Structure and function of the spermathecal complex in the phlebotomine sandfly *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae): I. Ultrastructure and histology

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Females of phlebotomine sandflies (Diptera: Psychodidae) possess highly variable spermathecae that present several important taxonomic characters. The cause of this diversity remains a neglected field of sandfly biology, but may possibly be due to female post-mating sexual selection. To understand this diversity, a detailed study of the structure and function of the spermathecal complex in at least one of the species was a prerequisite. Using scanning and transmission electron microscopy, described here is ultrastructure of the spermathecal complex in the sand fly, *Phlebotomus papatasi* Scopoli. The spermathecal complexes are paired; each consists of a long spermathecal duct, a cylindrical spermathecal body, and a spherical spermathecal gland. Muscle fibres, nerves, tracheoles, and vascular sinuses connect the spermathecal body and duct through the epithelial layers. Spermathecal gland is formed by a typical insect epidermis and consisting of an epithelial layer of class-1 epidermal cells and elaborate glandular cells of class-3 epidermal cells, each having both receiving and conducting ductules (i.e. "end apparatus") and a "cytological apodeme", which is a newly described cell structure. The spermathecal body and duct are lined by class-1 epidermal cells and a cuticle, and are enveloped by a super-contracting visceral muscular system. The cuticle consists of rubber-like resilin, and its fibrillar arrangement and chemical nature are described. A well-developed neuromuscular junction exists between the spermathecal gland and the spermathecal body, which are connected to each other by a nerve and a muscle. The spermathecal complexes of the sandfly are compared with those of other insect species. The physiological role and possible evolutionary significance of the different parts of spermathecal complex in the sandfly are inferred from the morphology and behaviour. Post-mating sexual selection may be responsible for the structural uniqueness of the spermathecal complex in phlebotomine sandflies.

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1. Introduction

The spermatheca plays a critical role in insect reproduction because sperms can be stored there in a viable condition for a long time, ensuring an efficient use of sperm during fertilization (Parker 1970). The ultrastructure of spermathecae in various insect species has been studied either as important reproductive structures in their own right or as specialized epidermal glands (Clement and Potter 1967; Gupta and Smith 1969; Jones and Fishman 1970; Happ and Happ 1970; Dallai 1975; Filosi and Perotti 1975; Grodner 1978; Kokwaro *et al* 1981; Sareen *et al* 1989; Fritz and Turner 2002). In recent years, however, attention has shifted from purely structural studies to functional morphological ones within the framework of post-copulatory sexual selection (i.e. female mecha-

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nisms influencing paternity success, Eberhard 1996). Post-copulatory sexual selection involves a wide range of male and female processes including sperm competition (i.e. the competition in a female's genital tract for ovum fertilization among the sperm of different males, Parker 1970); sperm displacement (i.e. the replacement at a subsequent mating of stored sperm from a previous mating, Walker 1980; Ridley 1989); cryptic female choice (i.e. non-random paternity biases resulting from the female morphology; physiology or behaviour that occur after coupulation, Pitnick *et al* 1999; Pitnick and Brown 2000); and evolutionary arms races (i.e. the conflict between sexes for the control of reproduction, Holland and Rice 1998; Arnqvist *et al* 2000; Ilango and Lane 2000).

For the medically important phlebotomine sandflies, of which some species cause leishmaniasis in Asia, Africa, the Middle-East, Southern and Eastern Europe, and Latin America, a deeper understanding of the spermathecal structure and function is relevant for two reasons. Firstly, in contrast to some other groups of Diptera, in which the spermathecal structure is highly conserved, sandflies exhibit an extraordinary morphological diversity, suggesting rapid divergence at the species level. Because of this, it is an important character for taxonomy (Theodor 1965) and phylogenetic systematics (Ilango 2004). There is great variation among subgenera, but very little within species of the phlebotomine sandflies. Secondly, as in other insect species, the spermathecal complex has a central role in reproduction as sperms are held in it, hyperactivated, and perhaps modified in some way so that they can subsequently gain entry through the micropyle and fertilize ovum (Davey and Webster 1967; Degrugillier and Leopold 1976; Miya 1982).

The objective of this study was to determine the structural characteristics (using light microscopy and scanning and transmission electron microscopy), and to deduce the function of different parts of the spermathecal complex in *Phlebotomus papatasi*, which is a proven vector of *Leishmania major* mostly in the sub-tropics of the Old World, but only rarely in the tropics.

2. Materials and methods

2.1 *Sandfly samples*

Materials used for this study came from a laboratory colony of *P. papatasi*, which was maintained at 26°C and 75% relative humidity at the London School of Hygiene and Tropical Medicine, London, UK, and came originally from Israel. Three-day-old adult female flies were used, which had been sugar-fed (30%, weight in mg/volume in ml) and had access to males, and two days later were given a blood meal. A detailed study of the spermathecae was made from freshly prepared specimens using a dissecting microscope and subsequently a Polyvar phase-contrast microscope.

2.2 *Preliminary processing*

All flies were immobilized and inactivated at 0°C for 5 min, dipped in 0·05% Tween 80 detergent soap solution to wet them, washed three-times in distilled water, and dried with tissue paper. All dissections were performed in normal saline (0.15 M NaCl) solution. The terminal abdominal segments were removed to study the spermathecae, using fine blades cut from razor blades or micro-entomological pins attached to wooden handles. The spermathecal complex was placed in a drop normal saline on clean slide and was pressed gently with cover glass to prepare squash. Movements and other physical properties of the spermathecae were also observed in normal saline. Subsequently, the materials were processed for either light microscopy or electron microscopy as detailed below.

2.3 *Light microscopy*

To resolve the three-dimensional structure of the spermathecae within the abdomen as a prelude to electron microscopy, a histological study by light microscopy was undertaken. The terminal abdominal segments were removed, serially dehydrated in an ethanol series, and embedded in wax. Transverse sections of 1 µm thick were cut, dewaxed, and stained with haematoxylin and eosin.

In addition, the toluidine blue test (Neville 1970; Jones and Fishman 1970) was used to detect the presence of resilin in the cuticle. The terminal abdominal segments were fixed in normal saline solution, dehydrated in ethanol, and embedded in paraffin wax. Sections of 1 µm thick were stained with 1% toluidine blue in 1% aqueous borax for 1 min, dehydrated, and mounted in Canada balsam or DPX. Photographs were taken with a polyvar phase-contrast light microscope using Kodak colour and black-and-white 35 mm film.

2.4 *Electron microscopy*

2.4a *Scanning electron microscopy*: Sandflies were dissected at room temperature in normal saline (0.15 M NaCl) to expose the spermatheca, furca, and overlying tissues, such as the accessory glands and ovaries, which were carefully removed. Specimens were fixed by gradually replacing the saline solution with a solution of 3% glutaraldehyde in saline at room temperature to avoid a sudden contraction of the spermatheca before finally fixing them in 3% glutaraldehyde in 0·075 M sodium cacodylate buffer at 4°C for 1 h. After washing in 0·075 M

sodium cacodylate buffer/0·2 M sucrose (pH 7·4) at 4°C for 1 h, specimens were post-fixed in 1% osmium tetroxide in 0·075 M cacodylate buffer followed by distilled water, each at 4°C for 30 min. The specimens were then dehydrated in graded acetone solutions at room temperature (70%, 80%, 90%, 100%, 100%: 10 min in each grade), before they were carefully transferred to small porous pots in acetone for critical point drying (CPD750 critical point drier). The specimens were then mounted on aluminium stubs with double-sided cellotape and sputter-coated on an Eduardo S150 Sputter coater with a thin-layer of gold. The spermathecae were examined with a JEOL25 III scanning electron microscope and photographed with Kodak Technical Pan Film TP120.

2.4b *Transmission electron microscopy*: Entire sandfly specimens were fixed in 3% glutaraldehyde in 0·075 M sodium cacodylate buffer (pH 7·4) at 4°C overnight. After fixation, they were washed in washing buffer (at 4°C); post-fixed in 1% osmium tetroxide in 0·075 M sodium cacodylate buffer at 4°C for 1–2 h; washed in distilled water, and block stained (2% uranyl acetate in 30% methanol). Specimens were then dehydrated in graded methanol (30%, 60%, 70%, 80%, 90%, 100%, 100%; 10 min in each grade) and transferred to propylene oxide (= 1,2-epoxy propane) for 20 min. Because the spermathecal complex is lined with an impermeable cuticular intima, which could prevent infiltration, the following combinations of epoxy resin and propylene oxide were used: 25% and 75% for 30 min, 50% and 50% for 1 h, 75% and 25% for 1 h before the 100% resin infiltration. Infiltration with 100% resin was done overnight (at 4°C), before embedding the specimens in fresh resin and polymerising them at 60°C for 48–72 h. Sections 35–40 nm thick were cut on an LKB Ultratome III using glass knives, placed on 200 mesh copper grids (with and without formvar film), and stained with Reynold's lead citrate for 5 min before examination with a JEOL 100CX microscope.

3. Results

3.1 *General description from the light microscopy study*

The spermathecal complex (figures 1A and 2) consists of a pair of golf club-like structures situated between the abdominal segments 7–9 and lying ventral to the rectum. Paired long spermathecal ducts originate separately from the bursa copulatrix, and each duct runs cranially to attach to the base of a small cylindrical chitinous structure, called the spermathecal body. A thin epithelial layer envelops each spermathecal body and spermathecal duct. A spherical mass of large secretory cells surrounds the apical end of each spermathecal body and forms the spermathecal gland. The spermathecal ducts are 0·13 mm long and 4 µm wide, the narrow lumen of each duct being constant over the duct's entire length. The spermathecal body is a chitinous, capsular and apparently segmented structure. Cuticular ductules are seen as hair-like processes on the head of the spermathecal body. The spermathecal duct, the spermatheca proper, and the presumed spermathecal gland collectively form the spermathecal complex. Their microstructure is described below.

3.2 *Scanning electron microscopy of the spermathecal complex*

The external features of the spermathecal complex cannot be resolved easily with light microscopy, but are well imaged by the scanning electron microscopy (SEM). For example, the well differentiated epithelial cells and muscle fibres of the spermatheca are contiguous with the bursa copulatrix, and, therefore, are of presumed ectodermal origin. The muscle fibres are alternately arranged with epithelial layers to form an interwoven network (figure 3A).

The spermathecal body is a sub-cylindrical, 18 µm long structure that is oriented at an angle of ca. 90° to the spermathecal duct. Both the epithelial layer and the muscle fibres are continuous with the spermathecal duct leading to the bursa copulatrix. The epithelial cells are spherical, 2·6–3·6 µm in diameter, and arranged in rows that run parallel to the muscle fibres. The basement membrane of the epithelial layer is deeply convoluted and has a nodular appearance. The thick, 2·5–5 µm wide muscle fibres run the length of the spermathecal body and are striated with repeating units at 2·5 µm intervals, which presumably indicate the state of contraction of the muscles.

A peripheral motor-nerve fibre, originating from the seventh abdominal nerve ganglion, is connected to each spermatheca near the junction of the spermathecal gland and spermathecal body (figure 3B). Each nerve fibre is divided into several branches, which spread over the spermatheca and spermathecal duct to connect with the muscle fibres. A network of tracheoles covers the spermathecal body and spermathecal duct. In addition, a tubular, 1 µm wide structure runs in a zig-zag course from the spermatheca to the base of the spermathecal gland. This tube is known as the "vascular sinus" and has also been found in grain weevils *Sitophilus granarius* (Tombes and Roppel 1971).

The spermathecal gland is a spherical structure of 30–36 µm in diameter. On the dorsal surface of the gland, the basement membrane is superficially convoluted and free of any tissue attachments. The ventral surface of the

spermathecal gland is closely applied to the apex of the spermatheca and is supplied by muscle and nerve fibres, tracheoles, and vascular sinuses that originate from the spermatheca.

3.3 *Transmission electron microscopy of the spermathecae*

3.3a *Macroscopic internal structure*: The spermathecal complex, like the integument of insects, is composed of a cuticle, which is a multilayered extracellular composite material, and an underlying epidermis, which secretes the several layers of the cuticle. The spermathecal complex, as revealed by electron microscopy, includes (i) a spermathecal gland with a cluster of elaborate secretory cells (figures 11 and 6A, B), (ii) a sub-cylindrical hollow spermatheca (figure 4B, D) with a spongy cuticular mass at its apex (figures 4B, 8A, B), and (iii) a long, narrow duct (figure 4A). Both the duct and spermatheca are lined by a cuticle of varying thickness with an epithelial covering

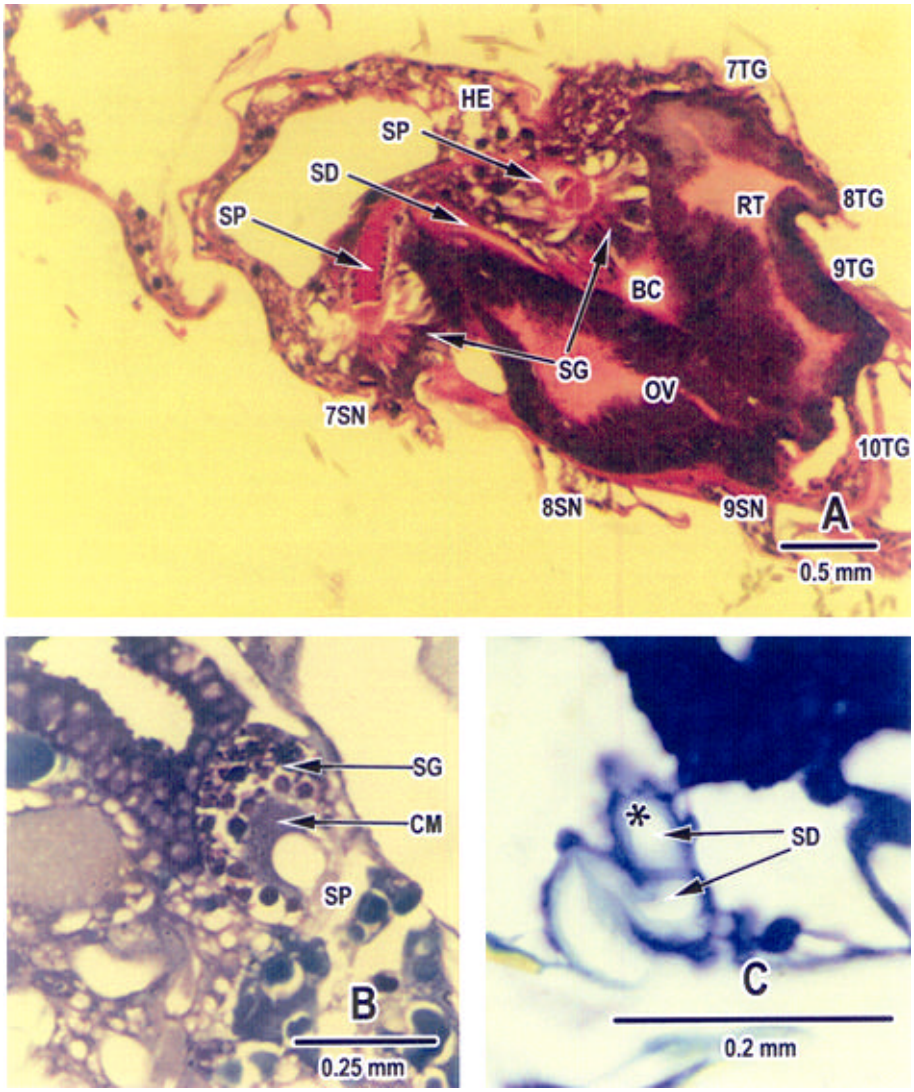

Figure 1. Light Microscopy of the spermathecal complex. (**A**) Longitudinal section of posterior abdominal segments, stained in haematoxylin and eosin, to show the internal organs. BC, bursa copulatrix; HE, haemocoel; OV, oviduct; RT, rectum; SG, spermathecal gland; SP, spermathecal body; SD, spermathecal duct; SN (7, 8, 9), sternites; TG (7, 8, 9, 10), tergites. (**B**) Longitudinal section of the cuticular mass (CM), which is located between the spermathecal glands and at the apex of the spermathecal body (SP); it is stained with toluidine blue for the presence of resilin. (**C**) Cross section of the spermathecal duct (SD) showing the presence of resilin (*) stained with toluidine blue.

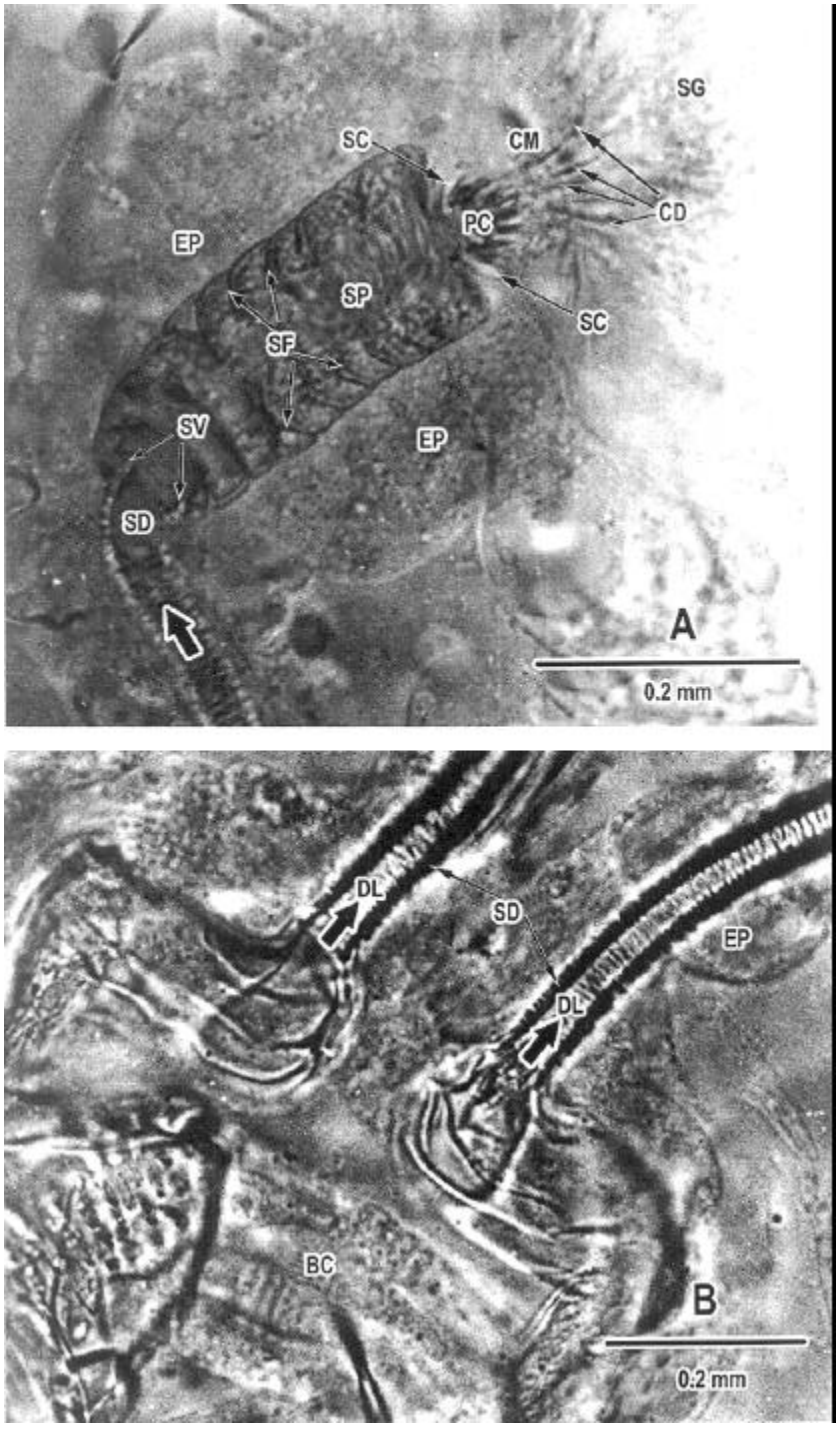

Figure 2. Saline squashes showing a single spermatheca and the paired spermathecal ducts. (**A**) In the spermathecal spermathecal gland (SG), a cluster of large cells with hair-like processes (CD) open into a large canal (PC) through the cuticular mass (CM) and flanked by narrow peripheral canals (SC). The cylindrical body of the spermatheca (SP) is segmented by a series of internal septal folds (SF), and its base is separated from the long narrow spermathecal duct (SD) by a sphincter valve (SV). The whole structure is supported by a thin epithelial layer (EP) and muscle fibres, which are difficult to differentiate. (**B**) A pair of spermathecal ducts (SD) lined by epithelial layer (EP) are attached to the bursa copulatrix (BC). The two ducts are separated and the duct lumen (DL, arrows) is of uniform width throughout.

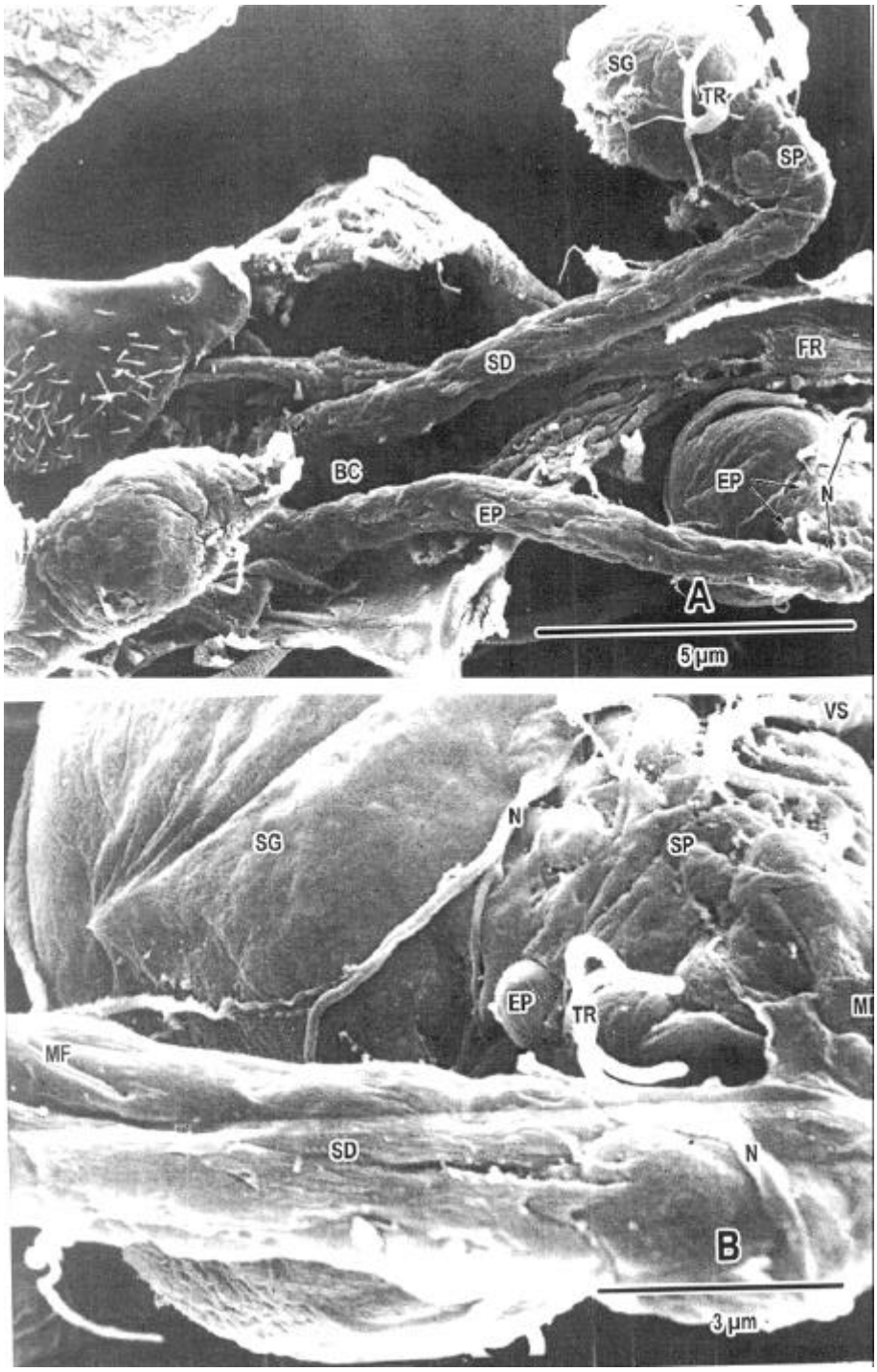

Figure 3. (**A**) SEM of the isolated spermathecal complex. The spermathecae (SP) are capped with spherical spermathecal gland (SG) and epithelial cells (EP) which also cover the spermathecal ducts (SD). Each spermatheca is supplied by corrugated tracheoles (TR), and a pair of straight motor nerves (N). The dorsal side, or roof, of the bursa copulatrix (BC) is connected with the spermathecal ducts, and its floor extends cranially as a large genital furca (FR). (**B**) SEM of the spermathecal gland and the spermathecal body. The outer surface, or basal membrane, of the spermathecal gland (SG) is smooth. The basal region of the spermathecal gland, the spermathecal body and the spermathecal duct are supplied by nerves (N), tracheoles (TR), and a dilated thick vascular sinus (VS). Note the thick visceral muscle fibres (MF) and globular epithelial cells (EP) supporting the spermathecal body and spermathecal duct (SD).

and a network of muscle fibres, nerve fibres, and tracheoles. The sandfly spermatheca shares many features with other insect spermathecae but has also distinctive features, such as the extraordinarily variable pattern of the cuticular intima, which differs from one part of the spermatheca to the other. Hence, the histological description of the spermathecal complex is organized in the following sequence.

(i) *Spermathecal duct*: Structure and physical properties of the cuticle in the spermathecal duct and spermatheca, including the structure of the surrounding epithelial layer and muscle fibres.

(ii) *Spermatheca*: The cuticular mass and its tubules and canals, the body of the spermatheca with its cuticular wall and its folding, and the basal sphincter valves.

(iii) *Spermathecal gland*: Secretory glandular cells, receiv-

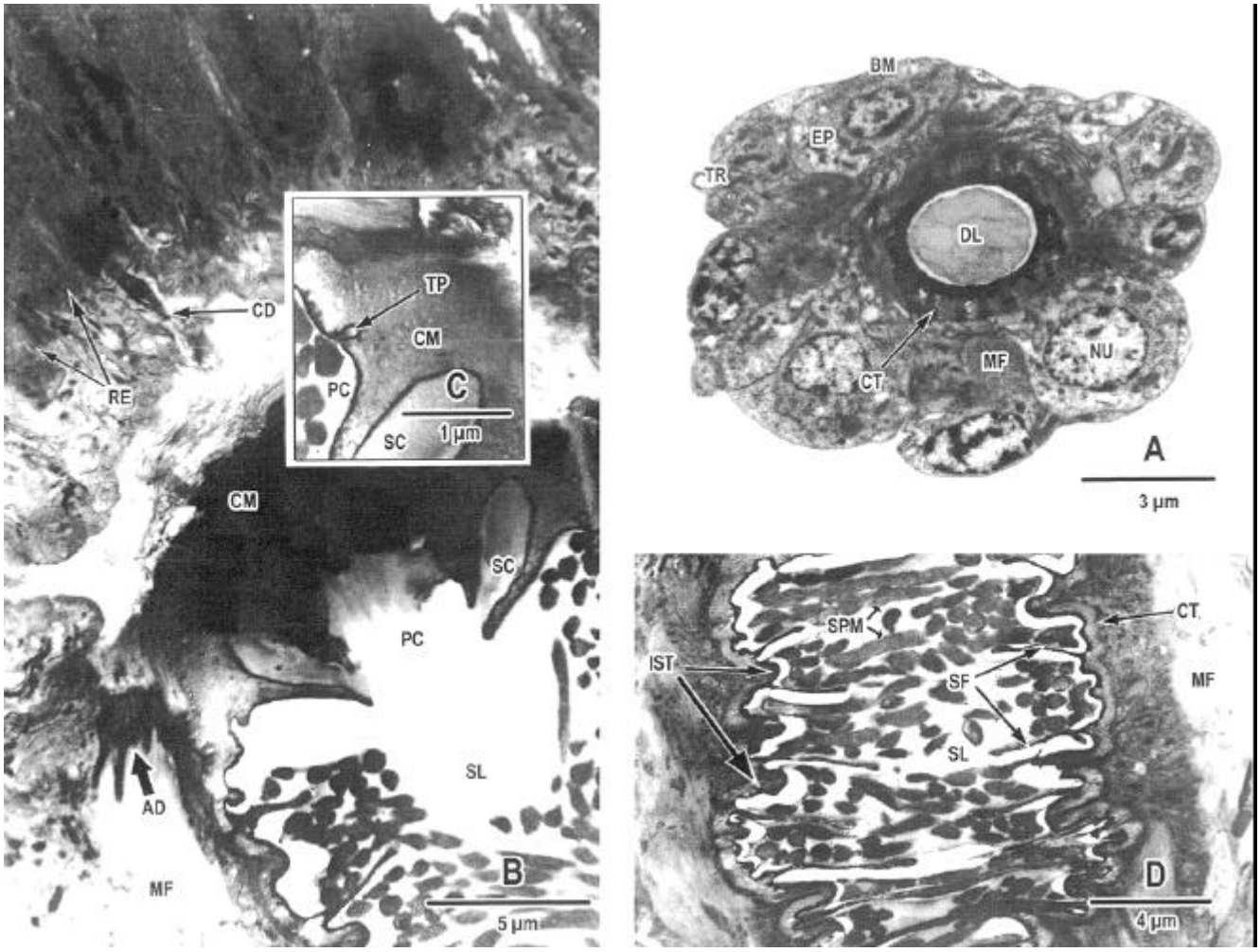

Figure 4. TEM of the spermathecal complex. (**A**) Cross section of the spermathecal duct showing the entire cuticle and epidermis. Note the circular lumen (DL) of the spermathecal duct lined by a thin cuticle (CT) and surrounded by a single layer of epithelial cells (EP) interlaced with muscle fibres (MF) and connections of tracheoles (TR). BM, basement membrane; NU, nucleus. (**B**) Longitudinal section of the apex of the spermathecal body. Note the row of the reservoirs (RE) of secretory glandular cells (see figure 11) connected to a hemispherical cuticle-mass (CM) through cuticular ductule (CD); underneath a dome-like primary canal (PC) is flanked by a secondary canal (SC). Beneath is the lumen (SL) of segmented spermathecal body packed with spermatozoa. On either side of the cuticle-mass is a cuticular apodeme (AD) to which longitudinal visceral muscle fibres (MF) are attached. (**C**) Longitudinal section of the cuticular ductules (DC). The cuticular ductules run from glandular cells through the cuticular mass (CM) and enter the primary canal (PC) via terminal pits (TP) (also see figure 8A). (**D**) Longitudinal section of the spermathecal body. The cuticle (CT) of the body wall is folded at regular intervals and compartmentalized the spermathecal lumen (SL) either as long septal folding (SF) or short interseptal foldings (IST). Thick, striated, visceral muscle fibres (MF) run along-side the cuticle wall, terminating at an apodeme at the apex (see **B**). At the base, the spermathecal body wall converges with a relatively thick folding of the cuticle supported by circular muscle fibres forming a sphincter-like valve separating the spermathecal lumen from the spermathecal duct (see figure 3D, Ilango 2005).

ing and conducting ductule (i.e. end apparatus), and cellular junctions.
(iv) *Associated structures*: The muscular system and neuromuscular junctions.

The terminology and classification of the cuticle, epidermal glands, cell junctions, muscle fibres, and neuromuscular junctions follow those of Filshie (1982), Noirot and Quennedey (1974, 1991), Satir and Gilula (1973), Edler (1975) and Osborne (1975).

3.4 *The spermathecal duct*

The spermathecal duct is circular or oval in cross-section (figure 4A) with a diameter of 4 µm. Its lumen is lined by a cuticle that is surrounded by an epithelial layer interlaced with muscle fibres. The duct cuticle and its epithelial layer and muscular system are actually an extension of the bursa copulatrix. The thickness of the cuticle, including its associated derivatives, differs from one part of the spermathecal complex to another: namely, (i) between the duct base and the base of the spermathecal body, the cuticle is even and smooth; (ii) between the spermathecal base and spermathecal apex, it forms septa and interseptal foldings; (iii) at the apex of the spermathecal body, the cuticle is cup-like and invaginated to form a primary cavity and secondary cavities; and (iv) the cuticular ductules of the glandular cells are specialized to form the "end apparatus".

3.4a *Structure of the cuticle*: The cuticle of the wall of the spermathecal duct (figure 5A) is 1 µm thick along its entire length up to the point where it joins the base of the spermathecal body. The ultrastructure of the cuticle appears similar to that of the sternite exocuticle of meal worm beetle *Tenebrio molitor* (Filshie 1982). There are two main horizontal layers in the cuticle of the spermathecal duct: The thin- and densely-stained epicuticle is a non-chitinous superficial layer facing the duct lumen; and the thick fibrous layer of procuticle is composed of protein and the polysaccharide α-chitin next to the epidermal cells. The epicuticle is 77 nm thick and can be differentiated, in order of their deposition, into the cuticulin layer: namely, the inner epicuticle; the outer epicuticle; and the superficial layers. The cuticulin layer is a 10 nm thick, electron-dense membrane made up of lipoprotein, and is the first layer of the cuticle to be secreted. The inner epicuticle is 25–90 nm thick, stains less strongly for protein than the cuticulin layer, and is situated between this and the procuticle. Furthermore, the epicuticle contains fine granular substances, which are presumably protein, lipid, lipoprotein, and oxidised phenols, and extends deeply into the procuticle which is an electron-lucent layer forming an alternate dark and light radiating pattern. The most superficial layer of the cuticle is the outer epicuticle, which is ca. 10 nm thick and lipid in nature.

The procuticle (figure 5A), lying deep to the epicuticle, consists of protein and polysaccharide α-chitin. The procuticle stained negatively (i.e. reduced staining) with uranyl acetate – a promising technique used by Rudal (1969) for the study of the fine structure of microfibrils in the inner layer of *Calliphora* puparium and exhibited a fibrillar pattern. These micro-fibres are composed of aggregates of micro-fibrils measuring 385 nm–500 nm in length with a uniform diameter of approximately 3 nm. The observed longitudinal orientation of the micro-fibrils along the axis of the spermathecal duct revealed that they are embedded in an electron-dense matrix (figure 5A, asterisk). This is consistent with the findings of Filshie (1982) that while the electron-dense material consists of protein, the electron-lucent micro-fibrils consist of chitin. Micro-fibrils were found only in the chitin-containing regions. The arrangements of the micro-fibrils and matrix are shown in detail in figures 5A and 8B.

The cuticle in the spermathecal wall (figure 5B) is less thick than that in the wall of the spermathecal ducts. It covers a large area because of the repeated infoldings, which increases the inner surface of the spermathecal body. Structurally, it includes a thin, electron-dense, 63 nm–156 nm thick cuticulin layer of the epicuticle and a relatively thick fibrous, 1 nm thick electron-lucent layer of the procuticle. The cuticulin layer of the epicuticle alone extends outwards to form a series of finger-like projections within the lumen of the spermathecal body. The heavy staining property with osmium uranyl acetate suggests that this layer consists of either lipoid or lipoprotein.

At the apex of the spermathecal body and lumen (figures 4B and 8B), a hemispherical block, or cuticular mass, is ca. 9 µm deep and 18 µm wide and is composed of soft procuticle containing rubber-like chitin and a 0·5 µm thin-layer of epicuticle. The cuticular mass appears to be composed of long micro-fibrils, and their arrangement within the matrix can be deduced from the arcuate patterns which appear as alternating light and dark bands running parallel or nearly parallel to the outer surface (i.e. the lumen of primary canals and secondary canals of the cuticular mass). To account for this arcuate pattern, there are two contrasting models proposed for the insect cuticle (Filshie 1982): (i) The dark bands may contain filaments that are oriented horizontally, and the light intermediate zones may contain arched filaments lying in parallel, oblique planes and meeting neighbouring dark bands; or (ii) arched, interband fibres may not exist, but the appearance of arcs in sections may be produced by the projections of short fibres lying in horizontal planes. Fibres are parallel to one another in a single plane, and the fibre orientation within successive planes rotates counter

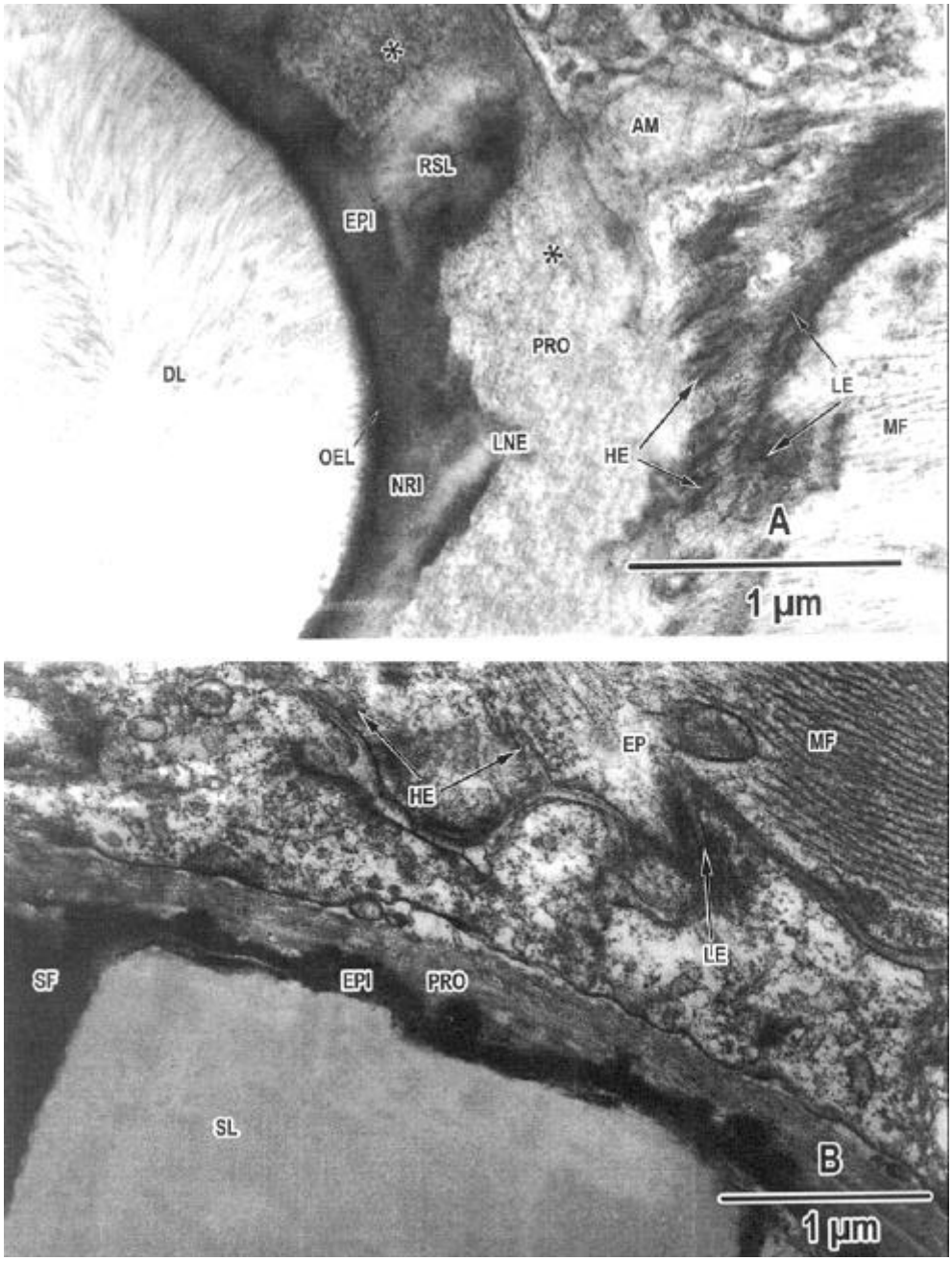

Figure 5. TEM of the spermathecal cuticle. (**A**) Cross section of cuticle of the spermathecal duct. The electron-dense, thin epicuticle (EPI) lying next to the lumen (DL) is differentiated into an outer epicuticle (OEL), a middle layer of inner epicuticle (NRI), and the cuticulin layer (LNE), which is continuous with the resilin layer (RSL). Note the thick layer of procuticle (PRO) in which the microfibrils of chitin appear as electron-opaque white patches surrounded by an electron-dense matrix of protein (*). Applied to the cuticle is the apical membrane (AM) of the epithelial cells supported by hemidesmosomes (HE), belt desmosomes (LE), and, below that, the myofibrils (MF). (**B**) Cross section of cuticle from the spermathecal body wall. The epicuticle (EPI) is relatively thicker than found in the duct and is thrown up from the procuticle (PRO) to form the finger-like projections of the septa (SF) within the lumen of the spermathecal body (SL). The procuticle (PRO) is relatively thin, within it the microfibrils can be seen running perpendicular to the axis of body of the spermatheca. Note the underlying epidermal cell (EP) and muscle fibres (MF) firmly attached to the cuticle by hemidesmosomes (HE) and belt desmosomes (LE).

clockwise by a small constant angle downward through the cuticle. An 180° rotation of the fibre orientation produces a single lamella. This is commonly known as the helicoidal model and can be found in many other biological materials such as in cholesteric liquid crystals, and is universally favoured as a description of insect and other arthropod cuticle.

Yet another important finding in the cuticular mass is the presence of networks of pore canals that maintain a direct connection between the epidermis and the lumen of the spermathecal body (figure 8B). The pore canals are connected to epicuticular channels in the epicuticle. They are initially direct cytoplasmic extensions of the epidermal cells, but often these extensions are withdrawn, leaving behind an apparently clear channel containing one or more electron-dense pore canal filaments. In some instances, micro-fibrils and matrix material partially or completely fill the channels so that these micro-fibrils become oriented perpendicularly to those in the cuticle.

The presence of resilin in the spermathecal complex is confirmed by alkaline toluidine blue stain – the cuticle in the wall of the spermathecal body and cuticular mass (figure 1B) and in the spermathecal duct (figure 1C) stained blue. Furthermore, the outer layer of the cuticle in the spermathecal duct appears pale blue, whereas the inner layer of the duct appears dark blue (figure 1C). The elasticity of resilin allows the spermathecal body and duct to adjust their shape under pressure from the muscular system. When the spermatheca is treated with chemicals, such as chloroform, or subjected to sudden significant temperature changes, an irreversibly contracted state result, which is often seen in taxonomic preparations. In response to milder temperature changes, the spermatheca readily recovers and returns to its original configuration. Under mechanical pressure (from a sharp-tipped needle, for example), the spermathecal duct lengthens about 1·5–2 times, because of the elasticity of the duct cuticle.

3.4b *Structure of epidermal cells in the spermathecal complex*: Three types of epithelial cells have been identified in the insect epidermis and spermathecae (Noirot and Quennedey 1974, 1991). The cells found around the spermathecal body and duct are typical class-1 cells, while the spermathecal gland (described below) is composed of class-3 cells, which are often associated or interspersed with class-1 cells.

The epidermal class-1 cells in the spermathecal duct (figure 4A) are simple relative to the glandular class-3 cells. The apical cell membrane is tortuously folded and attached to the base of the endocuticle. Belt desmosomes are often seen and act as anchoring points for microfilaments, which commonly occur between the apical membrane and the cuticle. The lateral walls of adjacent cells are linked by several kinds of specialized junctions. The most common contact sites between cells are septate junctions, but where two adjacent cell membranes are folded like loops at regular intervals, they are bound by hemidesmosomes (figure 5A).

The basal membrane exhibits a limited degree of infoldings and is covered by a network of tracheoles and nerve fibres. The cytoplasm of class-1 cells contains mitochondria of various sizes and membrane-limited vacuoles with unknown contents. No traces of Golgi bodies were observed. At the basal region of the cell is a relatively large, elongated nucleus with a well-defined nucleolus and nuclear membrane.

3.5 *Spermathecal body*

Light microscopy of potassium hydroxide (KOH)-treated specimens or of fresh preparations suggests that the spermathecal body is segmented and apically bears structures resembling a collar and a pit with hair-like processes (figure 2A). TEM in this study has revealed that the spermathecal body in fact has a hemispherical cuticular mass (figure 8B). In longitudinal (figures 4B and 8B) and transverse sections (figure 8A), the cuticular mass is penetrated by several tubules from the glandular units of the spermathecal gland. At the base of the cuticular mass (figures 4B and 6A), a deeply excavated circular cavity occurs – the primary cavity – which is surrounded by a collar-shaped peripheral cavity, the secondary cavity. Each tubule of the cuticular mass terminates in tiny, oval pits, the terminal pits (figure 4C), and open into the primary cavity. Both cavities are continuous with the lumen of the spermathecal body. Thus, the head of the spermathecal body seen in KOH-treated or fresh specimens is a solid cuticular mass and the collar is in fact a space, not a flange as often described (Theodor 1965) (see figure 11).

In a transverse section, the cylindrical spermathecal body (figure 4D and also see figure 3D of Ilango 2005) has a series of regular, finger-like foldings that protrude from the wall into the lumen. There are two types of cuticular wall foldings: (i) a slender septal folding that is 1 µm long with blunt tips; and (ii) stout, conical interseptal processes that are 0·25 µm long. The two types of projections are arranged in an alternating fashion. Under transmission light microscopy of either KOH-prepared or fresh specimens (figure 2A), the spermathecal body appears segmented with transverse septa. Clearly, these transverse septa are slender septal foldings that do not completely separate the spermathecal body into segments. In a three-dimensional model, the spermathecal body would resemble a stack of car tires with a large central lumen.

At the junction of the spermathecal duct and the main body of the spermathecae, the cuticle is folded and underlain by a ring of muscle that could act as a sphincter (figure 2A). Such a valve could control the passage of incoming or released sperm.

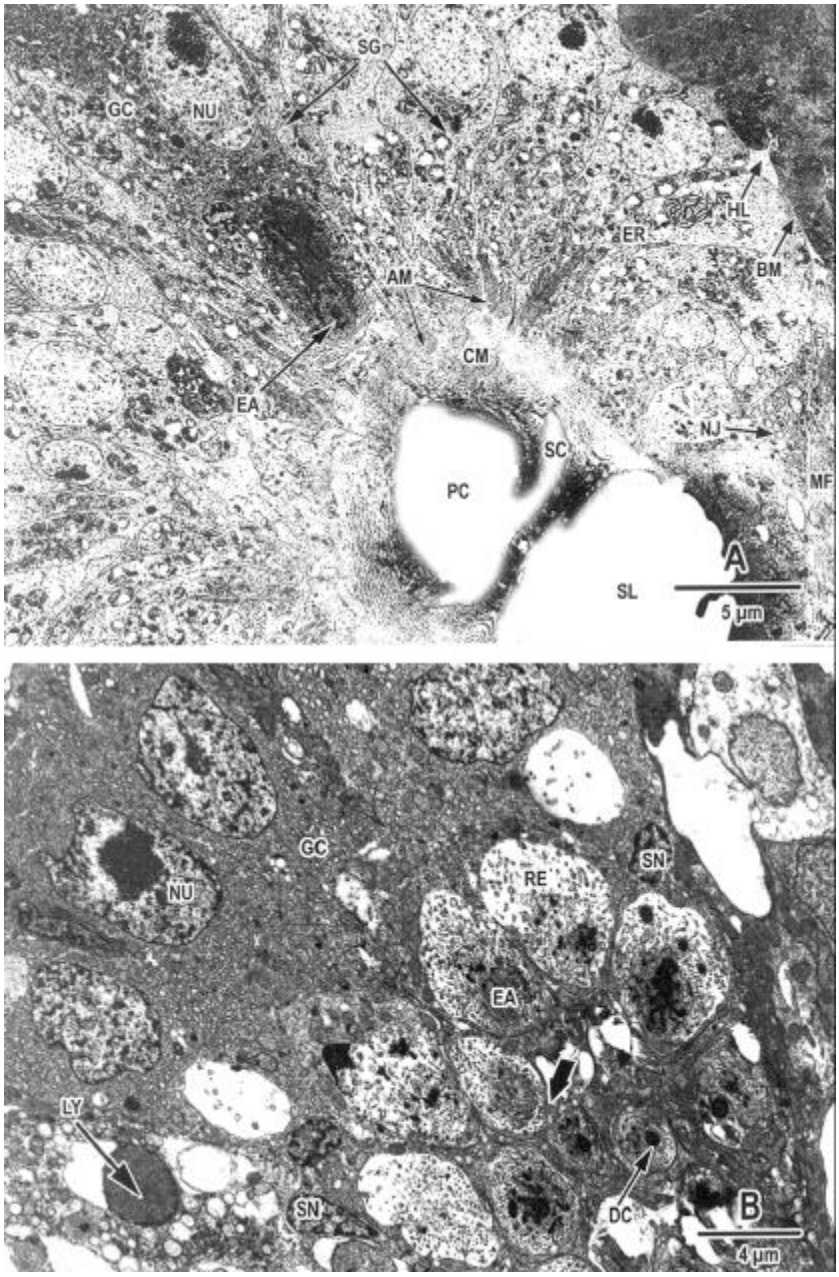

Figure 6. TEM of the spermathecal gland. (**A**) Transverse section at low magnification of the spermathecal gland. The spermathecal gland (SG) is composed of a cluster of type-3 glandular cells (GC) interspersed with simple epithelial cells. The basal membrane (BM) of the glandular cells is in direct contact with the haemolymph (HL). Below the gland is the cuticular mass (CM) in which the microfibrils are formed into arcuate patterns (for details see figure 8) and below that the primary canal (PC) of the spermatheca. The apical membrane (AM) of the gland cells develops into microvilli (AM). On the basal margins of the gland, motor nerves and muscle fibres (MF) form a neuromuscular junction (NJ). EA, end apparatus; ER, endoplasmic reticulum; SL, spermathecal lumen. (**B**) Transverse section of the spermathecal gland at about 45° showing glandular cells (GC). Each cell has well defined central reservoir (RE) and end apparatus consisting receving canal (EA), duct cell (DC). Between the glandular and duct cells are compact epidermal cells with a small nucleus (SN). A lysosome-like structure (LY) is found in the glandular cell that contains large nucleus (NU). Closed arrow indicates the marigin between the glandular cell and the duct cell.

3.6 *The spermathecal gland*

The spermathecal gland (figure 6) is a bulb-like structure consisting of clusters of glandular units that are lined with class-3 glandular cells and are 16–27 µm long. Between the glandular cells are a few small class-1 epidermal cells. Each glandular unit consists of a large primary terminal cell that is 22 µm long and a small duct cell that is 5 µm long and attached to the upper surface of the cuticular mass of the spermathecal body. A small cuticular ductule leads from the primary glandular cell through the cuticular mass to the lumen of the spermathecal body. The primary gland cell (figure 6) has an invaginated intracellular reservoir, which is 9–13 µm long and lined with microvilli, into which a cuticular ductule opens from the duct cell. They collectively form an "end apparatus" (Noirot and Quennedey 1974). The cuticular ductule that abuts the microvilli is a type of loose, sponge-like or perforated cuticle. The cuticular ductule comprises the receiving ductule that extends into the reservoir of the

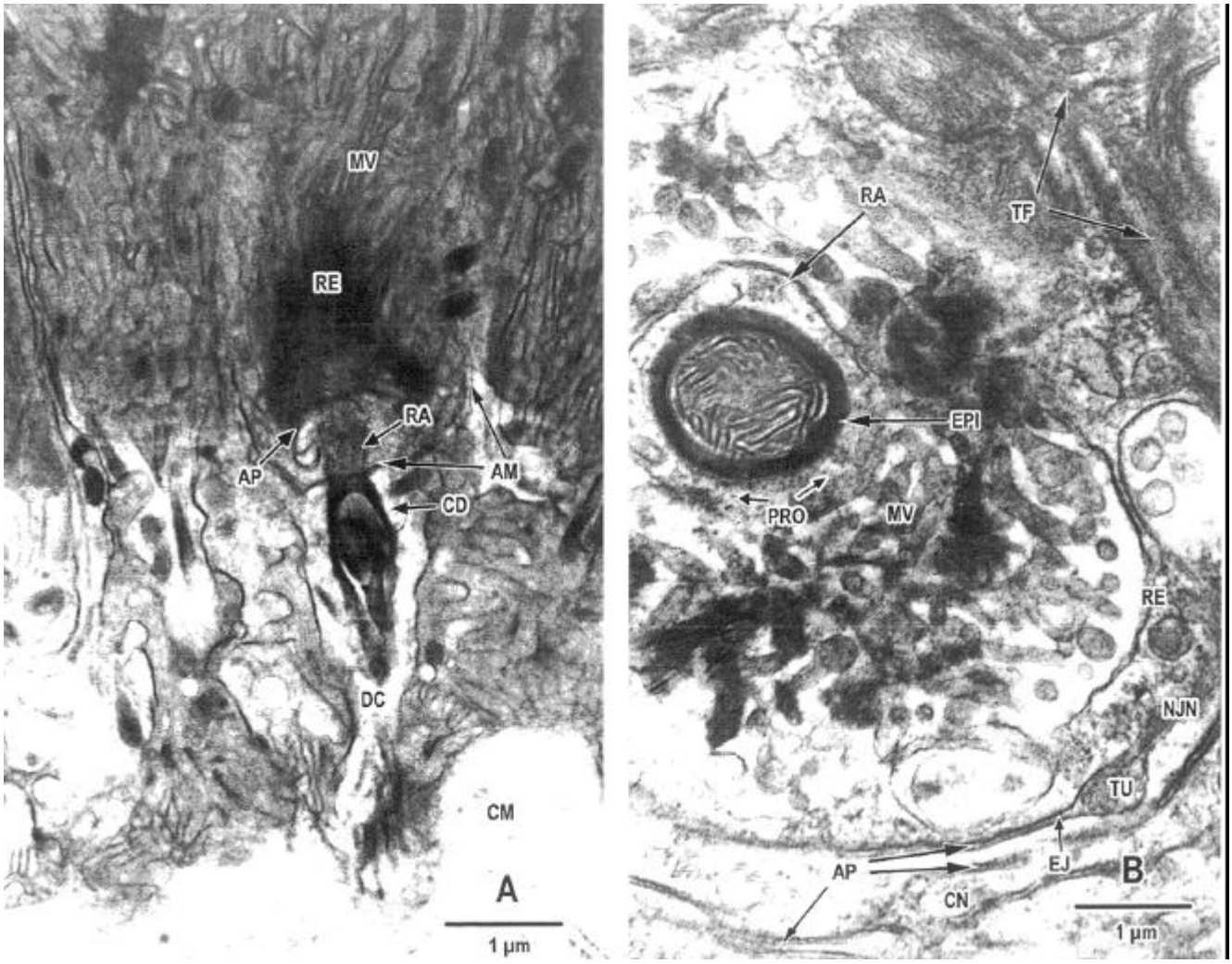

Figure 7. TEM of the end apparatus. (**A**) Longitudinal section of the receiving canal (RA) and conducting canal (CD) (see the text for revised terminology) of the glandular cell. The microvilli (MV) from the reservoir (RE) and the apical membrane (AM) unite to form a cytological apodeme (AP) in which the receiving canal (RA) is connected and leads to a conducting cuticular canal (CD). Note the cuticular ductule (CD) is distinctly enveloped in the duct cell (DC) and the latter is deeply inserted into the cuticular mass (CM) of the spermathecal body. (**B**) Cross section of the reservoir and receiving canal. The microvilli (MV) of the reservoir (RE) abut on the receiving canal (RA). Cuticle of the receiving canal is differentiated into an electron-dense thin-layer of epicuticle (EPI) and a thick filamentous electron-lucent layer of procuticle (PRO). The lumen is filled with a fibrous secretory material. The multilayered cell membrane of the cytological apodeme (AP) is supported by septate junction (EJ), continuous junction (CN), microtubules (TU), tonofibrils (TF) and NJN, desmosomes.

gland cell, and the conducting ductule surrounded by the duct cell leads up to the surface of the cuticle. The term "end apparatus" is currently being replaced by the original term, namely the "receiving ductule" and the "conducting ductule" (Noirot and Quennedey 1991).

Longitudinal and transverse sections of the "cuticular ductule" (figure 7) revealed that it has two distinct structural components, a thin electron-dense inner layer of epicuticle (3–7 nm) that lines the cavity, and a thicker electron-lucent fibrillar outer layer (8–15 nm). The thin superficial layer is interrupted at the junction where it meets the receiving canal; so the outer layer is continuous with the epicuticle of the cuticular mass. In the thick fibrillar layer, the constituent filaments are tubular, exhibit a tortuous course, and constitute a loose spongy meshwork. The relative resistance of this ductule to a corrosive medium, such a Berlese's or KOH, suggests the presence of unsaturated lipids (Filshie 1982) and is seen to bind with osmium as an electron-dense deposit. The diameter of the ductule is 40 nm, and the lumen is filled with fibrous secretory material irrespective of the physiological stage of the sandfly.

At the apical region (figure 9B) of the terminal cells abutting the cuticular mass, the plasma membrane, which forms an interface between the cytoplasm and the intracellular reservoir, is remarkably complex. There are two important cellular characteristics of this interface: the plasma membrane is repeatedly folded to form microvilli, which function as intracellular channels for the transportation of secretory substances, water and other metabolites from the cytoplasm into the reservoir; the plasma membrane along with the cuticle of the receiving ductule around the reservoir acts like an apodeme (i.e. an anchor point for the cuticular ductule), and, thus, a structure analogous to the muscular attachment of the cuticular apodeme (figure 7). Cross sections of the microvilli show that they have a central electron-lucent core covered by a thin electron-opaque layer. Longitudinal sections and cross sections of the apodeme revealed that the plasma membrane is folded into several layers and firmly attached to the opening of the receiving ductule. Plasma membrane contact and modifications within the interface indicate that the desmosomal elements are alternately arranged with the different cell junctions. Among the desmosomal elements is a girdle-like belt desmosome supported by an intracellular tonofibril. A patch of microtubules is present between the intercellular membranes. Next to the belt desmosome is a continuous junction characterized by parallel membranes with the intercellular space either filled with a homogenous, fine-grained material or septate junctions. Satir and Gilula (1973) suggested that the presence of a continuous junction is an indication of the presence of continuous epithelial regeneration. This finding of a complicated structure on the interface between the plasma membrane and cuticular ductule is reported here for the first time in an insect epidermal gland. Hence a new name is proposed to describe it; namely "cytological apodeme".

The lateral walls of the secretory cells are separated by cytoplasmic extensions with intercellular spaces (figure 9) that are 2–4 μm wide, often with septate junctions connected by microtubules. Hemidesmosomes are present on the apical halves of the cells. The basement membrane (figure 9A) is folded at regular intervals and extends only a short distance between the adjoining secretory cells. Vascular sinuses, which may exchange fluid between glandular cells and the haemolymph, occupy these spaces. The cytoplasm of the secretory cells is rich in microtubules around the reservoir (figure 9B) along the long axis of the cells. In addition to their role as cytoskeletal network and membrane component translocation, microtubules are possibly involved, in association with microfilaments, in the apical constriction and changing the volume of the cells (Fristrom and Rickoll 1982).

At the base of each glandular cell (figure 9A) is a large nucleus with a well-defined nucleolus double membrane. The cytoplasm around the nucleus is filled with cisternae of rough endoplasmic reticulum and with free ribosomes. On either side of the nucleus are the Golgi bodies, including stacks of the "classical apparatus" and two populations of very small multivesicular bodies (3 μm) and larger irregular vesicles with 5 μm in diameter. Between the nucleus and the apical area of the glandular cells are variously sized electron-dense secretory lysosome droplets (figure 6B) that presumably migrate towards the reservoir. The various shapes and sizes of mitochondria and vesicles, with evenly distributed electron-lucid particles, are typical of secretory cells. Between the class-3 cells and the rim of the spermathecal gland are a few non-secretory cells (figure 6B) with relatively smaller nuclei surrounded by thin cytoplasm.

The duct cells are 4 μm long (figure 7A) and are relatively much smaller than the gland cells and lie between the latter and the cuticle of the spermathecal body. The duct cells surround cuticular ductules (0·7 μm in diameter), which extend into the receiving ductule of the glandular cells at one end and penetrate basally through a massive block of cuticle to the lumen of the spermatheca at the other. The most conspicuous ultrastructural feature of the cuticular ductule is that it is devoid of an epicuticle and consists of an electron-dense thin outer layer and a thicker fibrillar or filamentous inner layer (figure 7B). The apical plasma membrane of the secretory glandular cell and the basal plasma membrane of the duct cells are tightly packed with desmosomes and well-defined tonofibrils. The apical plasma membrane of the duct cells is deeply folded in the microvilli and firmly anchored to the cuticle.

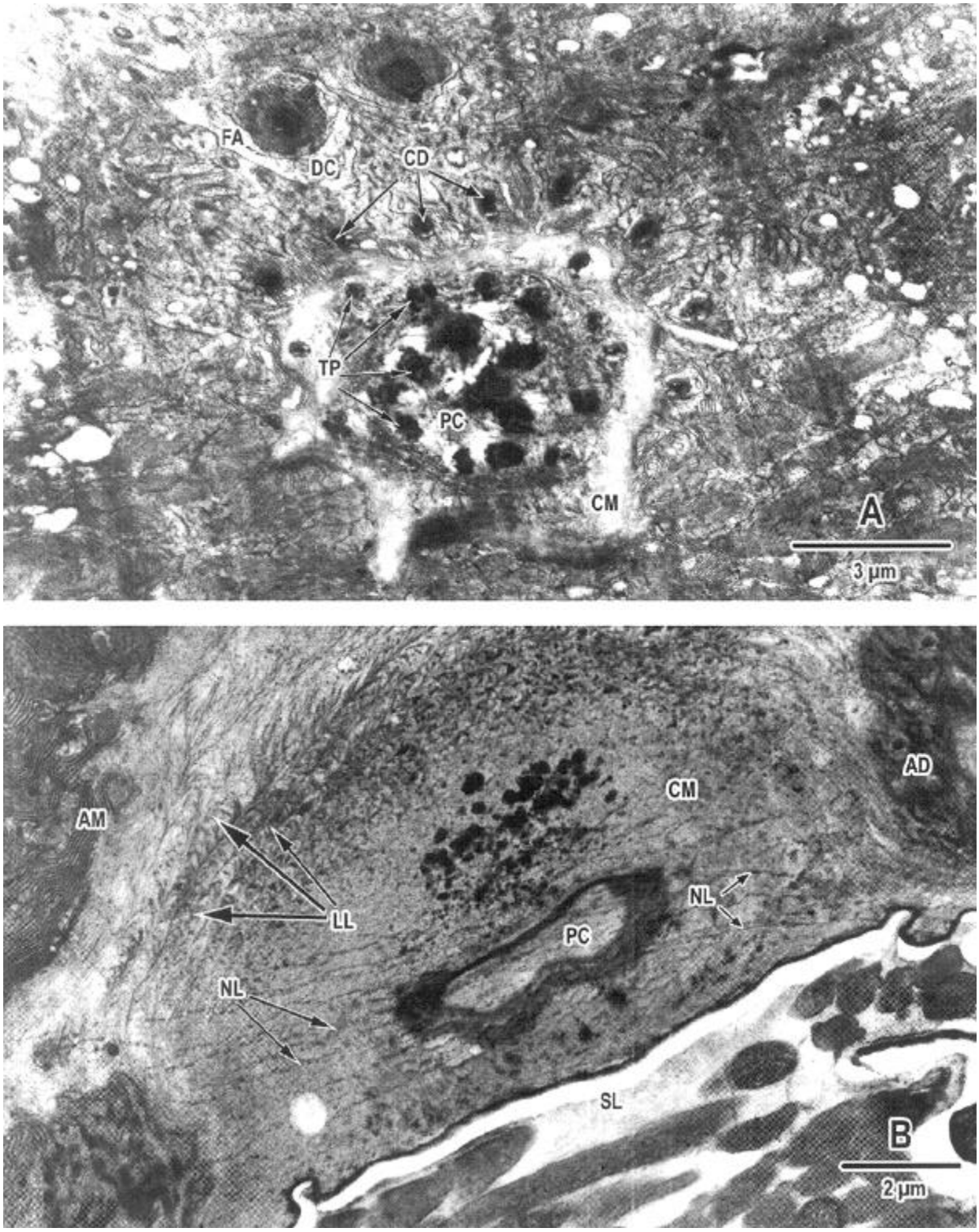

Figure 8. TEM of spermathecal apex. (**A**) Cross section of the apex of the spermathecal body (details as in the figures 4B, C and 7). (**B**) The apex of the spermathecal body showing the arrangement of microfibres in the cuticular mass (CM), cut at 45° to the vertical. Note the fibres are arranged in an arcuate pattern in lamellae (LL). The presence of pore canals (NL) in the cuticluar mass (CM), which lead to the spermathecal lumen (SL) through primary cavity (PC) emphasises the chitinous nature of the procuticle. The whole structure is supported by the muscle fibres, and cuticular apodeme (AD).

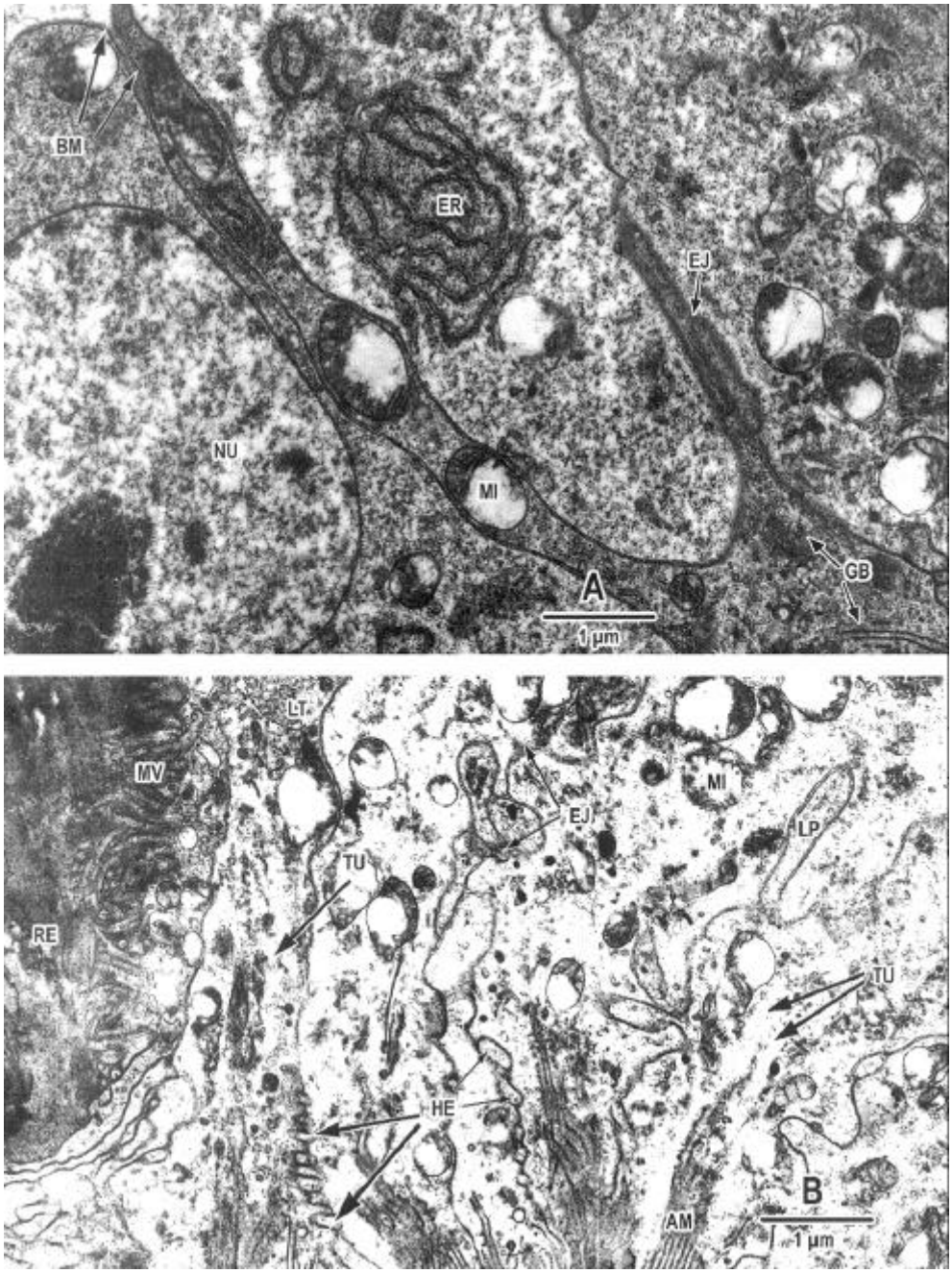

Figure 9. TEM of the spermathecal gland. (**A**) Basal region of glandular cells. The large nucleus (NU) is placed towards the base of the gland cells. On either side and anterior to the nucleus is the mass of rough endoplasmic reticulum (ER) and Golgi bodies (GB). A large number of mitochondria are randomly distributed (MI). The lateral wall of the plasma membrane is folded with a septate junction (EJ). The basement membrane (BM) of the glandular cells is deeply folded at the points where the vascular sinuses enter. (**B**) Longitudinal section showing the apical part of the gland cell and receiving canal. In addition to the microvilli (MV) found in the reservoir (RE) of the gland cell, the apical membrane (AM) is repeatedly folded for firmly fixing the cell into the cuticular mass. Below this interdigitating region, the apical membrane is modified into a series of hemidesmosomes (HE). The lateral plasma membrane of the cell is folded and looped (LP) and formed with septate junctions (EJ). Another important feature of this region is the presence of long microtubules (TU) and membrane limited tubules (LT) in patches, septate junction (EJ).

3.7 *The super-contracting visceral muscles of the spermathecae*

The muscular system (figures 4D, 10) consists of about 5–6 fibres that run longitudinally along the spermathecal structure intermeshed with the epithelial layer. At the head of the spermathecal body the fibres are attached to a cuticular apodeme. The muscle fibres are thin (2–3 nm diameter) with unusually short sarcomeres, in contrast to the long fibres of other insect visceral muscle fibres. They have irregular Z-lines and poorly defined A- and I-bands. There is a single large nucleolus, which is laterally placed in the sarcoplasmic reticulum. Mitochondria are few, relatively large, and randomly disposed. However, no endoplasmic reticulum or Golgi bodies were found.

The ultrastructure of the muscle fibres is of the super-contracting visceral muscle type reported by Rice (1970) in the oesophagus, midgut and aortic muscles of *Glossina morsitans*. In transverse and slightly oblique thin sections (figure 4D), each muscle fibre consists of a short 2·5 µm long and 2–4 nm wide sarcomere invested by a well differentiated sarcolemma, but the separation of myofibrils is incomplete, since the Z-discs are perforated with many holes, a characteristic feature of super-contracting fibres. Hence, a sarcomere of a super-contracting visceral muscle does not consist of isolated contractile units. During super-contraction, the myofilaments do not shorten, but slide through the holes in the Z-discs into the adjacent sarcomere. No M-bands are present, and the H-band is apparent in the middle of the A–band. The I-band is composed of thin myofilaments (i.e. 6 nm wide) that are bound into the Z-disc material from which they radiate, interdigitating with the thick myofilaments (6–10 nm wide). The visceral muscles appear, therefore, to be composed of thin filaments of myosin and thick filaments of actin. In cross section (figure 10A), the myofibrils are not aligned in the same direction, as some run circularly, some longitudinally, and some are diagonally oriented. The filament lattice shows myosin filaments in an approximately hexagonal array surrounded by 8–9 actin filaments. The transverse tubule system is well developed and is frequently associated with the sarcoplasmic reticulum forming the dyads. Hemidesmosomes attach Z-discs to the plasma membrane of the muscle fibre and overlie the thick basal lamina. The motor nerve terminal forms a neuromuscular junction with the muscle fibres near the junction of the spermathecal and its gland.

3.8 *The neuromuscular junction in the spermathecae*

Between the cuticular apodeme of the spermatheca and the spermathecal gland (figure 6A), the muscle is covered with nerve branches supplied by a pair of motor nerves from the 7th abdominal ganglion. A typical insect neuromuscular junction is present where these nerves contact muscle fibres (Gupta and Smith 1969; Tombes 1976). The classification and terminology of insect neuromuscular junction used here follow that of Osborne (1975).

In the neuromuscular junction (figure 10A), the axon lies in a shallow groove on the muscle surface and is not surrounded by a Schwann cell sheath. As the axon is naked, in effect, it is directly exposed to nearby haemolymph, which mixes with the blood meal taken by the sandfly, and the basement membrane offers little or no resistance to the flow of ions. The salient features of neuromuscular junction comprise synaptic vesicles, a presynaptic membrane and an electron-dense presynaptic projection (as reported in Diptera, Osborne 1975, 170), a synaptic cleft and a postsynaptic membrane. The axon terminal has a diameter of 2·5 µm, and the axoplasm contains a large number of spherical electron-lucent synaptic vesicles with an average diameter of 40 nm. According to Osborne (1975), these vesicles could arise either from the smooth endoplasmic reticulum or from the mitochondria and are the sources of acetylcholine or L-glutamate. A single large mitochondrion is found in the form of a vesicle. The presence of the presynaptic dense projection in the presynaptic membrane suggests that they are the attachment points for the synaptic vesicles and release sites for a neurotransmitter, either by means of a membrane-gating mechanism or by exocytosis. An intercellular gap known as the synaptic cleft, which is 12–15 nm wide, is between the axolemma and sarcolemma and is filled more or less uniformly by an electron-opaque substance. This substance usually consists of acid mucopolysaccharides and functions as a variable molecular sieve. Mucopolysaccharides are macromolecules with numerous anionic groups that reversibly bind positively charged irons and molecules. This binding alters the conformation of the macromolecules, which in turn affects not only its ability to link with more cations but also affects the electrical resistance of the space that the molecules occupy (Osborne 1975). The postsynaptic membrane is relatively straight compared with the rest of the sarcolemma and functions as an endplate containing enzymes such as acetyl cholinesterase for breaking down the transmitter substances.

To summarise, the spermathecae of the sandfly *P. papatasi* consist of a typical insect epidermal gland (reconstructed in figure 11). They include a cuticle, epithelial cells, glandular secretory cells, super-contracting visceral muscles, and neuromuscular junctions. The cuticle of the spermathecal body consists of septal and interseptal foldings and forms a cuticular mass rich in resilin at its apex. The glandular secretory cells contain receiving and conducting canals and an apical apodeme. The muscular system is characterised by perforated Z-disc for super-

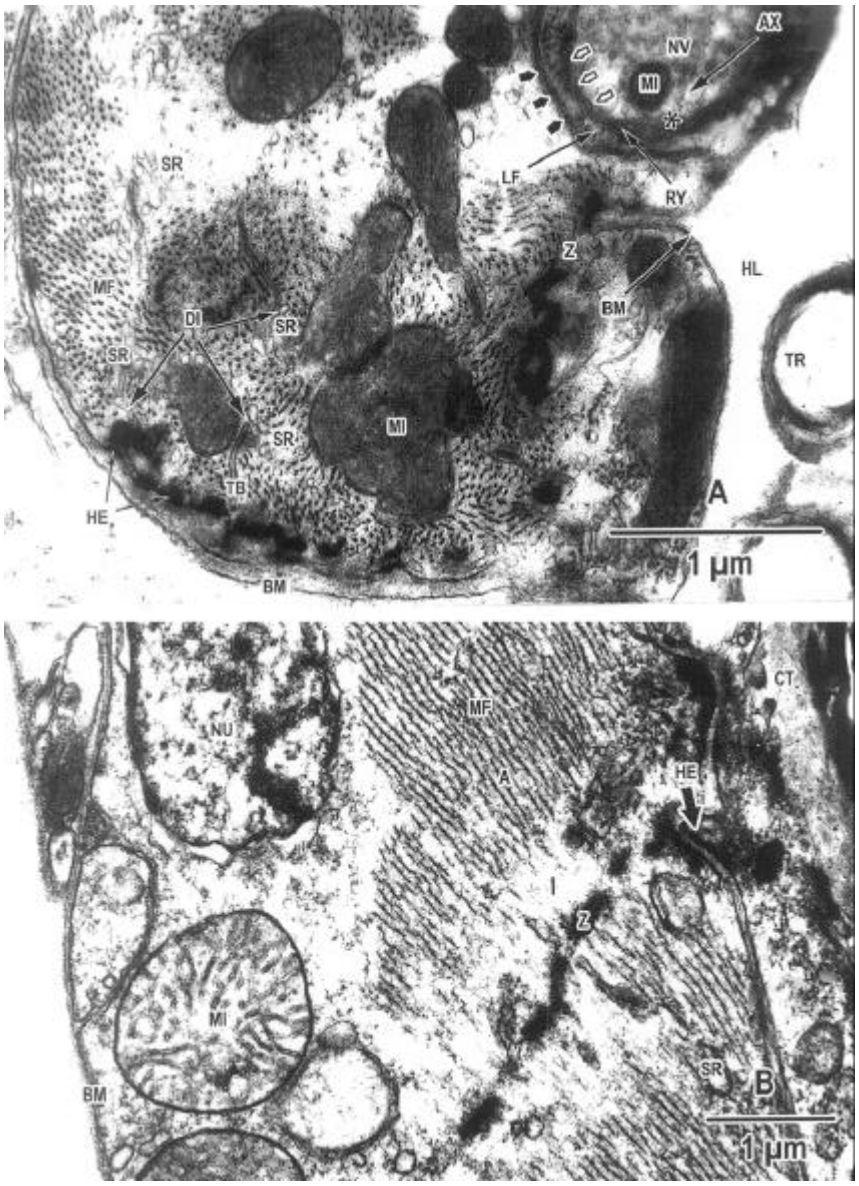

Figure 10. TEM of the muscle fibres and neuromuscular junction. (**A**) Cross section of the neuromuscular junction. The naked axon (AX) lies in a shallow groove on the surface of the muscle that faces the haemolymph (HL) and tracheoles (TR). Within the axon are electron-lucent, spherical, synaptic vesicles (NV) and a large mitochondria (MI). On the presynaptic membrane (open arrows) there is a presynaptic dense projection (RY), which is seen to be attached to the synaptic vesicles (*). In the synaptic cleft (LF), electron-dense particles can be seen. The postsynaptic membrane (closed arrows) is associated with sarcoplasm on one side and the basal membrane (BM) on the other. The muscle fibre shows the multidirectional arrangement of the filaments (MF), A- and i-bands (see **B**), the sarcoplasmic reticulum (SR), transverse tubules (TB), dyad (DI) and hemidesmosomes (HE). (**B**) TEM, Oblique section of visceral muscle on the spermathecal wall. Note the perforated Z-line, a characteristic feature of super-contracting intrinsic visceral fibres, which separates the sarcomere (MF). The I-band, representing the thin filaments of myosin, is close to the Z-discs and interdigitates with the neighbouring thick actin filaments (A). The sarcoplasmic reticulum (SR) is seen along the filamentous (MF) course and the transverse tubules (TB) are continuous with actin filaments and the hemidesmosomes (HE). The sarcoplasmic membrane is closely apposed (by hemidsmosomes, HE) to the body wall cuticle (CT). BM, basement membrane; MI, mitochondria.

contracting activity and is associated with a neuromuscular junction for neurosecretion.

4. Discussion

4.1 *Sandfly spermathecal comlex as typical insect epidermal glands*

The ultrastructure of insect spermathecae has been studied in nine species from four orders, namely the mosquito *Aedes aegypti* (Diptera) (Clements and Potter 1967); the Tsetse fly *Glossina morsitans* (Diptera) (Kokwara *et al* 1981); the vinegar fly *Drosophila melanogaster* (Diptera) (Filosi and Perotti 1975); the Cockroach *Periplaneta americana* (Dictyoptera) (Gupta and Smith 1969); the meal worm beetle *Tenebrio molitor* (Coleoptera) (Happ and Happ 1970); the granary weevil *Sitophilus granaruis* (Coleoptera) (Tombes and Roppel 1972); the cotton boll weevil *Anthonomus grandis* (Coleoptera) (Villavaso 1975a,b); and the honey bee *Apis mellifera* (Hymenoptera) (Dallai 1975). In all the species, the histology of the spermathecae is typical of insect epidermal glands, particularly in the ultrastructure of the procuticle. But the sandfly species studied here shows a remarkable variation from the basic structure, in particular in the cuticle of the spermathecal duct and body, in the presence of super-contracting visceral muscle fibres, and in the provision of a neuromuscular junction. The pattern of histological variation in the spermathecae of *P. papatasi*, suggests that the structure of the spermathecal complex in each species is adapted to meet the particular needs of sperm storage and may have evolved in relation to different mating systems. For example, the spermathecal body of *Sergentomyia babu* do not have segmentation and they are therefore unable to store sperms separately as in *Phlebotomus* spp., wherein the spermathecal body is segmented for storing sperms in different compartments.

The muscular system that supports the spermathecal body and duct consists of super-contracting visceral

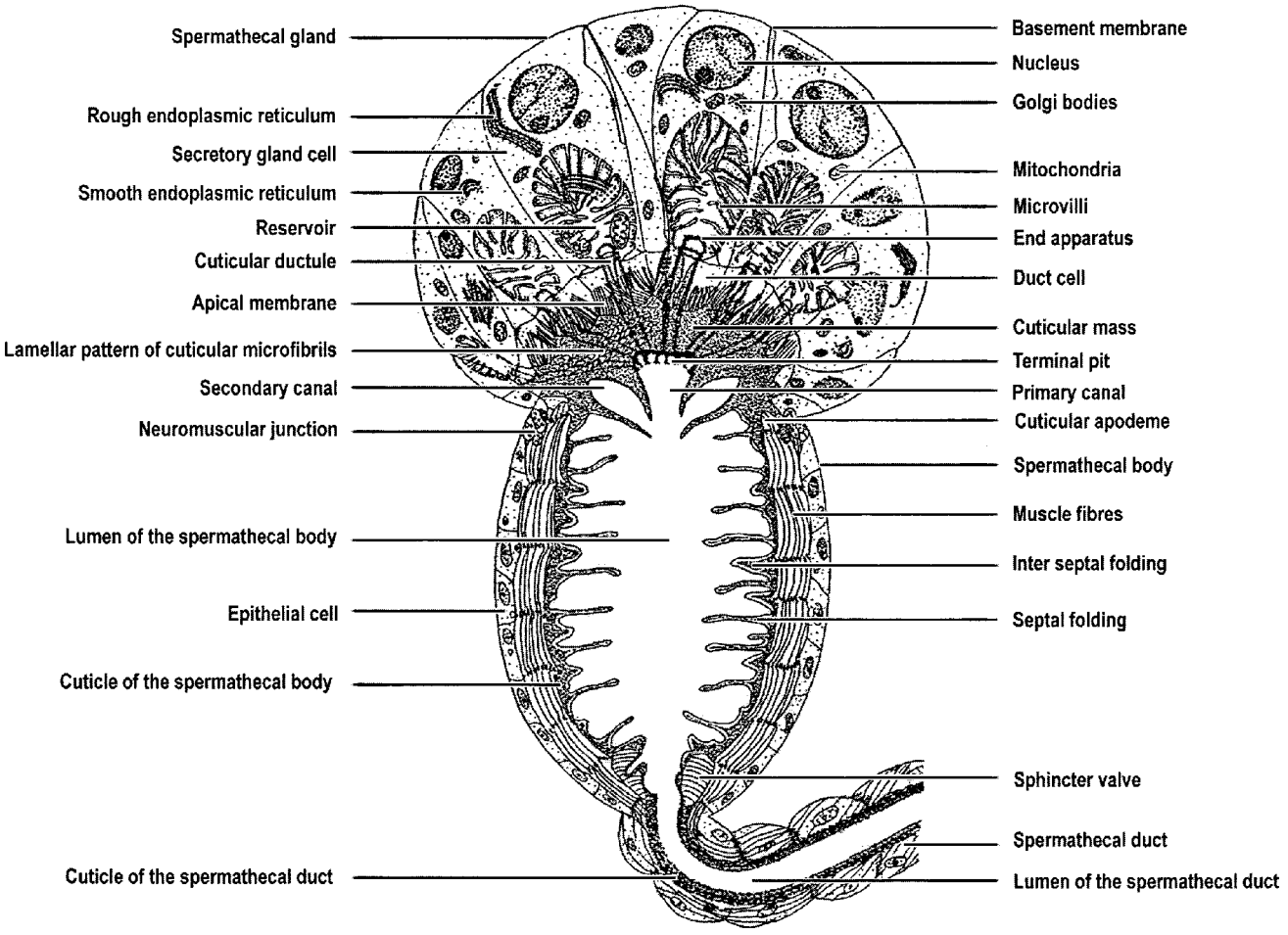

Figure 11. A diagrammatic reconstruction of the histological organization of the spermathecal complex in *P. papatasi* (not to scale).

fibres, which are characterized by their capacity for long, isotonic contractions around curved surfaces of visceral tubes and bags (Rice 1970). These muscle fibres are: (i) particularly well adapted to accommodate asymmetric contractions, which are bound to occur in muscles that operate around tubes, such the adeagal filaments of males; and (ii) they enable the compression of visceral tubes and bags. Such rhythmic and peristaltic movements were observed in spermathecal ducts of saline squash preparations. The muscular system of the spermathecal duct perhaps gives mechanical support during copulation. During mating, the resilin-rich cuticle of the spermathecal duct, which would allow great tightness together with the ability to maintain adequate tension over long contractions, are important factors in accommodating the non-flexible, aedeagal filaments of phlebotomine sandflies (Ilango and Lane 2000).

The existence of both neuromuscular junction and neurosecretory endings has been reported in the spermathecae of cockroaches (Gupta and Smith 1969) and granary weevils (Tombes 1976). In this study of *Phlebotomus* a similar neuromuscular system is present. The presence of neuromuscular junctions most likely promotes the secretion of substances from the glandular cells of the spermathecae immediately after blood-feeding, which would in turn encourage the sperm to migrate into the spermathecal body (Ilango 2005). Details of the ultrastructure of the neurosecretory endings indicate that Schwann cells are absent in *P. papatasi*. According to Osborne (1975), the absence of Schwann cells (i.e. naked terminal) in insect neuromuscular junctions is an adaptation of an insect species that reduces the transport of ions or molecules from the blood to the space surrounding the axon leading to high sodium ion content in the haemolymph. Thus, perhaps the blood meal ingested by the sandfly infiltrates into the haemolymph, so that action potentials are conducted in an abnormal ionic environments.

In contrast, insect species that feeds on plant sugar contain sodium ion poorly in the haemolymph. These insect's neuromuscular endings are provided with Schwann cells. The action potentials conducted in such a low sodium ionic fluids are therefore normal features of phytophagus insect species.

4.2 *Comparison of the spermathecal complex of the sandfly with that of other insects*

The spermathecal complex of *Phlebotomus* differs from some of the other insects in having considerable modifi-

Table 1. Summary of the different ultrastructures of the spermathecal complex in the sandfly *P. papatasi* compared with those of other insect species.

	Spermathecal duct				Spermathecal body						Spermathecal gland		
Taxa studied	Opens directly into the bursa copulatrix	Epithelial cell	Cuticle with resilin	Muscle fibres	Sphincter valve	Epithelial cell	Cuticular mass	Septal folding	Muscle fibres	Neuromuscular junction	Secretary gland	Receiving and conducting ductules	Cylogical apodeme
Diptera													
Sandfly, *Phlebotomus papatasi*	+	+	+	+	+	+	+	+	+	+	+	+	+
Mosquitoe, *Aedes aegypti*	+	+	–	–	–	+	–	–	–	–	+	+	–
Tsetse fly, *Glossina morsitans*	–	+	–	+	–	+	–	–	+	+	+	+	–
Vinegarfly, *Drosophila melanogaster*	+	+	–	–	–	+	–	–	–	–	+	+	–
Dictyoptera													
Cockroach, *Periplaneta americana*	+	+	–	+	–	+	–	–	+	+	+	+	–
Coleoptera													
Meal worm beetle, *Tenebrio molitor*	–	+	–	–	–	–	–	–	–	–	+	+	–
Granary-weevil, *Sitophilus granaruis*	+	+	–	+	–	–	–	–	–	+	+	+	–
Cotton boll weevil, *Anthonomus grandis*	+	+	–	+	–	–	–	–	–	–	+	+	–
Hymenoptera													
Honey bee, *Apis mellifera*	–	+	–	+	–	–	–	–	–	–	–	–	–

'+' Present; ' –' absent.

cations of the spermathecal duct, a compartmentalized spermathecal body, and super-contracting visceral muscles (table 1). Based on its ultrastructure, the cuticular mass at the apex of the spermathecal body appears to have a dual role: (i) It serves as a cuticular apodeme for the visceral muscle fibres running along the axis of the spermathecal body; and (ii) it provides support for the cuticular ductules of the glandular cells. Thus, the spermathecal body can change in volume depending on the requirements of the individual species for the reception and storage of spermatozoa. Some species of *Idiophlebotomus*, for example, have a large, balloon-like spermathecal body that might have the ability to store more spermatozoa than the narrow spermathecal body of *Phlebotomus* sp.

The presence of pore canals in the cuticular mass in *P. papatasi* is unique among the rubber-like cuticle of insects. The pore canals may have served as direct connections between the spermathecal gland and the lumen of the spermathecal body in an early embryonic stage and may have been retained as non-functional structures when the pharate stage was attained. The presence of primary and secondary canals at the base of the cuticular mass may not only increase the volume of the spermathecal body but may also collect the cuticular ductules and any secretion flowing through them.

The structure of the epithelial cells in the spermathecal duct and body shows exceptional diversity compared to other insect epidermal glands. Their morphology and location on the spermathecal body and duct correspond to the pattern of cuticular variation that may be designed to meet different needs for sperm nourishment and storage.

The structure of the spermathecal gland is much more complex and elaborate than the simpler organization of epithelial cells and indicates that the cells forming this gland are producing secretory products (detailed in the accompanying paper). The histology of the terminal cells with reservoirs and of the duct cells resemble that of cells in other insect spermathecae, but the spatial distribution of terminal cells differs among the species studied. In other insect species, such as the cockroach and the boll weevil, there are fewer cells than in *P. papatasi*. Whether this means that fewer sperm are stored, that sperm are stored for a shorter time, or that cells differ in their product is presently unknown.

In the glandular cells, the differentiation of the plasma membrane includes deep foldings as microvilli, apical apodemes, continuous belt desmosomes, septate junctions together with tonofibrils, and an extensive distribution of microtubules. These cellular differentiations and their inclusions are general properties of intercellular connection between insect epidermal glands but in the sandfly spermathecae, besides their adhesive role, they show extraordinary structural variation of the spermathecal gland cells.

To summarise, the histological variety of the spermathecal complex of *P. papatasi* may provide a source for sexual selection to act on. According to Eberhard (1998), sperm competition almost never occurs without females influencing the outcome, if only because their morphology determines how much sperm is stored and to what degree sperm from subsequent matings may enter and displace existing sperm stores.

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