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The fine structure of the *Anopheles aquasalis* spermatheca

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The mosquito spermatheca is the female organ responsible for storing and maintaining the viability of the male sperm after copulation. Taken that mosquitoes only mate once, understanding how the sperm in maintained throughout the female lifespan may provide clues on to how to control fertilization in mosquitoes [1,2]. Here, we investigated the fine structure of the spermatheca in the saltwater-tolerant mosquito *Anopheles aquasalis*, a vector of malaria in Brazilian coastal marshes.

Virgin and inseminated female spermathecae were dissected and fixed in paraformaldehyde 4% in PBS 0.1M and/or glutaraldehyde 2.5% 0,1M cacodylate buffer pH 7.2. After dehydration in ethanol series samples were embedded in Leica historesin, sectioned and stained with hematoxylin and eosin. For electron microscopy (SEM and TEM) analyzes, samples were post-fixed in 1% osmium tetroxide and dehydrated in ethanol (crescent series). Samples were critical-point dried using CO_2 and gold-coated by sputtering for SEM (LEO 1430VP) or embedded in LR White resin. Ultra-thin sections were stained with uranyl acetate and Reynold's lead citrate and analyzed under TEM (Zeiss EM109).

The spermatheca of *An. aquasalis* consists of a sac-like reservoir connected to the genital chamber by means the spermathecal duct (Figs. 1, 2). Externally to the duct, muscle fibers are found between the duct individual glandular cells (Fig. 3). Glandular cells are also seen in the reservoir epithelial wall. These cells have well-developed structures where glandular secretion is stored before being released into the reservoir lumen (Fig. 5). These cells also have microvilli packed tightly together, particularly where they converge with the cell ductule (Fig. 6). The ductules transport cell secretory products to the spermathecal lumen. The ductule openings form pores surrounded by a very thin epicuticle that is continuous with the spermathecal cuticle and project towards the reservoir lumen together with the laterally-associated epithelial cells (Figs. 4, 5).

The spermatheca in *An. aquasalis* share many features with the three spermathecae found in *Aedes aegypti* [3, 4]. However, significant differences such as the cell types that make up the spermatheca, as well as their numbers and distribution were observed. One of the most remarkable differences observed between these two mosquitoes is the absence of an individualized secretory glandular unit in *An. aquasalis*, which is present in *Ae. aegypti* [3,4]. In *An. aquasalis*, such secretory function is performed by glandular cells distributed along the spermatheca reservoir wall [5].

The spermathecae of anophelines and culicines display significant morphological differences that may be suggestive of species-specific variations. Such differences include the sperm storage capacity measured as the total volume of the organ, as well as the cell types, number and distribution, and the length of the spermathecal duct. In *An. aquasalis* females, the spermatozoa viability is likely maintained by isolating the spermatozoa inside the spermathecal reservoir, and by the spermathecal glandular secretions that provide nourishment to the spermatozoa in the spermathecal lumen. Understanding the spermatheca organization and function will contribute to unknown details of mosquito reproductive biology, and help answer questions related to the reproductive success of these major disease vectors [6,7]. Acknowledgments: Fapemig, CAPES and CNPq for financial support.

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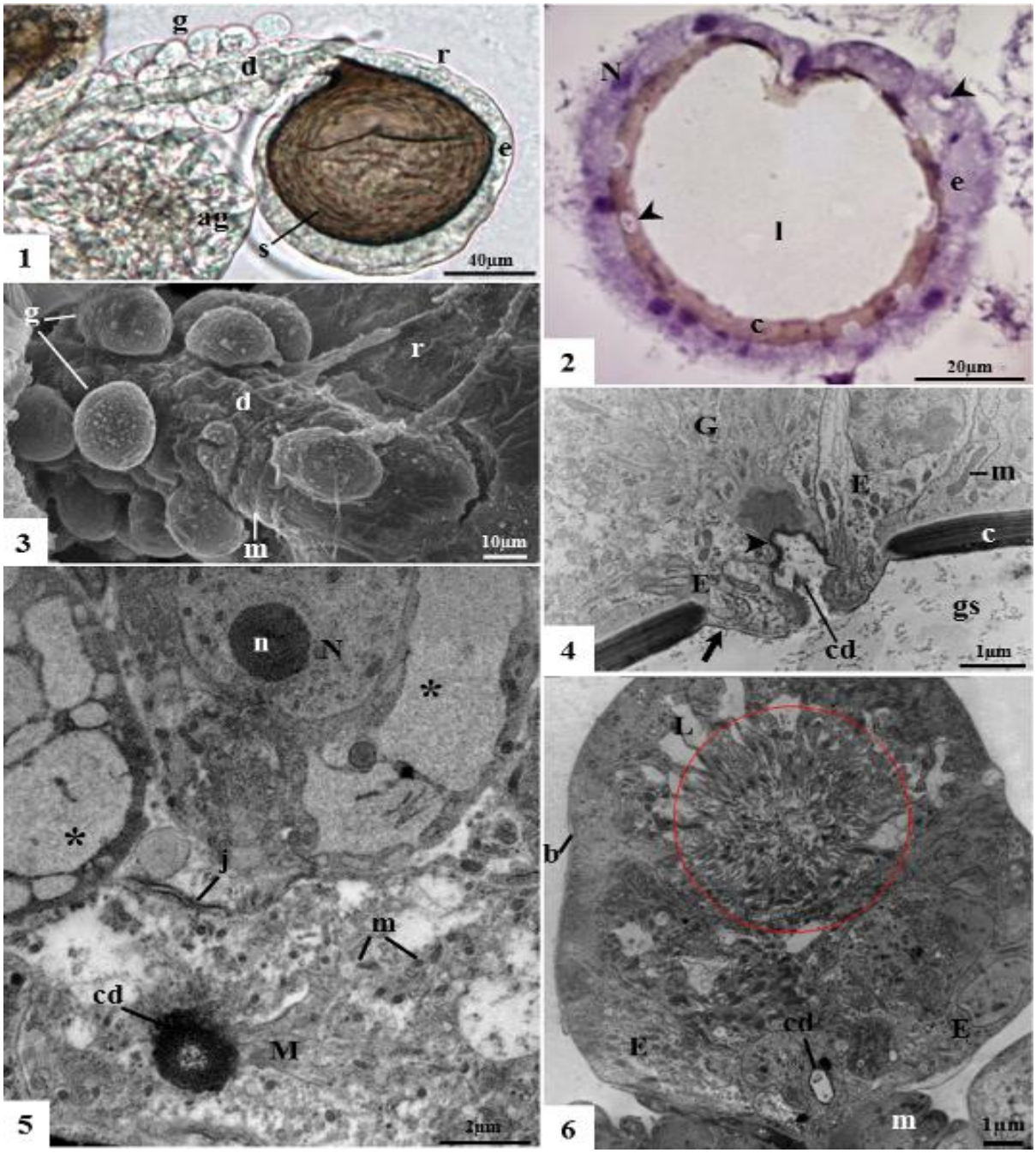

Figure 1. The round sac-like structure of the spermathecal reservoir (r) of the *An. aquasalis* female with a dark-brown cuticular capsule surrounded by the reservoir epithelium (e). The circular orientation of spermatozoa (s) in seen in inseminated female. Duct glandular cells (g) are seen attached to the spermathecal duct (d) surface; ag- accessory gland.

Figure 2. The reservoir lumen (l) is lined by the thick cuticle (c) and the epithelium with prominent glandular cells and their ductules (arrowheads), which co-localize with the cuticle pores; N- reservoir gland cell nucleus. The cell ductule (cd) is lined by the cuticle and is associated to microvilli (M) and mitochondria (m).

Figure 3. The spermathecal duct (d) with attached glandular cells (g) and muscle fibers (m).

Figure 4. The apex of a reservoir glandular cell (G). The cell ductule opens into the cuticle pores of the lamellate cuticle. The arrow points the very thin epicuticle that continues with the cuticle and lines the finger-like projection of the epithelial cells (E). This epicuticle (arrowhead) becomes thicker in the cell ductile bottom. gs- gland secretion.

Figure 5. TEM of the reservoir gland cells tightened by means of septate junctions (j). Note the presence of secretions/nutrients accumulated within cells (*). The cell nucleus and nucleolus (n) are prominent.

Figure 6. TEM of the duct glandular cell with densely package microvilli (red circle) and lacunae (L). Epithelial cells are associated the gland cell lateral sides and ductule; b- basal lamina; p- muscular pump.