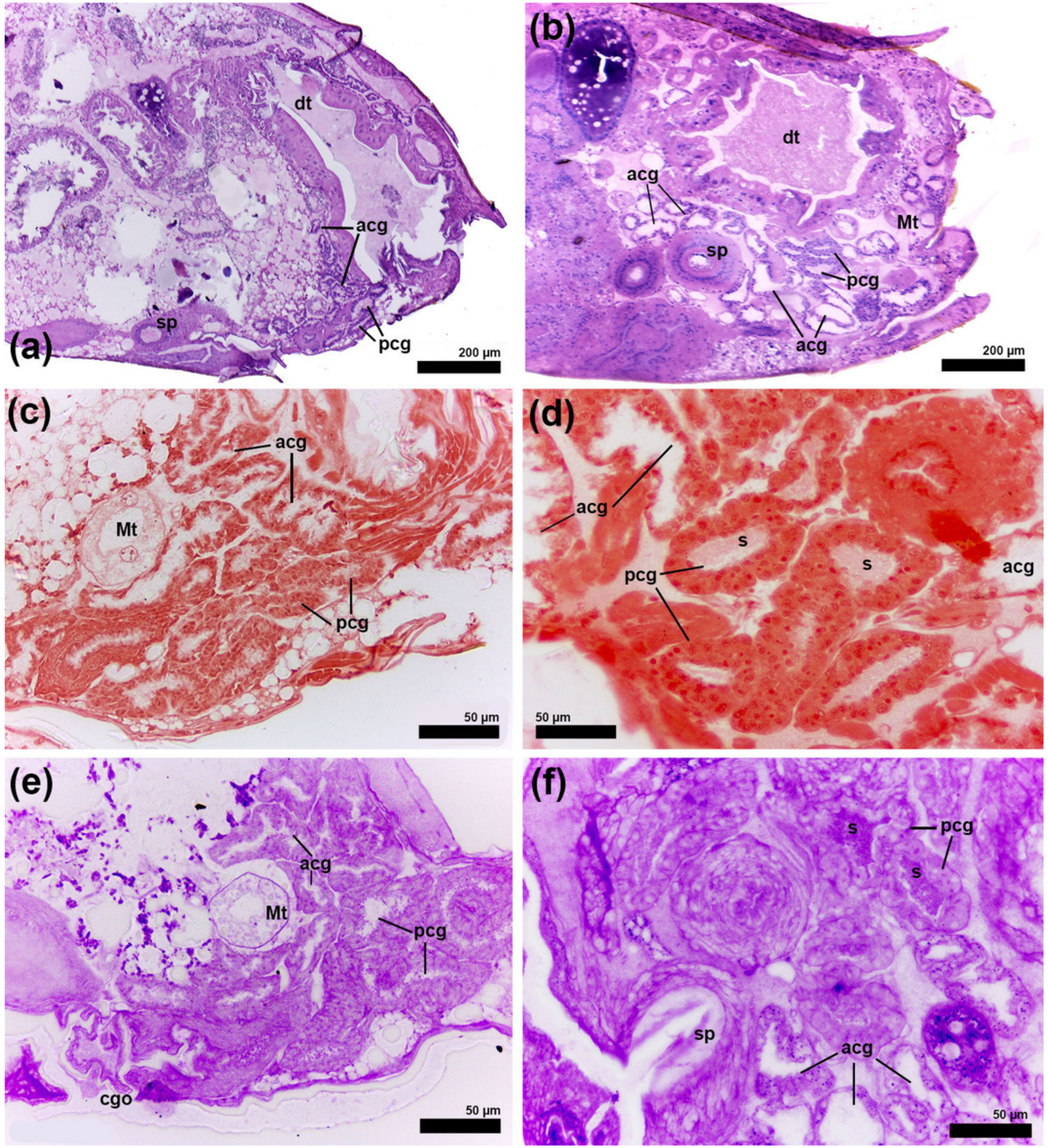

Figure 1. Histology and histochemistry of the colleterial glands in different-aged females of C. gestroi (a) General view of the colleterial glands in alate female; (b) General view of the colleterial glands in 4-yr-old queen. Stain: Hematoxylin-eosin. (c) Detail of the colleterial glands in alate female, showing the lumen of the anterior gland tubule devoid of protein. (d) Detail of the colleterial glands in 4-yr-old queen with secretion weakly stained in the lumen of the posterior gland tubule. Stain: xylidine-Ponceau. (e) Anterior and posterior colleterial glands in alate female. (f) Anterior and posterior colleterial glands in 4-yr-old queen with secretion strongly stained in the lumen of the posterior gland. Stain: PAS. acg = anterior colleterial gland, cgo = colleterial glands opening, dt = digestive tube, Mt = Malpighian tubule, pcg = posterior colleterial gland, s = secretion, sp = spermatheca.

Discussion

- The general morphology of the colleterial glands in C. gestroi females resembles those of *Kalotermes flavicollis* and *Cubitermes fungifaber*, in which both glands are distally ramified but join in a common basal trunk (Grassé, 1982; Soltani-Mazouni and Bordereau, 1987);

- Nevertheless, they differ from those of *Mastotermes darwiniensis*, *Zootermopsis nevadensis*, and *Pseudacanthotermes spiniger*, in which the anterior gland is paired and well-developed when compared to the posterior one (Courrent et al., 2008);

- The glandular opening also seems to vary among species. In *M. darwiniensis, Z. nevadensis,* and *P. spiniger*, the glands show a basal trunk each, which open separately into the genital chamber (Courrent et al., 2008).

Discussion

- The anterior and posterior glands did not show histological differences in alate females, similar to the observations described by Soltani-Mazouni and Bordereau (1987) for pseudergates of *K. flavicollis.* Nevertheless, the glands were easily distinguishable in egg-laying-queens;

- Our histochemical tests evidenced the occurrence of glycoproteinaceous secretion only in the lumen of the posterior gland in egg-laying queens, whereas no positive reaction to the histochemical tests was observed in the lumen of both glands in non-egg-laying females. These observations differ from Courrent et al. (2008), in which the posterior gland of the sampled termite were always less developed and empty;

- Secretion from different chemical nature may be synthesized by the colleterial glands of insects. Their defensive function ranges from the synthesis of antibacterial compounds to those related to ootheca formation (Stay and Roth, 1962; Marchini et al., 1991). Additionally, lubricating material may be secreted by these glands into the genital chamber during the oviposition (Ma et al., 2013).

Conclusions

- Colleterial glands in *C. gestroi* are composed of an longer and thinner anterior gland and a shorter and thicker posterior one. The glands join in a common basal trunk, which opens into the genital chamber and was already described for some termite species. Nevertheless, such a morphological feature is not universal in the termite literature, suggesting that it varies among and within Isoptera families.

- Morphometrical, histological, and histochemical data indicated developmental and physiological changes of the colleterial glands in non-egg-laying queens compared to those in oviposition. Such differences is based especially on the epithelial development, secretory activity, and the nature of the secretion.

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