

The Fine Structure of the Spermatheca in *Anopheles aquasalis* (Diptera: Culicidae)

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ABSTRACT The vectorial capacity of mosquitoes is related to the reproductive output, and dependent on the ability of male spermatozoa to survive within the inseminated female. Mosquito females mate once, and immediately after mating, the male spermatozoa are transferred to and maintained in the ectodermic spermatheca. Mosquito spermathecae in culicines, especially of the yellowfever mosquito *Aedes (Stegomyia) aegypti* (L.), have been characterized in detail. In contrast, not much is known about this organ in anophelines. Here, the morphology of the spermatheca in the saltwater-tolerant mosquito *Anopheles aquasalis* Curry was investigated for the first time using a combination of light, confocal, and scanning and transmission electron microscopy. The spermatheca in *An. aquasalis* share many features with the three spermathecae present in *Ae. aegypti*, including a round-shaped reservoir and spermathecal duct glandular cells. However, differences such as the volume and cell types, as well as their numbers and distribution, were observed. The most remarkable difference seems to be the absence of a separated glandular unit as seen in *Ae. aegypti*. In *An. aquasalis*, the glandular cells are distributed along the reservoir wall, possibly representing a feature exclusive of anophelines. Together, these glandular cell units constitute the main secretory apparatus of *An. aquasalis*, and are the main source of secretions to nourish and maintain the viability of the gametes during the female's reproductive life span. Understanding the spermathecal organization and function will contribute to understand details of mosquito reproductive biology, and help answer questions related to the reproductive success of these major vectors of pathogens.

KEY WORDS *Anopheles*, mosquito, reproductive system, spermatheca

The insect spermatheca is the female organ responsible for storage and maintenance of male sperm following mating, and thus is vital for insect reproduction. The organization of spermathecae may vary between different species of hematophagous mosquitoes. Whereas in anophelines the spermatheca consists of a single reservoir or capsule (Giglioli 1963), it is composed of three reservoirs in most, but not all, culicines (Jones and Wheeler 1965, Clements and Potter 1967), each consisting of an ectodermal organ with a sac-like reservoir connected to the proximal region of the female reproductive system (e.g., genital chamber) by a spermathecal duct (Giglioli 1963, Clements and Potter 1967, Pascini et al. 2012). Once insects copulate and the male sperm is transferred into the female, the spermatozoa migrate through the duct into the lumen of the spermatheca. Sperm is maintained via the secretion of exocrine glandular cells (also known as spermathecal exocrine glandular apparatus), which is released into the spermathecal lumen. In the spermathecae of the mosquito *Aedes (Stegomyia) aegypti*

(L.), the exocrine spermathecal glandular apparatus is made of individual duct-associated and reservoir-associated gland cells. These gland cells form a glandular unit that separately attaches to the reservoir (Clements and Potter 1967, Pascini et al. 2012).

In general, once insects copulate, spermatozoa migrate to the female's spermatheca and stay there until just before being released for egg fertilization. Hence, the reproductive success of the females depends on the maintenance of suitable conditions of the spermathecal lumen milieu that is believed to be crucial for the gametes protection, maintenance, and viability during the female reproductive life span (Collins et al. 2004, Klenk et al. 2004). The spermatheca also receives the content from the male accessory gland that was shown to alter female behavior, including a reduction in male acceptance by a female after copulation (for details see in Hartmann and Loher 1999, Gillot 2003 and Avila et al. 2010).

Mosquitoes are vectors of pathogens that cause many devastating diseases. In many cases, the ability to control pathogen transmission has been hampered by issues ranging from a complete lack of effective therapy to drug resistance by pathogens. Furthermore, of great significance is the emergence of insecticide-resistant mosquitoes (Foster and Walker 2009). Targeting mosquito reproduction as an alternate ap-

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proach to control mosquito-borne pathogens has been discussed elsewhere (Rogers et al. 2008, Sirot et al. 2011), and the reproductive biology of mosquitoes has been the focus of intensive research (Klowden and Chambers 2004, Catteruccia et al. 2005).

It is unequivocal that the mosquito reproductive output depends on the spermatozoa long-term survival within female spermatheca (Voordouw and Koela 2007, Rogers et al. 2008, Sirot et al. 2011). It is also generally accepted that the high reproductive rate among anophelines is one of the factors associated with their successful role as vectors of human malaria-causing *Plasmodium* sp. Despite this, little is known about either the morphology or the actual role the spermatheca plays in anophelines. Here we described for the first time the general and detailed structure of the spermatheca of the saltwater-tolerant *Anopheles aquasalis* Curry, a mosquito regarded as a potential *Plasmodium* vector in South and Central America (Deane 1986). Our results indicate that in *An. aquasalis*, the spermatheca displays a distinct cellular organization from what was previously reported in *Ae. aegypti* (Jones and Wheeler 1965, Clements and Potter 1967, Pascini et al. 2012), which may relate to taxon-associated differences.

Materials and Methods

An. aquasalis females were obtained from a colony maintained at the Laboratório de Malária (Instituto René Rachou—Fiocruz, Belo Horizonte, MG, Brazil). Three- and 10-d-old females were used. Adult mosquitoes were kept at 25–28°C, 70–80% relative humidity (RH), and a photoperiod of 12:12 (L:D) h. Adults also were maintained on sucrose solution ad libitum.

Fifty unfed females, including virgin and inseminated, were dissected under a stereomicroscope in 0.1 M sodium phosphate buffer (PBS), pH 7.2. The last two abdominal segments were removed from the abdomen with the spermatheca attached. Samples were transferred to fixative solutions containing 4% paraformaldehyde, 0.1 M PBS, pH 7.2 (for light and laser confocal microscopy), and 2.5% glutaraldehyde, 0.1 M cacodylate buffer, pH 7.2 (for electron microscopy) on ice. After dissection, samples were maintained at 4°C in the fixative solution until further use in the experiments described below.

For histology, fixed spermathecae from 10 virgin and 10 inseminated females were rinsed in PBS, dehydrated in crescent series of ethanol (70 to 100%), and embedded in Histoiresin (Leica, Heidelberg, Germany). Thin sections (3 µm) were stained with hematoxylin and eosin, and observed using an optical microscope (Zeiss Primo Star, Zeiss, Oberkochen, Germany). Pictures were taken using a digital camera (Zeiss AxioCamERc5s, Zeiss). Using the same microscope, the cells of the spermathecal glands were counted using whole-mounted spermathecae from six mosquitoes according to their distribution throughout the spermathecal duct and reservoir. Measurements were determined with the software Image ProPlus.

For confocal laser scanning microscopy (CLSM), three virgin and three inseminated spermathecae were washed in PBS and incubated overnight with 0.5% glycine, incubated for 8 h in fluorescent phalloidin-FITC (Sigma-Aldrich, St. Louis, MO) diluted 1:300 in 0.1% Triton X-100 and washed as before. Before analysis, samples were mounted with the antifading solution Mowiol (Fluka, Darmstadt, Germany). Images were obtained using multiple confocal sections from a Zeiss LCM 5100.

In addition, spermathecae from seven virgin and five inseminated, and from two inseminated females were used for scanning (SEM) and transmission electron microscopy (TEM), respectively. Samples were postfixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer at pH 7.2. Samples were dehydrated in increasing concentrations (70 to 100%) of ethanol. For SEM, samples were critical-point dried using CO₂ and sputter coated with gold for observation using a microscope (LEO 1430VP, Germany). Spermathecae from eight females were gently opened with the aid of thin entomological needles before metallization to observe their interior and content.

For TEM, spermathecae were dehydrated and embedded in LR White resin (EMS, Hatfield, PA). Ultrathin sections were stained with uranyl acetate and Reynold's lead citrate. After the staining, the ultra-thin sections were washed in distilled water and analyzed in a Zeiss EM109.

Results and Discussion

It has been shown that the number of reservoirs present in the spermatheca of hematophagous mosquitoes can vary according to species. Culicines such as *Ae. aegypti* display one large and two smaller reservoirs per spermatheca (Pascini et al. 2012), whereas in anophelines such as *Anopheles gambiae* Giles, a single reservoir is present (Giglioli 1963). Similarly, a single reservoir also is present in *An. aquasalis* (depicted in Fig. 1).

The round-shaped spermathecal reservoir (Figs. 1A and 2B) comprises a chamber where the sperm are kept after being transferred from the genital chamber after copulation (Jones and Wheeler 1965). As previously reported in mosquito species, the spermatozoa are stored in a circular fashion in the spermathecal reservoir (Catteruccia et al. 2005, Pascini et al. 2012). Not surprisingly, fertile females of *An. aquasalis* display a similar mode of storage (Figs. 1, 2A, and 3A).

Several factors may influence the number of eggs laid by mosquitoes, including body mass, bloodmeal size, and ambient temperature. Different mosquito species, strain, or both, and whether mosquitoes are maintained in laboratory colonies or originate from natural populations, all may also lead to differences in egg production. In culicines, under laboratory conditions, the number of eggs per gonotrophic cycle was reported to range between 71 and 124 in *Ae. (Stegomyia) aegypti* (Martins et al. 2012), 67–82 in *Aedes albopictus* (Skuse) (Xue et al. 2009), and 192–225 eggs per raft in *Culex quinquefasciatus* Say (Correia et al.

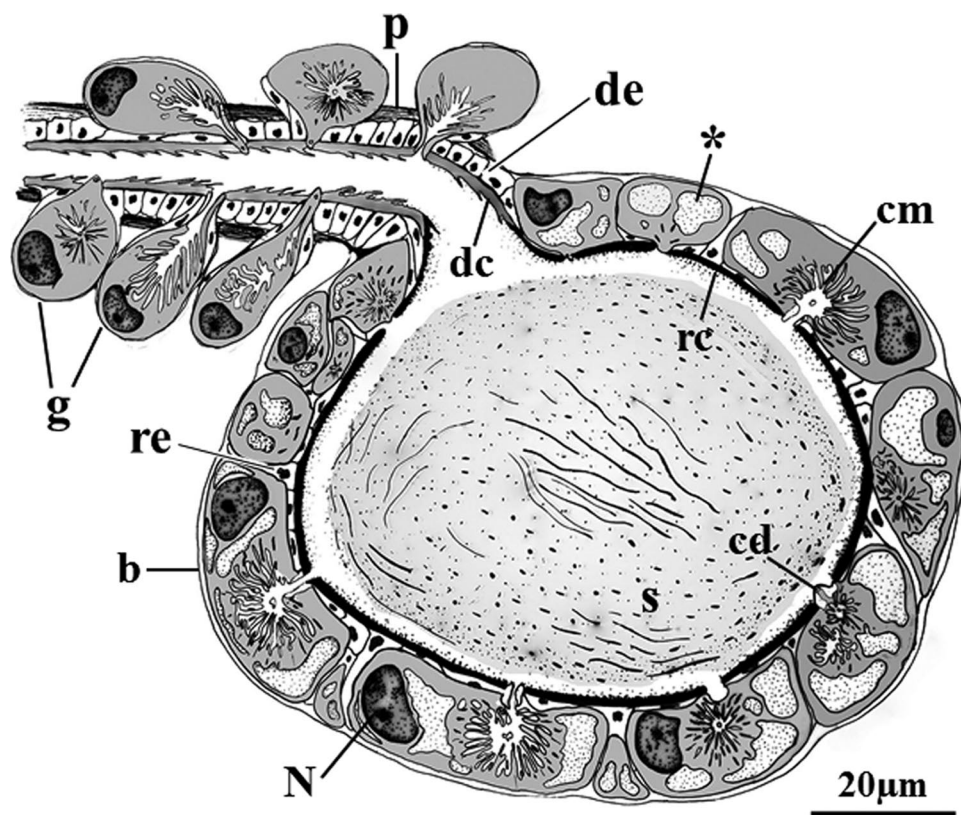


Fig. 1. Schematic drawings of a section of the spermatheca *An. aquasalis* showing the main spermathecal structures. b, basal lamina; cd, cell ductule; cm, cell microvilli; dc, spermathecal duct cuticle; de, spermathecal duct columnar epithelium; g, duct glandular cells; N, gland cell nucleus; p, spermathecal pump; rc, reservoir cuticle; re, reservoir epithelial cell; s, sectioned spermatozoa into the reservoir lumen. An asterisk (*) marks the content and nutrient stored inside the reservoir gland cell before being secreted.

2012). In anophelines, the number of eggs ranged from 57 to 62 per gonotrophic cycle in *Anopheles bellator* Dyar & Knab (Chadee 1999) and in *Anopheles homunculus* Komp (Chadee et al. 1998), and an average number of 64.4 eggs per female in caged house-collected *An. gambiae* (Fritz et al. 2008) and 96.8 in laboratory-reared *Anopheles stephensi* Liston (Suleman 1990). In wild-caught *An. aquasalis* females, 50–118 matured follicles were observed in the ovaries during each gonotrophic cycle (Chadee and Mohammed 1996), but the actual number of eggs laid by this mosquito still needs to be determined. Hence, the number of egg laid per gonotrophic cycle in culicines appears higher than in anophelines, possibly as a reflection of the higher storage capacity (of sperm) in the three spermathecal capsules of culicines.

The spermathecal reservoir of *An. aquasalis* has a diameter of $82.15 \pm 1.83 \mu\text{m}$, with a total volume of $\approx 2.9 \times 10^5 \mu\text{m}^3$. In other species of anophelines, such as *Anopheles merus* Dönitz, the estimated spermathecal volume is $7.3 \times 10^5 \mu\text{m}^3$ (White and Muniss 1972), and varies in different populations of the *An. gambiae* complex from 7.0×10^5 to $1.6 \times 10^6 \mu\text{m}^3$ (Clarke 1971, White and Muniss 1972). In the three spermatheca of *Ae. aegypti*, the combined volume is $9.6 \times 10^5 \mu\text{m}^3$

(Jones and Wheeler 1965). The capacity for storage of sperm in *Ae. aegypti* is significantly higher than in *An. aquasalis* (roughly 3.3-fold), and perhaps with the exception of some members of the *An. gambiae* complex, it also appears to be greater than in several anopheline species. Whether this represents a true distinction between culicines (*Ae. aegypti*) and anophelines still needs to be confirmed.

The spermathecae in *Ae. aegypti* and *An. aquasalis* differ with respect to epithelial cell types as well as the thickness of the reservoir wall. In *Ae. aegypti*, two cell types are observed in the reservoirs, the most common of which are flattened cells that cover the reservoir wall; furthermore, irregular shaped cells are located underneath the gland (Curtin and Jones 1961, Clements and Potter 1967, Jones and Fischman 1970, Pascini et al. 2012). The thicker wall of the reservoir of *An. aquasalis* has glandular (Figs. 2C and D and 3A) and long and flattened irregular-shaped epithelial cells that are located between and underneath the glandular cells (Fig. 3A and B).

As described for *Ae. aegypti* (Clements and Potter 1967) and other insects (Lawson and Thomas 1970, Dallai et al. 2012), the reservoir cuticle in *An. aquasalis* has a lamellate distribution, with five layers of elec-

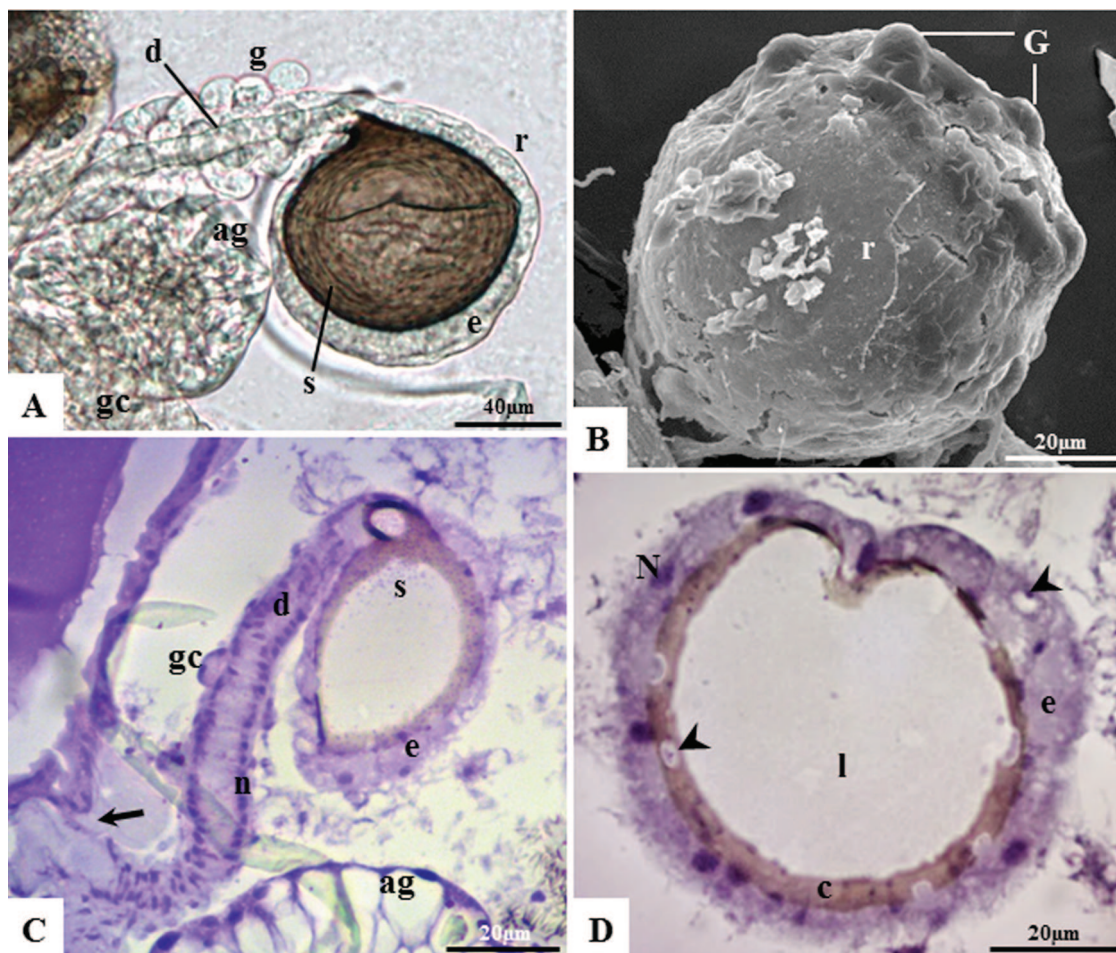


Fig. 2. Views of the spermatheca of *An. aquasalis*. (A) The round sac-like structure of the spermathecal reservoir (r) with a dark-brown cuticular capsule surrounded by the reservoir epithelium (e). The circular orientation of the spermatozoa (s) is seen in the interior of the reservoir. The spermathecal duct (d) connects the reservoir to the genital chamber (gc) and is in proximity to the accessory gland (ag). Duct glandular cells (g) are attached to the duct surface. (B) SEM of a spherical-shaped reservoir (r) with prominent glandular cells (G). (C and D) Histological sections. In (C), the spermathecal duct connects the reservoir to the genital chamber (arrow); in (D), the reservoir lumen (l) is lined by the thick cuticle (c) and the epithelium with prominent glandular cells and their ductules (arrowheads), which are associated with the cuticle pores. N, reservoir gland cell nucleus; n, duct epithelial cell nucleus; s, cross-sectioned spermatozoa. (Online figure in color.)

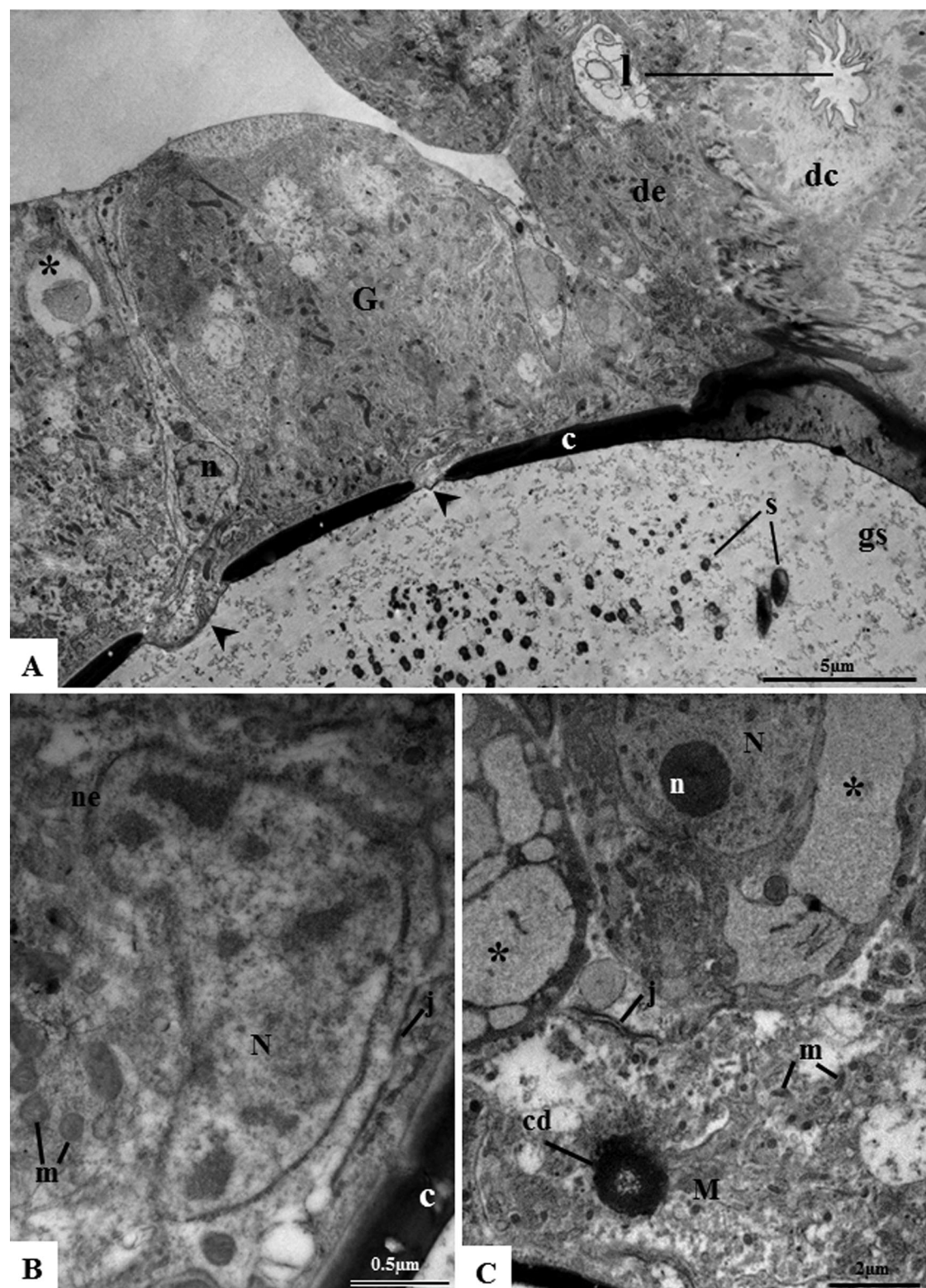
tron-dense materials that alternate with electron-translucent layers (Fig. 4A). The outer epicuticle deposited in the reservoir cuticular shell is thick; however, it becomes thin when it extends above the tips of the epithelial and glandular cell that project toward the reservoir lumen (Fig. 4A). We find it interesting that at the exact point of intersect with the ductile bottom of the gland cell the epicuticle thickens, and can be observed (in whole mounts) as brown dots in the center of the cuticular pores of the reservoir (Fig. 4A and B).

The spermathecal duct in *An. aquasalis* is shorter, at 142 μm in length, than the duct in *Ae. aegypti*, which is 265 μm in length (Jones and Wheeler 1965, Pascini et al. 2012), and it is roughly 2.58 μm thick. Such shorter length places the duct closer to the genital

chamber (Figs. 2A and 5A and B) and may be related to a faster migration of the male sperm into the spermathecal reservoir after copulation (Jones and Wheeler 1965).

The inner surface of the duct is lined with a thick cuticle ($1.27 \pm 0.03 \mu\text{m}$), with projections toward the duct lumen. This cuticle is narrower at the point of attachment of the spermathecal duct with the reservoir and lacks the lamellae organization seen in the cuticular reservoir shell (Figs. 1 and 3A). In addition, it is composed by two electron-dense layers separated by a thick electron-translucent layer (Fig. 3A), and as reported elsewhere (Clements and Potter 1967).

Similar to *Ae. aegypti*, the spermathecal duct epithelium in *An. aquasalis* is composed of columnar cells with a cytoplasm-filled nucleus (Fig. 2C). External to



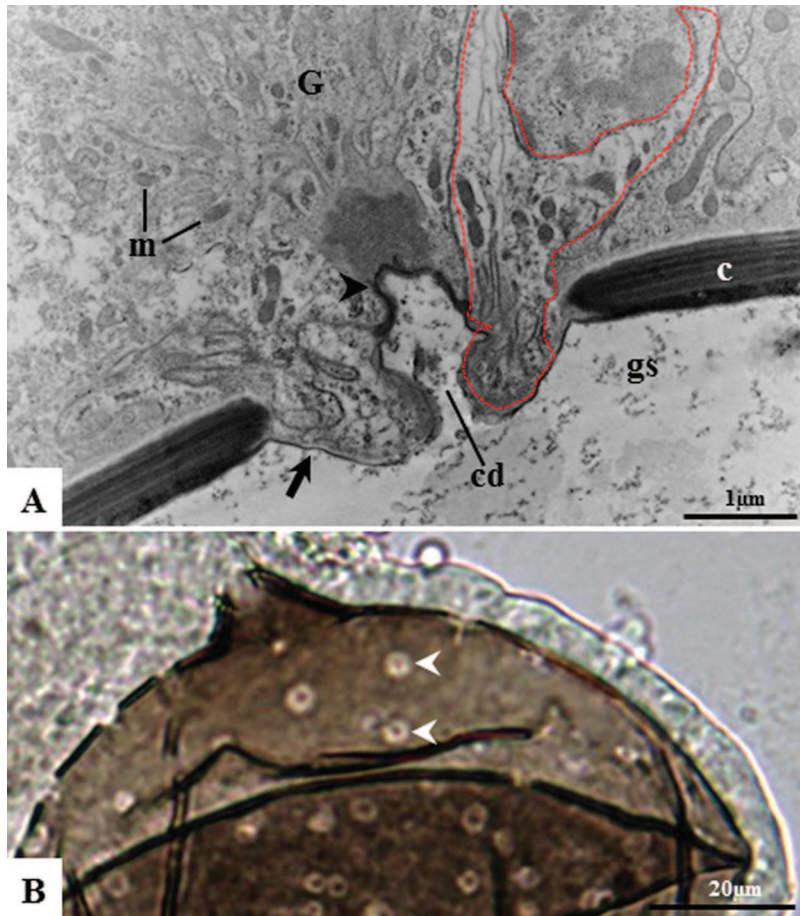


Fig. 4. Detailed views of exocrine glandular cells of the spermatheca of *An. aquasalis*. (A) Apex of a reservoir glandular cell (G). The cell ductule (cd) opens into the cuticle pores of the lamellate cuticle (c). The arrow points to the thin epicuticle that continues with the cuticle and lines the finger-like projection of the epithelial cells (red dotted line). This epicuticle (arrowhead) becomes thicker in the cell ductule bottom. gs, gland secretion; m, mitochondria. (B) Cuticular pores (white arrowheads) are seen with dots in their center in an empty spermathecal reservoir. (Online figure in color.)

the duct epithelium, a layer of elongated muscle fibers corresponding to the spermathecal pump is found between the glandular cells of the duct (Figs. 1 and 5A and B; Curtin and Jones 1961, Clements and Potter 1967, Pascini et al. 2012). These are organized in helical fashion (Fig. 5C). In *An. aquasalis*, this muscular apparatus runs along the length of the duct, as observed in *An. gambiae*, where it controls the filling of

the reservoir after mating and the release of spermatozoa for egg fertilization (Giglioli 1963).

The glandular cells of insect spermathecae were shown to be crucial in *Drosophila melanogaster* Meigen for sperm-supportive functions and activation before fertilization (Wolfner 2011). In *An. aquasalis*, these exocrine glandular cells can individually attach to the spermathecal duct, and they are also observed

Fig. 3. TEM of the spermatheca of *An. aquasalis*. (A) Aspects of sectioned reservoir and duct. The reservoir epithelium is composed of glandular (G) and thin epithelial cells (red dotted lines). The cross-sectioned spermatozoa (s) and the glandular secretion (gs) are seen in the reservoir lumen. The electron-dense reservoir cuticle (c) has pores that are lined by the epicuticle (arrowheads). The spermathecal duct is lined by the thick electron-lucent cuticle (dc) with a three-layer laminated aspect and the duct epithelium (de). Furthermore, this cuticle has many projections toward the duct lumen (l). Note the presence of secretions and nutrients accumulated within cells (*; also present in C). (B) Detail of the epithelial cell located just underneath the gland cell and in contact with the cuticle. j, septate junctions; m, mitochondria; N, nucleus; ne, nuclear envelope. (C) Detail of reservoir gland cells tightened by means of septate junctions. The cell nucleus and nucleolus (n) are prominent. The cell ductule (cd) is lined by the cuticle and is associated with the microvilli (M) and mitochondria.

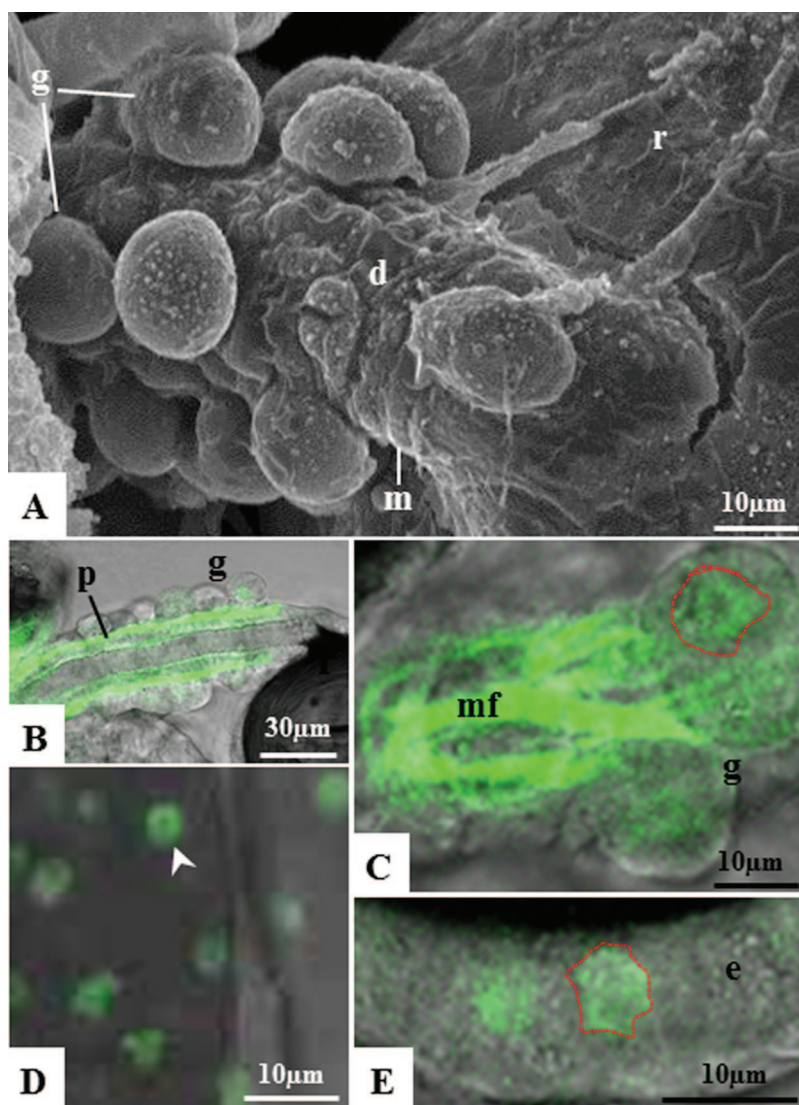


Fig. 5. The muscular and glandular apparatus in the spermatheca of *An. aquasalis*. (A) SEM of the spermathecal duct (d) with attached glandular cells (g) and muscle fibers in *An. aquasalis*. (B–E) CLSM analyses. (B and C) Cytoskeleton staining reveals the spermathecal duct muscle layer that constitutes the spermathecal pump (p). Muscle fibers (mf) are distributed in a helical arrangement. (D and E) Reservoir gland cells are positive for the staining similar to duct gland cells in (B). Arrowhead and areas encircled by red represent glandular cell regions that correspond to the cell ductule. e, reservoir wall epithelium. (Online figure in color.)

as part of the reservoir wall in addition to the epithelial cells (Figs. 1, 2D, and 3A). Either attached to the spermathecal duct, or a part of the wall reservoir, the spermathecal gland cells have well-developed nuclei (Fig. 3C).

The reservoir glandular cells can be seen protruding from the reservoir wall mainly near the point of attachment of the duct (Fig. 2B). In fractured samples, as well as in thin sections, glandular cell secretions originally extruded through cuticular pores can be observed inside the reservoir lumen (Figs. 4A and 6A and B). These secretions frequently accumulate as

flocculent deposits above the internal smooth surface of the reservoir (Fig. 6A).

In the spermathecal duct of *An. aquasalis*, an average of 14.12 ± 0.22 glandular cells are attached to the duct wall, and are mainly concentrated at the location where the duct attaches to the reservoir (Figs. 1, 2A, and 5A). A similar distribution of the glandular cells was observed in *An. gambiae* (Giglioli 1963). In *Ae. aegypti*, however, glandular cells are more abundant and spread farther from the duct-reservoir attachment point (Jones and Fischman 1970, Pascini et al. 2012). The large number of duct-associated glandular cells in

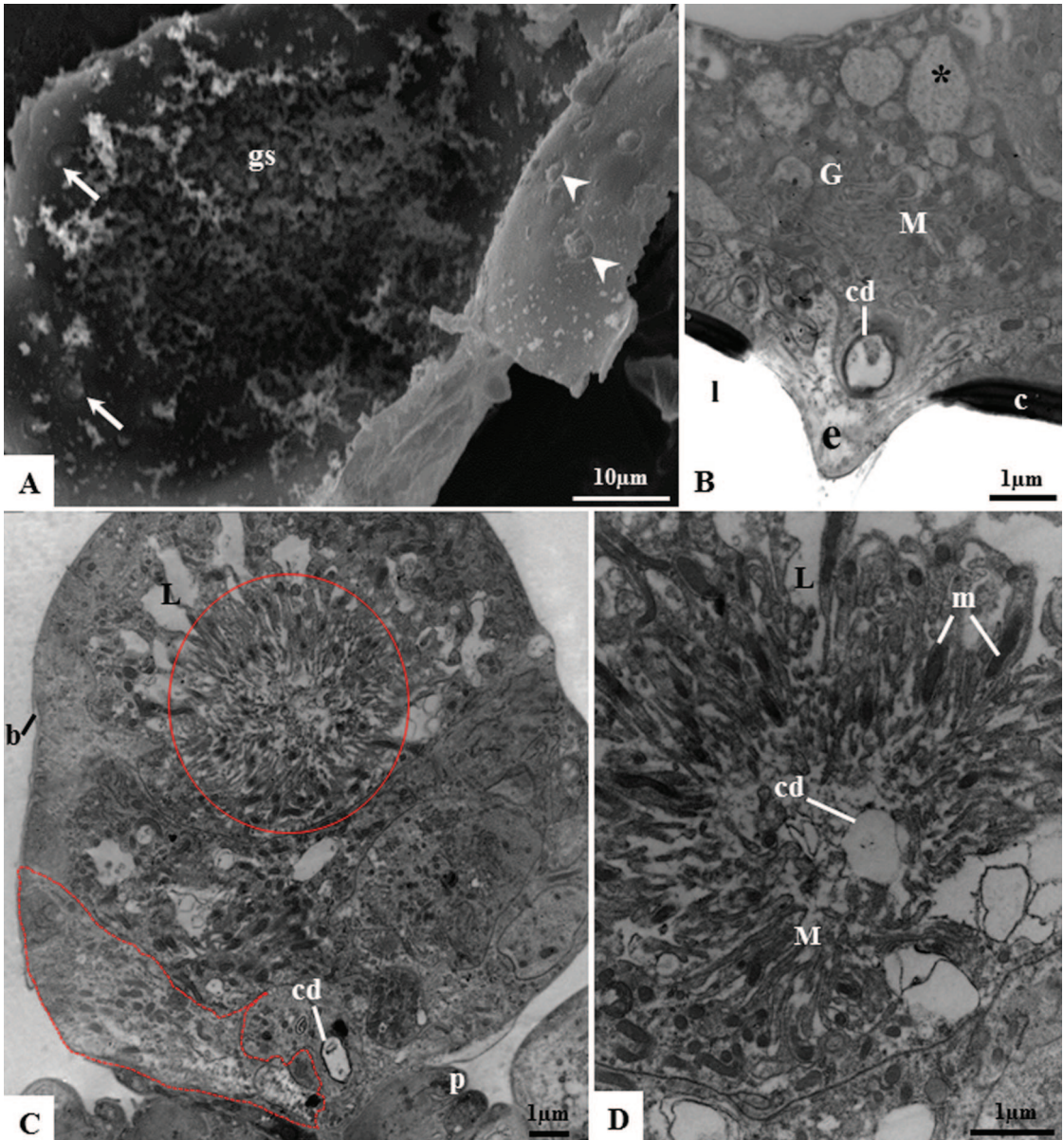


Fig. 6. Details of the reservoir and duct glandular cells in the spermatheca of *An. aquasalis*. (A) SEM showing the cuticular pores in the reservoir cuticle with (arrows) and without (arrowheads) extruding secretion in a virgin female. The glandular secretion (gs) accumulates inside the organ's lumen as flocculent material. (B–D) TEM detailed views of exocrine glandular cells. (B) Glandular cell apex with the ductule (cd) and the epithelial cell projection (e) toward the reservoir lumen. M, microvilli; *, stored cell content and secretion. (C) Duct glandular cell with densely package microvilli (red circle) and lacunae (L). Epithelial cells (red dotted line) are associated with the lateral sides of the gland cell and ductile. b, basal lamina; p, muscular pump. (D) Detailed view of the duct glandular cell apex with long and densely package microvilli and several mitochondria and the cell ductule. (Online figure in color.)

the spermatheca of *Ae. aegypti* and their wider distribution may be important for providing the gametes with their secretory products during the gametes migration inside the spermathecal ducts (Schoeters and Billen 2000), as they travel relatively longer distances from the reservoir lumen to the genital chamber in comparison with *An. aquasalis*.

The number and distribution of glandular cells in the spermathecal reservoir also varies between different mosquito species. Whereas *An. aquasalis* has an average of 95.37 ± 4.64 reservoir glandular cells, in *Ae. aegypti* this number ranges from 33 to 43 glandular cells in the two smaller spermathecal glands, and 70–85 in the large spermathecal gland (Pascini et al.

2012). In addition, in *An. aquasalis*, the glandular cells are distributed along the reservoir wall (Figs. 2D and 3A), in striking contrast to the well-defined spermathecal gland represented by a cluster of swollen cells located externally to the reservoir wall near the transition between the duct and the reservoir in *Ae. aegypti* (Curtin and Jones 1961, Clements and Potter 1967, Pascini et al. 2012). Both forms of glandular cell arrangement, either distributed on the wall of the spermatheca reservoir or as individualized in a glandular configuration, have been previously reported in insects (Lawson and Thomas 1970, Hartmann and Loher 1999, Lay et al. 1999, Schoeters and Billen 2000, Martins and Serrão 2002, Gobin et al. 2006, Dallai et al. 2008, Martins et al. 2008).

With respect to the ultramorphology, reservoir gland cells in *An. aquasalis* have well-developed structures where glandular secretion is stored before being released into the reservoir lumen (Figs. 1, 3C, and 6B). The gland cell secretory content appears with slightly electron-dense granules, as seen in the reservoir and gland cell ductule lumens (Figs. 4A and 6B). In addition, extensive septate junctions are observed at the attachment point of the reservoir gland and the epithelial cells, and between them (Fig. 3B–C). Cell ductules connect the gland cells to the duct and reservoir lumens. These ductules are lined by a thin epicuticle that is continuous with the reservoir cuticle by means of its own epicuticle (Figs. 4A and 6B and C). The cell ductules also extend toward the inside of the gland cells, and are associated with the microvilli (Figs. 4A and 6B–D). This feature is commonly found in glandular spermathecal cells in insects in general (Lawson and Thomas 1970, Stacconi and Romani 2011, Pascini et al. 2012).

The glandular cell microvilli are more developed in the duct gland cells and are packed tightly together, particularly where they converge with the cell ductule (Fig. 6C), in accordance with previous studies (Curtin and Jones 1961, Clements and Potter 1967, Pascini et al. 2012). These microvilli are also associated with the mitochondria (Figs. 3C, 4A, and 6D), described in a number of spermathecal glands in insect spermathecae to be related to the active transport of secretory substances toward the spermathecal lumen (Hartmann and Loher 1999, Lay et al. 1999, Fritz and Turner 2002). The presence of large amounts of actin in the gland cell apex, surrounding the secretory ductules (Fig. 5C–E), further supports the existence of such a mechanism of transport (Fritz and Turner 2002, Pascini et al. 2012).

The openings of the ductules into the reservoir lumen are associated with the translucent cuticle pores (Fig. 4B). Under the TEM, these pores appear to be lined by a thin epicuticle that is continuous with the spermathecal reservoir cuticle. The ductule openings project toward the reservoir lumen together with the laterally associated epithelial cells (Fig. 4A) that surround the gland cell ductules through “finger-like” cellular processes (Figs. 4A and 6B; Dallai et al. 2012). The outer epicuticle at the terminal end of the reservoir cell ductule thickens again (Fig. 4A and B), and

we suspect it may be important for the attachment of the glandular cell microvilli.

This study represents the first morphological description of the spermatheca of *An. aquasalis*. Despite the difference in terms of number of reservoirs observed in the spermatheca of anopheline vs. culicine mosquitoes (normally three in Culicinae and one in Anophelinae), *An. aquasalis* shares several features regarding the organization of its spermatheca with those from *Ae. aegypti* (Curtin and Jones 1961, Clements and Potter 1967, Jones and Fischman 1970, Pascini et al. 2012). In both species, the spermathecae are composed of an almost perfectly round dark-brown reservoir or capsule connected to the genital chamber by a translucent spermathecal duct. The reservoir is lined by a thick lamellate cuticle, continuous with the cuticle of the spermathecal duct, and the spermathecal duct is also lined by thick cuticle covered externally by an epithelium. The round-shaped glandular cells are individually attached to the duct, and in inseminated females, the male spermatozoa stored inside the spermatheca appear as parallel bundles. However, many differences in the architecture of the spermathecae were observed between mosquito species representative of anophelines and culicines. Such differences include the length of the spermathecal duct, the total organ volume, as well as cell types, number, and distribution. For instance, the analyses of the composition of the spermathecal cells in *An. aquasalis* revealed that the distribution of epithelial and glandular cells constitute the most remarkable difference between this species and *Ae. aegypti* spermatheca. Hence, in *An. aquasalis*, the release of glandular secretions occurs throughout the spermathecal reservoir and not in the specific regions of the glandular units as in *Ae. aegypti*.

In female insects, the viability of the spermatozoa is likely maintained by a combination of factors that include isolating the spermatozoa inside the spermathecal reservoir wall and by the spermathecal glandular secretions that provide nourishment to the spermatozoa in the spermathecal lumen (Dallai et al. 2012, Pascini et al. 2012). From the observations made in the study, these crucial steps also take place in *An. aquasalis*. In copulated *An. aquasalis* females, the spermatozoa are isolated inside the reservoir lumen by a thick cuticle and by a thick reservoir wall that likely is impermeable to the hemolymph. Thus, gametes receive secretions and nutrients produced and secreted into the reservoir lumen by the spermathecal glandular cells. However, although it is clear that the spermatheca is necessary for sperm maintenance, how this is actually accomplished by the many cells that are part of the spermatheca is not yet clear.

Mosquito control is crucial for prevention of many diseases caused by pathogens transmitted by vector species. While understanding details of mosquito reproduction may provide new mechanisms for control of mosquito populations, much remains to be clarified with respect to spermatozoa viability during the female life span. For instance, how the female spermatheca protects spermatozoa from free radicals (e.g.,

ROS) generated after the successive blood acquisitions is still unresolved. As understanding morphology can assist in understanding function, identifying aspects of spermathecal morphology in *An. aquasalis* may provide insights on how spermatozoa viability is maintained and possibly provide new clues on how to reduce mosquito fecundity.

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