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REVIEW ON ELECTRON MICROSCOPY IN TAXONOMY AND BIOLOGY OF PARASITIC NEMATHELMINTHES

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Abstract

Electron microscopy (EM) proved a very helpful means that solved a lot of information in different scientific aspects. EM is a very good tool in the hospitals and research centers. It was aimed to pile up available information on the biology in the descriptive morphology of nematodes and their immature stages by scanning (SEM) and transmission (TEM) electron microscopy.

Watson (1965a, b) studied *Euchromadora vulgaris* and *Ascaris* sp. by using TEM respectively. Lee (1969) investigated the ultra-structure of *Nippostrongylus brasiliensis* by SEM & TEM, as well as some nematodes by TEM (Lee, 1972). The topography of the adult *Baylisascaris procyonis* caudal end was illustrated by Snyder (1989). Male tail relatively long, smoothly attenuated, with a small button-like or mucronate termination. Pre-anal papillae situated ventrally in 2 slightly divergent and somewhat irregularly spaced rows. Anterior and posterior to anus 2 slightly raised roughened patches consisting of several rows of small spines. Just anterior to anus along outer margin of pre-anal roughened patch, a large double medio-ventral papilla. Five pairs of post-anal papillae with first pair just posterior to anus doubled and 4 pairs more closely associated in a group near tail end. Second pair with doubled papillae; but, in a few specimens fused as if 2 single closely associated papillae. Three pair single. Fourth pair of caudal papillae phasmids and in centers of each a ringed pore-like opening. Male spicules with a highly sculptured surface with a pincher-like terminal end.

Ashour (1990) described *Mastophorus muris* by TEM and considered; a- three layered cuticle of different nematodes, b- structure of each layer of cuticle (Lee and Atkinson, 1976). c- outer-most layer armed of a triple layered membrane. d- median layer homogeneous and less osmiophilic than outer one being somewhat, as an electron translucent zone containing a network of collagen fibrils, and fusion of outer (cortical) and median layers sometimes found. e- basal layer formed of three fiber layers, middle one crossed the other two layers, but sometimes formed of three layers of compact fibrils. Arrangement of fibrils in basal layer differed from species to species (Crofton, 1957). Each fiber layer consisted of parallel strands running in spirals at about 75^{o} to longitudinal worm axis and middle one crossed other layers at about 135^{o} to form mobile lattice.

SEM studies on nematodes are very scarce. Male head and tail of the majority of them are of taxonomic importance due to characteristic micotopography of these regions. Nembo *et al*. (1993) described the micro-topography of 3^{rd}, 4^{th} and adults of rat-hook worm; *Nippostrongylus brasiliensis* (Helig-mosomatidae) by SEM, and concluded that the main differences between 3^{rd} & 4^{th} stage larvae was the lip-like structures around oral aperture, presence of cephalic space with a cephalic cap on 4^{th} stage larvae, longitudinal ridges pattern and sexual differences. Pore-like papillae were in 4^{th} stages and adults. Deirids was in adults, and phasmids were poorly discerned. Neuhaus *et al*. (1996) studied cuticle development in *Oesophagostomum dentatum* by LM, SEM & TEM, found cuticle of 1^{st} 3 juveniles with a trilaminated epicuticle, an amorphous layer and a radial striated layer. In last juvenile and adults, radial striated layer replaced by a fibrous layer with 3 sublayers of giant fibers and a basal amorphous layer. Tkach and Swiderski (1996) described a rare species of bat *Pterygodermatites bovieri* by SEM found cephalic end structure and cuticular armament were of great systematic value in family Rictatuliriidae. Mouth opening sub-terminal and oriented dorsally with numerous sclerotized denticles in 2 rows in buccal cavity, better developed in its ventral part. Female had 12-14 ventral denticles. Cephalic papillae 2 rows: internal (6 papillae; 2 dorsal, 2 laterals & 2 ventral) and external (4 papillae), and 68 cuticular elements as combs and spines in each row. Ma-

les with 40-41 cuticular combs in each ventro-lateral plate row, and a short row of 4 ventral, unpaired precloacal fans. Koga (1996) found that few studies were done on surface morphology of *Gnathostoma* adults and eggs of 4 species by SEM. Worms with a sub-globular head-bulb armed with 7-10 rows of cephalic hooks. Mutidigitate cuticular spines spaced unevenly on transverse cuticular striations on anterior body half. Spines lengths varied with tridentate spines longer than bidentate ones, tridantate spines was a species-specific character. Posterior body half of *G. doloresi* & *G. hispidum* densely covered with long unidentate spines gradually shorter posteriorly. Male terminals ventral sides with different papillae shape (small & caudal ones) in species. Eggs in uteri covered with cuticular pits of different sizes, shapes and depths. Magambo (1996) by TEM found that albendazole caused prominent changes in adult *Ascaris suum* as necrotic dense bodies; myelin whorls represented different stages of lysosomal formation and autolysis, disruption and microvilli erosion confined to central part of intestinal epithelial cells.

Mobarak and Ryan (1999) used LM, SEM & TEM to study putative origins of immunogenic secretory-excretory product (ESP) of *Strongylus vulgaris*, found that they sharply delineated but superficial attachment to equine caecum by mouth leaves behind an oval area devoid of epithelial cells. Attachment not extending deeply enough to muscularis mucosa layer of intestine. The progressive digestion of ingested plug of tissue (epithelial cells, blood cells and mucous) was visualized. The coelomocytes, floating cells and membranous structures located in pseudo-coelom and intimately associated with digestive, excretory and reproductive systems, with somatic muscles. The ESP of 2 ventrally-located, secretory-excretory glands connected to tubular elements, glands synthesized granules of various sizes and delineated density. De Net *et al.* (2001) described fine structure of sheath and cuticle of *Litomosoides chagasfilhoi* microfilaria by TEM, especially on deep-etched replicas of fully developed intra-uterine microfilariae and mature stretched microfilariae by female in cultivation showed trilaminated sheath. In contrast in deep-etching replicas sheath had 2 layers: an inner layer with tightly arranged globular material, and an outer one with relatively smooth external surface. Both in thin sections, in

classical freeze-fracture and deep-etched replicas, cuticle had 2 distinct regions: an external one, corresponded to trilaminated epicuticle, and an inner one, corresponded to inner cuticle. Deep-etching replicas showed epicuticle with several structures on micro-filariae annulations and inner region with two parallel rows of globular structures. Muller *et al.* (2001) reported in the thick filaments of body muscle in *Caenorhabditis elegans*, myosin A and myosin B isoforms & a sub-population of paramyosin, a homologue of myosin heavy chain rods, organized about a tubular core. By SEM, thick filaments showed a continuous decrease in mass-per-length (MPL) from central zones to polar region that agreed with other authors; both content and structural organization micro-differentiated as a position function. Cores composed of a second distinct sub-population of paramyosin associated with alpha, beta and gamma-filagenins. MPL measurements showed cores of 7 sub-filaments with 4 strands of paramyosin molecules, rather than 2 formerly proposed. The periodic locations of filagenins within different regions and presence of a central zone where myosin A was located implied that cores were micro-differentiated with respect to molecular content and structure. The differentiation resulted from a novel induced strain, assembly mechanism based on interaction of filagenins, paramyosin and myosin A. Cores might serve as differentiated templates for assembly of myosin B and paramyosin in tapering, micro-differentiated polar region of thick filaments. Decraemer *et al.* (2002) reviewed ultra-structure of body cuticle and its phylogenetic interpretation within frame-work of DNA-sequence data in particular structure of median and basal zones. Several structural elements of cuticle seemed to arise independently several times in Nematoda and were highly homoplasious (e.g. cortical or basal radial striae, spiral fibre layers and a fluid matrix with struts). Identifying homology of cuticle ultra-structures was very difficult at deep taxonomic levels. So, cuticle was unreliable regarding resolution of deep-level relationships in nematodes. But, at less inclusive taxonomic levels (e.g. families, genera, etc…) cuticle was a dependable phylogenetic marker. Movsessian *et al.* (2003) studied micro-morphological and histological of *Trichinella spiralis* and *T. pseudospiralis* larvae in muscles, liver and small intestine of rat-host before and after

biostimulator administration of phyto-haemagglutinin and phyto-anthelminthes found that infected rats developed unspecific allergic angiomyositis, hepatitis, cholangitis, and erosiohaemorrhagic enterocolitis in a host 35th day after infection. Processes of compensatory hypertrophy supported rat's homeostasis on cell and tissue levels at histo-destructional and morpho-functional deficiency. The phyto-haemagglutinin; biostimulator injected in host before infection was of immuno-stimulating nature and partially destroyed *Trichinella* larvae. Phytoanthelminthes produced a significant trichinellocide effect: RNA synthesis and glycogen was intensified in treated animal's organs, the pathomicromorphogenesis weakened, compensatory and regenerative processes were seen. The combined use of the phyto-hemagglutinin and phyto-anthelminthic failed to intensify the effect. Otranto *et al.* (2003) stated that *Thelazia* caused ocular infection in several mammals and transmitted by dipterous flies. *Thelazia callipaeda* Railliet and Henry 1910 (Spirurida: Thelaziidae) caused infection in carnivores and humans in the Far East, and in dogs, cats and foxes in Italy. But, in China and other Eastern countries, data of *T. callipaeda* morphology was scanty. Eighty-three nematodes from the eyes of naturally infected dogs in the Basilicata region (Southern Italy) were examined by LM and SEM, and the most important features were illustrated. The diagnostic morphology for *T. callipaeda* was given. Han *et al.* (2003) studied surface ultra-structure of advanced 3rd larvae of *Gnathostoma nipponicum* by SEM recovered from the grass snake *Rhabdophis tigrina* in Korea. It had a globular head bulb with a pair of lips anteriorly and 2 labial papillae and an amphid on each lip. Head bulb armed with 3 transverse rows of hooklets averaging 36, 38, & 43 in number increasing posteriorly. A total of 213-232 minute unidentate cuticular spines were present along entire body length, forming transverse striations. Two pairs of cervical papillae located between 8th and 12th transverse striations and a pair of body papillae laterally on posterior body third, and pair of caudal phasmids found near posterior extremity. Surface ultrastructure of 3rd larvae unique compared with others. de Net *et al.* (2003) isolated cuticle of adult *Litomosoides chagasfilhoi*, and purity of fraction determined by LM & TEM, deep etching, TEM, atomic force microscopy, immunocyto-chemistry, gel ele-

ctrophoresis (SDS-PAGE) and Western blot. Epicuticle showed a ragouts surface with parallel rows and several globular particles involved in absorption of nutrients and secretion of products. Analysis by SDS-PAGE of purified cuticles showed 5 major polypeptides corresponding to 151, 41, 28, 13 & 11 kDa. A polyclonal anti-body against a synthetic 18 amino-acid peptide corresponded to sequence of domain E of *Haemonchus* contortus 3A3 collagen gene recognized many protein bands on Western blot of purified cuticle, and labeled all cuticular layers by immuno-cytochemistry. Oliveira *et al.* (2003) stated that *Hassalstrongylus epsilon*, an adult nematode found in intestinal micro-villi of water-rat, *Nectomys squamipes*, Brants 1827 (Muridae), had cuticle external appearance as transversal striations and longitudinal ridges by SEM. TEM of thin sections and replicas of quick-frozen, freeze-fractured, deep-etched and rotatory shadowed samples showed cuticle of struts raised from fluid median layer, extending outward to epicuticle. Cuticle had 5 layers: epicuticle, cortical, fibril-rich, fluid median & fibrous. Cuticle layers made of an assemblage of fibers that created compartments, larger in fluid region than in fibril-rich median layer. Naem (2004) used SEM for surface ultrastructure of adult *Spirocerca lupi*. Anteriorly in both sexes, mouth hexagonal, without definite lips, and with 2 pairs of submedian cephalic papillae, 2 lateral amphids and a pair of lateral cervical papillae. At body anterior-ventral an excretory pore. Vulva located anteriorly and tail blunt, with a pair of subterminal phasmids. Male tail spiral, with 4 pairs of preanal papillae, a single large median preanal papilla on anterior lip of cloaca aperture, 2 pairs of postanal papillae, 4 pairs of tiny papillae near tail tip and 2 subterminal phasmids. Cuticle showed an abnormal tumor-like mass in 2 cases. Fagerholm *et al.* (2004) presented for the first time in *Hysterothylacium auctum*, structural features of phasmids, paired sense organs, positioned in a bilateral manner close to tail point by SEM & TSM. Each phasmid had a single ciliated dendritic process in a phasmidial canal surrounded by 2 supporting cells, a socket and a sheath cell. Socket cell had clusters of electron-dense fibrous material at apical, covering phasmidial canal whole length. Sheath cell had a well-developed endoplasmic reticulum. Phasmidial canal lined with a thin layer of cuticle

incomplete at base of ciliated dendritic process, which consisted of a high number of microtubule singlets and some peripheral microtubule doublets. Dendritic process base had many striated rootlets with a large number of fingerlike off-shoots, villi, invading surrounding sheath cell. Systematic significance and functional implication of phasmid were given. Tseng *et al.* (2004) stated that matrix metallopro-teinase-9 (MMP-9) was implicated in eosinophilic meningitis patho-genesis caused by *Angiostrongylus cantonensis*. Such meningitis in mice was associated with elevated expression of MMP-9 mRNA, elevated MMP-9 concentrations and enhanced MMP-9 activity in cerebrospinal fluid (CSF). Immuno-cytochemistry showed an anti-MMP-9 antibody reacted with macrophages, neutrophils and eosinophils from CSF. As eosinophils were effectors cells in host defense against A. *A. cantonensis*, high-resolution immuno-electron microscopy confirmed MMP-9 localization in eosinophils from CSF. The method based on immunogold, showed that eosinophilic MMP-9 was mostly localized in small granules in cytoplasm and along the cell membrane, but not in crystalloid-containing secretory granules. So, MMP-9 was synthesized and/or stored in small granules of eosinophils and was released. Frantova and Moravec (2004a) studied intestinal epithelium of third stage larvae and adults of *Cystidicoloides ephemeridarum* from mayfly' haemocoel and stomach of brown trout by EM & cytochemistry. Intestine of both composed of a single layer of about 10 undifferentiated intestinal cells in a ring. A labyrinth of deep invagination was in each cell basal region. The apical surface modified into the well developed, regularly arranged microvilli. They together with many organelles engaged in metabolism and a well defined gut lumen filled with unidentifiable material suggested that intestine might function in digestion and absorption during both stages. Adults fed upon semi-fluid content of stomach of brown trout. Fortuitous oral infection with unknown bacteria *in vitro* led to degenerative changes in intestinal tissue and might cause death of infected specimens. Up to 75% of cell volume in L3 was occupied by glycogen deposits. In adults a minor portion of glycogen together with lipid droplets was seen. Adults relied more or less on aerobic metabolism, as anaerobic metabolism (glycolysis) might prevail in L3**.**

Frantova and Moravec (2004b) used SEM & TEM for tissue-dwelling third-stage larvae of *C. ephemeridarum* from intermediate host *Ephemera danica*were regarding body wall morphology in associated transferring from inter-mediate to definitive host. Cephalic end and zones of somatic cuticle of infective larvae basically corresponded with those of adults. Somatic cuticle composed of a fuzzy epicuticle, an outer and inner cortical zone, a median zone and a basal zone consisted of 3 sub-zones. Globular bodies absent in median zone of infective larvae. Lateral hypodermal cords of infective larvae cellular, consisted of a median cell enclosed by 2 sublateral cells. Excretory canal present within lateral cord in infective and adult stages. Excretory canals wall contained Golgiderived vesicles communicated with canal lumen. Large deposits of glycogen indicated anaerobic respiration in hypodermal cords and non-contractile parts of muscle cells in third-stage larvae in intermediate host. Frantova and Moravec (2003) by LM, SEM & TEM found that adult *C. ephemeridarum* in stomach of brown trout, *Salmo t. fario* L. had body wall composed of a cuticle, a hypodermis and longitudinally oriented somatic musculature. Body cuticle composed of trilaminated epicuticle outer and inner cortical zones and a median zone with globular bodies and a basal zone with 3 sub-zones. Massive cuticle had 3 zones and an osmiophilic lined in buccal capsule. Armament lacked from buccal capsule and head end. Body surface transversely striated with cortical annuli. Only specialized attachment devices of *C. ephemeridarum* were the flexible overlapping margins of the annuli that elongated in body first-third, together with oesophagus sucking pressure, they played a role in penetration and mechanical damage of host's tissue. Hypodermis appeared syncytial. Up to 300 worms were in bolus of food consumed by host, or attached by head ends in lamina epithelial resulting in localized disruptions of mucosal epithelium without inflammation. Infection with *C. ephemeridarum* was temporary with mild pathogenesis season-dependent event. Dabrowska *et al.* (2004) studied changes in muscles cells of mice infected with *Trichinella spiralis* larvae in 220 days in nurse-cell region in contact with its wall. EM revealed the continuity of muscle cell membrane adjacent to larva surface. Alves *et al.* (2005) reported that

use of large diethylcarbamazine (DEC) and its mechanism of action was controversy. Other authors' accepted the hypothesis that DEC had no effect on nematodes, but infective larvae of *Wuchereria bancrofti* treated in-vitro with DEC showed several behavior and morphological changes. After 2 hrs with 3, 5, 10 mg/ml of DEC was the reduction of motility, with 5, 10 m/ml severely affected organelles, formation of several vacuoles in the neurocytes, muscle cells, and cytoplasm dissolution. Some larvae had extreme cellular disorganization with many large and dense mitochondria and numerous large vacuoles with residual organelles. Lamellar bodies related to an assembly of hypodermal membranes were in some larvae. So, in-vitro DEC treatment gave similar therapeutic conditions regardless dose (Hawking, 1979). Peixoto (2005) found that confocal & EM revealed that some female *W. bancrofti*, from volunteers given DEC showed few or no embryos, and in gravid uterus was a fine granular, electron-dense bodies as pearls strings of about 70 nm in length around intra-uterine microfilariae and on eggshells probably secreted by embryos or uterine. Microfilaria intra-uterine surface with scattered structures as in egg and blood microfilariae sheath showed similar electron-dense projections as in uterus. Hobrg *et al.* (2005) reported polymorphism in tail structure of 1st stage larvae of *Parelaphostrongylus odocoilei* (Protostrogylidae) 2 distinct larvae with a characteristic dorsal spine included; a morphotype with a kinked conical tail marked by 3 distinct transverse folds or joints and a symmetrical terminal tail spike and a morphotype with a digitate terminal without folds or joints and with an asymmetrical subterminal tail spike. Larval forms were postulated as 2 distinct elaphostrongylines sp. Application of a multi-locus approach using ITS-2 sequences from nuclear genome and COX-II sequences from mitochondrial genome confirmed them to be the larvae of *P. odocoilei*. By SEM larval cephalic region contained a cuticular triradiate stoma surrounded by 6 single circumoral papillae of inner circle, 10 papillae of outer circle (4 paired & 2 single), and 2 lateral amphids, and that it was first demonstration of the structural polymorphism in larval con-specifics in the Metastrongyloidea and Strongylida. Basis of polymorphism was undetermined, but such phenomenon was the widespread contributed to continued conf-

usion in discriminating among 1st stage larvae for species, genera and subfamilies in Protostrongylidae. Hasegawa *et al.* (2005) re-described chimpanzee pinworm, *Enterobius* (*E.*) *anthropopitheci* (Gedoelst, 1916) (Oxyuridae), based on LM & SEM of both sexes from chimpanzee's feces, *Pantroglodytes* in Redondo Island, Tanzania. *E. anthropopitheci* had a small body (male 1.13-1.83mm, female 3.33-4.73 mm long), a rather straight spicule with a ventral membranous formation in male, double-crested lateral alae in female, small eggs (53-58x24-28 um), and a smooth eggshell with 3 longitudinal thickenings. Pilakasiri *et al.* (2005) studied the gravid uterus with zygotes and micro-filariae in *Brugia pahangi*, a rich source of antigen as revealed by IFA. Epithelial cells of uterus showed features of synthetically active cells. Secretions gave nutrients for eggs. Also, uterine epithelial cells secreted the substances forming basal lamina of uterus rather thick and irregularly fused with basal lamina lining body wall where pseudocoelomic cavity obliterated. For most part, uterine basal lamina contained uniform granular material of moderate electron density, with elongated visceral muscle cells embedded in, and around uterus, with adjacent cells overlapping. Gravid uterus contained several stages of developing microfilariae within the lumen, cleaving zygotes at another level. Morula of zygotes composed of several closely packed cells surrounded loosely by their own egg shell membranes. The egg shell became more convoluted as development proceeded surrounding developed micro-filariae in utero was secreted by uterine epithelium. This later on became sheath of circulating microfilariae, and highly antigenic as showed by intense labeling with IFA. Georgieva *et al.* (2005) by TEM studied contact surfaces of *Passalurus ambiguus*. The integument included a cuticle, hypodermis and a muscular layer. Specificity regarding epicuticle different number of cuticular sublayers in anterior, central and posterior body parts, lacked basal cuticular membrane. Intestinal wall consisted of epithelial cells with micro-villi, both contact surfaces functioned absorption, secretion, transport, protecttion, movement, etc. Tilney *et al.* (2005) found that *Trichuris muris*, an intracellular parasite living host intestinal epithelial cells. But, how *Trichuris* bored its way via mucosal epithelium, as its apical surface was stabilized by actinic cytoskeleton and

cell junctions, and remained intact over worm after cells entry. In contrast, the non-stabilized lateral membranes of host epithelial cells ruptured and cells were killed to form an inert syncytial tunnel. The worm ventral surface was studded by pores overlaid bacillary cells; pores penetrated via cuticle to be in the direct contact with host cytoplasm. From SEM of isolated worms, each adult contained approximately 50,000 bacillary cells. The apical surface of bacillary cells extensively folded into plicae 40 nm in diameter, thereby increasing surface area many-fold. Bacillary cells lack organelles for enzyme synthesis and secretion and fail to export protons. By confocal LM, it was seen that fluorescent macromolecules in excess of 100,000 Da can penetrate into the pores. Taken together, so bacillary cells were essential for living inside host epithelium and function predominantly in absorption of soluble molecules from the host mucosal cytoplasm, in essence behaving as an external gut epithelium that is protected from abrasion by the cuticle that surrounds the bacillary openings. Cardenas *et al.* (2005) described the ultrastructure of the camallanid nematode *Procamallanus (Spirocamallanus) halitrophus*, a parasite of flounder, was described for the first time by TEM. Body wall composed of an outer cuticle, a hypodermis, and a muscular layer. Cuticle comprised the epicuticle, cortical, median, fibrous, and basal layers. The cortical layer subdivided into an outer and inner zones, median layer subdivided into an outer layer, rich in electron-dense fibrils, and an inner layer, lacking fibrils. Fibrous layer was subdivided into 3 regions delimited by the electron-dense lines; basal layer, electron-dense sustaining structures**,** underlined by hypodermis with many organelles. The muscles striated, each cell consisted of the individualized contractile & non-contractile regions. Bodies inclusion in muscle fiber, hypodermis, hypodermal chord and intestine. Cardenas and Lanfredi (2005) redescribed *P.* (*S.*) *halitrophus* Fusco and Overstreet, 1978 in the intestines of flounders (*Syacium papillosum* and *Citharichthys macrops*) in coasts of Rio de Janeiro, Brazil. LM and SEM gave morphology and taxonomic features as cephalic structures, cuticle, vulva, caudal papillae localization on males, and excretory pore. *C. macrops* was a new host, and Rio de Janeiro as a new habitat. Frantova *et al.* (2005) stated that somatic cuticle of

female *Philometra obturans* (Dracunculoidea) had 5 zones and an over-lying prominent fuzzy epicuticle, closely adherent to host's gill arterial wall. The cuticle fenestrated and infiltrated with electron-dense substances, protruded into hypodermis forming numerous protuberances. The hypodermal' plasma membrane formed prominent folding in sub-cuticular region, many endosomes bud-off from cuticle to hypodermis. Glycogen deposits found in hypodermis and muscle cells. Muscle cells well developed polymyarian (up to 30 in each quadrant) and coelomyarian in shape. Female body wall had features common in family Filarioidea. Terenina *et al.* (2006) described *Aspidodera sogandaresi* (Heterakoidea: Aspidoderidae) from *Dasypus novemcinctus* by LM & EM. It parasitized armadillos in south Panama Canal north via central Mexico to southern USA, formerly called *A. fasciata* (Schneider, 1866). *A. sogandaresi* n. sp. had blunt projections on lips and lateral expansions at spicules' distal tips, but *A. fasciata* had conspicuous digitiform projections on lips and a terminal round at spicule' tips, and in north America were *A. binansata* Railliet and Henry, 1913; *A. vazi* Proenca, 1937.

Weimer (2006) studied *Caenorhabditis elegans* by EM and immunocytochemistry using high pressure freezing and freeze substitution technique. HPF converted liquid water to a depth of about 0.6 mm, into amorphous ice nearly instantaneously. At mid-body, an adult *C. elegans* hermaphrodite approached its widest girth of about 0.1 mm. Theoritically, an entire live adult had physically immobilized instantly in amorphous ice thus, providing a unique opportunity to examine cellular architecture with exquisite spatial preservation. He gave procedures for freezing *C. elegans* under high pressure, for embedding frozen samples in resin after a freeze-substitution step, and for post-embedding immunogold labeling of proteins contained within thin sections of embedded animals enabling high-resolution analysis of morphology and molecular domains in *C. elegans* tissues.

Falcon and Lamoth (2006) by LM & EM described *Sciurodendrium bravohollisae* (Heligmonellidae) from intestine of squirrel, *Sciurus aureogaster* Cuvier, 1829; in Los Robles, Municipio de Tlalnepantla, Morelos State, Mexico. This 7th n. sp. had a well-developed and sacciform genital cone.

Glockling and Beakes (2006) studied *Myzocytiopsis vermicola*, a holocarpic parasite of Rhabditis, by TEM for infection development, asexual and sexual reproduction. Rhabditis was infected by attachment of apical cystospore buds to cuticle. Apical buds packed with vesicles with dense fibrillar contents, but absent from thallus. Some of the thalli developed into sporangia, others became paired gametangial cells. Zoospore cleavage often intrasporangial, but during early stages of an epidemic partially differentiated zoospores released via an exit tube into a fine vesicle. Packets of tripartite tubular hairs absent in cytoplasm of developing or mature sporangia. Sections in zoospores showed biflagellate zoospores, some without hairs and others with a proximal row of very short hairs on anterior flagellum. Gametangial contact via a short, walled fertilization tube and surplus antheridial and oogonial nuclei remained in respective gametangial cells until periplasm disintegration. Mature zoospores had a scalloped, electron opaque, epispore wall layer.

Bouamer, and Morand (2006) described *Maracaya africana* n.sp. (Cosmocercidae) in large intestine of lizard *Chamaeleo inturensis* in Democratic Republic of Congo, as species record of Maracaya Diaz (1963) in Africa. LM and SEM showed the morphological difference in male caudal end and cephalic end, enabled to identify a n. sp. by a new genus key.

Nadler *et al.* (2006) estimated phylogentic relationships in 25 entomopathogenic nematodes of genus *Steinernema*, parasite used as biological control agents of several lepidopteran, dipteran and coleopteran pests. They used ucleotide sequences from 3 genes and 22 morphological characters. The parsimony analysis of 28S (LSU) sequences yielded a well-resolved phylogenetic hypothesis with reliable bootstrap support for 13 clades. Parsimony analysis of mitochondrial DNA sequences (12S rDNA & Cox 1 genes) yielded phylogenetic trees with a lower consistency index than for LSU sequences and with fewer reliably supported clades. Combined phylogenetic analysis of 3-gene dataset by Parsimony and Bayesian methods gave well-resolved and the highly similar trees. The bayesian posterior probabilities were high for most clades; bootstrap (parsimony) support was reliable for about half of internal nodes. Parsimony analysis of morphological dataset yielded a poorly resolved tree, whereas

all evidence analysis (molecular & morphology) yielded a phylogenetic hypothesis consistent with, but less resolved than the trees inferred from combined molecular data. Parsimony mapping of morphology of 3-gene trees showed most structural features of the steinernematids highly homoplastic. Distribution of nematode foraging strategies on trees predicted that *S. hermahroditum, S. diaprepesi* and *S. longicaudum* had cruise forager behaviors.

Sukontason *et al.* (2006) by SEM reported varied egg-shell of *Paracapillaria (C.*) *philippinensis* from a human sample. Two distinct egg shapes; a typical peanut-shaped and swollen peanut-shaped. Both thick and thin eggshells present. Thick one either fairly smooth or with a beam-like network in relation to the pillars in surface ultra-structure. Thin eggshells transparent showed coiled larva. Presence of thin shell gave evidence of autoinfection. Morimoto *et al.* (2006) found rare non-human parasite and eggs in faeces of an 8-year-old Japanese female with Henoch-Schonlein purpura. The worms were rhabditiform females measured 325.6-441.2 x18.3-26.5 mum with a pair of labia oris notched with many spiny projections, and another strongly curved outwards. By LM & SEM they were free-living *Diploscapter coronata* (Cobb). Eggs were cultured on a filter-paper technique; 7-days later, male and female emerged. Adult survival time and eggs' hatchability was determined *in vitro* after gastric or intestinal fluid treatment. Adult survived for less than a minute, eggs hatched after treatment. So, eggs accidentally ingested or deposited by adult caused human infection.

Tejada *et al.* (2006) stated that ingestion of fish parasitized with *Anisakis* larvae produced infection and/or allergy in consumers. Technological and food processing treat-ments were applied to fish to kill larvae and avoid human infection. Four lots of the hake (*Merluccius merluccius*) steaks artificially parasitized with *Anisakis* larvae were subjected to 2 storage chilling ($5^oC \pm 1^oC$) and freezing ($-20^oC \pm 1^oC$) and 2 food processing of heat (86.3^oC) and microwave (66.9^oC) and studied by SEM, and environmental scanning electron microscopy (ESEM) (acid [pH=2] and water preparations), and emission of fluorescence. *Anisakis* larvae resisted acids, and remained alive. Larvae in heat and microwave-treated lots presented coagulated and dis-

rupted zones in cuticle with release of fluids. Cylindrical shape changed to a dehydrated one. Fluorescence was only noticable in frozen larvae. Larvae without apparent changes together with dehydrated ones seen by ESEM in frozen lot but, without cuticle disruptions. More studies might elucidated if changes reduced parasite resistance to gastric enzymes and to determine allergens released to flesh by live larvae during chilled storage of fish.

Naem (2007) by SEM studied adult of *Thelazia skrjabin*i (Spirurida, Thelaziidae) an ocular parasite of cattle. Anteriorly both sexes had orbicular buccal opening, mouth surrounded by 2 circles of cephalic papillae; inner one with 6 papillae and outer circle with 4 submedian. On head lateral sides, 2 amphids, and a pair of lateral cervical papillae. Vulva anteriorly, and female tail carried an anal pore and 2 phasmids near tip. Male tail blunt curved ventrally without caudal alae. 31 to 38 unpaired preanal papillae; 2 paired postanal, and 2 phasmids at posterior end. Cuticle had fine scarcely visible transverse striations. At anterior end of 2 cases an abnormal mass was seen.

It can be concluded that electron microscopy contributed valuable in taxonomy and biology of helminthes.

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