Journal of the Egyptian Society of Parasitology, Vol.49, No. 3, December 2019
J. Egypt. Soc. Parasitol. (JESP), 2019 (3), 479- 486

TRANSMISSION ELECTRON MICROSCOPY OF THE BODY WALL OF *PORRORCHIS INDICUS* (ACANTHOCEPHALA) WITH SPECIAL REFERENCE TO LEMNISCI

By
HEWAYDAH E. ABOU SHAFEEY
Department of Zoology, Faculty of Science, Tanta University, Egypt
(E-mail hewaydah@yahoo.com)

Abstract

The study reported the ultrastructure features of the different tegumental layers, musculature and lemnisci of *Porrorchis indicus* (Das, 1957) Schmidt and Kuntz, 1967 (Acanthocephala, Plagiorhynchidae) from the Egyptian *Centropus senegalensis aegyptius* from Sharkia Governorate**.** Different semi-thin and ultrathin sections of the middle portion of the preasomal part of *P. indicus* were cut using transmission electron microscopy (TEM). The results show that the body wall is formed of five successive layers; epicuticle, striped, felt, radial and basal layer, the later rests on basement membrane (basal lamina) which is followed by a layer of circularly oriented muscle fibers. Peripherally, body wall is covered by a very thin glycocalex.

Key words: Ultrastructure; TEM, Tegument, lemnisci, *Porrorchis indicus,* Acanthocephala.

Introduction

The acanthocephalan tegument is a syncytium with many functions, including protecttion, inactivation of the host's digestive enzymes by charge effects, osmoregulation, concentration of nutrients, ion transport. The acanthocephalan body wall is a unique structure and serves a number of functions. One of its functions is to take up nutrients (Hammond, 1967). The acanthocephalan lacunar system has been studied (Miller and Dunagan, 1977, 1985); they concluded that the lacunar system acts to facilitate waste removal from the muscles as well as to provide nutrients, also it is a very effective fluid transport system and possibly serves as a hydrostatic skeleton. And the possible roles of the wall of the praesoma and the lemnisci in fat excretion or uptake were discussed (Hammond, 1967; 1968b).

Closely packed pores at the tegument surface lead to pore canals that branch and anastomose. This fluid filled system of channels is called the lacunar system (1). The absorptive surface is considerably increased by invaginations of the outer plasma membrane (pore canals and vesicles) (Graeber and Storch, 1978). Pores have been found which penetrate the cuticle. Thus, the structure of the surface layers is such as would facilitate the absorption of nutrients (Crompton and Lee, 1965).

Lemnisci are specific metabolic centers, where lipids are accumulated and utilized. Lipids get to these centers either by means of absorption by praesomal tegument or from the pseudocoel (Nikishin, 2003a). The fine structure of trunk and that of praesoma of different acanthocephalan species have been studied by the light and electron microscope (Crompton and Lee, 1965, Nicholas and Mercer, 1965, Dezfuli and Sbrenna, 1990, Nikishin, 2003b).

The organization and ultrastructure of the subsurface (cutaneous) musculature of different acanthocephalan worms was studied (Crompton and Lee, 1965, Dunagan and Miller, 1974, Wong *et al,* 1979, Herlyn, 2002, Herlyn and Ehler, 2001, Herlyn and Taraschewski, 2017, Nikishin, 2004). The subsurface musculature has similar structure in all acanthocephalans whose morphology was described, including two main components: the circular muscles underlying the basal plate and the longitudinal muscles actually limiting pseudocoelom (Miller and Dunagan, 1985). The evolutionary novelty of acanthocephalans could be the existence of only a single layer of musculature under the presomal tegument while the possession of circular and longitudinal muscle layers under metasomal tegument should be evolutionarily older (Herlyn and Taraschewski, 2017).

Structurally wall of the trunk and praesoma were similar and the lemnisci resemble the 'inner layer' of the praesoma wall. But, physiologically; the wall of the trunk differed from that of the praesoma and the lemnisci (Hammond, 1967).

Fine structure of the early stages of tegument of \some acanthocephalans was studied (Herlyn *et al*, 2001) and discussed the similarities between them and wall structure of cestode cysticercoids (Nikishin, 2011). The superficial morphological and histochemical descriptions assembled all developmental stages of the Acanthocephala (Taraschewski, 2000). Also, Nikishin, 1989, 2003b, described the fine morphology of surface complex of young and mature acanthocephalan species. The outer surface coat is a carbohydrate rich glycocalyx. Outermost glycocalyx is morphologically described (Nicholas and Mercer, 1965, Dunagan and Bozzola, 1989). The morphology of glycocalyx on acanthoce-phalan tegument surface was analyzed (Nikishin, 2018).

The present study studied in details the ultrastructure of *P. indicus* body wall for data on characterize morphologic features of the tegument in comparison with previous reports. With special attention was given to the ultra-structural of the lemnisci.

Materials and Methods

Alive worms of *P. indicus* were collected from the small intestine of naturally infected *Centropus senegalensis aegyptius* from Sharkia Governorate, Egypt and treated for transmission electron microscopy (TEM) as follow. Alive specimens were rinsed in 0.9 Na Cl solution and immediately 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 20 min., ultrathin sections were examined in a Joel - JEM/ 1010 transmission electron microscopy made in Japan at 80 Kv.

Results

Semi-thin section (Fig.1a) and TEM micrographs showed that the body wall of the preasoma middle portion of *P. indicus* formed of five successive layers; epicuticle, striped, felt, radial and basal layer, the later rests on basement membrane (basal lamina), followed by a layer of circularly oriented muscle fibers (Fig.1b). Peripherally, the body wall was covered by a very thin glycocalex detected only with high magnification (fig. 1c).

The outer most layer; epicuticle was an electron opaque layer separated from the deeper layers of the tegument by apical plasma membrane. Epicuticle was differentiated into four layers (tetra laminate). Epicuticle outermost external part appeared as an electron-dense smooth layer followed by a thin electron-opaque layer overlying a thick moderately electron-dense heterogeneous layer; and divided into two layers; its outer layer was less dense than the inner that was underlined by apical plasma membrane (Fig. 1c). Apical plasma membrane extended internally; in integumental syncytium perpendicular on the surface and lines the lacunar system channels and pores. Underneath the apical plasma membrane was a thin lucent cytoplasmic zone lined the channels of the second striped layer (Fig. 1d).

The second striped layer is characterized by presence of numerous parallel membrane-bounded channels immersed in thin crypts of homogeneous electron-dense material. The channels were uniformly distributed and ended with micropores on the surface. Some channels contained numerous spherical or ovoid electron opaque granules

(Figs.1d, e).

Third tegumental layer; felt layer contains channels of lacunar system that are narrower than that in the second layer extend, their terminal portions appear no longer separated by the dense material as in the second layer (Fig.1f). In transverse sections, the branches look like ovoid vesicles with variable sizes and sometimes they were crowded. They are filled with a fine loosely packed granular material. This layer was crossed by bundles of fine cytoplasmic filaments; these bundles were irregularly distributed and extended to the basal region without a precise localization and orientation. Few aggregations of glycogen granules, electron opaque lipid depositions (sometimes surrounded by dense membranes), and electron dense depositions were seen in this layer (Fig.1f).

Radial layer represents the largest part of the integument (nearly 80/of tegument thickness, Fig. 1g). It consisted of a finely granulated opaque matrix that contains numerous filaments with dissimilar length; which extended solitary or in bundles without an obvious definite arrangement. In transverse section these filaments appeared hollow. Numerous bundles of short filaments extended in different directions (Figs. 1h, i), few bundles were longer and wavy extended along till reaching the matrix of the external tegumental layers (Fig. 2a).

Vesicles, glycogen granules, lipid droplets and clusters of mitochondria with different sizes and shape (spherical and ovoid) with few cristae and a typical dense compact matrix could be detected (Figs 1i, 2a-b). Giant nuclei, with irregular shape are observed internally in this layer; some appeared surrounded with double nuclear membrane and having a round compact electron dense nucleolus (Figs.1g, 2b). Near nuclear membrane numerous clusters of opaque large elongated mitochondria were detected (Fig. 2b). The basal layer was thin with electron lucent matrix which contains numerous cytoplasmic filaments; arranged in parallel manner or irregularly distributed in the matrix (Fig. 2 c). Mithocondria with dense highly compacted matrix and vesicle-like structures were also visible in this layer (Figs. 2c, d).

The basal layer overlies a thick basal lamina (Figs. 1b, 2e, f). The basal lamina appears consists of finely granulated opaque connective tissue contains electron dense bodies with different shapes; rounded or ovoid, the ovoid shaped ones appear electron opaque (figs. 2 e, f) while the rounded ones have different sizes, some of them were small and electron dense (Figs. 2f, g) others appear as large membrane-bounded vesicles. The matrix of some bodies appeared tri-lamellate; has peripheral opaque layer followed by moderately dense layer then central opaque. Others look tetra lamellate with circular dense zone in the center of the inner opaque layer (fig. 2h).

The plasma membrane is electron dense intensity folded with feather- like protrusions drawn out to form membrane-bounded vesicles (fig. 2c). In some areas, these vesicles resemble those found through tegumental layers, some vesicles with electron dense particles (Fig. 2d). The electron dense basal membrane overlied the muscle layer; in some parts the basal membrane appears extended straight; parallel to the plasma membrane (Fig. 2f) and in other parts and extended internally in between the muscle fibers forming canal like structures (Fig. 2i). The canal like structure contains finely granulated opaque connective tissue enclosed few electron dense granules with different sizes and shapes (Fig. 2i).

Musculature of praesoma consisted of only circulatory oriented muscle fibers (Figs. 2i, 3a, b). Glycogen aggregation, opaque mitochondria, lipid droplets and dense membrane-bounded bodies were scattered in between fibers of this layer (Figs. 2e, f, 3b, c).

Irregular open sac like cavities with different sizes was within the inner zone of the muscle layer. They bounded with the electron dense membrane open to the pseudocoel and contain electron lucent matrix filled with crowded rounded vesicles with different sizes and electron intensity (Figs. 3a, c). Some were small and electron dense, others are large and membrane-bounded (Fig. 3d), and much similar to these in the basal lamina.

In transmission electron micrographs, the lemnisci appeared in cross section as sac like shape (Fig. 3e). It has an irregular central cavity bounded with lemnisci wall. In some parts the wall is completely surround the cavity in others it is partially surround the cavity (Fig. 3f). The wall consisted of finely granulated connective tissue that contains electron dense fibrous strands; parallel extend, in other parts they extended in different orientations, small electron opaque depositions scattered between fibers (Fig. 3g). In some areas vesicles contained electron dense particles scattered between the crowded fibers, they resembled those found within tegumental layers (Fig. 3h) most of the vesicles were double membrane (Fig. 2h). Cavity of lumnisci enclosed elongated mitochondria and dense granules (Figs. 3e, f, g).

Discussion

The ultra-structural organization of the body wall of *P. indicus* in the present study resembles in its basic pattern that of the other reported acanthocephalan species, (Nicholas and Mercer, 1965, Hammond, 1968a, Beermann *et al*, 1974, Storch, 1979, Whitfield, 1984, Olsen, 1986, Dezfuli and Sbrenna, 1990, Heckmann *et al*, 2013). It is consisted of five consecutive layers; epicuticle, striped layer, felt layer, radial layer and basal layer. Also, it showed a trilaminar basal lamella that followed by a layer of circularly oriented muscle fibers.

In the present study, the body wall organization has an outer electron opaque laminated epicuticle comparable to the epicuticle as in *Polymorphus minutus* (Crompton and Lee, 1965) and in (Olsen, 1986) and was defined in *Echinorhynchus truttae* and in *Pomphorhynchus laevis* (Hammond, 1968a) and in *Telosentis exiguous* (Dezfuli and Sbrenna, 1990), also comparable to a glycocalyx in (Beermann *et al,* 1974). It undoubtedly contributes in protection and defense function of the body wall region. Presence of numerous pores that penetrated the integument to the channels within the surface layers and uniformly distributed on surface certainly facilitate the nutrients absorption.

In the present study, the outermost region of the tegument was divided into an outer part, exhibits a cytoplasmic matrix, which is filled by moderately electron- dense material and an inner part having opaque and lucent cytoplasm. The electron dense matrix may contribute into increase the supporting function of the body wall region, where it enclosed fine filaments bundles to build up a skeletal network with greatest possibly constitute an integumental cytoskeleton; this agreed with (Hammond, 1968b, Byram and Fisher, 1973, Graeber and Storch, 1978 and Whitfield, 1984).

All acanthocephalans lack a digestive tract, so body wall was very well adapted for carry out different functions; structural and protective also it performs an absorptive function. The present results showed that the plasma membrane penetrates into the integumental syncytium, producing an intensive organized arrangement of tubular channel system. Channels are irregular in shape and branch out having micropores on the surface. The presence of this system increases the amplification of membrane surface area of the parasite, resulted the increase of absorptive surface area. This result agreed with descriptions of Byram and Fisher (1973), Graeber and Storch (1978) and Dezfuli and Sbrenna (1990) for *Moniliformis dubius*, *Echinorhynchus gadi* and *Telosentis exiguous* body wall respectively.

In the present study, the striped layer contained numerous branched lacunar channels, many electron opaque depositions with different sizes and shape could be detected within these channels reach up to the pores. This structure of the striped layer supports the theory that the praesoma body wall and lemnisci are involved in the absorption of fat (Hammond, 1968b), and agreed with the report that, lacunar system is a very effective fluid transport system and serves as a hydrostatic skeleton (Miller and Dunagan, 1985).

The present data showed that the lacunar channels in felt layer were filled with fine, loosely packed materials, glycogen granules and lipid depositions, this structure agreed with Nicholas and Mercer (1965) that the vacuole formation at the surface of the tegument proposes that pinocytosis plays a part in assimilation.

Mitochondria were detected in the felt and radial layers of the body wall and in the circular muscles (Crompton and Lee, 1965).

In the present study, the radial layer contained numerous bundles of filaments; woven into a network within opaque matrix with nume- rous clusters of mitochondria. This is attributed to the possibility that this layer plays a role in the constriction of the wall of the body and the worm movement. The structure of the surface layers facilitated the absorption of nutrients (Crompton and Lee, 1965). The present trilaminar basal lamella was detected before in different acanthocephalan species (Whitfield, 1984; Olsen, 1986; Dezfuli and Sbrenna, 1990). The body wall musculature consisted only of circular muscles, this agreed with that of *Acanthocephalus anguillae* (Herlyn and Ehler, 2001). Within the musculature; large particles that it may be glycogen aggregated also dense membrane-bounded bodies and lipid droplet present; this may be indicate as effect for metabolite the results of absorbed nutrients, which agreed with the body wall description of Fayed *et al*. (2019) for *Acanthogyrus niloticus*.

In the present study the presence of huge numbers of vesicles within the basal lamella and muscle of *P. indicus* may be as indication that they represented an integumental specialization to increase the metabolism and transport function of the body wall.

Lemnisci were paired organs that extend into the body cavity from the neck region. The central canal of each lemniscus is continuous with the lacunar system. The lemniscus serves as a fluid reservoir when the proboscis is invaginated. They may also have a function in fat metabolism. The lemnisci resemble the 'inner layer' of the praesoma wall (Hammond, 1967). In the center of each lemniscus are large nuclei surrounded by a common cytoplasm with large protein granules (Marchand and Grita-Timoulali, 1991). Lipids may withdraw to the centers either by praesomal tegument of absorption or by pseudocoel via the system of invaginations of cytoplasmic membrane limited lemnisci (Nikishin, 2003).

Conclusion

In the present study the observation of numerous vesicles, protein granules, mitochondria, lipids with different shapes, sizes and electron intensity; within the lumnsci wall and cavity probably proposes that they play a part in lipid metabolism. So, the lemnisci could be specific metabolic centers, where lipids are accumulated and utilized.

References

Beermann, I, Arai, HP, Costerton, JW, 1974: The ultrastructure of the lemnisci and body wall of *Octaspihifer macilentus* (Acanthocephala). Canad. J. Zool. 52:553-5.

Byram, IE, Fisher, FM, 1973: The absorptive surface of *Moniliformis dubius* (Acanthocephala). I. Fine structure. Tissue and Cell 5:553-79.

Crompton, DWT, Lee, DL, 1965: The fine structure of the body wall of *Polymorphus minutus* (Goeze, 1782) (Acanthocephala). Parasitol. 55, 2:357-64.

Dezfuli, BS, Sbrenna, G, 1990: A morphological and ultrastructural study of *Telosentis exiguus* (Acanthocephala, Palaeacanthocephala. Boll. Zool. 57:225-32.

Dunagan, TT, Bozzola, JJ, 1989: Fine structure of anterior terminus of apical sense organ in *Macracanthorhynchus hirudinaceus* (Acanthocephala). J. Parasitol. 75, 2:297-302.

Dunagan, TT, Miller, DM, 1974: Muscular anatomy of the praesoma of *Macracanthorhynchus hirudinaceus* (Acanthocephala). Helminthol.

Soc. Wash. 41, 2: 199-210.
Fayed, RH, Khalil, AI, El-Gamal, MM, Radwan, NA, 2019: Acanthocephala from Nile fish. In Press.
Graeber, K, Storch, V, 1978: Electron microscopic and morphometric investigations of the integument of Acanthocephala (aschelminthes). Parasitol. Res. 57, 2:121-35.
Hammond, RA, 1967: The fine structure of the trunk and praesoma wall of *Acanthocephalus ranae* (Schrank, 1788), Luhe, 1911. Parasitol. 57: 475-86.
Hammond, RA, 1968a: Observations on the body surface of some acanthocephalans. Nature (London), 218:872-3.
Hammond, RA, 1968b: Some observations on the role of the body wall of *Acanthocephalan ranae* in lipid uptake. J. Exp. Biol. 48:217-25.
Herlyn, H, Ehlers, U, 2001: Organisation of the praesoma in *Acanthocephalus anguillae* (Acanthocephala, Palaeacanthocephala) with special reference to the muscular system. Zoomorphol. 121, 1:13-8.
Heckmann, RA, Amin, OM, EI-Naggar, AM, 2013: Micropores of Acanthocephala, a scanning electron microscopy study. Parasitol. Sci. 14:105-13.
Hehn, N, Ehlers, U, Herlyn, H, 2001: Ultrastructure of the acanthella of *Paratenuisentis ambiguous* (Acanthocephala). Parasitol. Res. 87: 467-71.
Herlyn, H, 2002: The musculature of the praesoma in *Macracanthorhynchus hirudinaceus* (Acanthocephala, Archiacanthocephala): Re-examination and phylogenetic significance. Zoomorphol. 121:173-82.
Herlyn, H, Taraschewski, H, 2017: Evolutionary anatomy of the muscular apparatus involved in the anchoring of Acanthocephala to the intestinal wall of their vertebrate hosts. Parasitol. Res. 116:1207-25.
Marchand, B, Grita-Timoulali, Z, 1991: Comparative ultrastructural study of the lemnisci in larvae and adults of *Centrorhynchus milvus* Ward, 1956 (Acanthocephala, Centrorhynchidae. Canad. J. Zool. 69, 11:2918-23.
Miller, DM, Dunagan,TT, 1977: The lacunar system and tubular muscles in *Acanthocephala*. Proc. Helminthol. Soc. Wash. 44, 2:201-5.
Miller, DM, Dunagan, TT, 1985: New aspects of acanthocephalan lacunar system as revealed in anatomical modeling by corrosion cast method. Proc. Helminthol. Soc. Wash. 52, 2:221-6.
Nicholas, WL, Mercer, EH, 1965: The ultrastructure of the tegument of *Moniliformis dubius* (Acanthocephala). Quart J. Micro. Sci. 106, 2: 137-46.
Nikishin, VP, 1985: Ultrastructure of cover tissues in late acanthella of *Arhythmorhynchus petrochenkoi* (Acanthocephala: Polymorphidae). Parazitologiya 19, 4: 306-13.
Nikishin, VP, 2003a: Ultrastructure of lemnisci in *Polymorphus magnus* (Acanthocephala, Polymorphidae. Tsitolo. 45, 7:722-9.
Nikishin, VP, 2003b: Ultrastructure of the surface complex of acanthocephalans with the example of *Filicollis anatis* (Palaeacan-thocephala: Polymorphidae) and *Neoechinorhynchus crassus* (Eoacanthocephal. Parazitol.37, 5:394-401.
Nikishin, VP, 2004: Subsurface musculature of spiny-headed worms (Acanthocephala) and its role in formation of intercellular matrix. Biol. Bull. 31, 6:598-612.
Nikishin, V, 2011: Convergence of the cyst in cysticercoids (Cestoda, Hymenolepidata) and the tegument in cystacanths (Acanthocephala). Biol. Bull. 38, 2:195-202.
Nikishin, VP, 2018: Glycocalyx modifications in acanthocephalans. Biol. Bull. 45, 1:35-46.
Olsen, OW, 1986: Animal Parasites the Life Cycles and Ecology: Phylum Acanthocepha. Dover Publications, Inc., New York.
Storch, V, 1979: Contributions of comparative ultrastructural research to problems of invertebrate evolution. Amer. Zool. 19:637-45.
Taraschewski, H, 2000: Host-parasite interacttions in *Acanthocephala*: A morphological approach. Adv. Parasitol. 46:1-179.
Whitfield, PJ, 1984: Acanthocephala. In: A.G. Matoltsy, K. Richards (eds.), Biology of Integument (Invertebrates). Springer-Verlag, Berlin.
Wong, BS, Donald, M, Miller, Dunagan, TT, 1979: Electrophysiology of Acanthocephalan body wall muscles. J. Exp. Biol. 8:273-80.

Legend of figures

Fig. 1: (a) Semi-thin section and (b- i) TEM micrographs of middle portion body wall of preasoma of *P. indicus* showing: (a) Tegument (T), lemnisci (Le), muscle (Mu), pseudocoel (P), (b) Tegumental layers (I, II, III, IV, V), basal lamina (BL), muscle fiber (Mu) bar= 3000, (c) Epicuticle layers (arrows), glycocalex, granule (G) (Gx) bar=120000, (d) Apical plasma membrane (APM), lucent cytoplasmic zone (arrow head), granule (Gr) micropores (Mp) bar= 40000, (e) Striped layer, channels (Ch), micropores (Mp), granules (Gr) bar=25000, (f) Felt layer; vesicles (V), filaments (F), glycogen (G), lipid (Li) bar=25000, (g) Low magnification of tegument; radial layer (RL), Nucleus (N),

nucleoulus (Nu) bar=3000, (h) Enlarged radial layer, short filaments (F), glycogen (G), Vesicle (V), lipid (Li), mitochondria (Mi) bar=25000, (i) Enlarged radial layer, short filaments (F), Vesicles (V), lipid (Li), mitochondria (Mi) bar=40000

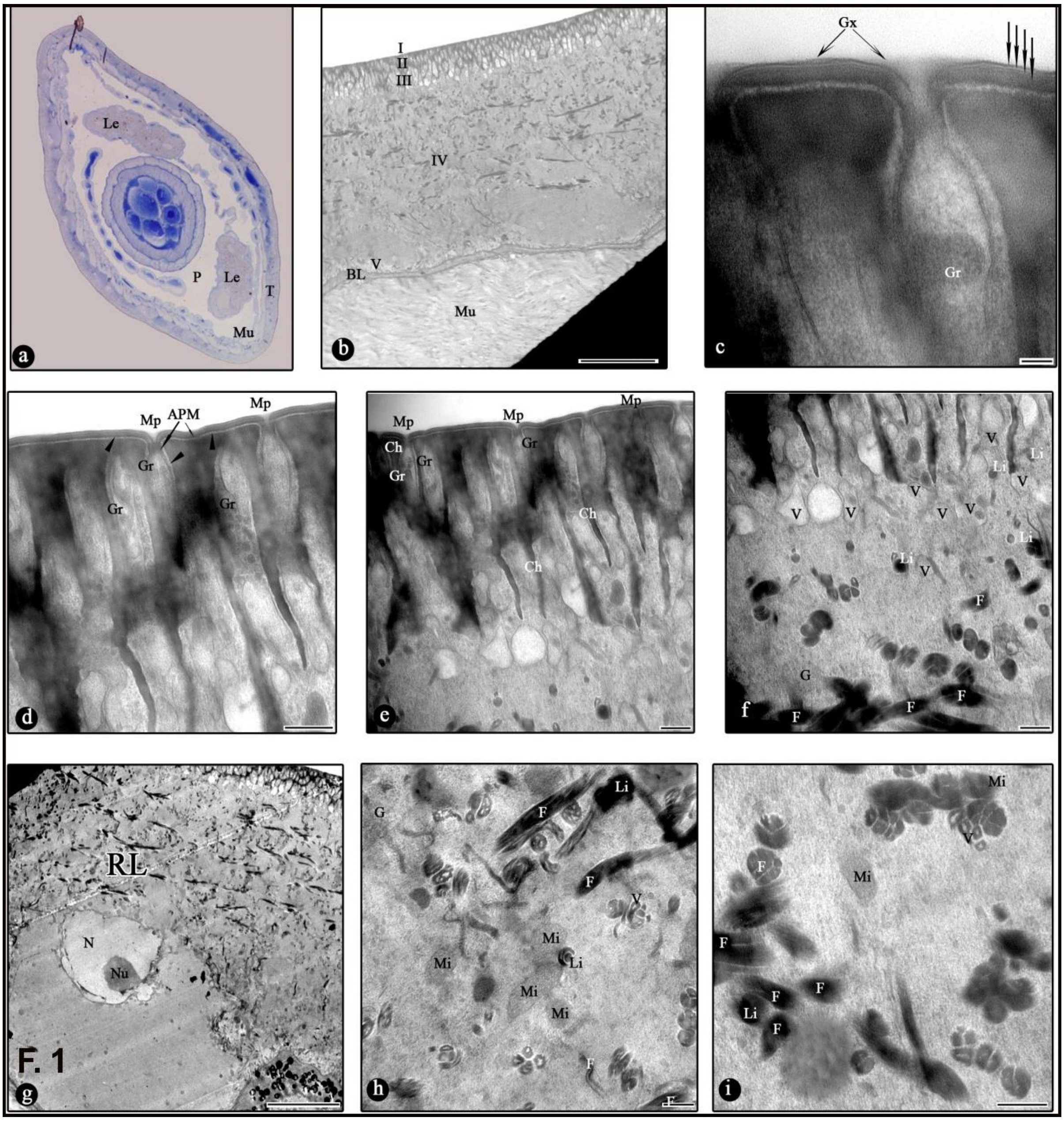
Fig. 2: (a- h) TEM micrographs of the body wall of middle portion of the preasoma of *P. indicus* showing: (a) Radial layer; wavy filaments (WF), Vesicle (V), mitochondria (Mi), lipid (Li) bar= 15000, (b) Enlarged Nucleus (N), nuclear membrane (NM), nucleoulus (Nu), mitochondria (Mi) bar=20000, (c) Basal layer; filaments (F), Vesicles (V) bar= 30000, (d) Enlarged basal layer; mitochondria (Mi), Vesicles (V) bar , muscle (Mu) =25000, (e) Basal lamina (BL), connective tissue (CT), lipid (Li) bar=10000, (f) Basal lamina (straight) (BL), rounded bodies (RB), lipid (Li), mitochondria (Mi), straight basal membrane (BM), muscle fibers (Mu), ovoid opaque bodies (OB) bar=5000

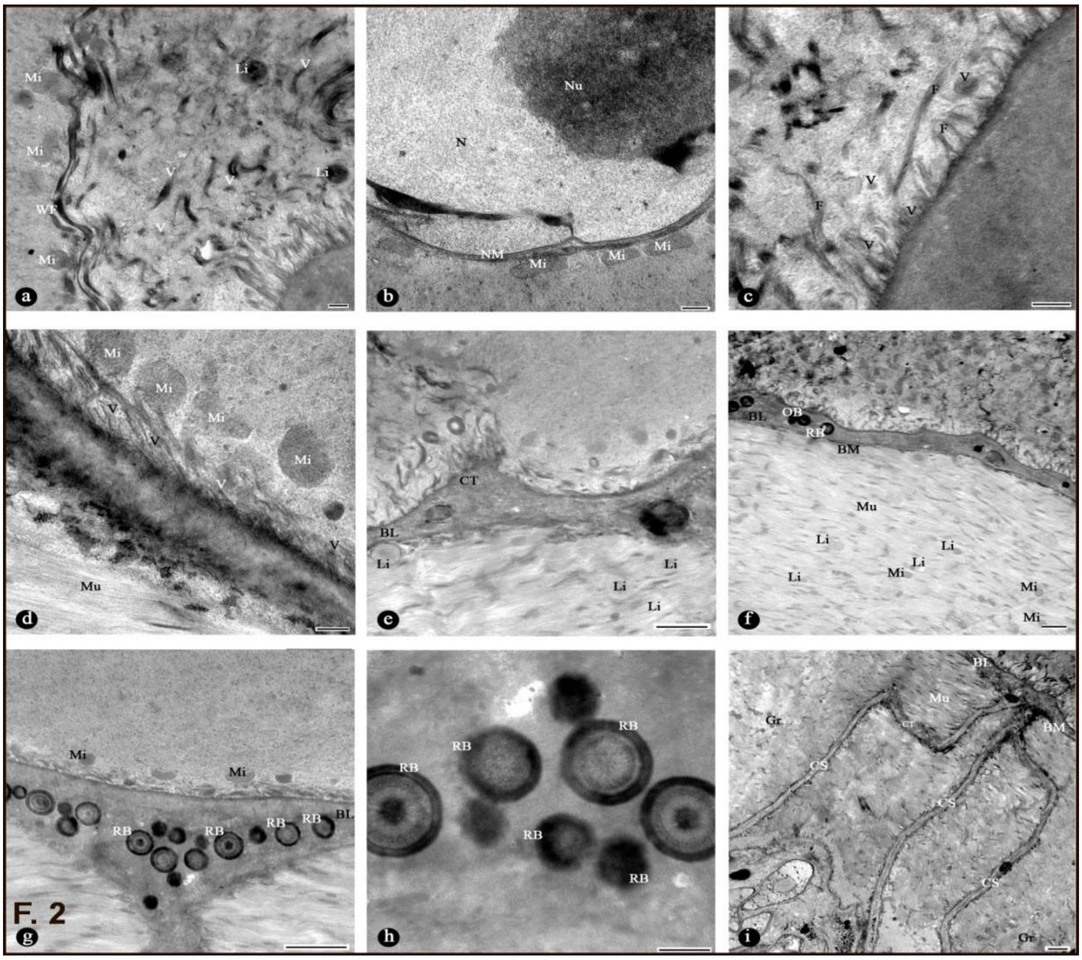
(g) Enlarged basal lamina (branch) (BL), rounded lucent bodies (RB), mitochondria (Mi) bar=12000, (h) Enlarged rounded bodies (RB) bar=4000, (i) Basal lamina (BL), branched basal membrane (BM), canal like structures (CS), connective tissue (CT), granules (Gr) bar=4000

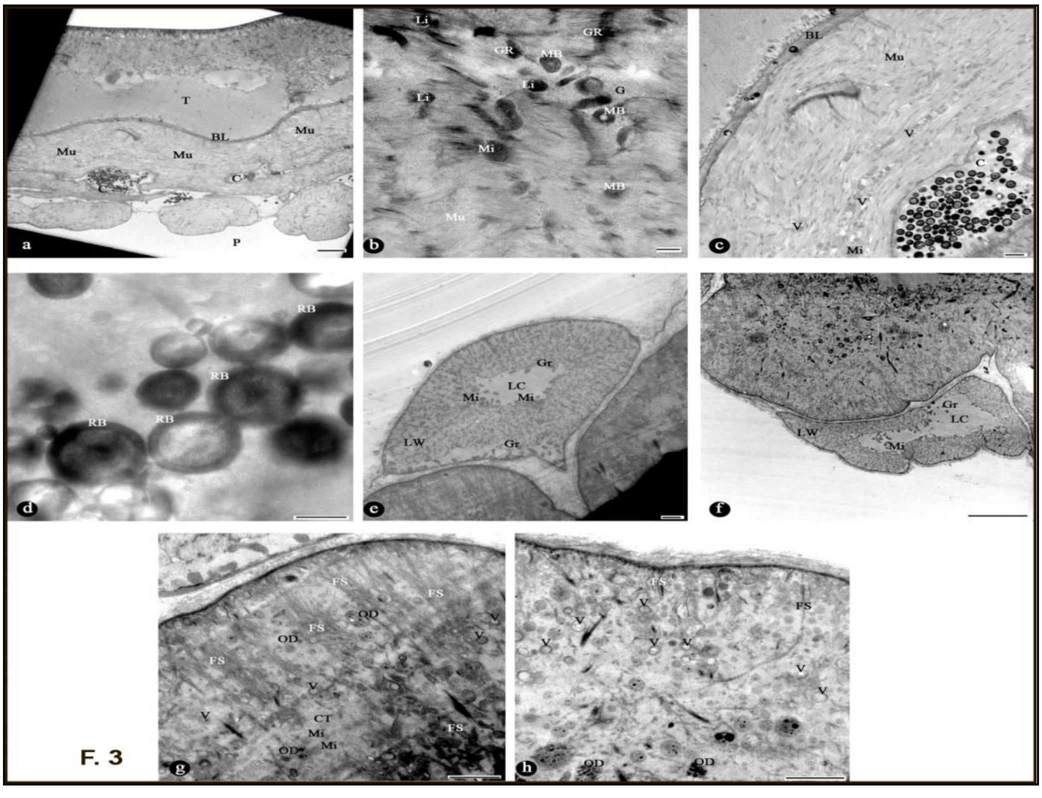
Fig. 3: (a- g) TEM micrographs of the body wall and lemnisci of *P. indicus* showing: (a) Body wall; tegument (T), circular muscle layer (Mu), lemnisci (Le), pseudocoel (P), cavity (C), basal lamena (BL) bar=1200

(b) Enlarged part of circular muscle layer (Mu), lipid (Li), mitochondria (Mi), glycogen (G), membrane-bounded bodies (MB), dense granule (Gr) bar=20000, (c) Circular muscle layer (Mu), mitochondria (Mi), cavity (C), basal lamina (BL), vesicles (V) bar=4000

(d) Enlarged rounded vesicles (RB) have different sizes and shape bar=40000 , (e) Section of lemniscus shows lemnisci wall (LW) completely surround cavity (LC), granules (Gr), mitochondria (Mi) bar=4000, (f) Section of lemniscus shows lemnisci wall (LW) partially surround cavity (LC), granules (Gr) mitochondria (Mi) bar=2500, (g) Enlarged part of lemniscus, connective tissue (CT), fibrous strands (FS), mitochondria (Mi) opaque depositions (OD), double membrane vesicles (V) bar=10000, (h) Enlarged part of lemniscus wall, double membrane vesicles (V), fibrous strands (FS), opaque depositions (OD) bar=12000




a
Mi
Li
V
WF
b
Nu
N
NM
c
F
d
Mu
e
CT
BL
f
OB
RB
BM
g
h
i
Gr
CS
F. 2


a
T
BL
Mu
C
P
b
Li
GR
MB
G
Mi
c
V
d
RB
e
LC
LW
Gr
f
g
FS
OD
CT
h
F. 3