A pair of Malphigian tubules emerged from the junction between the mid gut and hind gut, just before the pylorus (Figures 5, 10A). Each tubule diverged to two long tubes. Investigation using SEM revealed that the Malphigian tubules were composed of long chain globular structures (Figure 15A), and the thick section confirmed that each globular structure was separated, but adjacent to each other (Figure 15B). Each globular structure comprised compact columlar epithelium cells and had individual lumen (Figure 15B). TEM sections displayed the smooth basal lamina, and the columnar cells contained large area of content (Figures 15C,D). Cytoplasm of the cells was relatively loose.

3. Alimentary tract of females

3.1 LM observation

The alimentary canal of the female *C. megacephala* showed similarity in appearance to that of males. It consisted of the anterior (foregut), median (midgut) and posterior (hindgut) portions, with the midgut being the longest one (Figure 16). The salivary gland, crop and rectum displayed the same morphology as those in males. The salivary glands presented a coil-shaped, long tubular gland at the apex, but swelling of the gland was detected before it constricted to be a slender tube of the salivary duct. The cardia appeared as a globular structure lying between the end of the esophagus and the anterior midgut. The midgut was cylindrical in structure, occupying the main part of the body. The Malpighian tubules emerged from the midgut-hindgut junction. Each tube diverged into two long tubes. The junction between the ilium and colon was noticeable, being constricted. The rectum of females was the most distinctive organ of the hindgut, appearing as a long bulb shape.

3.2 SEM and TEM observations

Foregut

The salivary glands were paired organs lying in the thorax on either side of the esophagus. Each gland presented a coil-shaped long tubular gland (Figure 17A). The thick section revealed that the gland comprised compact columnar cells surrounding the central lumen (Figure 17B). Cells contained a large nucleus.

The crop appeared as a thin sac (Figure 16). The external surface of this organ was covered with muscle (Figure 17C). The semi-thin cross section displayed the covering of muscle and convoluted cuticle centrally adjacent to the lumen containing food particles (Figure 17D). Using higher magnification of TEM, epithelial cells, with a large nucleus, were found lying on the basal lamina. The cuticle was clearly evident.

Regarding the cardia, it was a bi-lobe globular structure, of which the posterior portion connected to the anterior midgut (Figure 18A). The thick section clearly indicated two

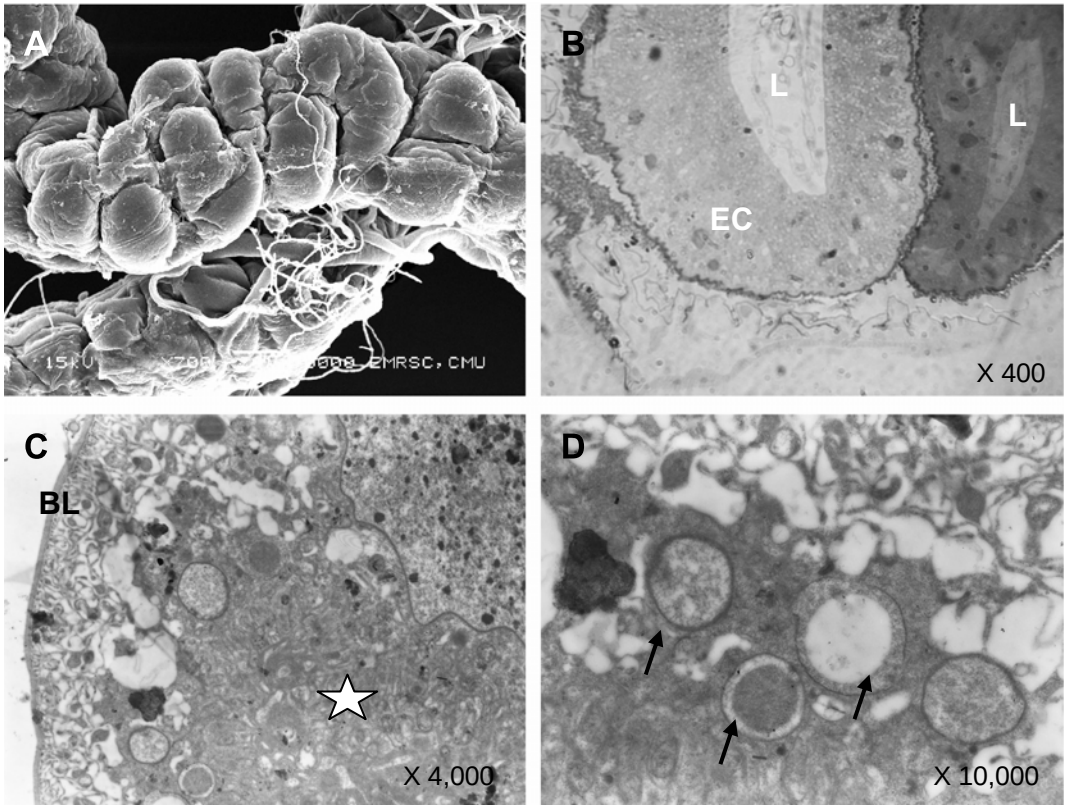

Figure 15. Malphigian tubules of male *C. megacephala*. (A) SEM image of Malphigian tubules. (B) Thick section showing the separated globular structures. Each structure comprised columnar epithelium cells (EC) and individual lumen (L). (C) TEM image of columnar cell displaying a smooth basal lamina (BL), and large area of content (star). (D). TEM image of cells with loose cytoplasm and much content (arrows).

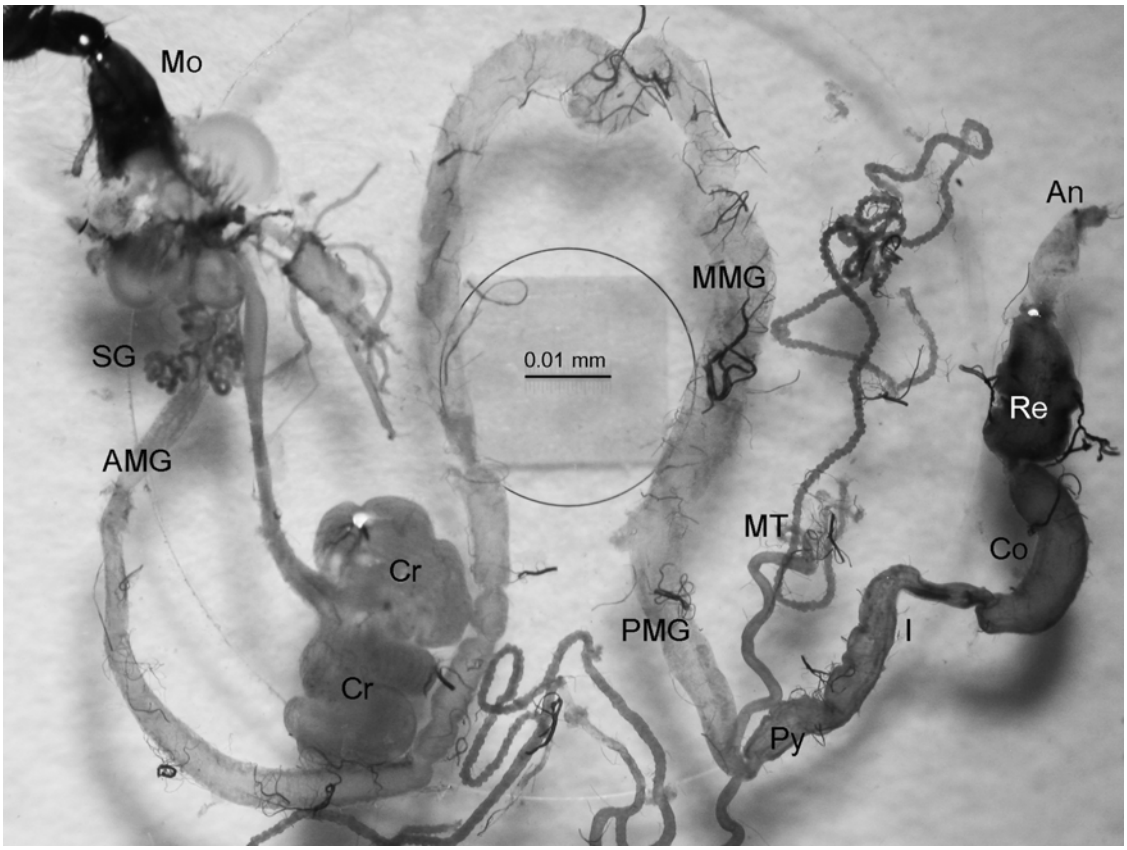

Figure 16. Alimentary canal of female *C. megacephala,* indicating the foregut consisting of mouth (Mo), salivary glands (SG), esophagus, and crop (Cr). The cardia represents the junction of the foregut and midgut. The midgut consists of anterior midgut (AMG), middle midgut (MMG), and posterior midgut (PMG). The hindgut begins with the pylorus (Py), Malpighian tubules (MT) arise, ileum (Il), colon (Co), rectum (Re) and anus (An).

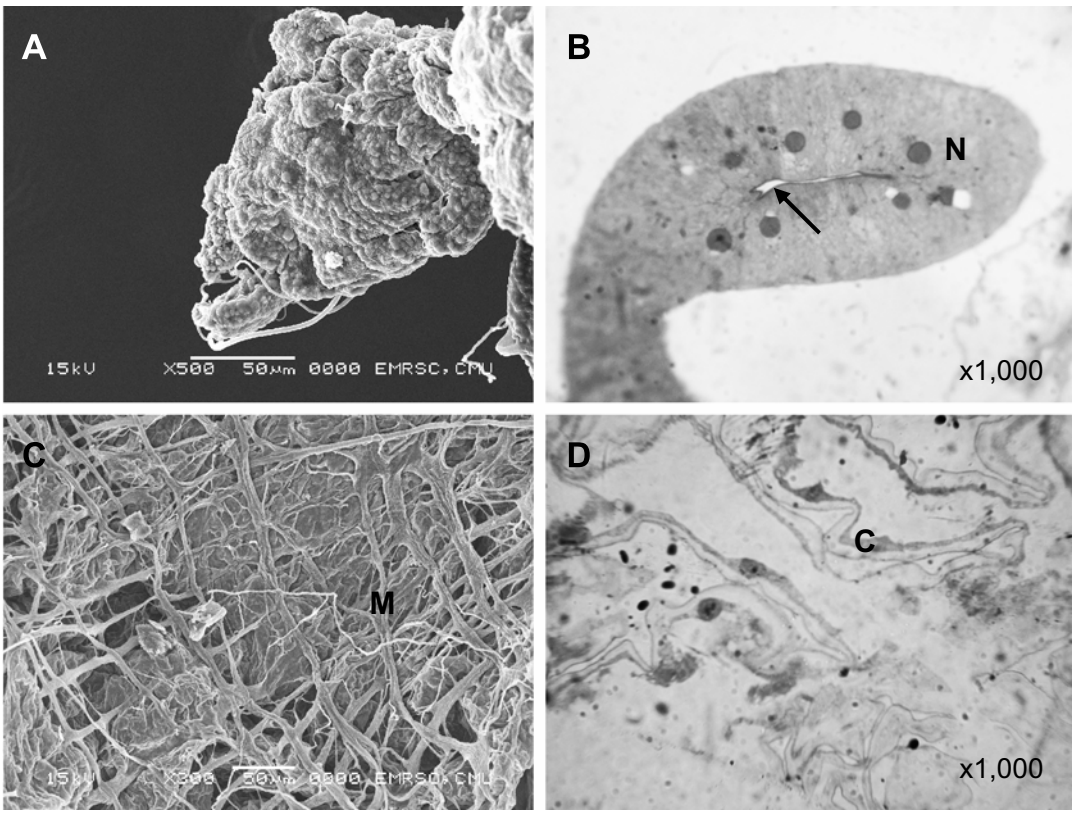

Figure 17. Foregut of female *C. megacephala*. (A) SEM micrograph of the salivary gland. (B) Thick section of the salivary gland showing the whole gland comprising columnar epithelium cells with a large nucleus (N) and central lumen (arrow). (C) SEM micrograph of the crop displaying the network of muscle (M) externally. (D) Thick section of the crop showing cuticle (C).

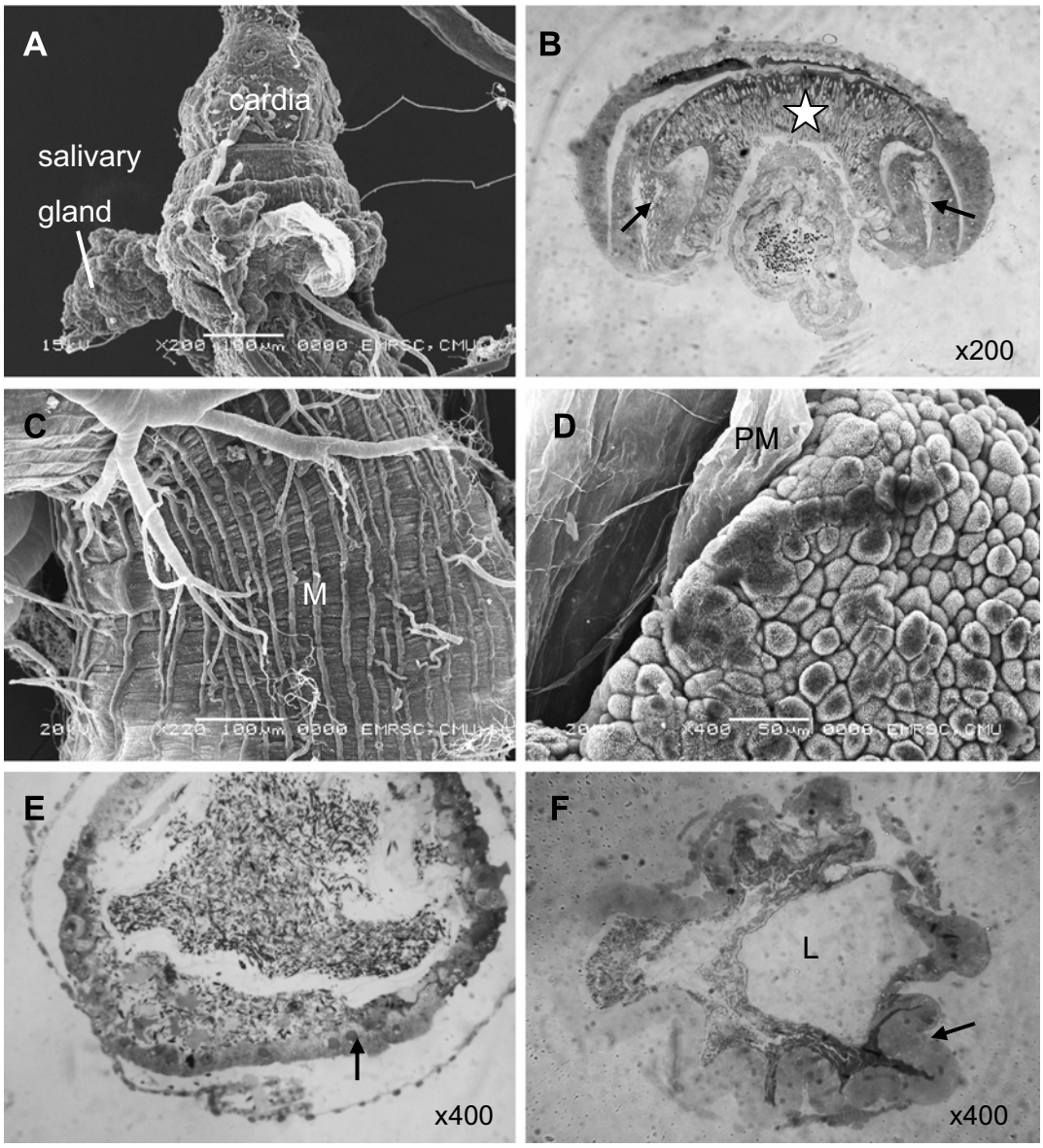

Figure 18. Alimentary canal of female *C. megacephala*. (A) SEM image of cardia. SG, Salivary gland. (B) Thick section of cardia displaying the anterior (star) and posterior portion (arrows). (C) SEM image of the mid midgut showing longitudinal fine muscle (M) along the outer surface. (D) SEM image of a ruptured midgut revealing compact epithelium cells, with microvilli and peritrophic membrane (PM). (E) Thick section of the posterior midgut showing a single layer of columnar cells (arrow). (F) Thick section of the pylorus displaying epithelial cells (arrow) and a centrally large lumen (L).

compartments; the anterior and posterior portion (Figure 18B). The anterior portion was located centrally, and received food content directly from the esophagus.

Midgut

Upon SEM, the pouch-like protrusions of the circular muscle occurred on the external surface of the anterior midgut; however, longitudinal fine muscle tended to distribute quite uniformly along the outer surface of the mid midgut (Figure 18C). Rupture of the midgut presented the compact midgut epithelium cells and central peritrophic membrane (Figure 18D). The midgut epithelium lay on a thin basal lamina, consisting of a single layer of columnar microvillated cells (Figure 18D). These cells contained a large nucleus (Figure 18E).

Hindgut

The hind gut started at the pylorus, and connected with the ilium, colon, rectum and anus, consecutively (Figures 16, 19A). The pylorus comprised epithelial cells with a large nucleus (Figure 18F).

Upon SEM view, the junction between the ilium and colon was noticeable (Figure 19A). The external surface of the ilium was covered with muscle (Figure 19B). The epithelium cells contained a large nucleus, which was more or less ovoid to elongated. As for the colon, the bundle of muscle was evident by covering the epithelium cells (Figure 19C, 19D). The epithelium cells contained a large nucleus (Figure 19D). Food particles were found along the lumen. Regarding the rectum, the morphology of the females was similar with those in the males, appearing as a muscular cone-shaped sac-like structure, with the anterior region being enlarged (Figure 19E). Four rectal pads were obvious at the anterior region. A cross section through the rectum revealed that the thick muscle covered the whole rectum (Figure 19F).

A pair of Malphigian tubules emerged from the junction between the midgut and hind gut, just before the pylorus (Figure 16). Each tubule diverged to two long tubes. Investigation using SEM revealed that the Malphigian tubules were composed of a long chain globular structure (Figure 20A), similar to that observed in the males (Figure 15A). The thick section confirmed that each globular structure was separated, but adjacent to each other. Each globular structure comprised compact columlar epithelium cells containing a large nucleus (Figure 20B).

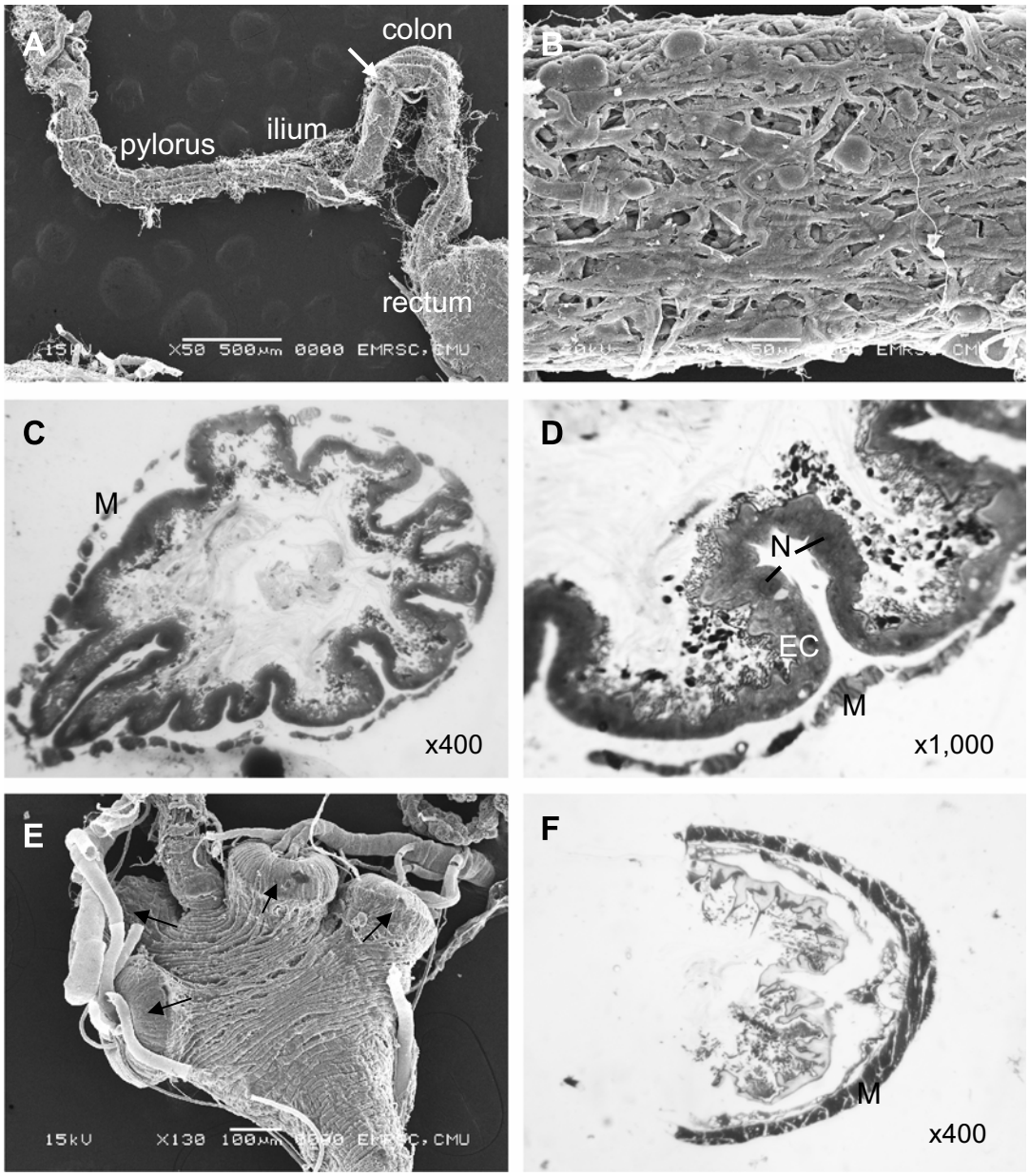

Figure 19. Hindgut of female *C. megacephala*. (A) SEM micrograph of the hindgut revealing the pylorus, ilium, colon and rectum. Arrow indicates junction between ilium and colon. (B) SEM image of the ilium. (C) Thick section of the colon showing muscle (M). (D) Higher magnification of colon showing epithelium cells (EC) containing a large nucleus (N). M, muscle. (E) SEM image of the rectum showing four rectal pads (arrows). (F) Thick section of the rectum revealing thick muscle (M).

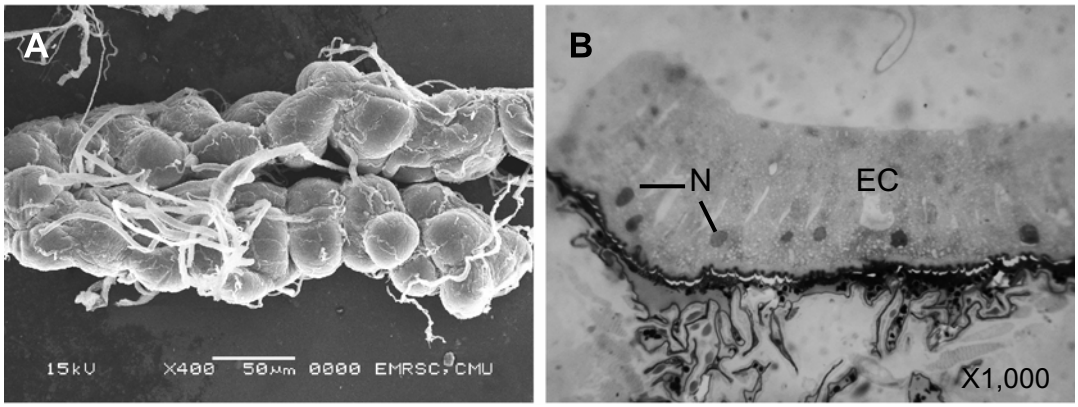

Figure 20. Malphigian tubules of female *C. megacephala*. (A) SEM image showing the long chain globular structure. (B) Thick section showing compact columlar epithelium cells (EC) containing a large nucleus (N) at the base.

4. Reproductive tract of males

The internal reproductive organs in male *C. megacephala* comprised pairs of testes, vas deferens and accessory glands, one ejaculatory duct and one sperm pump (Figure 21). The testes were oval-shaped, orange-brown in color, with the terminal end connecting to the vas deferens. The vas deferens was a thin, transparent and long simple tube that opened into the anterior end of the thin medial ejaculatory duct, which was relatively long in length. The terminal end of the ejaculatory duct was inserted into the sperm pump, connecting to the external genitalia. The anterior end of the ejaculatory duct was slightly enlarged and globular; where the terminal ends of the paired vas deferens and paired accessory glands opened into opposite sites.

Testis

LM observation: Dissection revealed that the testes of *C. megacephala* were located in the posterior region of the abdomen and had an overall oval shape. They maintained their ellipsoidal form for a day or more after emergence, and then a characteristic constriction occurred about a third of the length from the base of the testes (Figure 21). Moreover, this constriction extended apically and changed the form of the testes remarkably (Figure 22).

Measurements comparing the left and right testis revealed that no significant difference in either width or length at $P = 0.315$ and $P = 0.684$, respectively. Since there was no significant difference between the testes, the median of the two sides is illustrated in Figures 23A and 23B. There was an increase in testis length from day three until day five, when the testis length declined until day seven. This pattern of increasing and decreasing length was repeated for the duration of the study (Figure 23A). Median testis width fluctuated in a less predictable manner than median length, but the widest testis width was observed on day seven (Figure 23B).

The color of fly testes just after emergence was a pale orange. The color changed to become a reddish orange in 1-day-old flies. The color of the testes changed continuously from reddish orange to brown or to fuscous (Figure 24).

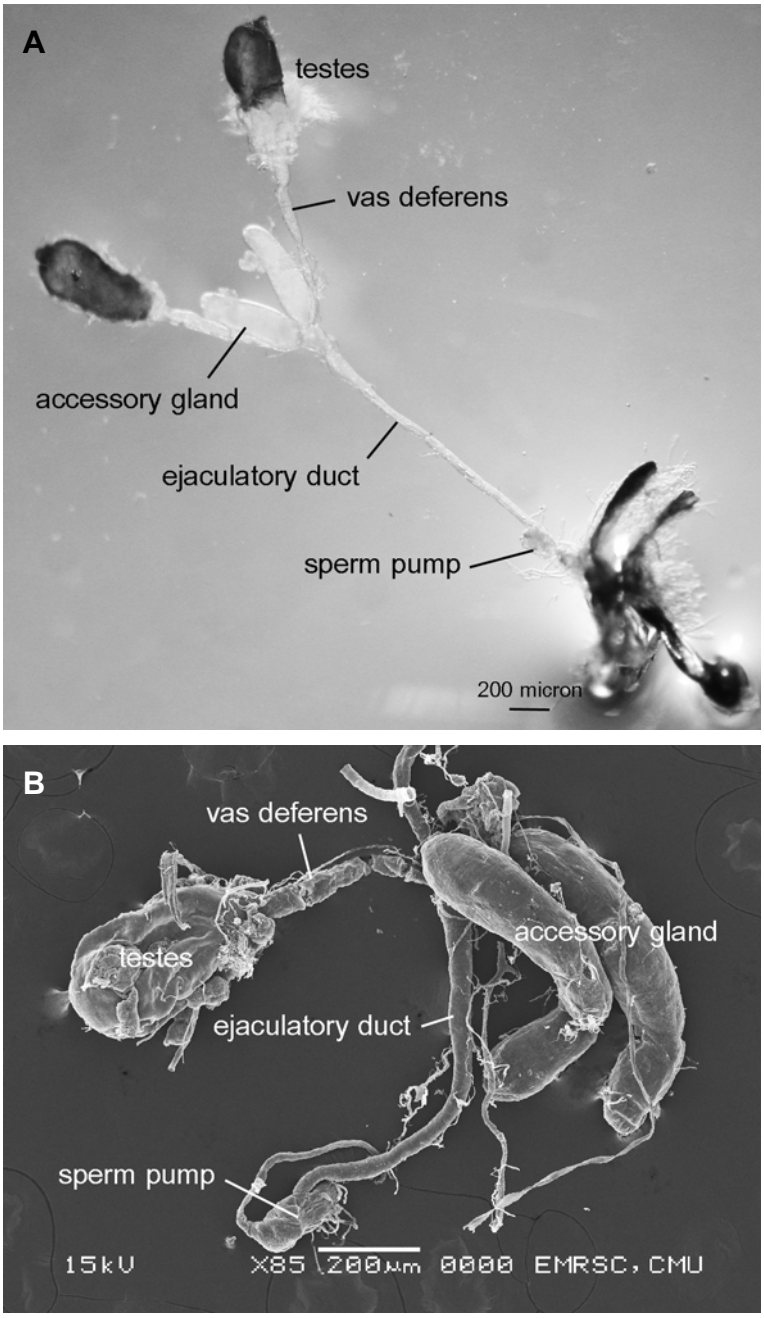

Figure 21. (A) Whole internal reproductive tract in male *C. megacephala* showing paired testes, paired vas deferens, paired accessory gland, medial ejaculatory duct and sperm pump. (B) SEM image.

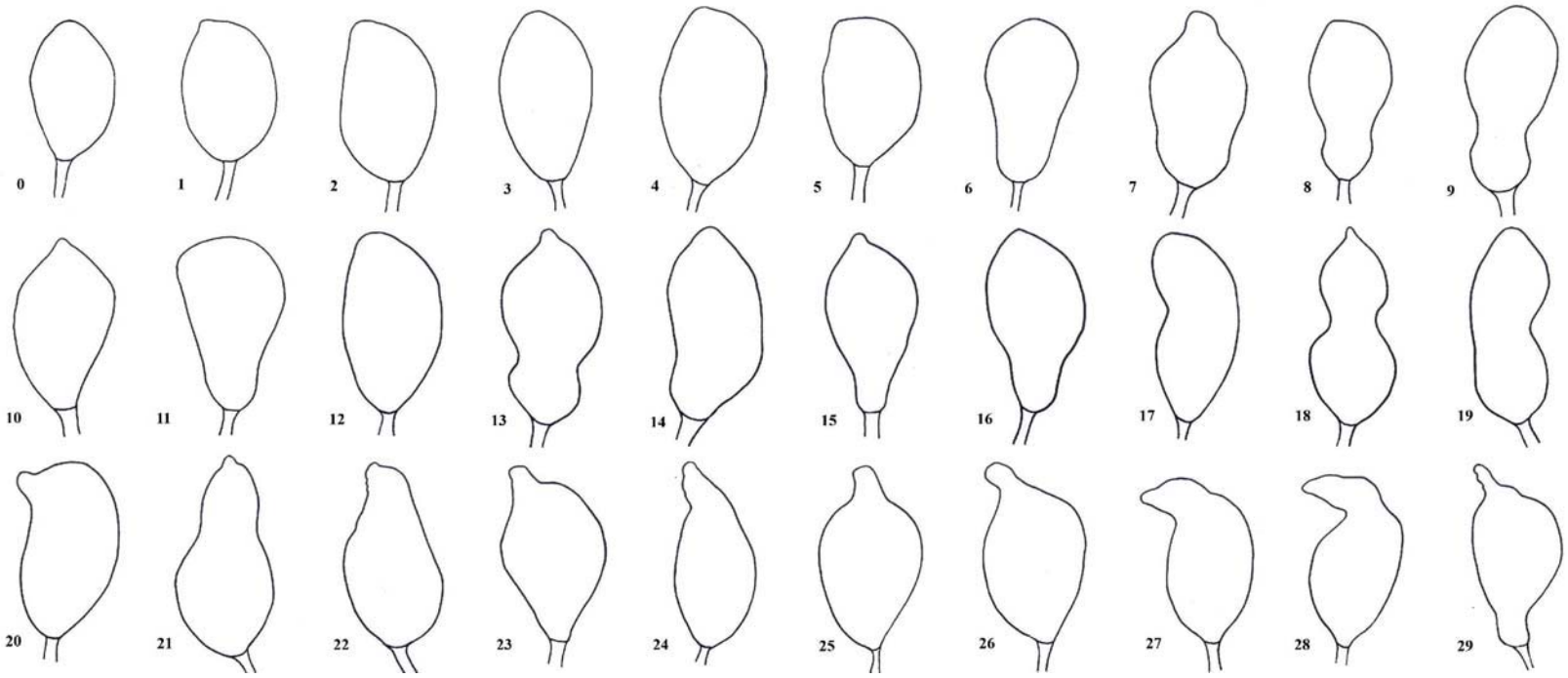

Figure 22. Illustrated change in shape of *C. megacephala* testes from just after emergence (day 0) to 29 days old. The first row shows day 0 to 9 day-old flies (from left to right), the second row displays 10 day-old to 19 day-old flies and the third row illustrates 20 day-old to 29 day-old flies. The increase in length from day 0 to day 3 and development of a constriction on day 7 is of interest. (*n* = 150; selected images used here best illustrate maturation and development of the testes).

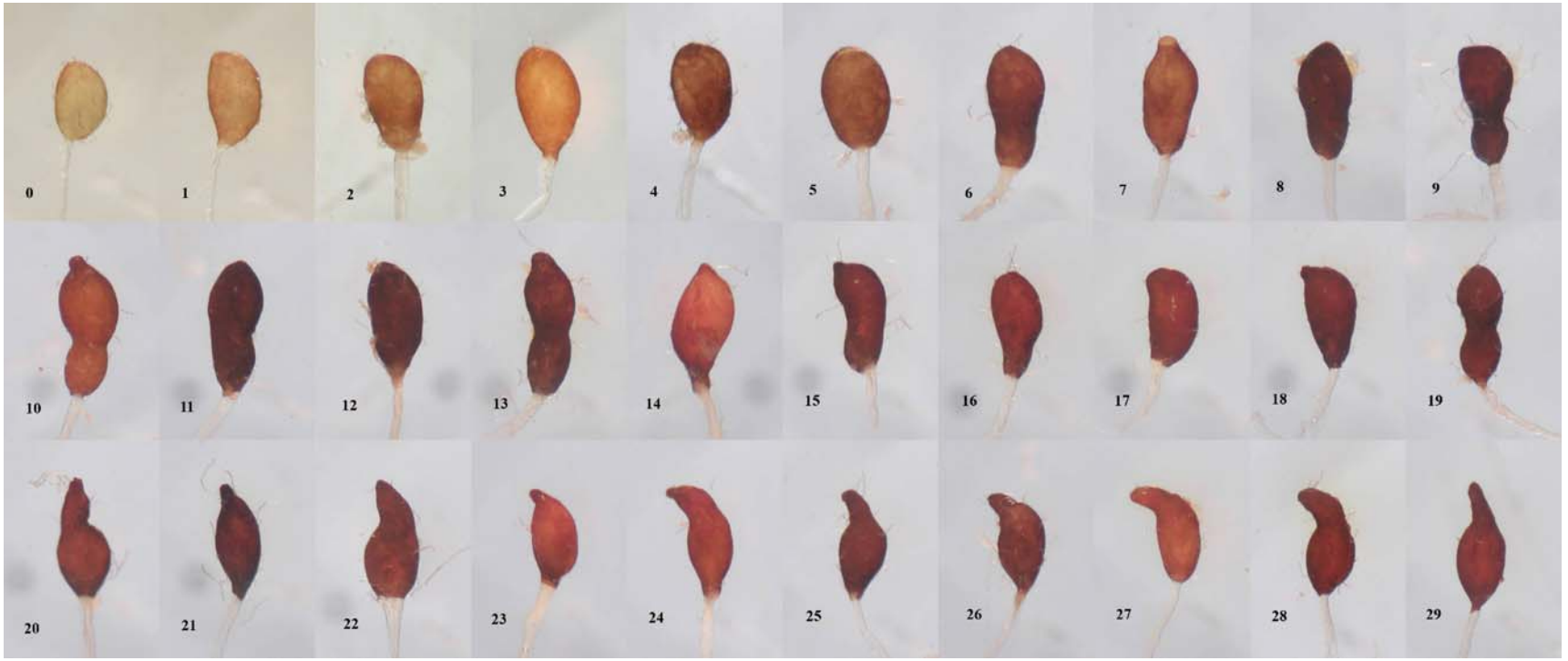

Figure 24. Light microscopy showing the progressive color change of *C. megacephala* testes just after emergence (day 0) to 29-days-old flies. The first row shows the color of the testes on emergence day (day 0) to 9 days old (from left to right), the second row 10 days old to 19 days old and the third row 20 days old to 29 days old. (*n* = 150; selected images used here best image maturation and development of the testes).

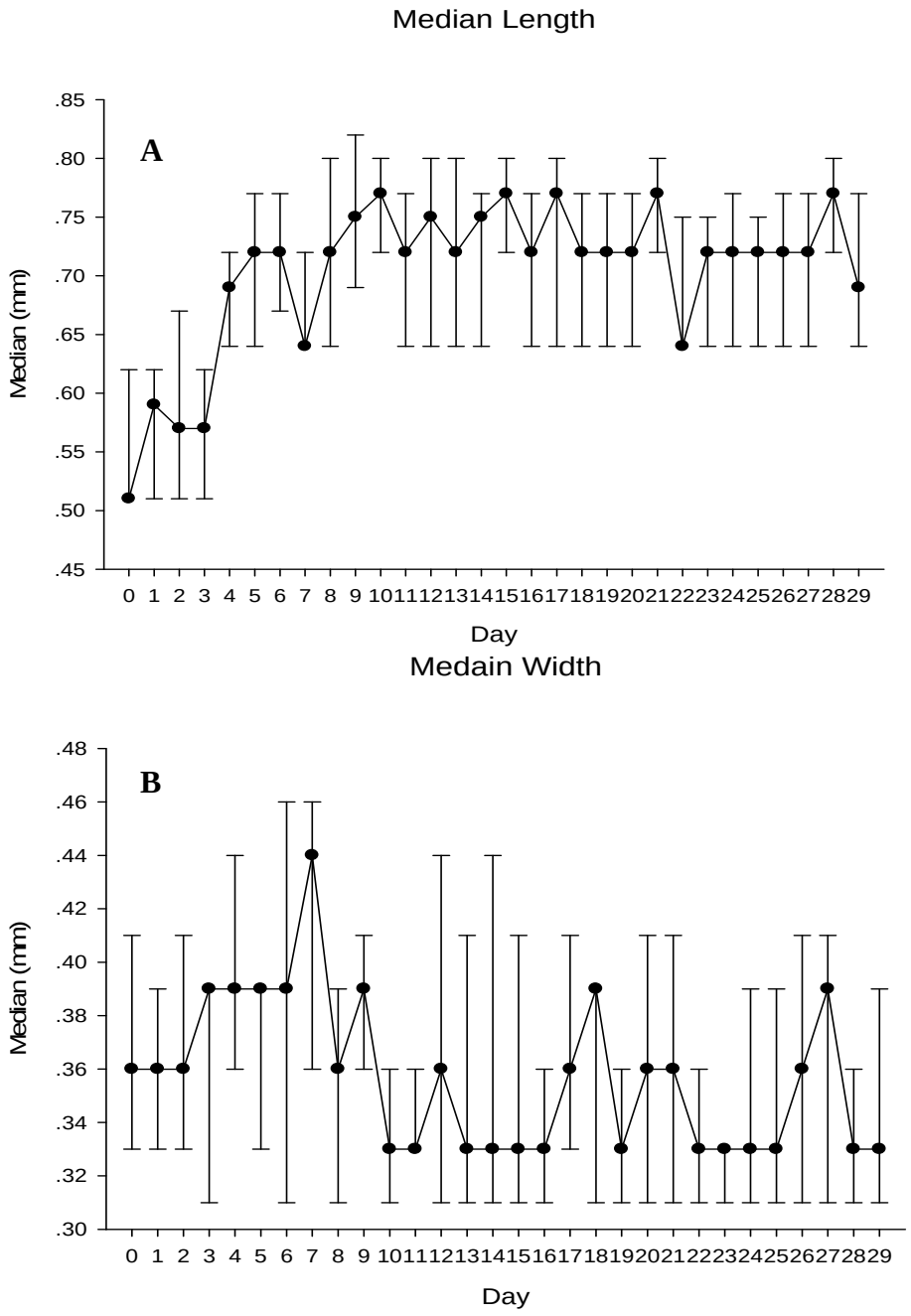

Figure 23. Median length (A) and width (B) of *C. megacephala* testes from just after emergence (day 0) to 29 days old, illustrating the change in both length and width of the testes throughout the male fly's lifetime.

SEM and TEM observations

An SEM micrograph of testes showed a smooth surface that was occasionally penetrated by the tracheoles. TEM micrographs showed that the testes wall of 3 day-old *C. megacephala* was actually formed by an external layer, a peritoneal sheath, basement membrane, and follicular epithelium (Figure 25A). Rounded grains containing pigment, with varying density levels, were spread in the cytoplasm of this layer.

The testes of 3 day-old adult males were characterized by three stages of developing spermatozoa. The first stage was commencement of the first division of maturation, the primary spermatocyte. The testes were pear-shaped with large nuclei (Figure 25B). The ratio of nucleus to cytoplasm was 1:1, with chromatin dispersed inside the nucleus (Figure 25B). An alternate appearance of chromatin in the spermatocyte nucleus was also present (Figure 25B). In the second division of maturation, the secondary spermatocyte was characterized by the presence of elongated clusters of chromatin in the globular-shaped cells. The primary and secondary spermatocytes could be differentiated in the transmission electron micrograph (Figure 25B), where the third division of maturation was recognized by the appearance of bundled nuclei, with a visible nuclear membrane and loose cytoplasm (Figure 25C).

Accessory gland

LM, SEM and TEM observations:

The accessory glands, which were separated from the vas deferens, appeared as a thick, white paired structure and the terminal end opened into the anterior end of the ejaculatory duct. TEM analyses of the 3-day-old gland showed that the basal region was vacuolated; recognized by variable sizes of subcuticular cavities, whereas, the apical region was connected to the gland lumen (Figure 25D). When focused on, the glandular cells appeared columnar, bearing a large, oval-shaped nucleus (Figure 25E) with a prominent eccentric nucleous. The cytoplasm of the glandular cells comprised several organelles, including numerous rough endoplasmic reticulum (RER), mitochondria, variable sizes of secretory vesicles, free polyribosomes and large numbers of vacuoles. A large nucleus contained one pack of chromatin, with a visible double-membrane nuclear envelope, which was occasionally porous. Mitochondria, mostly oval and spherical in shape, were numerous but variable in size, bearing apparent cristae and membrane. The RER looked active in the functional phase, appearing in two forms; mainly in the short swollen cisterns and less often in parallel stacks (Figure 25F). A group of electron-translucent content, probably the primary secretory granules, was frequently observed adjacent to the stacks or between the short swollen RER cisterns. RER, which comprised the secondary secretory granules, characterized by varying electron-condensed aggregation from light to medium density within the membrane-bound inclusions, were occasionally found. In some sections, large areas filled with, most probably, tertiary secretory

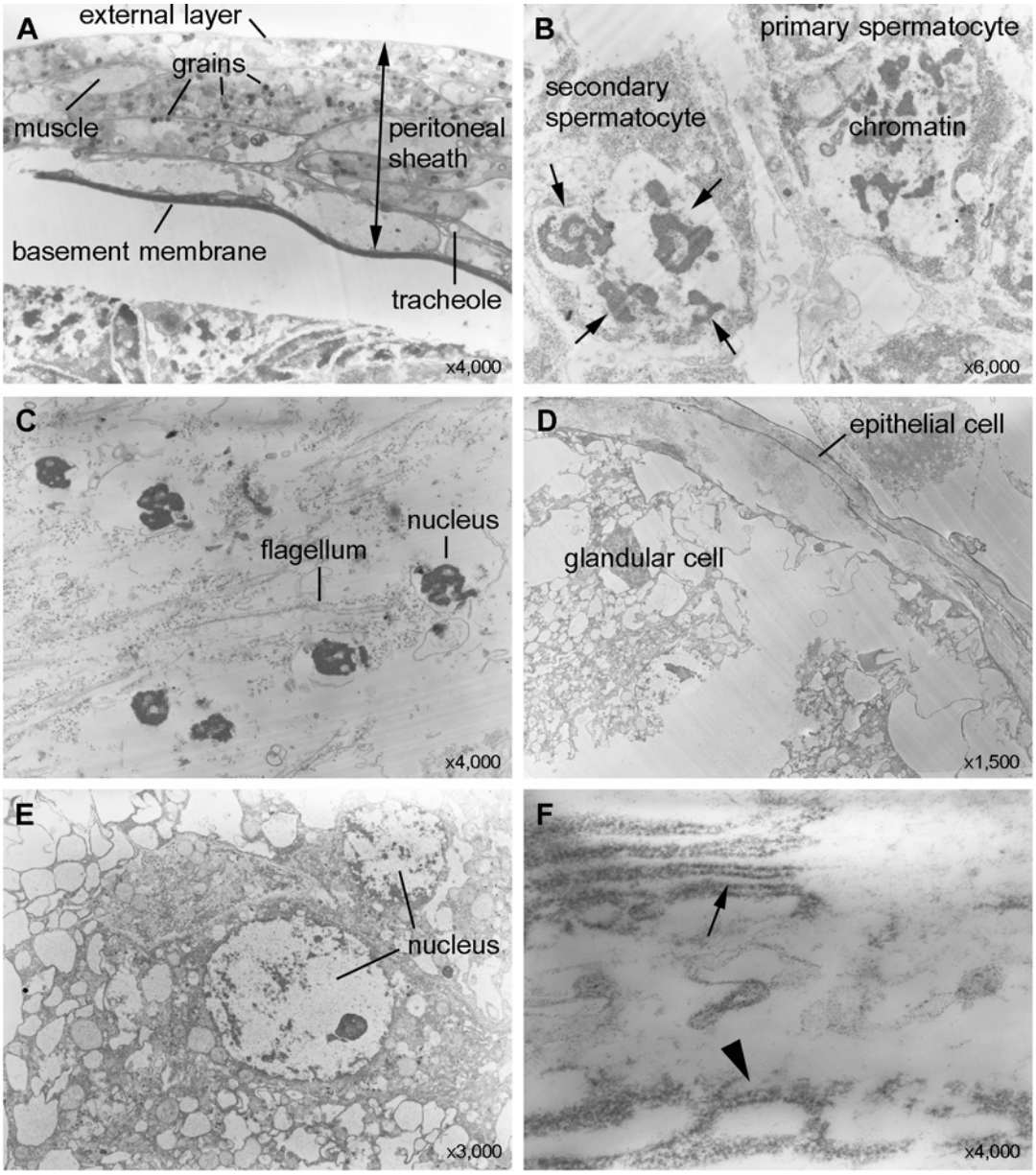

Figure 25. TEM micrographs of a 3 day-old testis and accessory gland of *C. megacephala*. (A) Testis showing the peritoneal sheath. Different densities of the grains in the peritoneal sheath were detected. (B) First division maturation of the testis, displaying a primary spermatocyte characterized by group chromatin material inside the nucleus, and secondary spermatocyte characterized by four clusters of chromatin (arrows) within the nucleus. (C) Third division maturation, characterized by individual spermatozoa, each having a nucleus and an elongated flagellum. (D) Accessory gland showing epithelial and glandular cells. (E) Glandular cells with a large nucleus and vacuolated cytoplasm. (F) Two forms of rough endoplasmic reticulum; mainly in the short swollen cisterns (arrowhead) and less often in parallel stacks (arrow).

granules were discernable in a commonly tight pack within or among glandular cells, but more evidently near the lumen. When focusing on the lumen, TEM sections of the 3-day-old glands revealed variable morphology of the tertiary secretory granules, giving rise to a progressive development. First, the lumen was mostly filled with moderated electron-dense materials with variable holes, and its rim internally lined by small, heavy electron-dense materials. Second, the moderately compact electron-dense materials disintegrated into numerous scattered small chains. Some sections exhibited fusion of these secretory materials to form polymorphic electron-dense materials within a delicate sac.

Vas deferens

LM, SEM and TEM observations:

The vas deferens of *C. megacephala* were a simple paired set of ducts that connected the testes with the ejaculatory duct (Figure 21A). The terminal portion of the ejaculatory duct terminated at the exterior via the external organ, the aedeagus.

Comparison of left and right vas deferens measurements revealed a significant difference in length at $P = 0.039$ for the overall median (paired *t*-test). The left vas deferens (median = 0.91 mm) was longer than the right (median = 0.86 mm), but the original anatomical position, with respect to left and right of the vas deferens, was not preserved during dissection. Therefore, it was not possible to be certain of this result corresponding to the internal orientation within the fly. The width of the vas deferens remained constant, measuring 0.06 mm for the entire observation period.

SEM images showed that the vas deferens and the ejaculatory duct were occasionally penetrated by tracheoles (Figure 21B). Investigation using TEM demonstrated that the vas deferens consisted of an epithelial cell layer, which was the most external layer, and a plasma membrane layer adjacent to the duct lumen (Figure 26A). The muscular layer and tracheoles were visible among the epithelial cells (Figure 26A). The plasma membrane layer adjacent to the duct lumen (Figure 26A) was rich in rough endoplasmic reticulum. The plasma membrane image showed a cell with a visible nucleus, numerous rough endoplasmic reticulum, Golgi complexes, mitochondria and secretions. The nucleus of the cell imaged in the plasma membrane revealed irregularly shaped, scattered chromatins in the cytoplasm. The nuclear membrane or nuclear envelope was visible as a double-membrane (Figure 26B) surrounded by mitochondria, with visible cristae (Figure 26C) and free polyribosomes. The Golgi complexes were composed of saccules and had more or less electron-dense vesicles of visible size. The plasma membrane layer of the vas deferens also contained myelin figures and rough endoplasmic reticulum. The secretions were loosely packed fibrous materials, which were visible in the lumen as well.

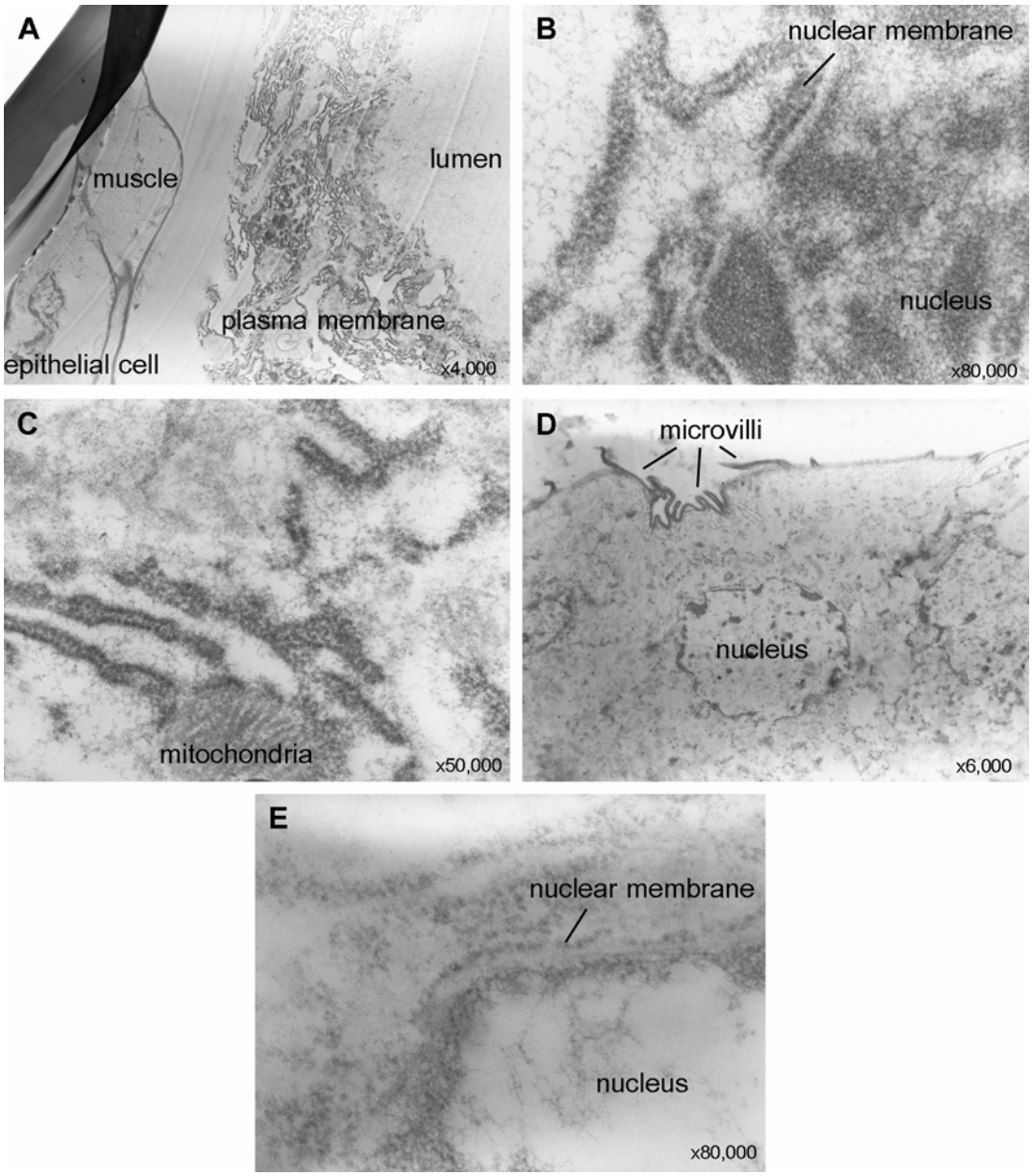

Figure 26. TEM micrographs of vas deferens and ejaculatory duct of 3-day old male *C. megacephala*. (A) Vas deferens displaying the epithelial cell layer, and plasma membrane layer adjacent to the duct lumen. (B). Nuclear membrane at the nucleus of the vas deferens. (C) Mitochondria in the cytoplasm of the vas deferens. (D) Ejaculatory duct showing epithelial cells with microvilli. (E) Nuclear membrane at the nucleus of the ejaculatory duct.

Ejaculatory duct

The median ejaculatory duct length fluctuated, but slowly increased similarly to the vas deferens with a peak in length on about day 5. Median duct width did not fluctuate and remained 0.078 mm for the duration of the study.

In the overall view, transverse sections of the ejaculatory duct of *C. megacephala* showed that it comprised the epithelial and luminal cuticle. The epithelial cells were flat, with round nuclei, and the outer epithelial layer formed the concave of microvilli (Figure 26D). The luminal cuticle was beneath the epithelial cells. An image of the region between the epithelial cells showed that it contained a microfilament and secretions, with visible fibrous materials. The double-membrane of the nuclei is visible in Figure 26E. Numerous mitochondria surrounded the rounded nuclei. However, despite evidence of a muscular layer in the SEM images, no sign of this layer was found in the ejaculatory duct of *C. megacephala* using TEM imaging. Besides nuclei, mitochondria, microvilli and microfilament between the epithelial cells, the ejaculatory duct of this blow fly did not have any special features. Interestingly, the spermatozoa were not present in either the vas deferens or the ejaculatory duct of *C. megacephala*.

5. Reproductive tract of females

The internal female reproductive organs of *C. megacephala* consisted of 2 ovaries, 2 lateral oviducts, a common oviduct, 3 spermathecae and 2 accessory glands. The round ovaries were placed dorsalateral to the alimentary canal, enclosed in a peritoneal sheath. The surface of the ovary was a mesh surface; usually penetrated by tubular tracheoles. The highly convoluted short lateral oviducts were fused to form a common oviduct leading to the genital chamber or vagina. There were 3 rounded spermathecae, of which 2 were loosely bound together. The spermathecae with tubercular surface consisted of a pyriform shape with ducts entering the vagina dorsally. A pair of long tubular accessory glands, opened into the dilated anterior vagina. Each accessory gland showed numerous papillae (Figure 27A,B).

Ovary and ovariole

The two ovaries of 3-day-old *C. megacephala* are small round bodies of white in color (Figure 27A), and the ovarioles could be observed within each ovary during egg development. Each ovary in the 3-day-old fly was sheltered by an ovarian envelope, which consisted of a tough outer epithelial sheath surrounding the entire ovary. The ovaries of 3-day-old blow flies were also tightly enveloped by a large and well developed tracheal system (Figure 27B), which became looser as the eggs matured.

The ovaries of 9-day-old females appeared larger and more developed than those of 3-day-old females. They reached a maximum size observed at nearly 4 mm in diameter by nine days of age, and by then the eggs fully filled the ovary (Figure 28). The ovarioles remained

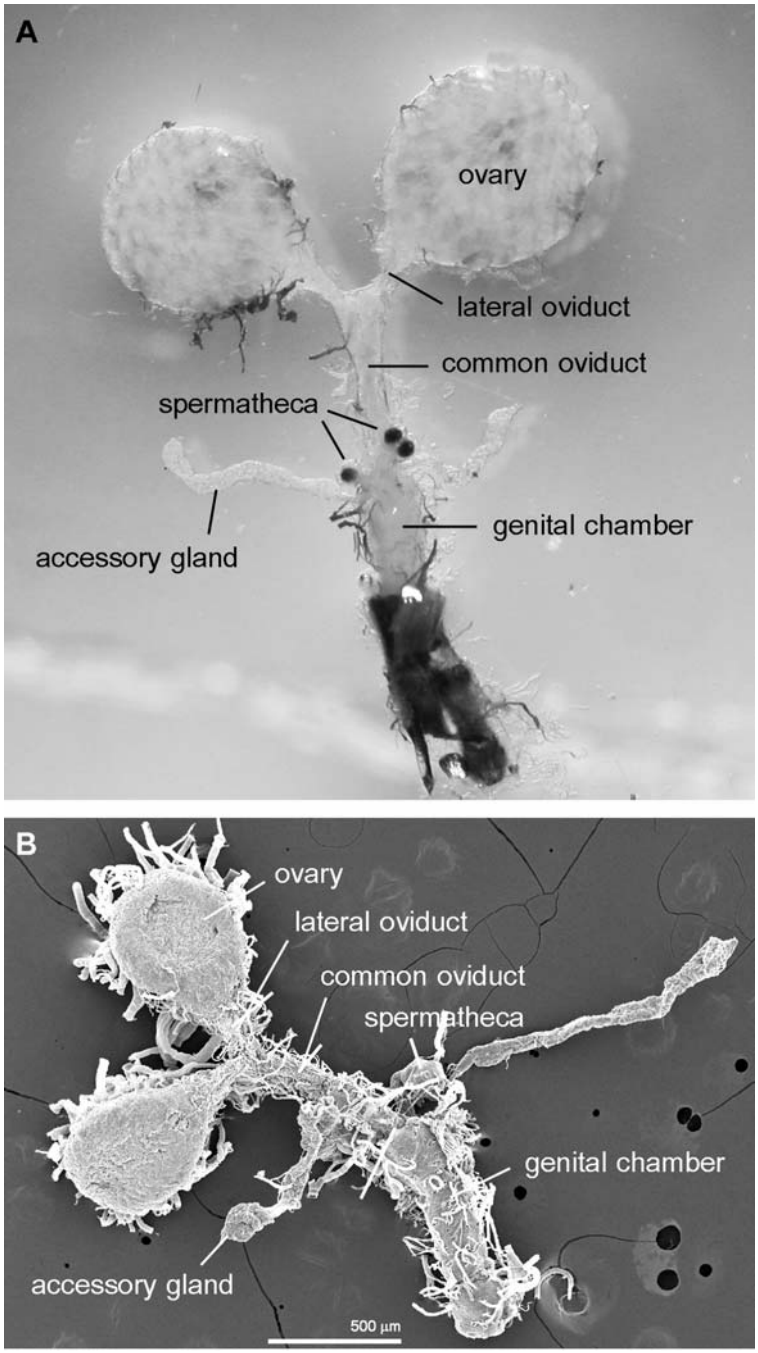

Figure 27. (A, light micrograph; B, SEM micrograph) Internal female reproductive organs of *C. megacephala* showing ovaries, lateral oviducts, a common oviduct, 3 spermathecae and accessory glands.

sheltered by the ovarian envelope, however, the whole structure appeared thin with many holes. The tracheal system also appeared to be greatly reduced in 9-day-old blow flies.

The median ovary length and width of the left and right sides were approximately equal, which was confirmed by no significant difference between sides in length or width at $P = 0.260$ and $P = 0.159$ (paired *t*-test), respectively. The relationship between the morphometric characters of the ovaries and female age are presented in Figures 28A, 28B. There was a slow increase in length and width of the ovaries at day 0 (just after emergence) until day five. Then, there was a rapid increase in size of the ovary from day five to nine (Figure 28A, 28B). These features correlated with changes in ovariole size during egg development, which is described in the following section.

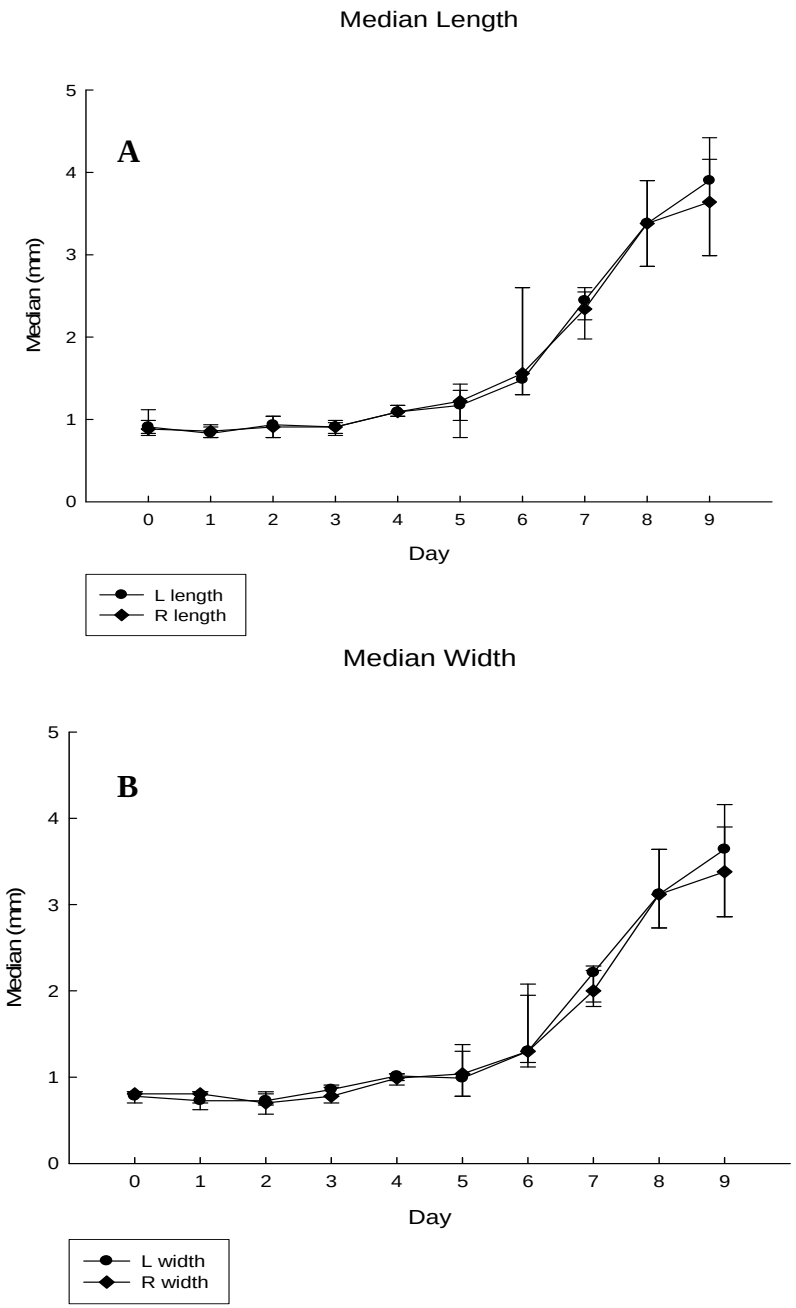

Figure 28. Median (+/- range) length (A) and width (B) of *C. megacephala* ovaries of from just after emergence (day 0) to 9 days old, illustrating the change in both length and width of the ovaries.

Changes in the ovariole during egg development:

Changes in the ovariole: The stages described in this study for *C. megacephala* were from the basis of an aging technique that could be used to differentiate the ages of field collected female blowflies. In this experiment, the ovarioles of *C. megacephala* were observed for changes from day 0 (just after emergence) to 9-days-old under ambient temperature conditions of 18-27^{O}C during the study period. The observations of the ovarian stages were conducted using fresh preparations, in which the ovarioles were placed on a slide and covered with a cover slip. They were then examined and measured under an ordinary light microscope. The ovarioles of *C. megacephala* were of the polytrophic ovariole type, and the characterization of each stage of ovariole maturation was conducted in a similar manner to the way in which these stages have been outlined in other blow fly species; including *Lucilia cuprina*, *Chrysomya bezziana*, *Cochliomyia hominivorax*, *Chrysomya putoria* and *Chrysomya albiceps*. The correspondence between the classifications used to describe the ovarian stages of other blowflies with that of *C. megacephala* is provided in the following section.

Stage I (Day 0-2: Figure 29): Flies from emergence to 3-days-old displayed the same ovariole stage, in which each ovariole haa a piriform germarium with a developing cyst. It was assumed that the oogonium divided into a cystoblast that would continue this division process. The follicles were not yet well differentiated from the germarium.

Stage II (Day 3: Figure 29): The follicle was nearly spherical and distinct from the germarium. The cystocytes were clearly visible and situated in the basal region of the follicle, which was surrounded by the follicular cells.

Stage III (Day 4: Figure 29): The follicle was now completely separated from the germarium, with its only connection by interfollicular stalk. It was nearly spherical in shape. The cystocytes were completely surrounded by the follicular epithelial cells and a more distinct separation had appeared between the germarium and the follicle in the formation of the first egg chamber.

Stage IV (day 5-6: Figure 29): The follicle was spherical and the cystocytes had differentiated into nurse cells. The follicle had enlarged considerably and the nurse cells dispersed in the follicle chamber. However, no yolk was visible in the follicle chamber.

Stage V (Day 7: Figure 29): By this stage the follicle had increased in size and was slightly ellipsoidal in shape. Yolk deposition had increased in the oocyte and was visible at the basal region of the follicle. The follicle epithelium was clearly visible and a second follicle had started to form in the germarium.

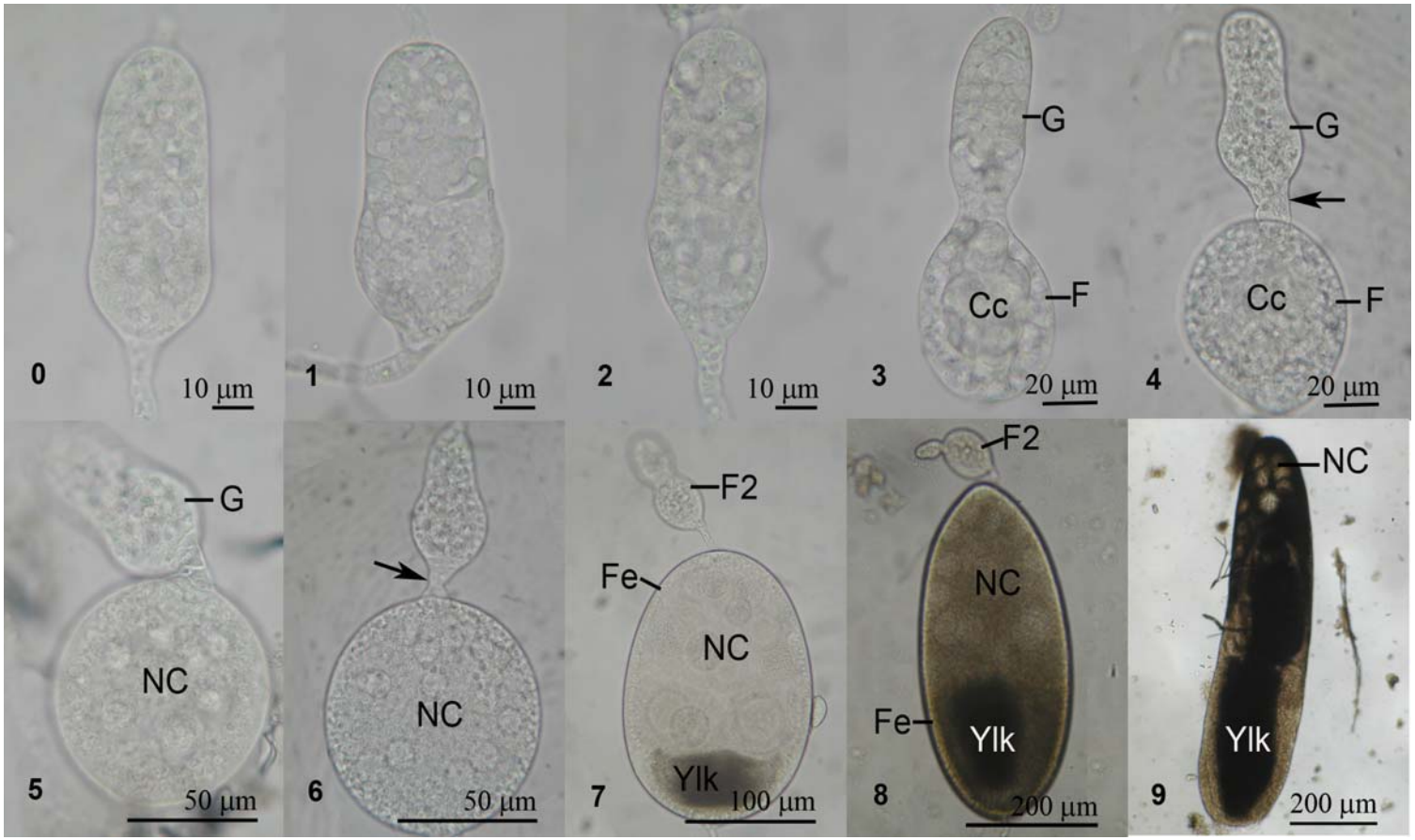

Figure 29. Light microscope images showing the ovariole development of *C. megacephala* from just after emergence (day 0) to 9 days old, and illustrating the successive ovarian stages. The first row illustrates flies of 0 to 4 days-old (from left to right), and the second row displays flies of 5 to 9 days-old. Stage I (day 0-2) shows the follicles not well differentiated from the germarium. Stage II (day 3) with the follicle (F) showing the germarium and cystocytes (Cc) clearly visible. Stage III (day 4); the first follicle is almost fully separated from the germarium (G) by an interfollicular stalk (arrow). Stage IV (day 5-6) with the follicle showing the nurse cell (NC). Stage V (day 7); the follicle considerably elongated the yolk (Ylk) at the basal region. The follicular epithelium (Fe) is clearly visible. Stage VI (day 8); one-third of the follicle is occupied by yolk (Ylk). Stage VII (day 9); Two-thirds of the follicle is occupied by yolk (Ylk). Note the secondary follicle (F2) at stage IV is visible in 7- and 8-day-old blow flies.

Stage VI (Day 8: Figure 29): The follicles were now oval in shape, with yolk (oocyte) occupying up to one-third to one-half of the total follicle length. The epithelium around the oocyte had become columnar and the appearance of the follicular material around the nurse chamber remains similar to that in stage V.

Stage VII (Day 9: Figure 29): The follicles were elongated, and had greatly increased in length, while only slightly increasing in width. The yolk (oocyte) now accounted for two-thirds of the total follicle length. The degenerating nurse cells occupied only the anterior cone of the follicle. The follicular cells were still present, but of squamous shape and they were actively secreting the chorion.

Stage VIII (Day 10: Figure 29): In this final stage, the eggs were full size with the oocyte occupying the entire follicle, and the nurse cells having disappeared. The eggs of *C. megacephala* were elongated, about 1.4 mm in length and 0.40 mm in width.

Development of the follicular epithelium in *C. megacephala*

This study presented the first transmission electron microscopic study of follicular epithelium development in 3-day-old and 7-day-old *C. megacephala*. The follicular epithelium surrounded the developing egg and was responsible for secreting the chorion. The cellular changes of the follicular epithelium were documented during egg development for 3-day-old and 7-day-old females in the following section.

In 3-days-old flies, three layers of the developing chorion were discernable, including the basal lamina, follicular cell layer and a transparent electron-dense space between this layer and the occyte with nurse cells (Figure 30A). The follicular cells contained large nuclei that were infiltrated with numerous tracheoles and a muscle layer, which was also visible (Figure 30A). The nuclei were elongated along the cell axes and contained large nucleoli (Figure 30A). The cytoplasm of the oocyte contained visible mitochondria and rough endoplasmic reticulum (Figure 30B).

In 7-days-old flies, the formation of the chorion appeared more complete, as evidenced by presence of the basal lamina, follicular cell layer, and the outer and inner layer of chorion (Figure 30C). The inner chorionic layer at the posterior pole of the egg formed a shallow pit surrounded by a collar (Figure 30C). The bottom of this pit was the site of attachment for the egg. The components of the oocyte were visible, including the yolk body surrounded by the vitelline envelope (Figure 30D). The vitelline envelope was a fine granular layer, quite close to the chorion.

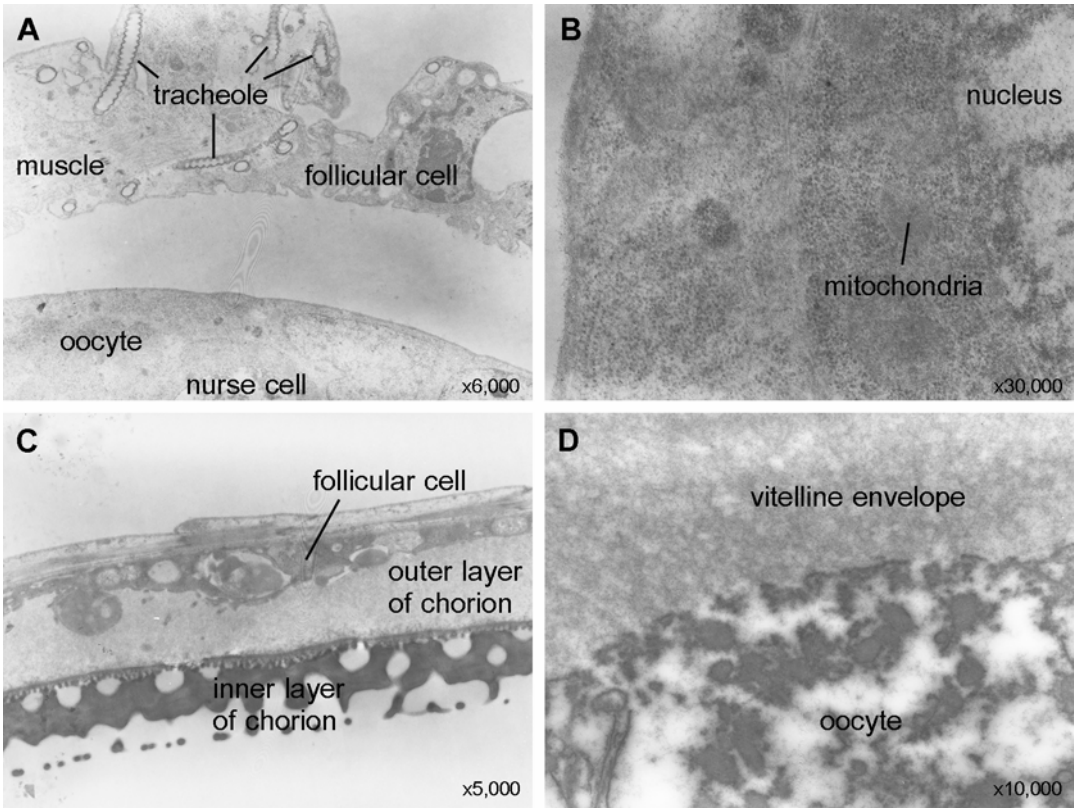

Figure 30. TEM micrographs of ovarioles of 3-day-old (A,B) and 7-day-old (C,D) *C. megacephala*. (A) The chorion mainly consists of follicular cells, which are inserted by tracheoles. The occyte with nurse cells is beneath the transparent electron-dense space. (B) Oocyte showing nucleus and mitochondria. (C) Chorion showing follicular cells, and an outer and inner layer of chorion. (D) The oocyte illustrating the yolk body surrounded by the vitelline envelope.

Accessory gland

The accessory glands of *C. megacephala* were long and tubular, opening into the dilated portion of the anterior genital chamber (Figure 27). SEM micrographs confirmed that the accessory glands were a paired structure opening to the proximal end of the genital chamber (Figure 27B). The level of detail revealed by the SEM images was much higher than that obtained with light microscopy. These images showed that each gland has two distinct portions including an apical bulb and a tubular duct (Figure 31A). The apical region was a dilated region forming an elliptically shaped apical bulb. This area was characterized by numerous papillae and occasionally penetrated by tracheoles. The region of the accessory gland, adjacent to the genital chamber, was a long and tubular gland duct. The surface of the tubular duct was also covered in papillae with penetrating tracheoles (Figure 31A).

The epithelial cells of the apical bulb and tubular gland duct of 3 day-old flies are displayed in Figure 31B. The transverse section through the epithelial cells of the apical bulb of the gland at 3 days old displayed a single cell, with a large central nucleus and Golgi apparatus visible in the cytoplasm. The lining of the duct-forming cells and thin cuticle was deep in and projected toward the gland lumen (Figure 31C). The cistern cells, where large secretions are stored, were also visible adjacent to the epithelial cell (Figures 31B, 31C). The lumen of the cistern was apparently empty in this young female accessory gland and appeared transparent electron dense. Figure 31C displays a transverse section through the tubular portion of the gland, comprising the duct. This region contains secretory granules and vesicles of variable sizes that are present in the cytoplasm of the secretory cells. The cistern cells contain secretory materials for delivery directly to the gland lumen.

Replicated transverse sections along the long axis of the accessory gland revealed cells in various stages of maturation, which could be divided into three main stages. The first stage of maturation was characterized by the presence of numerous rough endoplasmic reticulum, secretory granules in the cytoplasm and cistern cells filled with secretory materials (Figure 31D). Each rough endoplasmic reticulum was characterized in this stage by having distinctly long channels. The lumen of one of the imaged cistern cells contained fine fibrous material, which suggested a pore in the cuticle lining of the lumen and, thus, a connection with the lumen of the gland. The second stage of maturation was characterized by the presence of a large cistern cell in the cytoplasm, which was surrounded by mitochondria and secretory granules. In the lumen of the cistern cell, fibrous material was also observed, as in the first stage. The secretory granules seemed to be connected with the lumen of the cistern cell, suggesting that they released secretions into the lumen of the cistern cell, which was, in turn, released into the gland lumen through pores in the cuticle. In the third stage of maturation, secretory granules, mitochondria, and rough endoplasmic reticulum were still observed in the cytoplasm, but the

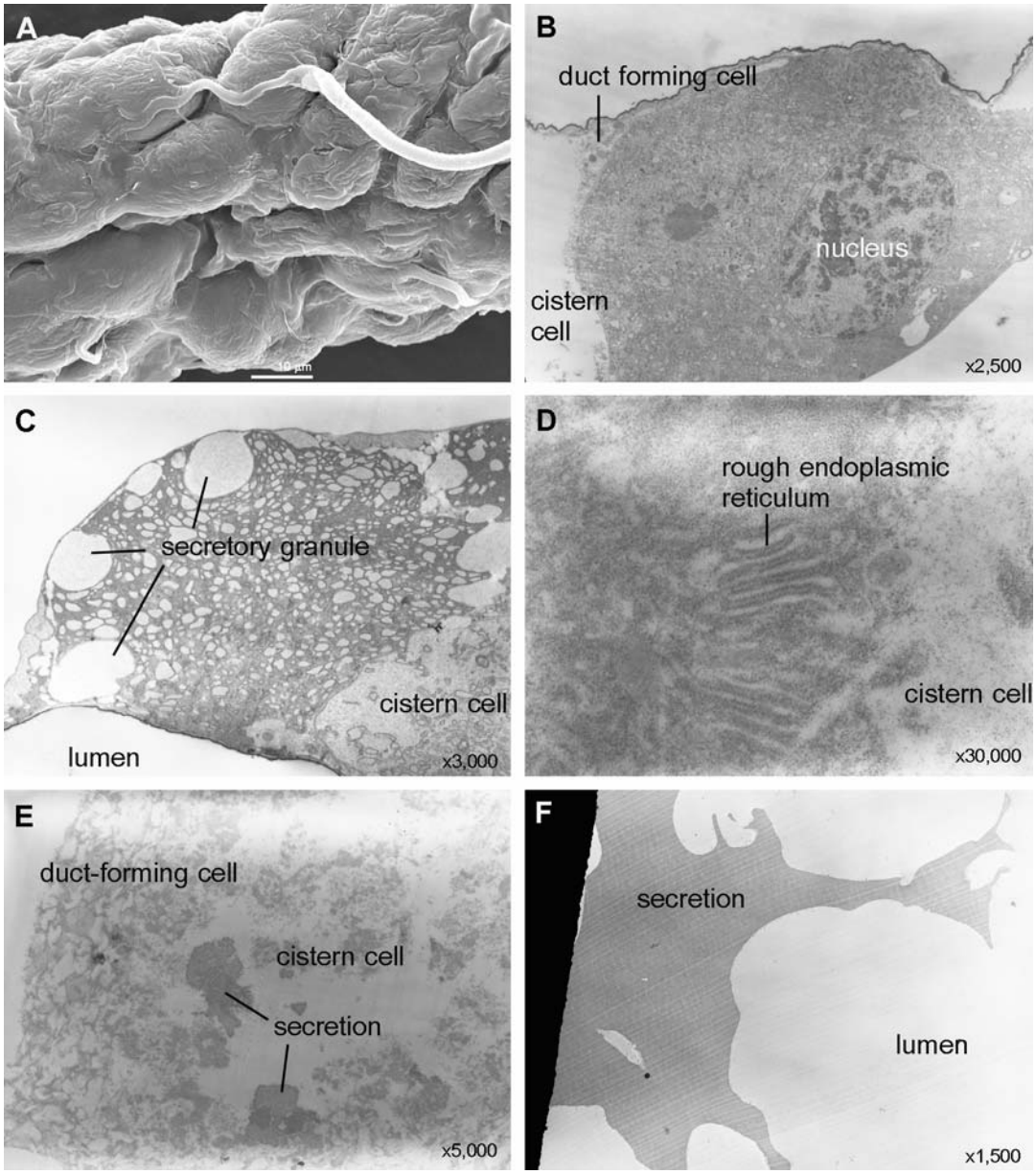

Figure 31. Accessory gland of 3 day-old female *C. megacephala*. (A, SEM; B-F, TEM) (A) Morphology of the gland. (B) Gland with epithelial cells containing large nucleus, duct-forming cells. (C) Epithelial cells with large secretory granules (D) Rough endoplasmic reticulum and cistern cells. (E) Large cistern cell and large area of secretion. (F) Gland lumen with homogenous dense secretions.

rough endoplasmic reticulum of the third stage was more swollen in shape (Figure 31E). The cistern cells in this stage appeared to contain two types of secretions, including one of dark color and one that was pale color. In the main accessory gland lumen, homogenous dense secretions were present in low volume, with about 15% of the total area comprising secretions. There was also a transparent electron dense space with between the epithelial cells and gland lumen (Figure 31F).

Spermathecae

The spermathecae of *C. megacephala* were black and pyriform shaped, with a duct entering the genital chamber dorsally (Figure 27A). The spermathecae of this blow fly were arranged in 1:2 or 2:1 bilaterally. The two spermathecae on one side were loosely bound together, while the one on the other was not attached.

SEM micrographs of the three spermathecae showed that they had a tubercle covered surface, which was penetrated by tracheoles. The basal region of each spermatheca was connected via a spermathecal duct to the genital chamber. Longitudinal muscles were clearly visible on the surface of the spermathecal duct. TEM observations of 3 day-old female *C. megacephala* revealed that the cells of the spermathecae (Figure 32B) produced secretions that might aid in the maintenance of stored sperm and, thus, have many similarities in structure to the cells found in secretory gland structures. The spermathecal epithelial cells were characterized by the presence of a rounded nucleus, mitochondria, numerous vacuoles, rough endoplasmic reticulum and tracheoles. In the lumen of the cistern cells, a fine fibrous material was present, surrounded by rough endoplasmic reticulum, Golgi apparatus and dense droplets of different sized secretion (Figure 32C). In the cytoplasm of the epithelial cells, numerous vacuoles of variable size were present, and penetrated by mitochondria. The mitochondria in the cytoplasm were of various shapes (Figure 32D). The fact that 3 day-old females were not filled with spermatozoa was an important observation.

Genital chamber

The genital chamber of *C. megacephala* was a single, long tubular organ. It was located in the posterior region of the abdomen in the female fly (Figures 27A, 27B), and the internal conduit for the reception of sperm and passage of eggs. At its anterior end, the genital chamber received the common oviduct and spermathecal ducts (Figure 27A). It was an invagination of the body wall and thus lined with cuticle.

SEM images showed that the genital chamber was also occasionally penetrated by tracheoles (Figure 33A). When viewed with SEM, the genital chamber was a tubular shaped duct, having an external surface that had a distinct pattern consistent with longitudinal muscle interfacing with circular muscle (Figure 33A). Detailed images of the circular muscle showed

tracheoles penetrating between muscle fibers into the spaces between the cell layers. Neither of these features were observable under light microscopy.

The morphology of the genital chamber was observed in 3-day-old flies using semi-thin sections and TEM micrographs. Semi-thin cross sections through the tubular genital chamber demonstrated approximately five epithelial cell layers encircling the lumen (Figure 33B). A semi-thin section of the genital chamber lumen showed that a thin convoluted cuticle lines the lumen (Figure 33C). TEM images confirmed that the central lumen of the genital chamber was surrounded by distinct cell layers. Moving from the center outward, the first-layer was the cuticle-layer, which projected into the lumen, and the fifth-layer was the outermost layer. The fine structures of each layer were described by using TEM magnifications in the following sections.

The first-layer of the genital chamber: A transverse section of the first-layer, located adjacent to the lumen, showed that it consisted of a cuticle and cells (Figure 33C). Higher magnification revealed that the cuticle of the genital chamber was composed of an inner epicuticle and an overlying endocuticle. The inner epicuticle was thin, with an electron-dense layer and double membrane (Figure 33D), while the endocuticle was a less electron-dense layer, but much thinner. The secretion material perforated to the lumen through the epicuticle layer (Figure 33D).

The second-layer of the genital chamber: A transverse section of the genital chamber, showing the second- and third-layer, revealed that a membrane junction existed between both layers, as shown in Figure 33B. The membrane junction contained numerous tracheoles (Figure 33E). In the second-layer, numerous mitochondria of variable size and shape were visible as well as numerous vacuoles. At the highest magnification, elongated mitochondria were observed. Approximately 10-15 mitochondria were found in each cell in the second-layer (as counted under TEM).

The third-layer of the genital chamber: The one cellular chamber of the third-layer was surrounded by a cell membrane with tracheoles. The third-layer was rich in mitocondria, with much higher densities in each cell (≈75-85 mitochondria/cell) than those found in the second-layer. Higher magnifications of the third-layer revealed mitochondria of variable shape, including round and elongated mitochondria. An elongated nucleus was also present with two visible nucleopores. Next to the nucleus, a pack of ribosomes was visible, which occasionally penetrated by mitochondria. Additionally, a muscle layer was visible in the third-layer.

The fourth-layer of the genital chamber: TEM images of the fourth-layer showed that it consisted of multi-layer cells, which each one was elongated and contained numerous mitochondria. Approximately 100 mitochondria/cells were found in the cells of this layer (Figure 33F). Each layer of cells was separated by a membrane junction, shown as a line of electron-

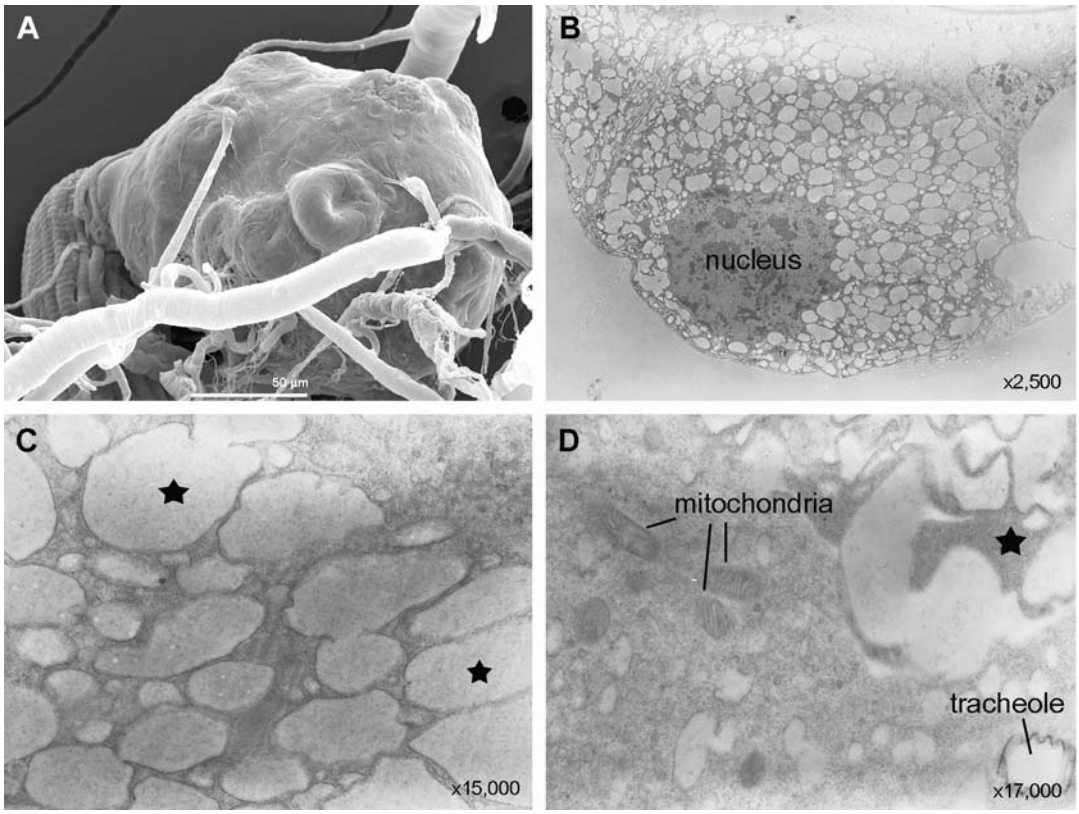

Figure 32. Spermatheca of 3-day-old female *C. megacephala*. (A, SEM; B-D, TEM) (A) Globular structure of spermatheca connected via a spermathecal duct. (B) Epithelial cells containing large nucleus and vacuolated cytoplasm. (C) Variable size of secretions (stars). (D) Secretion (star) and mitochondria inside the cytoplasm. Tracheole is penetrating into the cells.

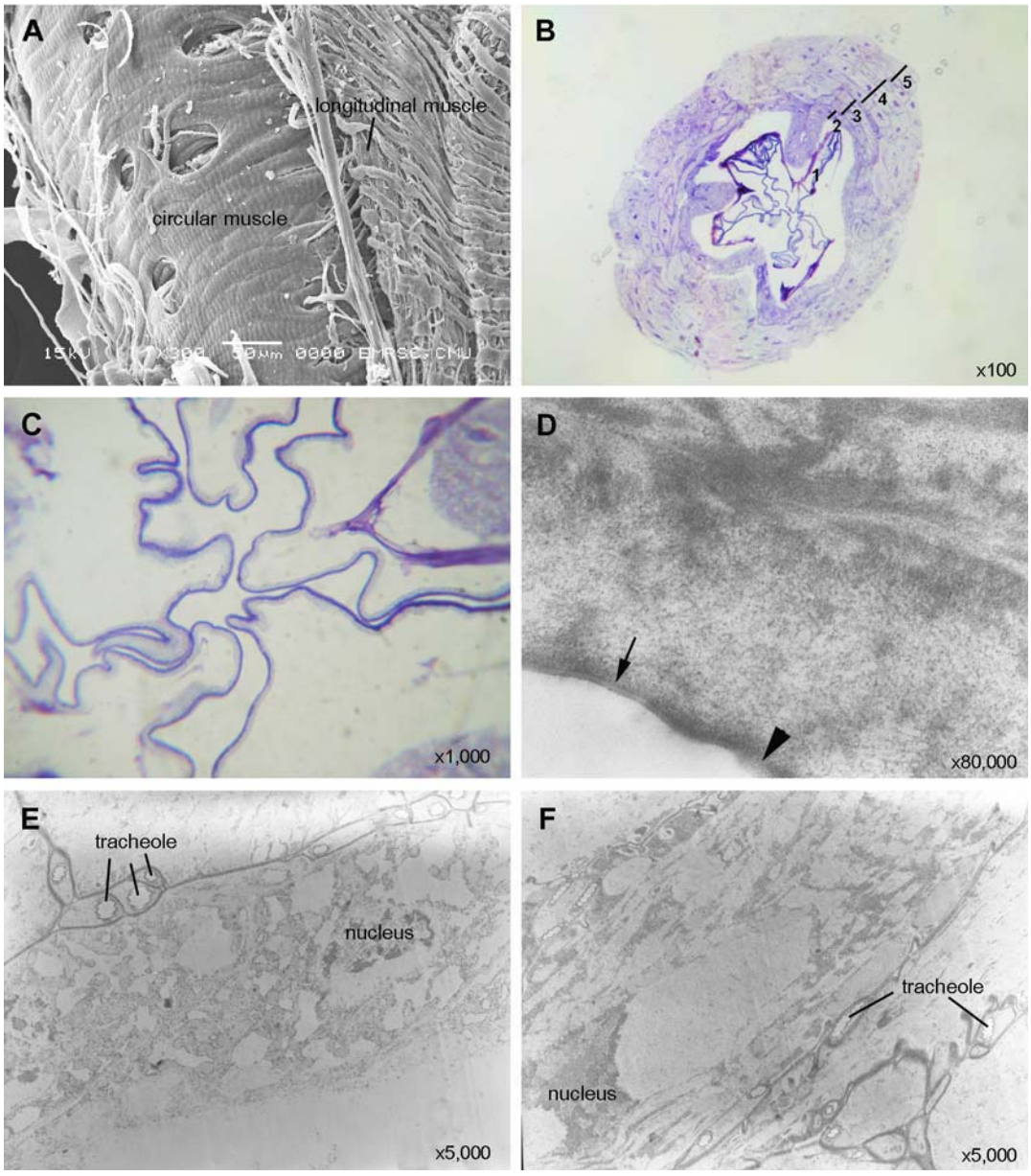

Figure 33. Genital chamber of 3-day-old female *C. megacephala*. (A) SEM image showing dense circular muscle covering the chamber. (B) Thick cross section showing approximately 5 layers of the genital chamber covering the central lumen. (C) Thick cross section showing the first-layer of the cell with internal cuticle. (D) TEM image of the first layer revealing the double layer of the epi-cuticle (arrow) and perforation of secretion into the lumen (arrowhead). (E) Cells of the second-layer. (F) Cells of the fourth-layer.

dense material with tracheoles. Multi-layer cells in the fourth-layer of the genital chamber had two different types of nuclei, characterized by either compact chromatin or dispersed chromatin in the nucleus. The compact chromatin nuclei had a compact homogeneous dense chromatin within the nucleus, whereas, the dispersed chromatin nuclei displayed a dispersed dense chromatin.

The fifth-layer of the genital chamber: The fifth-layer consisted of cells with bundles of ribosomes located centrally. Each columnar cell was separated by a cell membrane, with tracheoles present in between the cell membranes. The nuclei of the fifth-layer cells were elongated, with packs of chromatin peripherally located near the nuclear-membrane. A bundle of ribosomes were visible at the pole of the nucleus, which was also penetrated by numerous mitochondria. The number of mitochondria in the cells of the fifth-layer was much higher than 100 mitochondria/cell of variable size, which were located within the ribosome packs.

The cytoplasm of the epithelial cells (the second-layer to fifth-layer) was high in cellular organelles, with most cells occupied largely by mitochondria. However, the muscle layer was only visible in the third-layer, and not observed in any other layer of the genital chamber of *C. megacephala*.

6. The external genitalia of males and females

The male genitalia of *C. megacephala* revealed characters of the cercus, surstylus, epandrium, phallus, ejaculatory apodeme, and aedeagal apodeme (Figure 34A). The epandrium was a broad structure, similar to a crescent shape. The ejaculatory and aedeagal apodeme were resembled by their lengths. The cercus was significantly longer than the surstylus (Figures 34B, 34D). The apical end of the cercus was more or less rounded laterally (Figure 34C). The external portion of the cercus was endowed with long bristles; the sensilla chaetica and sensilla trichodea along the lower half area (Figure 34B), and short bristles, sensilla basiconica, which existed at the proximal area (Figure 34C). The cercus was enlarged in the upper half, united at its base, and separated distally into two arms, which gradually tapered at the lower half, with the terminal end of each being abruptly truncated (Figure 34D). The surstylus was stoutly triangular shaped, and the proximal half was covered immensely with sensilla chaetica and sensilla trichodea (Figure 34E).

The aedeagus was a clavate shape formed by two main parts; the base theca and the elongated phallus (Figure 35A). The theca expanded proximally, forming a slight central ridge. The distal end of the theca was connected to the proximal part of the phallus, called the corpus (Figure 35B). The corpus was an elongated part of the structure, having gently curved microtrichia inside (Figures 35A, 35B). The end of the phallus was bilobed, with smooth-surfaced vesica (Figure 35C). The juxta lay medially between the vesica, with the outer edge of

both the juxta and juxta process covered with many rows of strong spines (Figure 35C). The stylus formed by a membranous tube lay between the juxta processes. The corpus was connected to the harpe, which was recurved anteriorly, and distally pointed (Figures 35D, 35E, 35F). Some specimens revealed that the anterior end of the harpe was either inserted between the juxta and vesica (Figure 35C). or beneath the vesica (Figures 35E, 35F). The juxta was found to either lie between the vesica (Figure 35D). or be movable beneath the vesica (Figure 35E).

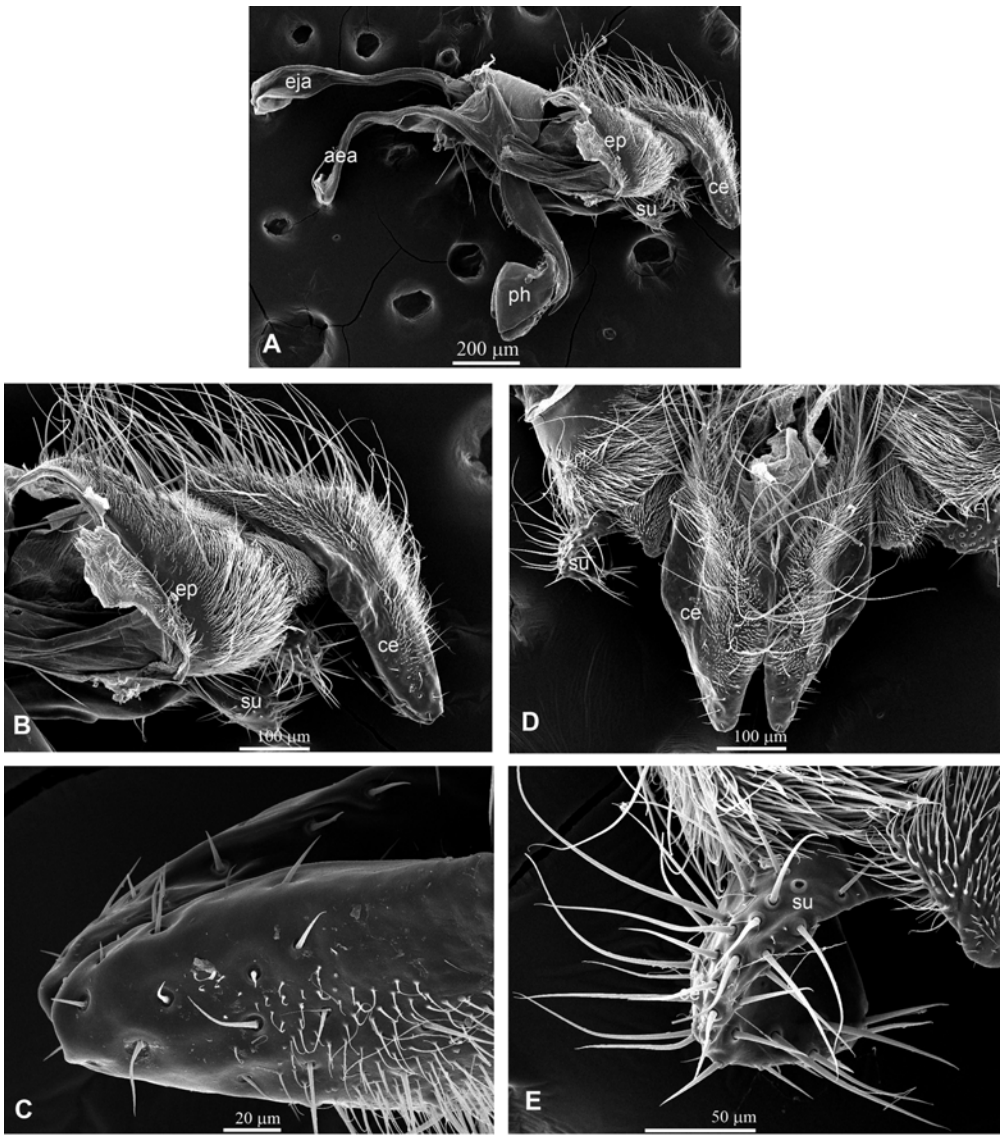

Figure 34. SEM micrograph of the male genitalia of *C. megacephala*. (A) The entire genitalia showing the cercus (ce), surstylus (su), epandrium (ep), phallus (ph), ejaculatory apodeme (eja), and aedeagal apodeme (aea). (B) The lower part of the male genitalia points to a longer cercus (ce) compared to the surstylus (su), which lies underneath the base of the epandrium (ep). (C) The cercus. (D) Enlargement of the upper half of the cercus (ce), laterally lying with the surstylus (su). (E) The surstylus (su) endowed with sensilla chaetica and sensilla trichodea.

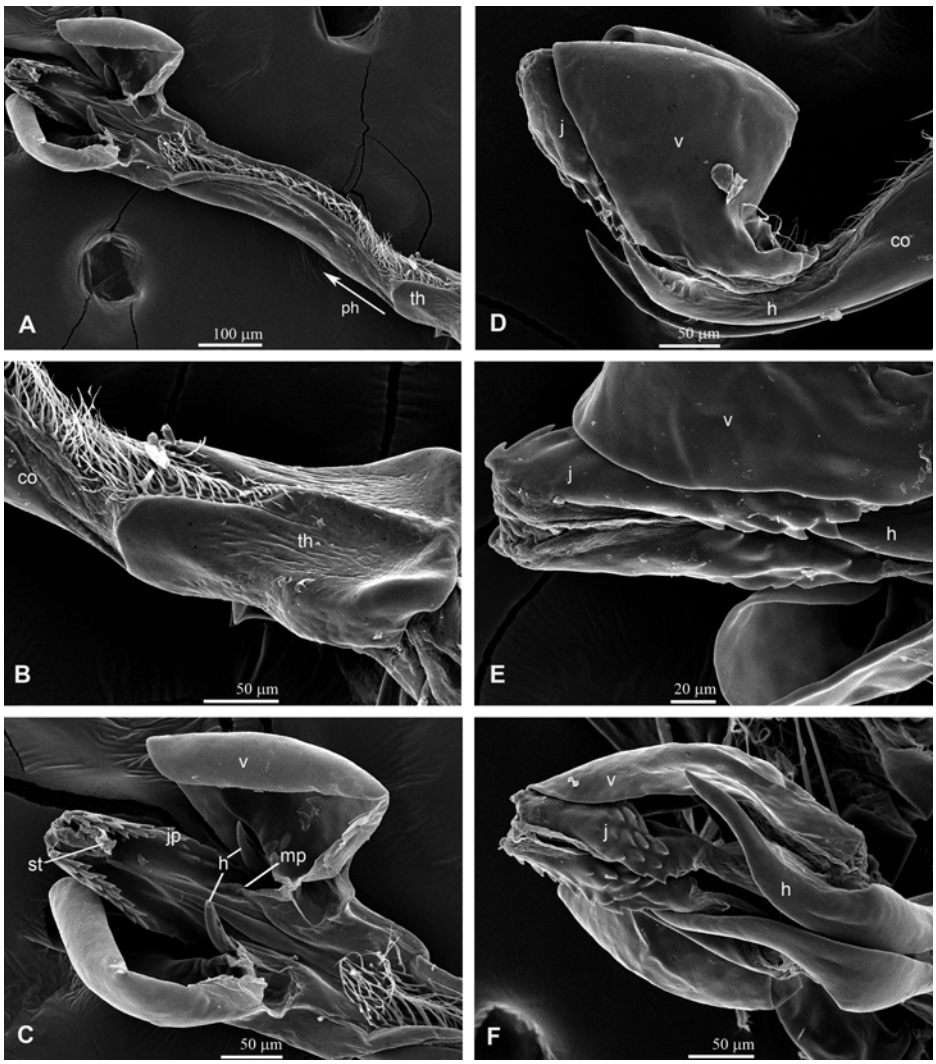

Figure 35. SEM micrograph of the aedeagus of male *C. megacephala*. (A) Aedeagus appearing as a clavate shape formed by the base theca (th) and the phallus (ph). (B) Theca (th) connected to the corpus (co). (C) The apical end of the phallus showing bilobed vesica (v), juxta process (jp), stylus (st), and harpe (h) recurved between the median processes (mp). (D) Aedeagus with elongated corpus (co), harpe (h), juxta (j), and vesica (v). (E) Apical aedeagus showing vesica (v), harpe (h), and juxta (j). (F) Ventral view of the apical aedeagus showing vesica (v), harpe (h), and juxta (j).

Female genitalia or ovipositor of *C. megacephala* are presented in Figure 36. The ovipositor extended from the last pre-abdominal segment and comprised four visible segments (Figure 36A). The dorsum of the last segment “supraanal plate” or “epiproct” was more or less triangular-shaped (Figure 36B). Both the supraanal plate and cercus had a scarcity of sensilla (Figure 36B). Short sensilla basiconica (Figure 36C) and sensilla placodea were found on the supraanal plate.

The ventral view of the female genitalia revealed elongated ovipositor (Figure 36D). The subanal plate or paraproct was a stout maple leaf, whereas the cercus was a clavate shape (Figure 36E). Different sensillum types were observed on the ventrolateral cercus. The sensilla trichodea bore longitudinal grooves externally, which were of variable lengths. The sensilla basiconica comprised short hair shafts bearing longitudinal grooves or having smooth surfaces inserted into cuticular sockets. Stout hair shafts at the base or subapical region of the sensilla basiconica were also observed. Globular sensilla that were probably sensilla styloconica were also observed. This sensillum was revealed as a ball-shaped peg, with rugose surface. The sensilla placodea appeared as a plate-like cuticle that was usually recessed in a shallow pit with a characteristic central pore.

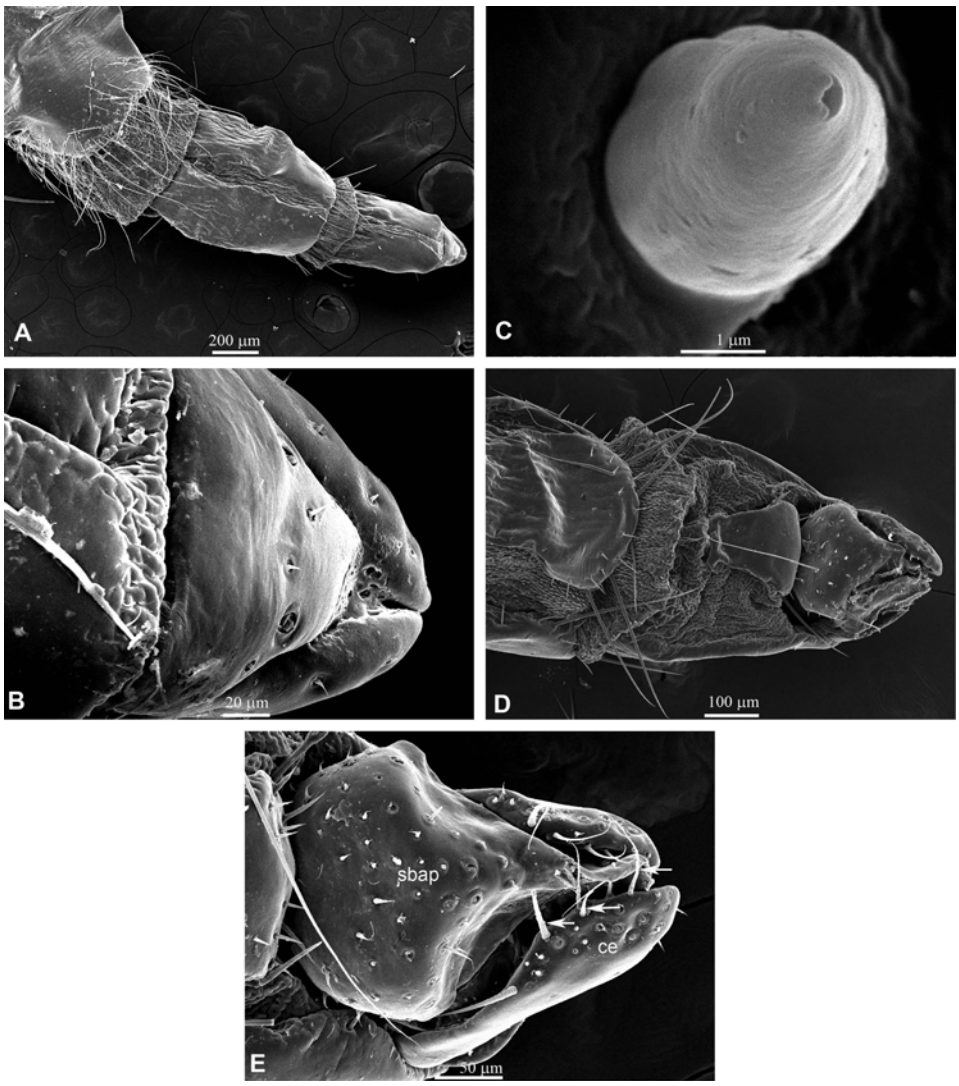

Figure 36. SEM micrograph of the female genitalia of *C. megacephala*. (A) Ovipositor in dorsal view. (B) Ovipositor showing the supraanal plate lying between the short cerci. (C) The short length sensilla basiconica observed at the supraanal plate. (D) Ventral view of the ovipositor. (E) Enlargement of the ventral view showing the subanal plate (sbap) and cercus (ce).

DISCUSSION

Alimentary tract

An overview of gross morphology of the alimentary canal of the blow fly larvae, *Calliphora vicina*, based entirely on LM, was previously provided by Greenberg (1973). Otherwise, works of this type on blow fly larvae are lacking. Results of this study came from the combined use of light microscopy, SEM and TEM to provide a more detailed examination of the anatomy and histology of the digestive tract of the larval blow fly, *C. megacephala*.

In Diptera, the crop is an alimentary structure that functions in the storage and flow of ingested food (Stoffolano et al., 1995). The cuticle (epi- and endocuticle), clearly observed in both the crop and esophagus of the third instar larva of *C. megacephala*, was quite similar in structure to that described in the adult fruit fly, *Bactrocera dorsalis* (Lee et al., 1998). The morphology of the salivary glands of larvae observed in this study closely resembled that in the first instar larva of the bot fly, *Dermatobia hominis* (Evangelista and Leite, 2005), with simple, tubular glands opening into narrow efferent ducts on each side, which converge to form a single median deferent duct leading to the oral cavity. The composition of the salivary gland of *C. megacephala* larvae appears to be comparable to that of the secretory region of the lateral duct in the larval salivary gland of the ant, *Pachycondyla villosa* (Hymenoptera: Formicidae) (Zara and Caetano, 2003).

The cardia is a distinctive organ in Diptera that encompasses the posterior end of the foregut and anterior end of the midgut. The longitudinal section of the organ in *C. megacephala* larvae clearly showed two compartments: the anterior foregut and posterior midgut tissue. This is the same as seen in the adult fruit fly, *B. dorsalis* (Lee et al., 1998). Semi-thin and ultra-thin investigations revealed that the peritrophic membrane first appears in the posterior midgut tissue of the cardia, thereby indicating type II PM that actually forms in the cardia (Lehane, 1997). Synthesis of PM constituents in the cardia of the adult tsetse fly, *Glossina morsitans morsitans* has been reported (Hao et al., 2003). In that same study, the cardia was also found to play a crucial role in immunity in this tsetse fly species. The results in this study *C. megacephala* larvae showed that the PM occurs continuously along the alimentary canal from the cardia to the anus. The function of the PM as a barrier against pathogens invading the midgut epithelium, or for mechanical protection of the midgut epithelium from damage by food particles, has been previously described (Lehane, 1997).

The long blind end tubes of the gastric caeca, which were apparent in the larvae of *C. megacephala* in this study, resembled those seen in the third instar larva of *Calliphora vicina* (Greenberg, 1973). However, this was in contrast to the absence of gastric caeca previously reported in the third instar larva of the bot fly, *D. hominis* (Evangelista and Leite, 2003).

The presence of microvilli along the midgut epithelial cells observed in *C. megacephala* larvae corresponds with similar findings reported in other insects such as the sand flies, *Lutzomyia longipalpis* (Leite and Evangelista, 2001); fruit fly, *Drosophila auraria* (Dimitradis, 1991); mosquitoes, *Aedes (Stegomyia) aegypti* (Zieler et al., 2000); tick, *Haemaphysalis longicornis* (Matsuo et al., 2003); bee, *Melipona quadrifasciata anthidioides* (Neves et al., 2003); or the wingless firebrat, *Thermobia domestica* (Rost et al., 2005). Occurrence of microvilli on cells typically indicates regions where large amounts of absorption and/or secretion take place.

A large number of secretory granules were evident in the midgut cells of *C. megacephala* larvae. They were not only observed inside the cells, but seen outside the cells on many occasions often bound to the apical cell membrane. This suggests the secretory role these cells must play. Secretory granules are most likely released from their vesicles by a process known as exocytosis, in which the secretory vesicles move to the inner apical surface of the cell, fuse with the cell membrane and release the secretory granules into the gut lumen (Chapman, 1998).

It is quite obvious that microvilli line the entire lumen of the Malpighian tubules of *C. megacephala* larvae. This situation structurally resembles that described for the fire ant, *Solenopsis saevissima* (Arab and Caetano, 2002) and the Malpighian papillae of the dipluran, *Campodea (Monocampa) quilisi* (Pigino et al., 2005).

In the hindgut, the morphological feature of the rectum was markedly different between the third instar and adult *C. megacephala*, in both males and females. The rectum of the third instar appeared as a long, slender tube, whereas that of males and females was similar in appearance, showing a muscular cone-shaped structure and four protruding muscle-free rectal pads at the anterior end of the gland. In this regard, no sexual dimorphism is observed. Appearance of the rectal gland of adult *C. megacephala* is comparable with that of the male fruit fly, *Bactocera papayae* (Khoo and Tan, 2005). In this study, the histological section of females revealed the presence of rectal pads that extended into the gland as rectal papillae. This observation corresponded with Khoo and Tan (2005), who described the rectal gland of *B. papayae*, in which the rectal pads extended into the gland as rectal papillae having large nuclei in the cell. In this study, TEM section revealed a large oval nucleus within the cells of rectal papillae, which resembled the area of rectal papillae previously reported in *B. papayae* (Khoo and Tan, 2005). Also, a large amount of mitochondria was similar to *B. papayae*. Only circular muscle covering the rectum was detected in both males and females in *C. megacephala*. Contrary to this observation, Khoo and Tan (2005) demonstrated that the rectum of male *B. papayae* showed both circular and faint longitudinal muscles; whereas, that of females was covered by only circular muscle. The presence of only circular muscle in the rectal gland

suggests the ability of this organ to contract only in the dorsal-ventral direction (Khoo and Tan, 2005).

A rich supply of tracheae was observed in the rectal pad of male and female *C. megacephala*. This was in accordance with the previously reported rectal gland of the blow fly, *Calliphora erythrocephala* (Gupta and Berridge, 1966); when male and female *B. papayae* (Khoo and Tan, 2005) strongly suggested that these glands were involved in active aerobic activity (Khoo and Tan, 2005). It has been suggested that water, chloride and probably other ions were absorbed at the ractal pad (Greenberg, 1973).

The muscular fibers observed within the walls of the alimentary canal were most apparent in the hindgut region of *C. megacephala* both third instar and males. Musculature was apparent, beginning in the ileum, and becoming more pronounced when moving posteriorly down the hindgut, with the greatest intensity of myo-epithelial cells being found in the anal tube. These observations strongly suggest that intense activity of muscle contractions is performed in the hindgut region of the alimentary canal.

Reproductive tract

Males

The internal male genital organs of *C. megacephala* consist of various parts that are commonly found in insects. The testes are located in the posterior abdomen and contain all sperm stages from primary spermatocytes to spermazoa. In almost all other Diptera, the testes are enveloped by two tissue layers consisting of the outer epithelium or tunica externa and the inner epithelium or tunica interna (Hori, 1960; Snodgrass, 1993). In species where color has been observed in the testes, pigment granules are deposited in the outer epithelium, contributing to the apparent visible color (Hori, 1960). As in other Diptera, TEM micrographs of the testis wall of *C. megacephala*, show that the outer epithelium is full of rounded grains containing pigment, which give the organ its characteristic color. This characteristic has also been observed in the Mexican fruit fly, *Anastrepha ludens* (Valdez, 2001). The coloration of *C. megacephala* testes is probably due to the pigment granules being deposited in this layer as maturation progressed. Likewise, the muscle layer in the testis wall, between the outer and inner epithelium, was first reported in the Mexican fruit fly, *A. ludens* (Valdez, 2001). This feature could be related to the unifollicular structure of this blow fly testis.

Previously, a report was carried out on the color change of the testes in six species of calyptrate muscoids during the pupal and adult stages (Hori, 1960). The change in color of the testes in these flies, during the course of pupal life, was progressive from greenish-yellow through orange to reddish-orange. In the muscids, *Ophyra nigra* and *Musca domestica vicina*, the testes continue to change successively from reddish-orange to brown or fuscous after

emergence. However, in *Scopeuma stercorarium*, *Calliphora grahami*, *Lucilia sericata* and *Sarcophaga simislis*, the color remained reddish-orange throughout their entire adult stage (Hori, 1960). In this study, the color of *C. megacephala* testes changed from pale orange at emergence to a reddish-orange color in 1-old-day flies, and continued to change from reddish-orange to brown or fuscous as the flies aged.

The testis shape of calyptrate muscoid flies has been classified into five types including oval, oblong, fusiform, banana-shaped and lamp-shaped (Hori, 1960). *C. megacephala* appears to have a pair of oval shaped testes. They maintain their ellipsoidal form for a day or more after emergence, then a characteristic constriction occurs at about one third of the length from the base of the testes. This is probably caused by the discharge of spermatozoa into the vas deferens. Daily measurements suggested that this may occur around the seventh day post-emergence. The lower third of *C. megacephala* testes seems to function as a pump, forcing the developed spermatozoa into the vas deferens. This characteristic changes the shape of mature male testes, and has been described previously in three other fly genera including: *Musca*, *Lucilia* and *Calliphora* (Hori, 1960). The testis constriction in *C. megacephala* extends apically, and changes the form of the testes remarkably as the flies age. Consequently, the change of the testes shape of this blowfly species closely correlates to the age of the fly, and could be used to differentiate the age of individual flies.

Scanning electron microscopy studies of the testes of various insects have shown both similarities and differences in *C. megacacephala*. Investigation of the morphological characters of testes in four *Plecoptera* species (Fausto et al., 2001; 2002) consisted of a number of separate follicles, each enclosed by its epithelium and open into the vas deferens. In *Leuctra fusca*, each of the two testes consisted of 9 to 10 follicles, almost tubular in shape; in *Brachyptera risi*, the follicles were ovoid; in *Taeniopteryx stankovichi*, the follicles were more rounded than those in *B. risi*; and in *T. kuetreiberi*, the two testes were united in the median proximal portion, forming a single arc sac. In this study, SEM micrographs of male *C. megacephala* revealed a pair of oval shaped testes, with a smooth surface that was occasionally penetrated by tracheoles.

The presence of the primary and secondary spermatocytes observed in the developing spermatozoa of 3 day-old *C. megacephala* males was similar to that seen in the pupal stage of the thrips, *Haplothrips simplex* (Thysanoptera: Phlaeothripidae) (Paccagnini et al., 2006). The tail region ultrastructure of spermatozoa in 3 day-old adult *C. megacephala* was not clearly seen in this study, and the axoneme pattern was indistinguishable. In an example from another dipteran, it was found that the testes of the 4 day-old adult mosquito, *A. aegypti* (Owusu-Daaku et al., 2007), and had multi-organelled spermatozoa with extra tail elements (axonemes and mitochondrial derivatives). Nevertheless, an electron micrograph of the testes in 3 day-old adult

C. megacephala revealed that the developing axoneme pattern had yet to be transformed into the typical 9+2+2 microtubule pattern.

The gross morphology of the male accessory glands is very diverse in Calyptrate muscoid flies. Based upon the morphology investigated by Hori (1960), the accessory glands of male muscoid flies constitute six types; (1) no accessory glands, (2) papillary, (3) spherical or ellipsoidal, (4) banana-shaped, (5) coiled, and (6) rod-shaped; suggesting one of the taxonomic traits that might be useful for taxonomic analysis. Banana-shaped male accessory glands have been observed in many genera of blow flies (*Chrysomya, Lucilia, Hemipyrellia, Phormia, Protophormia, Calliphora, Stomorhina*, *Triceratopyga, Onesia*) or flesh flies (*Metopia*) (Hori, 1960). Despite the same banana shape, the slender tubular structure of *C. megacephala* in this study was different from the stout tubular structure observed in *Phormia regina* (unpublished data of authors), indicating the variability in morphology within the group of blow flies. Indeed, SEM results presented in this study revealed that even in the same species of *C. megacephala*, the sub-apical constriction of male accessory glands in some specimens was noticeable and unexpected. Still, the reason for this sub-apical constriction remains uncertain; variation in the morphology of this organ is questionable.

In this study of *C. megacephala*, ultrastructural change has been noticed in the accessory gland of 3 -day-old males; in the glandular cells and gland lumen. With regard to the cells, the presence of numerous RER and primary and secondary secretory granules was the prominent feature in 3-day-old glands. In *C. megacephala*, whether females can successfully mate with younger or older males has not been fully explored. However, investigation in the fruit fly, *Drosophila pseudoobscura*, suggested that females preferred mating with older males, which may relate to the transfer of larger volumes of sperm and possibly accessory fluids as well (Avent et al., 2008). Regarding this, investigation of successful mating related to the development of accessory glands of *C. megacephala* merits further study.

In the male accessory glands of insects, there may be cellular diversity, even within a single type (Davey, 1985). Regarding those of *C. megacephala*, the results obtained from this study confirmed the multicellular gland, appearing as a simple tubular structure in morphology. Based on the difference in staining used in the light microscopic study, the glands of *C. megacephala* exhibited one type of cell, but a difference in functional state. Active glandular cells were predominantly observed, but only a few inactive ones were seen. The former were characterized by bearing large secretory granules within the cytoplasm or at the apical end adjacent to the lumen; while the latter showed no remarkably large secretory granules.

With respect to the structure of the accessory glands of *C. megacephala per se*, they were recognized as ectodermal in origin or “ectadenia”, on the basis of the gland that opened into the ejaculatory duct (Chapman, 1998). The simple tubular structure observed in males in

this study was different from the multi-chamber organization investigated in females (unpublished data of author). Interestingly, based on histological examinations, the thick epithelial cells of the accessory glands observed in males were similar to those in females (Bansal and Murad, 1987). Vacuolated cytoplasm of the glandular cells in males was similarly observed in females (Bansal and Murad, 1987).

At the ultrastructural level, this study revealed that the glandular cells of the accessory glands of male *C. megacephala* showed typically common structures associating with the production of secretory granules. These included richness in the active phase of RER, extensive mitochondria and a large number of secretory granules, indicating high activity in secretory function. Such typical features of cells engaged in high secretory activity has been previously described in the triatomine bug, *T. rubrofasciata* (Freitas et al., 2007). Increasing the secretory activity of male accessory glands was related to maturation (Freitas et al., 2007).

With respect to the role of male accessory glands in insects, they primarily ensure that the reproductive success has been addressed, including insemination, barriers to reinsemination, contributions to survival and fecundity (Leopold, 1976; Chen, 1984).

The vas deferens of *C. megacephala* are simple in structure and connect the testes with the ejaculatory duct. The vas deferens are cylindrical ducts, which do not form seminal vesicles in this species. In some insects, a portion of the vas deferens may be enlarged, as the seminal vesicle serves as a storage reservoir for sperm before it is transferred to the female (Snodgrass, 1993). In other Diptera such as the sandfly, *Phlebotomine perniciosus* (Diptera: Psychodidae), a portion of the vas deferens forms a pear-shaped seminal vesicle (Fausto et al., 2000).

The shape of the ejaculatory duct of Calyptrate muscoid flies has been divided into three main groups, including N-shaped winding, short or C-shaped winding and long or coiled. *Chrysomya* species are considered to be of the short form of ejaculatory duct (Hori, 1960). The vas deferens in some species of *Sarcophaga* and *Musca,* especially in which the ejaculatory duct is short, the right ducts are often longer than the corresponding left ones (Hori, 1960). This study also documented a difference in length between left and right ducts; however, the original anatomical position was not retained during dissection, so the result cannot make an assumption about how this relates to the actual internal anatomy of the fly.

SEM micrographs in this investigation showed a pattern consistent with longitudinal muscle on the surface of the ejaculatory duct of *C. megacephala*, in which, however, the transverse sections did not show evidence of a muscular layer. Conversely, the ejaculatory duct of other Diptera including the house fly, *Musca domestica* (Diptera: Muscidae) (Riemann, 1973) and lovebug, *Plecia nearctica* (Diptera: Bibionidae) (Trimble, 1974) demonstrated muscle layers that were visible under TEM observation. The absence of a muscular layer in the ejaculatory duct has been reported in the flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae)

(Dallai et al., 1997) and *M. bicolor bicolor* (Hymenoptera: Apinae) (Dallacqua and Cruz-Landim, 2003).

These results also demonstrated the presence of secretory cells in the plasma membrane of vas deferens and epithelial cells of the ejaculatory duct of *C. megacephala*. Secretory cells are usually associated with the production of secretions that support the functions of accessory reproductive glands, such as spermatopore production, mating plug formation, and sperm activation (Fausto et al., 1997). The function of secretory cells in this species has yet to be investigated.

Females

This study examined the development of ovaries and ovarioles of female *C. megacephala* from emergence (0-day-old) until 9-days-old using several methods. The results of this study presented a method for age determination of female flies that can be easily adapted to field studies, as it is based on practical light microscopy methods. This study gave the first TEM images of the developing embryonic chorion in this insect, which provided interesting insight into the development of the chorion as the egg matures. Measurements of the ovaries also detected significant increases in their size during the period of study, both in length and width, based on measurement analyses that also have potential utility in age determination of female *C. megacephala*. The morphometric characters of ovarian length and width have been used as a tool in determining the ovarian development of the female Caribbean fruit fly, *Anastrepha suspense* (Diptera:Tephritidae) (Kendra et al., 2006).

This study was the first to report the changes in ovarioles during egg formation in *C. megacephala*, and form the basis for the aging technique, as created for other blow flies, e.g., *Lucilia cuprina* (Vogt et al., 1974). The cellular changes observed during oogenesis in other calliphorid flies have been documented for *L. cuprina* (Vogt et al., 1974; Beattie and Cheney, 1979), *C. bezziana* (Spradbery and Sands, 1976), *C. hominivorax* (Adams and Reinecke, 1979) and *C. putoria* (Avancini and Prado, 1986). Slight differences have occurred between studies when comparing ovarian stages, and many of the descriptions vary in the number of ovarian stages presented. For example, the results of this study showed that in *C. megacephala*, stage I is present at day 0 (just after emergence) of the adult female, as also found in *C. putoria* (Avancini and Prado, 1986), however, in *L. cuprina, C. bezziana* and *C. hominivorax* these features occur during the pupal stage.

In this investigation, *C. megacephala* females completed egg development in eight stages and about ten days under ambient temperature fluctuations (18-27^{O}C). Previous studies have documented the completion of oogenesis in *C. megacephala* in 10-13 days for females reared at 25±2^{O}C, while being maintained on raw beef liver (Linhares and Avancini, 1989). The

total time for egg development in this study corresponded well with temperature controlled studies, which suggests that the differences in the number and description of stages is largely dependent upon small differences in author interpretation rather than temperature dependent processes.

The development of follicular epithelium in the 3- and 7-day-old blow fly passes through several distinct stages of oognesis. TEM micrographs illustrated the differentiation of the developing chorion layers in young and old adult females. The fine eggshell structure of *C. megacephala* showed several layers similar to those observed in *Drosophila melanogaster* (Margaritis et al., 1980). The characteristic changes in the development of the follicular epithelium or process of chorionogenesis in this blow fly are similar to those described in the stoneflies, *Perla marginata* and *P. pallida* (Plecoptera: perlidae) (Rosciszewska, 1995). The *C. megacephala* oocyte is largely composed of yolk protein, which has presented the same follicular changes as other insects in the vitellogenesis (Raikhel and Snigirevskaya, 1998).

The reproductive status or parity of flies can be used to approximate the generation time or time between egg batches. This information has been used to estimate the population size of the bush fly, *Musca vetustissima* (Diptera: Muscidae) (Matthiessen et al., 1986), and is often used in disease surveillance studies of vector mosquitoes(Woodbridge and Walker, 2002). Hence, the stages of ovarian development in *C. megacephala* can be a useful tool for determining the age, approximate generation time and population potential of this blow fly. The light microscopy based age grading system, presented herein, can also be used to determine the age of flies collected in the field. In addition, the basic information provided by this study can be used to understand the ovarian development and fine structure of the ovaries and ovarioles of *C. megacephala*, which might prove useful in developing new methods to control this blow fly in the future.

The accessory glands of *C. megacephala* are paired long and tubular glands that open into the dilated anterior genital chamber. Each gland is composed of two distinct regions, including an apical bulb and a tubular gland duct, which differentiate during maturation of the fly, as evidenced by TEM micrographs of 3-day-old flies. This two-part accessory gland is similar to those observed in other insects such as the thrips *Frankliniella occidentalis* and *Heliothrips haemorrhoidalis* (Thysanoptera: Thripidae) (Dallai et al., 1996; Bene et al., 1998) and blow fly, *C. putoria* (Diptera: Calliphoridae) (Tirone and Avancini, 1997).

The basis or head of the glandular portion of female blowfly accessory glands has been grouped into three types based on overall shape including: (1) oval or elongated oval, (2) banana shaped or (3) clavate (Hori, 1961). The glandular portion shape of the female accessory glands of *C. megacephala* was classified as clavate (Hori, 1961). The SEM micrographs in this study show that the surface of the female accessory glands of this blow fly are covered in

papillae and occasionally penetrated by tracheoles, which is the first report of this morphology in this species. This feature is similar to the globular cell wall shape of the female accessory glands of the sandfly, *Phlebotomus perniciosus* (Diptera: Psychodidae) (Fausto et al., 1997).

The epithelial cell structure of the female accessory gland of *C. megacephala* consists of both secretory and duct forming cells, which is similar to that of many insect ectodermic glands (Dallai et al., 1996). These features have been described in the accessory glands of other insects such as *F. occidentalis*, and *H. haemorrhoidalis* (Thysanoptera: Thripidae) (Dallai et al., 1996; Bene et al., 1998). The cistern cells that serve as storage organs for secretion products of the secretory cells for production of fluid secretions, correspond to that of type 1 insect ectodermal glands (Noirot and Quennedey, 1974; 1991).

The cellular level on the morphology of 3-day-old female accessory glands in this study revealed that the cistern cells contained transparent electron dense material, where large amounts of secretions are normally stored. This suggested what was missing or had not yet been formed. The tubular duct portion of the gland had secretions present in the cistern cells. A low volume of secretions was present in the central lumen. These characteristics are similar to those observed in the development of the female accessory gland cells of *Orchesella villosa* (Collembola: Hexapoda), where the epithelial cells of virgin females rarely showed secretions, whereas, inseminated females showed large amounts of secretions (Dallai et al., 2008).

The secretion function of female accessory glands is a typical adhesive material that serves to cement eggs to the substratum or hold them together in a mass (Chapman, 1998). A histochemical test determined that secretions of the accessory glands of *C. putoria* were composed mainly of a glycoprotein, which is consistent with an adhesive material (Tirone and Avancini, 1997). A cytochemical analysis of the cellular secretions of *C. megacephala* would be necessary to determine the nature and functions of the secretions of this gland with more accuracy.

The spermatheca is a sac for the reception and storage of the spermatozoa in a female insect (Hori, 1961; Snodgrass, 1993; Chapman, 1998). In Diptera the spermathecae vary in number; there are none in the family Cloropinae, one in *Anopheles* (Culicidae) and *Simulium* (Simuliidae), two in Drosophilidae, and three in *Culex* (Culicidae), *Stegomyia* (Culicidae) and Tabanidae (Hori, 1961). There are three in the blow flies, *L. cuprina* (Clift and McDonald, 1973), *C. bezziana* (Spradbery, 1976) and *C. megacephala* (Bansal and Murad, 1987). The type of spermathecae arrangement in calyptrate muscoid flies is divided into three kinds including 1:2, 0:2 and 1:1:1 (Hori, 1961). This study confirmed that *C. megacephala* has three spermathecae, which are arranged in the 1:2 configuration, with the two on one side loosely bound together and the one on the other side unattached.

This work described the fine structure of spermathecae in this blow fly for the first time. SEM images showed that tubercles cover the surface of all three spermathecae and that each is penetrated by tracheoles. The basal region of spermathecae is connected via a spermathecal duct, and longitudinal muscle is clearly visible on the surface of the spermathecal duct. The muscle fibers of the spermathecal duct have been investigated under SEM in other Diptera such as the medfly, *Ceratitis capitata* (Diptera: Tephritidae), Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae), phlebotomine sandfly, *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae), and four species of *Dysmachus* (*D. fuscipennis, D. picipes, D. praemorsus and D. transcaucasicus*) (Diptera: Asilidae) (Marchini et al., 2001a; Fritz and Turner, 2002; Ilango, 2005; Dallai et al., 2008).

The spermathecae of many insects are used for the storage of sperm once the females are inseminated, and the spermathecal duct may contain glycogen deposits that can serve as an energy source for the sperm as they pass through to the egg (Chapman, 1998). TEM images of the cistern cells of *C. megacephala* show secretion materials inside the lumen. This secretion could be used as nutrition for the spermatozoa stored in the spermathecae, but it was not examined in this investigation. The ultrastructure of the spermathecae of *C. megacephala* could be useful for determining the physiological mechanisms responsible for changes in behavior, occurring before and during mating, and oviposition as well as how fertilization of the eggs occurs in this blowfly species.

The genital chamber of *C. megacephala* is a single long, tubular organ, which is located in the posterior region of the female abdomen, in the same orientation that it is generally found in the reproductive tracts of other female insects (Hori, 1961; Snodgrass, 1993; Chapman, 1998). The histology of the vagina or genital chamber of *C. megacephala* has been studied previously under light microscopy (Bansal and Murad, 1987). However, in this study, a more detailed description of the genital chamber structure of *C. megacephala* was presented by using LM, SEM and TEM. High magnification imaging revealed that the genital chamber consists of a central lumen wrapped by approximately five distinct cell layers, with each layer characterized by a difference in structure and cellular organelles. Interestingly, TEM images showed a muscle layer that was only visible in the third-layer, while longitudinal and circular muscles were seen on the surface in the SEM micrographs. In previous studies, muscle layers in the vagina epithelial cells of other arthropods have been observed, including *C. megacephala,* consisting of both circular and longitudinal muscle(Bansal and Murad, 1987a). A similar muscular structure has been observed in the medfly, *C. capitata* (Diptera: Tephritidae) (Marchini et al., 2001b), and ticks, *Ornithodoros (Pavlovskyella) erraticus* (Acari: Argasidae) (Shoura, 1988) and *Ixodes ricinus* (Acari: Ixodidae) (Roshdy, 1969). Additionally, the epithelial cells of the second-layer

through to the fifth-layer are rich in mitochondria, which was not observed in the previous study (Bansal and Murad, 1987).

Results indicating that numerous mitochondria in the muscle layer and lumen are lined with cuticle of *C. megacephala*, suggest similarity in function with the genital chambers of other insects, which serve for both copulation and the discharge of eggs (Snodgrass, 1993). In a study of the medfly, *C. capitata*, sperm bundles can reach the egg micropyle in the fertilization chamber by their own movement in conjunction with the muscle contractions of the chamber pushing the spermatozoa toward the eggs (Marchini et al., 2001). The muscular layers found in that study suggest this might occur in *C. megacephala*, but it was not observed in this study.

The external genitalia: males and females

This study describes the ultrastructure of the male and female genitalia in *C. megacephala*. Regarding males, the morphological features of the genitalia, in particular the components of the aedeagus, show distinctive characteristic. The prominent, bilobed vesica observed in this fly show similarity with those of some other chrysomyine blow flies, such as *C. pinguis*, *C. defixa* (Senior-White et al., 1940), and *C. bezziana* (Spradbery and Sands, 1976), but are markedly different from the blow flies *Cochliomyia hominivorax*, *Cochliomyia macellaria* (Leite, 1995), or *L. cuprina* (Merritt, 1989) for instance. However, the thorny appearance of the juxta in this study was similar to that previously described as the ventrolateral process in *C. hominivorax* (Leite, 1995). With regard to specific observation of the male *C. megacephala* cercus, the abrupt truncation at the terminal end differs from those shown for other blow flies via SEM, like *C. hominivorax* (Leite, 1995), or in illustrations of many other blow fly species such as *Calliphora fulviceps*, *Calliphora pattoni*, *Calliphora vicina* [=*C. erythrocephala*], *Calliphora vomitoria*, *Chrysomya albiceps*, *C. nigripes*, *Chrysomya phaonis*, *C. pinguis*, *Chrysomya rufifacies*, *Hemipyrellia pulchra* and *L. cuprina* (Senior-White et al., 1940). However, the cercus, being longer than the surstylus in *C. megacephala*, was similar to the illustrations (Senior-White et al., 1940) of *C. bezziana*, *C. defixa*, *C. pinguis*, *C. phaonis*, *Lucilia sericata* and *Lucilia porphyrina*. As indicated by many researchers, the features of the cercus and surstylus are often used in the morphological identifications of insects and flies (Senior-White et al., 1940; Leite, 1995).

Ultrastructural observation of the surstylus and cercus of male *C. megacephala* indicated the endowment of numerous sensillae, which are comparable to those found by SEM on the structures of other flies, such as the blow flies, *C. hominivorax* and *C. macellaria* (Leite, 1995); flesh fly, *Parasarcophaga* (*Liosarcophaga*) *dux* (Chaiwong et al., 2007); and bot fly, *D. hominis* (Fernandes et al., 2004). Evidence for the diagrammatical display of dense lines of sensillae on these structures exists in numerous fly species; for instance the blow flies, *C. bezziana* (Senior-

White et al., 1940; Spradbery and Sands, 1976), *Chrysomya defixa*, *C. phaonis*, *Calliphora vicina*, *C. pattoni*, *Hemipyrellia ligurriens*, *H. pulchra*, *Lucilia fumicosta*, *L. ampullacea*, *L. sinensis*, *L. papuensis* and *L. porphyrina* (Senior-White et al., 1940); and syrphid fly, *Copestylum alberlena* (Marcos-Garcia and Perez- Banon, 2002). Based on the existence of long bristles, which are morphologically similar to the sensilla chaetica and sensilla trichodea, and short bristles, morphologically similar to sensilla basiconica, observed in both the surstylus and cercus of *C. megacephala*, the functions of mechanosensitivity, dual mechanosensitivity and contact chemosensitivity, olfactory sensitivity, or thermosensitivity, have been proposed (Zacharuk, 1985). The location of these sensillae on the external surfaces of the surstylus and cercus suggests that these sensillae are probably involved in copulation with females; however, further behavioral investigation is needed to verify this suggestion.

The sensillae of the female genitalia and/or ovipositors of insects has been recorded for the blow fly species, *Phormia regina* (Wallis, 1962), *L. cuprina* (Rice, 1976) *C. nigripes* (Ngern-Klun et al., 2007); and face fly, *Musca autumnalis* (Hooper et al., 1972). Based on the specimens examined in this study (n = 10 for each sex), it appears that the type, number, and distribution of various sensillae observed in female genitalia of *C. megacephala* are very similar to those present in another blow fly species, *C. nigripes* (Ngern-Klun et al., 2007). These include the sensilla trichodea with variable length; sensilla basiconica with short length, which are either longitudinally grooved or smooth-surfaced; plate-like sensilla placodea; and ball-shaped peg with a rugose surface.

As for *C. megacephala per se*, males had significantly more sensilla trichodea than females, which may explain greater mechanosensitive or dual mechanosensitive and contact chemosensitive response in males than females (Zacharuk, 1985). Similar findings have been recorded for the sand fly*, Lutzomyia* spp. (Diptera: Psychodidae), with their sensillae being used by males in establishing contact with females during copulation (Spiegel et al., 2000). In contrast, considerably greater densities of sensilla basiconica, sensilla placodea, and probably sensilla styloconica were observed on the genitalia of females than *C. megacephala* male. Indeed, a higher number of sensilla were found clustered along the ventral surface than the dorsal surface, implying more responsiveness to hygrosensitivity, chemosensitivity, or mechanosensitivity and chemosensitivity, as previously suggested (Zacharuk, 1985). This interpretation is consistent with observation of the paired lateral leaflets of *L. cuprina* (corresponding with the cercus in this study), where it was suggested that the ovipositor of *L. cuprina* plays a role as an organ of both taste and smell (Rice, 1976). A comparable observation of taste sensilla occurring along the opening of the ovipositor has been described for the moth, *Plutella xylostella* (Qui et al., 1998). The mechanosensitive function of the longer hairs near the base of the cerci, which were morphologically similar to that of the sensilla

trichodea in this study, has also been indicated (Zacharuk, 1985). Sensilla on the ovipositors of insects provides information pertaining to the elicitation of oviposition behavior(Rice and McRae, 1976). Likewise, sensillae on female terminalia of the mosquito, *A. aegypti*, also suggested their function in copulation and oviposition behaviors (Rossignol and McIver, 2005).

CONCLUSION

In conclusion, results obtained from using a combination of LM, SEM and TEM in this study have provided thorough information on the ultrastructure of *C. megacephala* in both the alimentary canal (the third instar, adult males and females) and reproductive tract (for males and females). The ultrastructural characteristics of each component within the foregut, midgut and hindgut were found to have differences from both morphological and functional viewpoints, which helped this study to clarify and understand better each particular organ in the alimentary canal of mature larvae of this species. The ultramorphology of the reproductive organs of *C. megacephala* provides basic information that can be applied to develop better control strategies.

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OUTPUT จากโครงการวิจัยที่ได้รับทุนจากสกว. (19 July 2006-19 July 2009)

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

1.1 Boonsriwong W, Sukontason K, Olson JK, Vogtsberger RC, Chaithong U, Kuntalue B, Ngern-klun R, Upakut S, **Sukontason KL**. Fine structure of the alimentary canal of the larval blow fly *Chrysomya megacephala* (Diptera: Calliphoridae). *Parasitology Research* **2007**;100:561-74.

Impact Factor ปี 2007 = 1.512

1.2 Chaiwong T, **Sukontason KL**, Chaithong U, Olson JK, Kurahashi H, Sukontason K. Male genitalia of flesh fly *Parasarcophaga (Liosarcophaga) dux* (Diptera: Sarcophagidae) revealed by scanning electron microscopy. *Journal of the American Mosquito Control Association* **2007**;23:80-83.

Impact Factor ปี 2007 = 0.894

1.3 Ngern-klun R, Sukontason K, Methanitikorn R, Vogtsberger RC, **Sukontason KL**. Fine structure of *Chrysomya nigripes* (Diptera: Calliphoridae), a fly species of medical importance. *Parasitology Research* **2007**;100:993-1002.

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1.5 Sukontason K, Narongchai P, Kanchai C, Vichairat K, Sribanditmongkol P, Bhoopat T, Kurahashi H, Chockjamsai M, Piangjai S, Bunchu N, Vongvanich S, Samai W, Chaiwong T, Methanitikorn R, Ngern-klun R, Sripakdee D, Boonsriwong W, Siriwattanarungsee S, Srimuangwong C, Hanterdsith B, Chaiwan K, Srisuwan C, Upakut S, Moopayak K, Vogtsberger RC, Olson JK, **Sukontason KL**. Forensic entomology cases in Thailand: a review of cases from 2000 to 2006. *Parasitology Research* **2007**;101:1417-1423.

Impact Factor ปี 2007 = 1.512

1.6 **Sukontason KL**, Chaiwong T, Piangjai S, Upakut S, Moophayak K, Sukontason K. Ommatidia in blow fly, house fly, and flesh fly: implication of their vision efficiency. *Parasitology Research* **2008**;103:123-131.

Impact Factor ปี 2008 = 1.473

1.7 Sukontason K, Piangjai S, Siriwattanarungsee S, **Sukontason KL**. Morphology and developmental rate of blowflies *Chrysomya megacephala* and *Chrysomya rufifacies* in Thailand: application in forensic entomology. *Parasitology Research* **2008**;102:1207-1216.

Impact Factor ปี 2008 = 1.473

1.8 Sukontason K, Methanitikorn R, Kurahashi H, Vogtsberger RC, **Sukontason KL**. External morphology of *Chrysomya pinguis* (Walker) (Diptera: Calliphoridae) revealed by scanning electron microscopy. *Micron* **2008**;39:190-197.

Impact Factor ปี 2008 = 1.839

1.9 Chaiwong T, **Sukontason K**, Olson JK, Kurahashi H, Chaithong U, Sukontason KL. Fine structure of the reproductive system of *Chrysomya megacephala* (Diptera: Calliphoridae): the external sexual organ. *Parasitology Research* **2008**;102:973-980.

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1.10 **Sukontason KL**, Sribanditmongkol P, Chaiwong T, Vogtsberger RC, Piangjai S, Sukontason K. Morphology of immature stagers of *Hemipyrellia ligurriens* (Wiedemann) (Diptera: Calliphoridae) for use in forensic entomology applications. *Parasitology Research* **2008**;103:877-887.

Impact Factor ปี 2008 = 1.473

1.11 Chaiwong T, Sukontason K, **Sukontason KL**. Two new species of *Sarcophaga* s. lat. from Thailand with a key to species (Diptera: Sarcophagidae). *Journal of Medical Entomology* **2009** (in press)

Impact Factor ปี 2008 = 1.967

1.12 **Sukontason KL**, Chaiwong T, Chaisri U, Vogtsberger RC, Sukontason K. Ultrastructure of male accessory glands of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). *Journal of Vector Ecology* 2009 (submitted)

Impact Factor ปี 2008 = 1.057

หมายเหตุ Reprint ผลงานวิจัยเรื่องที่ 1-10 อยู่ในภาคผนวก

ผลงานวิจัยที่คาดว่าจะตีพิมพ์เพิ่มเติม

- Ultrastructure of alimentary tract of adult blow fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)
- Fine structure of male reproductive tract of adult blow fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)
- Fine structure of female reproductive tract of adult blow fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)

2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงสาธารณะ

มีการสร้างเครือข่ายความร่วมมือกับหน่วยงานอื่นคือ

- หน่วยจุลทรรศน์อิเล็กตรอน คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
- ภาควิชาพยาธิวิทยาคลินิค คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล
- Department of Entomology, Texas A&M University, USA
- Department of Biology, Midwestern State University, USA
- Department of Medical Entomology, National Institute of Infectious Diseases, Japan

- เชิงวิชาการ

มีการผลงานวิจัยไปพัฒนาการเรียนการสอน ในกระบวนวิชากีฏวิทยาการแพทย์ ในระดับบัณฑิตศึกษา ภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ และมีการสร้างนักวิจัยใหม่จากโครงการนี้ โดยร่วมมือกับโครงการปริญญาเอกกาญจนาภิเษก คือ อ.ดร. ธารินี ไชยวงศ์ และดร.

วรโชติ บุญศรีวงศ์

3. การเสนอผลงานในที่ประชุมวิชาการ

- ไปเสนอผลงานวิจัยเรื่อง Number of the ommatidia in flies of the families Calliphoridae, Muscidae and Sarcophagidae; implication of their vision efficiency ในการประชุมวิชาการนานาชาติ The 21st Pacific Science Congress เมืองโอกินาวา ประเทศญี่ปุ่น วันที่ 12-18 มิถุนายน 2550
- เข้าร่วมประชุมและนำเสนอผลงานวิจัย ในการประชุม “นักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว.” ในวันที่ 16-18 ตุลาคม 2551 ณ โรงแรมฮอลิเดย์อินน์ รีสอร์ท รีเจนท์ บีช ชะอำ จังหวัดเพชรบุรี ผลงานวิจัยที่นำเสนอชื่อ Ultrastructure of male accessory glands of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)