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ULTRASTRUCTURAL STUDY OF SPERMIOGENESIS AND SPERMATOZOON OF *PORRORCHIS INDICUS* (DAS, 1957) SCHMIDT AND KUNTZ, 1967 (ACANTHOCEPHALA, PALAEACANTHOCEPHALA, PLAGIORHYNCHIDAE) FROM THE EGYPTIAN *CENTROPUS SENEGALENSIS AEGYPTIUS* (AVES: CUCULIDAE)

By

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Abstract

Porrorchis indicus is an endoparasite of the Egyptian birds, *Centropus Senegalensis aegyptius*. This parasite causes damage to the gut wall of the infected birds. Spermiogenesis and sperm ultrastructure have been widely used in the phylogeny of parasitic platyhelminthic and acanthocephalan worms. However, there are limited ultrastructural data available on spermiogenesis and the spermatozoon on the acanthocephalan family, plagiorhynchidae to which *P. indicus* belongs. The present study investigated the ultrastructure features of the different stages of *P. indicus* spermiogenesis. The morphology and anatomy of the spermatozoa was described in comparison with the previous reports. Different ultrathin sections of the body of *P. indicus* (testes and vas deferens) were cut using transmission electron microscopy (TEM) to describe the different stages of the spermiogenesis as well as both morphology and anatomy of spermatozoa.

The results showed that the primary spermatocystes of *P. indicus* was rounded shape with large rounded nuclei and high nucleocytoplasmic ratio. The secondary spermatocystes showed nearly ovoid shape; each possessed a small nucleus and numerous mitochondria. The early spermatid possessed a large nucleus and contained a single centriole. The early spermatid showed a posterior bulb in which the centriole had a flagellum with 9+0 pattern surrounded by a plasma membrane it changed into 9+2 pattern. The cytoplasmic canal is characterized by a ring form which appeared around the axoneme. The anterior extremity of the spermatozoa showed the presence of the centriolar derivative that had three different stages. The first is made of nine peripheral elements. The second is of nine peripheral and one central element. The third showed nine peripheral and two central elements. The centriolar derivative is followed by a flagellum with a 9+2 pattern. In mature spermatozoa, protein granules different sizes, numbers and shapes were recognized.

Keywords: Acanthocephala, *Porrorchis indicus*, Spermatozoon, Spermiogenesis, TEM, Ultrastructure

Introduction

Few studies have been conducted on the reproduction of phylum Acanthocephala. Hyman (1951) and Guraya (1971) reported their studies for the first time using light-microscopy but, Whitfieds (1971), Marchand and Mattei (1976a, b, c, 1977a,b, 1978a, b) also Zhao and Liu (1992), Carcupino and Dezfuli (1995) investigate mainly the reproduction of the Acanthocephala using electron microscopy. The reproduction in this phylum was reviewed by Carcupino and Dezfuli (1999).

Crompton and Nicol (1985, 2009) revised the biology of the acanthocephalans.

Recently, a new species of acanthocephalan Cavisomidae was described based on the phylogenetic data analysis (based on 18S, 28S rDNA & mtDNA data) after Costa-Fernandesm *et al.* (2019).

A rotifer-acanthocephalan relationship was recorded during the study of spermiogenesis of a Rotiferan species (Ferraguti and Melone, 1999). Some studies performed based on the phylogenetic analysis of ribosomal DNA sequence data; moved Acantho-

cephala into a position closer to Rotifera than to Myzostomida (Garcia-Varela *et al*, 2000, 2002; Zrzavy *et al*, 2001; Herlyn and Rohrig, 2003).

The accomplished studies concerning the process of spermiogenesis in Acanthocephalans were relatively few. The first ultrastructural study was done by Whitfied (1971) on *Polymorphus minutus*. Later, Marchand and Mattei (1976a) showed a reversed orientation of the spermatozoon of *Illiosentis furcatus*. More studies concerning the ultrastructure features on spermiogenesis and spermatozoa of different Palaeacanthocephalan species; *Leptoryhynchoides plagicephalus, Acanthocephaloides incrassatus, Cavisoma magnum* respectively (Foata *et al*, 2004, 2012a, b) and of the Archiacanthocephalan species; *Macracanthorhynchus hirudinaceus* (Foata *et al*, 2005) have been performed.

There are limited ultrastructural data available on spermiogenesis and the spermatozoon on the Plagiorhynchidae family to which *P. indicus* belonged. In Egypt, there were limited ultrastructural data available on *P. indicus*; Ashmawy and El-Sokkary (1991) described morphobiological characters of *Pseudoprrorchis species*. Abd-El-Moaty and Taeleb (2011) re-described *P. indicus* using ultrastructure study on its body surface.

The process of spermiogenesis and the organization of the spermatozoon of *P. indicus* have not been previously observed. The present study aimed to describe for the first time the ultrastructure of spermiogenesis and the spermatozoon of *Porrorchis indicus*; collected from the small intestine of the Egyptian *Centropus Senegalensis aegyptius*. The results were compared with available data of other acanthocephalan species.

Materials and Methods

The worms of *P. indicus* were collected alive from the small intestine of naturally infected *Centropus senegalensis aegyptius* from Sharkia Governorate, Egypt and treated for transmission electron microscopy as follow. Alive male specimens were rinsed in 0.9 Nacl solution, fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer, PH 7.3, for 2h at 4°C following a buffer wash, post fixed in 1% osmium tetroxide in 0.1M phosphate buffer at PH 7.3 for 1h. Samples were dehydrated in ethanol and propylene oxide, embedded in resin, at 60°C for 48h. Semithin sections were cut on a Leica ultramicrotome and stained with toluidine blue for light microscopy. For TEM, ultrathin sections (60-90)nm at different levels in the body (testes and vas deferens) was cut using a diamond knife of an LKB 4800 Ultramicrotome. Sections were mounted on uncoated copper grids and double stained with alcoholic uranyl acetate and aqueous lead citrate for 20min.; ultrathin sections were examined in a Joel-JEM/1010 transmission electron microscopy made in Japan at 80 Kv.

Results

The primary spermatocystes of *P. indicus* were rounded cells with large rounded nuclei and high nucleocytoplasmic ratio (Fig. 1a, c). The secondary spermatocystes of *P. indicus* with nearly ovoid shape; each possessed a small nucleus and numerous mitochondria (Fig. 1b, c). The early spermatid of *P. indicus* was nearly rounded cell which possessed a large nucleus and contained a single centriole (Figs. 1d & 5a).

At the beginning of the spermiogenesis the spermatid centriole laid out against the plasmic membrane and the nuclear pores were observed in the periphery of the nucleus; the nuclear pores are great proteinic complexes allowing the exchanges between the core and the cytoplasm (Figs. 1e, f; 5b).

The early spermatid has a posterior bulb in which the centriole gives rise to a flagellum with 9 ± 0 pattern surrounded by a plasma membrane (Fig. 1g, h). It changed into 9+2 pattern; nine peripheral doublets and two central singlets (Figs. 1i; 5c). The flagellum follows the centriole in its migration and then the cytoplasmic canal is formed (Figs. 1j; 5d). The cytoplasmic canal is characterized by a ring form which appeared around

axoneme (Fig. 1k, l). Chromatin starts to condense and appears as electron-dense thickening (Fig. 2a) in transverse section. Centriolar derivative are two parts; one anterior which is electron-lucent while the posterior part is electron-dense (Fig. 2b, c).

The cytoplasmic canal stopped its progression when the centriolar derivative reached the nuclear anterior extremity (Figs. 2d; 5d). Then the centriolar derivative continued its anterior migration pushing in front of it the plasma membrane of the anterior extremity of the spermatid (Figs. 2b-d and 5e). Thus, it passes beyond the anterior extremity of the spermatid. During the flagellar migration the anterior and the posterior parts of the flagellum appeared temporary free (Figs. 2e & 5f).

When the posterior extremity of the flagellum passes the posterior extremity of the spermatid; the flagellar migration stopped. The nucleus extended towards the back of the spermatid (Figs. 2f and 5g); simultaneously, the chromatin started to turn into an amorphous electron-dense mass in contact with the nuclear groove (Fig. 2g). The chromatin becomes gradually lamellar (Fig. 2h). Then, it appeared as lamellar anastomoses (Fig. 2i) that forming a network (Fig. 2j). The chromatin reached maximum condensation.

At the end of spermiogenesis, the nuclear envelope opened widely and appeared like a pentalaminate structure which is consistent to a remnant of the nuclear groove (Fig. 2h, k). So, the nucleocytoplasmic derivative in which the chromatin was in close with the cytoplasm and protein granules appeared. Mitochondria are reduced in the residual cytoplasm. In the transverse section, the posterior part of the flagellum was marked by the disorganization of the axoneme, which was recognizable by both reduction in number of microtubules and the transformation of doublets into singlets (Fig. 2l).

The spermatozoa of *P. indicus* were detected in the seminal vesicles (Figs. 3, 4, 6). The transverse sections of the anterior extremity of the spermatozoa showed the presence of the centriolar derivative grouped into three different stages; the first was made of nine peripheral elements (Figs. 3a, b; 6a). The second was made of nine peripheral and one central element (Figs. 3c; 6b) and the third one was made of nine peripheral and two central elements (Figs. 3d; 6c).

The centriolar derivative is followed by a flagellum with a 9+2 pattern (Figs. 3e, f; 6d, e). A pentalaminate remnant of nuclear envelope is appeared. The pentalaminate remnant of the nuclear envelope occurs in the concave side (equivalent to a ventral side) of the nucleocytoplasmic derivative. Then, the nucleocytoplasmic derivative appeared like a reniform section. The chromatin showed developed condense and it appeared in an anastomosed shape. The chromatin located in the dorsal side of the nucleocytoplasmic derivative (Fig. 3g-i). Transverse section of the mature spermatozoon after the middle part showed that the chromatin reached its maximum condensation it appeared as a horseshow shape against the axoneme. The nuclear envelope was still present (Figs. 3j; 6g).

Transverse section of the mature spermatozoa of *P. indicus* showed protein granules distributed heterogeneously between the chromatin and the pentalaminate remnant of the nuclear envelope (Fig. 4a). Protein granules showed different sizes, numbers and shapes. They appeared alone with elongated (Fig. 4b), spherical (Fig. 4c) or lengthened (Fig. 4e) in shape full of their content. Some of these granules appeared partially empty with its contents (Fig. 4d, f). Also, they appeared two; with either nearly ovoid (Fig. 4g) or spherical (Fig. 4h) and nearly equal in shape, some of them looked elongated in shape, one longer than the other (Fig. 4i) and they are full of their content; while on the other hand, one appeared partially empty and the other full of its contents (Figs. 4j & 6f). Mitochondria were not observed in the spermatozoon.

The posterior extremity was characterized by disorganization of the flagellum, which was represented by noticeable with the interruption of the microtubules marked by the reduction in number of microtubules and the transformation of doublets into singlets (Fig. 4k-n). Transverse sections of posterior part obtained behind the distal extremity of spermatozoon showed different shapes of the chromatin, with an extended lamina (Figs. 4o; 6h). Some appeared as a closely curled thin lamina (Figs. 4p; 6i) and other as a horseshoe shape (Fig. 4q).

Discussion

The spermatid of *P. indicus* possessed only a single centriole. The centriole of the young spermatid gives rise to a flagellum. Presence of only single centriole in the spermatid of an acanthocephalan was recorded in different acanthocephalan species (Marchand and Mattei, 1976a, b, c, 1977a, b, 1978a, b; Foata *et al*, 2004, 2005, 2012a,b).

At the beginning of the spermiogenesis the centriole was laid out against the plasmic membrane. The nuclear pores were observed in the periphery of nucleus. Those were great proteinic complexes allowing the exchanges between the core and cytoplasm. The findings agreed with Foata *et al.* (2012a). During its migration, the centriole followed by the axoneme drags the posterior plasmic membrane, and cytoplasmic canal was made up. The progression of the cytoplasmic canal ended in front of the anterior nuclear extremity. Foata *et al.* (2012a) noticed the presence of a ring form in the anterior part of the cytoplasmic canal of *Acanthocephaloides incrassatus* spermatid and the transformation of a centriolar derivative.

In the present study, a ring form appears in the anterior part of the cytoplasmic canal of *P. indicus* spermatid around the axoneme, and the transformation of a centriolar derivative. It appeared to be made up of two zones of different densities followed by the axoneme and seems to progress simultaneously with the axoneme. During the flagellar migration, the anterior and the posterior parts of the flagellum were temporary free. The axonemal migration continued till the posterior end of the flagellum passes beyond the nuclear posterior end. The present findings agreed with those of Marchand and Mattei (1976a) who was the first to suggest that the anterior migration of the flagellum must occur in all acanthocephalan species. After that it was regularly shown in different acanthocephalan species Marchand and Mattei (1976a, b, c) and Foata *et al.*(2005, 2012a).

In the present study, when the flagellar migration ended, the nucleus extended towards the back of the spermatid. Simultaneously, the chromatin started to turn into an amorphous electron-dense mass in contact with the nuclear groove; it became gradually lamellar and appeared as lamellar anastomoses and like a network of lamellar anastomoses. The protein granules were elaborated while the chromatin reached maximum condensation. The degree of the chromatin condensation was variable in different classes of *Acanthocephala*. Foata *et al.* (2004) observed that, during spermiogenesis, the chromatin started to condense near the nuclear groove but rapidly gave rise to a structure of anastomosed layers like those observed in other Paleacanthocephala, e.g. *Leptorynchoides plagicphalus*. This structure progressively aggregated and reached maximum condensation in the mature spermatozoon. Marchand and Mattei (1978a, b) and also Foata *et al*. (2012b) observed that during the elongation of the nucleus, mitochondria stay in a cytoplasm which would be eliminated by sliding along the flagellum and the authors suggested that; in acanthocephalans, the elaboration of the nucleocytoplasmic derivative occurred when residual cytoplasm slides and is eliminated. At the same time, the opening of the nuclear envelope and insertion of protein granules between chromatin and nuclear envelope remnant occurred, and then, the posterior lamina was set up.

The present results were in line with these

opinions when the opening of the nuclear envelope and the insertion of protein granules between chromatin and nuclear envelope remnant occur. Then, the posterior lamina was set up. At the end of spermiogenesis the nuclear envelope opened wide and appeared like a pentalaminate structure which was consistent to a remnant of the nuclear groove. Then, the nucleocytoplasmic derivative appeared; in which the chromatin was in close contact with the cytoplasm and the protein granules but, the mitochondria were reduced in the residual cytoplasm. This feature was usual of other acanthocephalans (Foata *et al*, 2005, 2012).

In the present study, the flagellum of *P. indicus* spermatozoon has been a 9+2 pattern. The flagellum showed different patterns in the acanthocephalan spermatozoa, according to the class. Species belonged to the first class, Eoacanthocephalans, showed 9+ n patterns where no. ranged between 0 and 5 (Marchand and Mattei, 1977b). In the described species belonging to the second class; Paleacanthocephala, the spermatozoon flagellum was described of the 9+2 pattern such as in *A. incrassatus* (Foata *et al*, 2012a) and in *C. magnum* (Foata *et al*, 2012b) except in *I. fucatus* (Marchand and Mattei, 1976d) and in *L. plagicephalus* (Foata *et al*, 2004),

The spermatozoon flagellum of *P. indicus* was described as a 9+2 pattern approving with most of the studied Paleacanthocephala. While in the Archiacanthocephala the flagellum has a 9+2 pattern, similar to those *Moniliformis cestodiformis* (Marchand and Mattei, 1978b) and in *Macracanthorhynchus hirudinaceus* (Foata *et al*, 2005).

The anterior extremity of the spermatozoa of *P. indicus* showed that the presence of the centriolar derivative changed into three different stages; first was made of 9 peripheral elements, the second of nine peripheral and one central element and the third was made of nine peripheral and two central elements. The nine peripheral with two central elements was the last stage before the transformation in nine peripheral doublets with two singlets of the flagellum. This agreed with Foata *et al.* (2012a) who observed in *A. incrassatus* that the spermatozoon was distinguished by an original centeriolar derivative consisting of two zones: one electron-dense and the other electron-lucent.

In the present study, the mature spermatozoon of *P. indicus* presented many protein granules organized heterogeneously between the remnant of the nuclear envelope and the chromatin. They appeared to different sizes, numbers and forms and sometimes empty of their content. The present findings agreed with Foata *et al.* (2012a). The granules evacuation of their content were characteristic of an old spermatozoon (Marchand and Mattei, 1978a, b), these authors observed small and large granules laid out without the specific order in in *I. furcatus*. In *P. indicus* a thin chromatin layer extended on the dorsal side of the NCD. The present findings agreed with those of Marchand and Mattei, 1978a; Foata *et al.* (2012a). The nucleocytoplasmic derivative *P. indicus* rapidly evaluated. A pentalaminate remnant of nuclear envelope was formed, and then the pentalaminate remnant of the nuclear envelope occurs in the ventral side of the nucleocytoplasmic derivative and the chromatin was plated in the dorsal side, as reported by Foata *et al.* (2012a).

Transverse section of mature spermatozoon of *P. indicus* near after the middle part shows that the chromatin appeared as horseshow shape against the axoneme; this feature was not observed in other studied Paleacanthocephalans; then this conformed mainly the reversed anatomy of the spermatozoon of *Acanthocephala* (Marchand and Mattei, 1976d; Foata *et al*, 2012a.)

The free anterior part of the flagellum was observed to be longer than the NCD. The length of the free part of the flagellum was variable according to Paleacanthocephalan species. Marchand and Mattei (1976d) re-

ported similar observation for *I. furcatus* (a Paleacanthocephalan species). Also, Foata *et al.* (2004, 2012a) gave similar conclusions for another two Paleacanthocephalan species; *L. plagicephalus* and *Acanthocephaloides incrassayus* respectively. But, these data could not be generalized to all paleacanthocephalans because the flagellum anterior part was very short in two reported species: *C milvus* (Marchand and Mattei, 1976c) and *S. socialis* (Marchand and Mattei 1976b).

The posterior part of the flagellum of *P. indicus* extends in the contact with the NCD till the disorganization of the flagellar occurred; represented by the interruption of the microtubules. The NCD ends as a thin chromatin lamina such as in most *Acanthocephala*, e.g.: *M. hirudinaceus* and *A. incrassatus* (Foata *et al*, 2005, 2012a). Ultrastructural studies of spermiogenesis in *P. indicus* conform to those observed in all *Acanthocephala* species. The general organization of *P. indicus* spermatozoon agreed with previous description in other recorded *Acanthocephala* species; mainly a reversed anatomy and the mitochondria were not observed in the spermatozoon (Marchand and Mattei 1976d; Foata *et al*, 2012a). But, this work has been done for the first time in Egypt.

Conclusion

The present study reported for the first time the point of spermatogenesis, spermiogenesis and the ultrastructure of sperm in acanthocephalan parasite in birds. Spermatological features of *P. indicus* were compared to those of other acanthocephalan.

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Explanation of the figures

Fig. 1: [a-d, f, h-i, k-l (Cross), e, g, j (Longitudinal) section showing spermiogenesis of *P. indicus*]: a. primary spermatocystes (P), nucleus (N), secondary spermatocyste (S) Bar= 500nm, b. secondary spermatocystes (S), mitochondria (M) Bar=2nm, c. enlarged primary spermatocyste (P), nucleus (N), secondary (S) spermatocyste, mitochondria (M) Bar=2nm, d. young spermatide nucleus (N), centriole (C) Bar=500nm, e. spermatid centriole (C) Bar=2nm, f. spermatid nuclear pores (arrows heads) Bar=500nm, g. flagellum of spermatid centriole (F) Bar=2nm, h. flagellum; 9+0 pattern Bar=100nm, i. flagellum; 9+2 pattern (arrow head) Bar=500nm, j. centriole (C), cytoplasmic canal (CC), nucleus (N), centriolar derivative (double arrow) Bar=2nm, k. spermatid nucleus (N), cytoplasmic canal (CC) Bar=500 & l. enlarged cytoplasmic canal (CC), axoneme (arrow head) Bar=500nm

Fig.2: [(a, h-l (cross) , b-f (longitudinal) section] showing spermiogenesis of *P. indicus*: a. Chromatin (Ch), flagellum (F) Bar=500nm, b. Centriolar derivative; anterior part (arrow head), posterior part (double arrow head) Bar=500nm, c. Centriolar derivative; anterior part (arrow head), posterior part (double arrow head Bar=500nm, d. Cytoplasmic canal (CC), nucleus (N), centriolar derivative (arrow head) Bar=500nm, e. Anterior (AP) and posterior (PP) parts of flagellum (F) are free Bar=500nm, f. flagellum (F), anterior extremity (AP) Bar=500nm, g. Chromatin (Ch) Bar=500 nm, h. Lamellar chromatin (double arrow head) Bar=100nm, i lamellar anastomosed chromatin (arrow head) Bar=100nm, j. Chromatin anastomose like a network (arrow head) Bar=500nm, k. Nuclear envelope opens wide (arrow) Bar=500nm &l. Posterior extremity of axoneme; singlets (arrow head) Bar=500

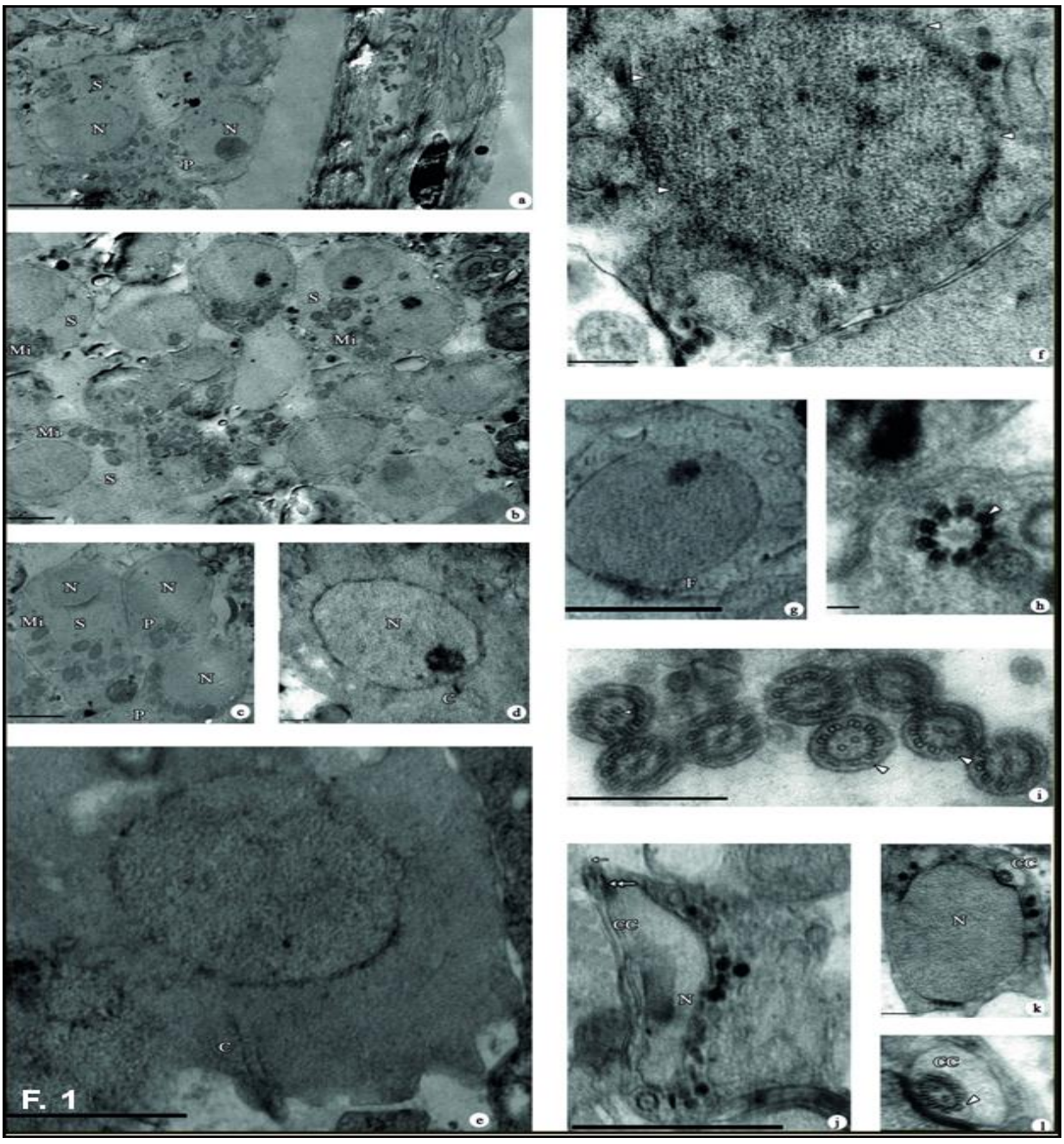
Fig.3: (a- j) Cross section of mature spermatozooa in the seminal vesicle of *P. indicus*: a. anterior extremitries of spermatozoa (arrows heads) Bar=2nm, b. anterior extremity of spermatozoon 9+0 pattern; a in Fig. 6 Bar=500nm, c. anterior extremity of spermatozoon 9+1 pattern; b in Fig. 6 Bar=500nm, d. anterior extremity of spermatozoon 9+2 pattern; c in Fig. 6 Bar=500nm, e. flagellum of 9+2 d in Fig. 6 Bar=500nm, f. flagellum of 9+2 e in Fig. 6 Bar=100nm. g. anastomose chromatin (double arrow head), nucleocytoplasmic derivative (arrow head), flagel-

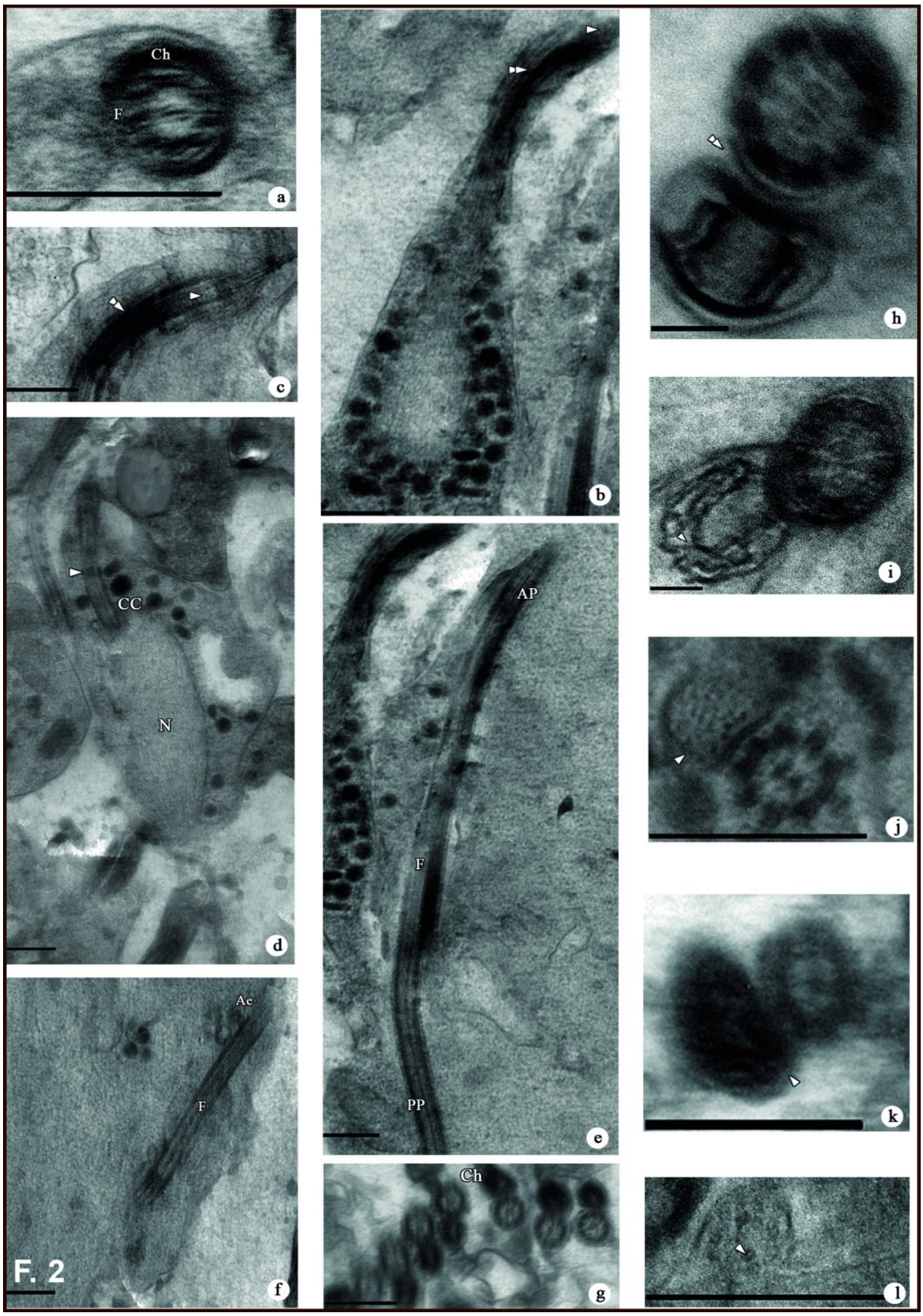
lum (F) Bar=500nm, h. middle extremity; nucleocytoplasmic derivative (arrow head), chromatin (Ch), flagellum (F) Bar= 500nm, i. middle part; nucleocytoplasmic derivative (arrow head), flagellum (F) Bar=500nm &j. middle part; flagellum (F), chromatin (Ch), remnant of nuclear envelope (arrow head) Bar=100nm

Fig. 4: (a- q) Cross section of mature spermatozooa in the seminal vesicle of *P. indicus* [(b-f; single protein granule), (g-j; two protein granules), (k-q; posterior extremity)]: a. Protein granules with different sizes and shapes (arrow heads) Bar=500nm, b. oval and full with its contents (arrow head) Bar=500nm, c. spherical and full with its contents (arrow head) bar=500nm, d. spherical and partially empty with its contents (arrow head) Bar=500nm, e. lengthened and full with its contents (arrow head) Bar=500nm, f. elongate and partially empty of its content (arrow head) Bar= 500nm, g. elongate and full with their contents; equal in shape (arrow head) Bar=500nm, h. spherical and full with their contents; equal in shape (arrow head) bar=500nm, i. elongate and full with their contents; one longer (arrow head) than the other Bar=500nm, j. spherical; one partially empty (arrow head), other full with its contents Bar=500nm, k. interruption of microtubules (arrows) Bar=500nm, l. transformation of doublets into singlet (arrow head) Bar=500nm, m. reduction of number of microtubules (arrow head) Bar=500nm, n. reduction of number of microtubules (arrow head) Bar= 500nm, o. Chromatin; straight thin lamina (arrow head) Bar=500nm, p. Chromatin; coiled thin lamina (arrow head) Bar=500nm & q. Chromatin; horseshoe shape (arrow head) Bar=500nm

Fig. 5: (a- h) Diagram showing ultrastructural organization of main stages of spermiogenesis of *P. indicus*

Fig.6: (a- i) Diagram showing ultrastructural organization of mature spermatozoon of *P. indicus.*

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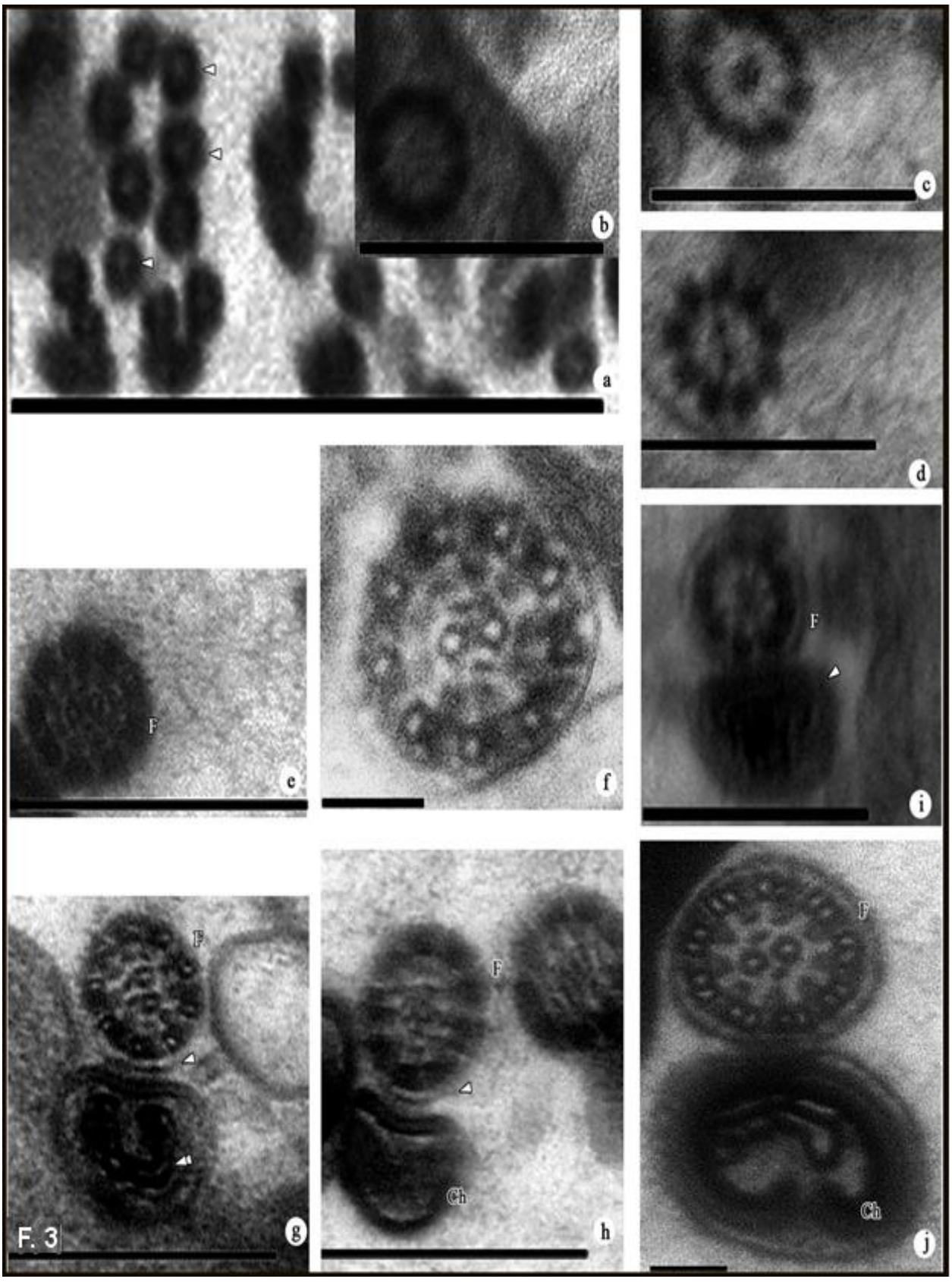
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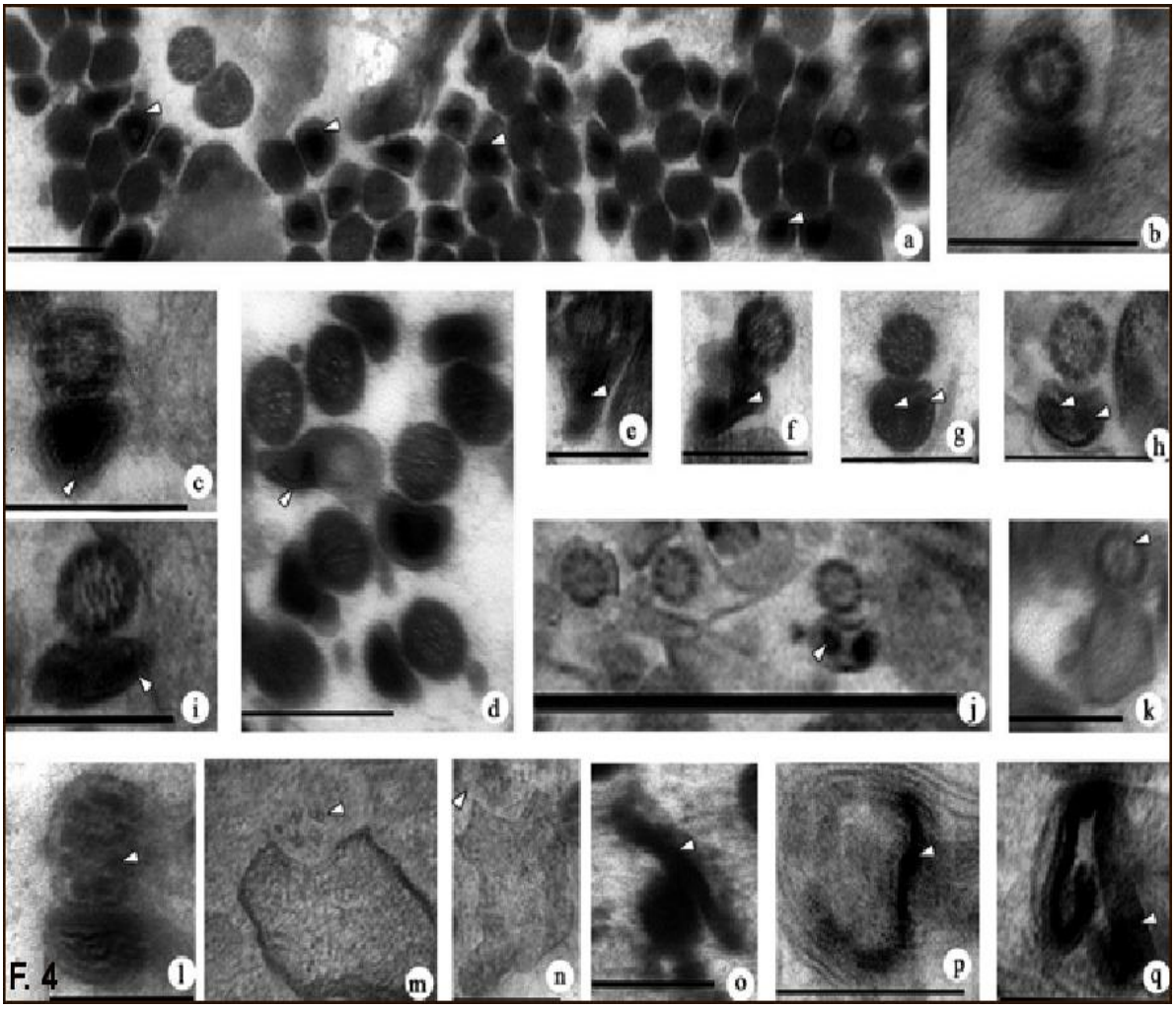
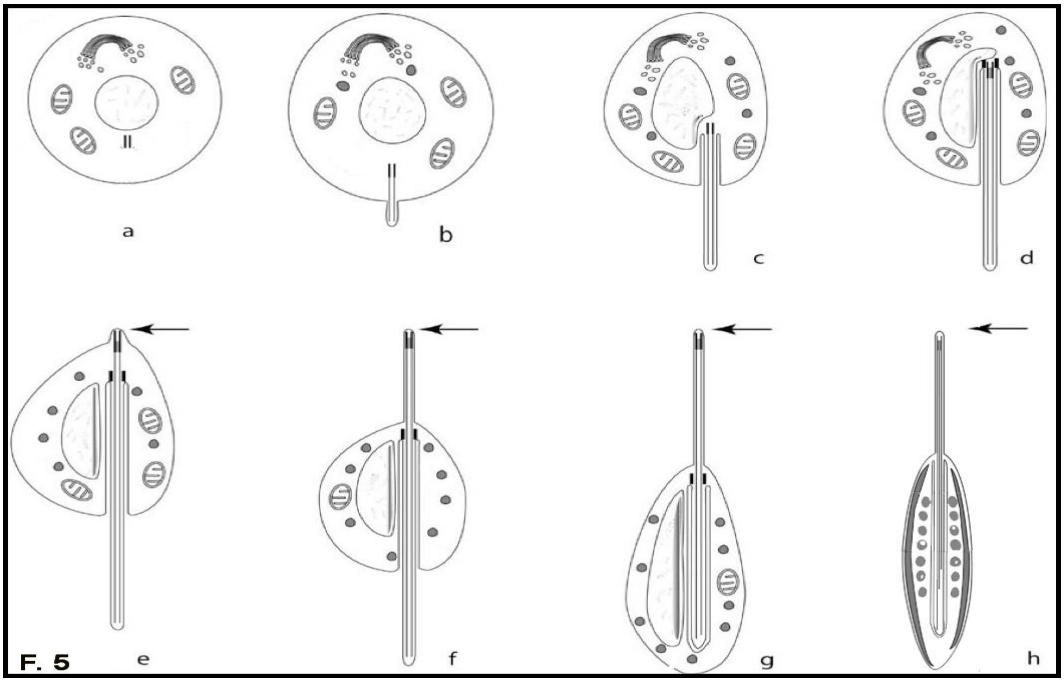
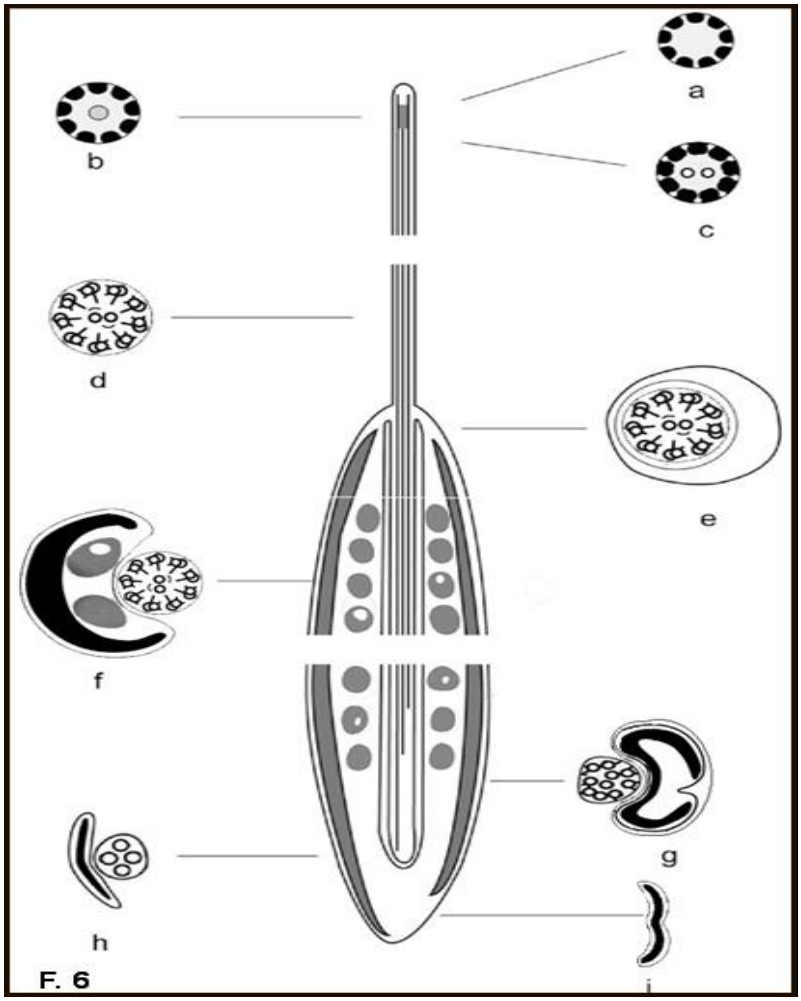


F. 4



F. 5



F. 6