The Contribution of Electron Microscopic Studies to the Taxonomy and Biology of Parasitic Trematodes

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INTRODUCTION

Electron microscopy has proved to be a very helpful instrument in the study of biology in general. Since it has been invented a lot of information has accumulated in different aspects of biology. Studying the ultrastructure of the different animals has added a great deal of knowledge to biologists. Electron microscopy is also a very good diagnostic instrument used in hospitals and medical centers. The present review aims to pile up the available information concerning the biology and taxonomy of trematodes, platyhelminthes. The review deals with studies on the taxonomy of this group of helminthes, using the scanning (SEM) and transmission (TEM) electron microscopy. Also it deals with the studies on the developmental stages of these worms. Other works deal with the studies of drug efficacy on these worms as seen under electron microscopy.

Members of the platyheminthes phylum include the various dorsoventrally flattened animals that are commonly known as the flatworms. The body shap of these heminthes varies from a broad, dorsoventrally flattened leave like-worms to that of a long chain of proglottids as in tape-worms. The phylum includes three classes: the exclusively free-living organisms; rarely commensals or parasites Turbellaria, the parasitic Trematoda and the totally parasitic Cestoidea (Beaver *et al*., 1984).

All trematodes are obligatory parasites as adults. The class Trematoda is divided into three subclasses:

I-Subclass: Aspidogastrea: This is a unique group of trematodes. Members of this group are parasites on or in the viscera of molluscs (or in the intestinal canal of cold-blooded vertebrates); adult stage with or without change of host; oral sucker poorly developed, but ventral provided with a powerful sucking disk, frequently divided into a series of sucking cups. No representative in man (Beaver *et al*., 1984).

II-Subclass: Monogenea: Ectoparasitic or in the bladder or respiratory organs of the host; suckers usually present, those at posterior end consisting of powerful adhesive disks usually provided with chitinoid hooks or anchors; with single host, parasites of cold-blooded aquatic vertebrates (fishes, amphibians and reptiles). No representative in man (Beaver *et al*., 1984).

III-Subclass: Digenea: Almost all species endoparasitic; organs of attachment consisting of one or more suckers, of which one is circumoral; with one or two changes of hosts, of which that harboring the intermediate stages is a mollusc; larva hatched from egg has ciliated epithelium (Beaver *et al*., 1984). They are regarded as the first trematodes in terms of the amount of electron microscopic investigations. This is because it is the most diverse group of trematodes or even of all helminthes. Digenean trematodes include several groups of helminthes, such as: liver flukes, pulmonary flukes, intestinal flukes and blood flukes. They parasitize a wide range of vertebrate hosts.

The trematodes have been the subject of diverse investigation using electron microscopy and the improvement of high resolution apparatus has permitted the detailed study of some structures so far overlooked. This review is intended to draw attention to all the studies that contributes a great deal about these important organisms.

The ultrastructure studies on aspirogasridea: This subclass is the least trematodes, investigated by electron microscopy. The only electron microscopic study found on representatives of this group is the work of Rohde and Watson (1992), who described the ultrastructure of the tegument, ventral sucker and rugae of *Rugogaster hydrolagi*, using (TEM). The tegument possesses surface microtubules longer than those of aspidogastrids. The ventral sucker and rugae have a typical tegument with microtubules. The ventral sucker and the rugae must be considered to be homologous with

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the ventral disc of the Aspidogastridae and the ventral sucker of the Digenea. Rohde and Watson (1992) stated that, this species is of particular interest because it resembles digenean trematodes in the presence of two caecae and a ventral sucker.

The ultrastructure studies on monogenea: It ranks second after Digenea in terms of the amount of electron microscopic studies. El-Naggar and Khidr (1985) redescribed the monogenian gill parasite *Cichlidogyrus halli typicus* (Price and Kirk, 1967) Paperna, 1979 from *Tilapia* spp in Egypt, usining light microscopy (LM) and (SEM). They described the haptor sclerites with (SEM). Kearn (1986) pointed out that the monogeneans are probably the least specialized of the three major groups of parasitic Platyhelminthes. El-Naggar (1987) described a new species of monogenean trematodes namely, *Protancylodiscoides mansourensis*, isolated from the gills of a freshwater fish. The description was based on (LM) and (SEM). He produced a good electronmicrograph of the species described, showing the dorsal surface and part of the ventrolateral surface of the haptor. El-Naggar and Kearn (1989) described the haptor glands of *Cichlidogyrus hallitypicus*, using (TEM). They stated that, this monogenean has at least four, small uninucleate gland cells with long narrow ducts, which open on the dorsal and ventral haptor surfaces. They concluded that, the function of such glands may be histolytic. El-Naggar *et al.* (1990, 1992) made some (TEM) observations on the monogenean *Cichlidogyrus hallitypicus*. He also described the head lobes and haptor of the monogenean *Gyrodactylus groschafti* Ergens, 1973, using (SEM). Each of the two head lobes bears a ventrally situated, cup-shaped adhesive sac and terminates with spike sensilla, lying dorsal to the sac. The adhesive sac possesses 8-12 emerging papilla, the surface of which is thickly covered with microvilli and penerated by gland duct openings. The haptor is disc-shaped and its inner surface is thrown up into radially arranged folds. In detached specimens, the hooks of the hamuli do not emerge over the surface of the haptor while the blade is enclosed between two U-shaped sucker-like folds. El-Naggar (1993) was investigated the head lobes and haptor of the monogenean *Macrogyrodactylus clarii* Gussev, 1961, using (SEM). He noticed that each head lobe of the monogenean bears a single cup-shaped adhesive sac on its ventral surface. Two types of presumed sensory structures are closely associated with each sac, single spike sensilla on the anterior border and three dome-shaped papillae on the posterior border. Each sac contains at least 12 emergent papillae which are thickly covered with microvilli and perforated by gland duct openings. Justine *et al.* (1994) described the ultrastructure of the tegument, tegumentary receptors of the monogenean *Tetraonchoides*, using (TEM). They stated that, the syncytium of this species has one of the simplest structures found in the Monogenea. Tegumentary receptors are of the uniciliary type. The tegumentary syncytium bears microvilli which form a crown around the receptor. Watson and Rohde (1994; 1995) described two new sensory receptors in the monogenean *Gyrodactylus* sp. and described the ultrastructure of the buccal complex of the monogenean, *Polylabroides australis*. Ramasamy *et al.* (1995) described the ultrastructure of the surface structures of the monogenean *Allodiscocotyla diacantha* from the gills of marine teleost fish, using (SEM) and (TEM). Observations of the haptor region have revealed the presence of cushion-like supports at the base of each clamp, together with a pair of anchor-hooks. Other topographical features observed included a mid-ventrally positioned genital atrium and a ventrolateral vaginal pore. Tegumental serrations, composed of electron-dense bars, partially cover the hindbody. The tegumental syncytium contains numerous dense granules and lucent vesicles, the contents of which are released into the cytosol of the syncytium. The clamp sclerites are invested with tegument which, in some specimens contains inclusions resembling bacteria. Each jaw of the clamp is distinguished by the presence of approximately 175 non-ciliated putative sensory endings and a pair of cone-like sensillae occurs on the ventral surface of the lappet. Additionally, the entire body surface of the worm is covered with some 3000-4000 inoculated structures that are presumed to be sensory in nature. Williams and McKenzie (1995) described the monogenean *Polystoma integerrimum*, using (SEM). The adult stage occurs in two stages; the bladder and branchial. The tegumental surfaces of both stages are elevated into ridges forming a honeycomb-like pattern of hexagons. In some region, upright microvilli are located at the angles of the hexagons.

Agrawal *et al.* (1996) Described a new species of Monogenea namely *Pseudohexabothrium taeniura* from the gills of marine fish, using (LM) and (SEM).

The ultrastructure studies on digenea: Of all Digenea, schistosomes are the one group that received and still achieve the focal attention in EM studies.

The ultrastructure studies on schistosomes: Through the scanning electron microscopy (SEM), the descriptive analysis of *Schistosoma mansoni* Sambon, 1907 adult worms was achieved. It was established that small spines cover the inner surface of the oral and ventral suckers, while the outer surface lacks these structures (Silk *et al*., 1969; Race *et al*., 1971). The inner surface of the ventral sucker presents sensorial papillae and a marginal ring of bigger spines (Hockley, 1973). On the dorsal surface of adult male worms there are tubercles randomly distributed along the body: more numerous at the middle region than at the anterior and caudal portions and less numerous toward the lateral and posterior margins of dorsal surface (Hockley, 1973). The surface of the worms is strongly wrinkled and between the folds and the tubercles there are numerous grooves, that in sections appear as a great number of sinuous, deep, branched and interconnected canals (Hockley, 1973). Between the grooves there are spines and sensorial papillae. The ventral surface is distinguished by the formation of the gynaecophoric canal with lateral margins covered by numerous and big spines, suggesting that these structures are related to the mating of the worms (Miller *et al*., 1972). The interior of this canal is also grooved, with little spines, irregularly distributed (Hockley, 1973). The sensorial papillae of the surface of adult male worms are present all over the body and are, also, more numerous in the anterior region of the gynaecophoric canal (Morris and Threadgold, 1967; Silk *et al*., 1969; Smith *et al*., 1969). Voge *et al.*, (1978a) studied the changes in tegumental surface of *Schistosoma mansoni*, during development in mice, using (SEM). They described that the tegumental surface of immature worms bear little, if any, resemblance to the surface of patent males and females. The tegumental surfaces of immature males are indistinguishable from females. Differentiation of the adult surface begins several days after copulation. Senft *et al.* (1978) made (SEM) observations on the tegument maturation in *S. mansoni* grown in permissive and non-permissive hosts. They stated that, in permissive hosts (hamster and mouse) the surface of the worm alters rapidly during early maturity and is characterized by fusing of a highly undulate surface network into smooth folds and spine-covered tubercles. In non-permissive hosts (rat) maturation of the tegument is both delayed and incomplete and the tubercles are aspinous. Hicks and Newmann (1977) and Kuntz *et al.* (1979) claimed that, (SEM) data, combined with currently used criteria for grouping species may be useful in recognizing small morphological differences between them. They also stated that, the male *S. haematobium* tegument is essentially similar to that of *S. mansoni*, except that the dorsal tubercles appear larger and the degree of spination is more pronounced, while the female has only small non-spiny bosses throughout its surface. Voge and Mansour (1980) noticed an unusual structural feature of the Egyptian strain of *S. mansoni*, using (SEM). Basch and Basch (1982) studied the *in vitro* development of *S. mansoni* with the aid of (SEM). They concluded that, *in vitro* cultivated specimens do not differ from those grown *in vivo* in terms of tegumental foldings, spines, sensory structures or other components. However, they reported that, eggs produced by worm pairs grown *in vitro* were grossly abnormal, usually composed of loosely packed vitelline granules and lacking of shell. They also noticed that, cultured worms differ from normal worms in two major respects: they are smaller and they develop more slowly. Vongpayabal *et al.* (1982) studied the surface topography of *S. mekongi,* by (SEM). They also compared the surface topography of this species with other human schistosomes. They concluded that, the male *S. mekongi* tegument lacks tubercles and spines, except for the gynaecophoral canal. The surface of this species is formed of tubercles of highly perforated ridges which give it the spongy appearance. In addition, there are three kinds of papillae interspersed on the surface among the ridges. The first is a doughnut-shaped papilla with a central crater which is most abundant on the ventral surface of the anterior part, on the floor of the gynaecophoral canal and on the dorso-lateral aspect of the tail. Similar papillae have been reported to be present in the dorsal tegument of male *S. mansoni* but their function is not known (Miller *et al*., 1972). The second is a pleomorphic papilla with irregular shape and size, which is scattered throughout the dorso-lateral aspect of the middle part of the body. The third type of papilla has a uniform hemispherical shape, possesses a cilium projecting from the apex and probably corresponds to the sensory papilla found in other species. The tegument of the female differs from that of male by having numerous short spines over the whole surface; however, the pleomorphic papillae are much fewer in number and the ridges are much less developed than those of the male tegument; complex trabeculae are absent. The closest species to *S. mekongi* is apparently *S. japonicum.* Leitch *et al.* (1984) described the ultrastructure of the tegument of *S. haematobium,* using (TEM) and (SEM). They revealed that, the tegument of *S. haematobium* (Nigerian strain) is similar in ultrastructural appearance to

that of *S. mansoni* and Iranian and Egyptian strains of *S. haematobium.* TEM studies revealed the same finding, except for that, *S. haematobium* appears to have relatively large number and slightly more complex mitochondria than *S. mansoni*, suggesting a higher energy requirement. Sobhon *et al.* (1986) compared between Chinese, Philippine and Indonesian strains of *S. japonicum,* together with *S. mekongi*, using (SEM). They stated that the surfaces of all strains are highly spongy and consist of ridges and papillae. They also observed certain differences among males of the three strains in the number and distribution of papillae and the pattern of ridges. They described three kinds of papillae: 1-The large fungiform papillae (most without cilia) are more numerous in Philippine and Indonesian strains; They concentrate on the lateral aspect of the anterior and middle parts close to the edges of the gynaecophoral canal and on the latero-dorsal aspect of the posterior part and the tail; 2-The small ciliated hemispherical papillae are numerous in all strains, especially in and around the suckers, the gynaecophoral canal and on the tail, elsewhere they are evenly distributed; 3-The crated papillae(about half bearing cilia) are most numerous in the Indonesian strain but are very few in the Chinese strain; they concentrate on the lateral aspect of the middle part and on the edges of the gynaecophoral canal. Ridges are long, thin, wavy and least branching in the Chinese strain. They are thicker and more branching in the Philippine and Indonesian strains. Furthermore, in the latter two there are leaf-like projections and microvilli on the posterior half of the body surface; they cover a greater area of the tegument in the Indonesian strain. Spines are present only on the tail end; they are large and more numerous in the Philippine and Indonesian strains. In contrast to males, the surface of the females is relatively similar. There are spines on all areas except the most anterior end where a large number of

long cilia are present. Fungiform papillae are more numerous on females of the Philippine and Indonesian strains and they concentrate on the latero-dorsal aspects of the middle and posterior parts and around the excretory pore. Ridges in all females are not so well developed and are present only in the middle part of the body. They also stated that males of all three strains lack spines over most of their surfaces except on the posterior region close to the tail tip. It is also reported by Sobhon *et al.* (1986) that the extensive formation of ridges and microvilli on the body surface of males *S. japonicum* is a unique characteristic. They are absent in both *S. mansoni* and *S.*

haematobium (Voge *et al.*, 1978a; McLaren, 1980; Basch and Basch, 1982). Voge *et al.* (1978b) made some observations on the surface of four different geographical strains of adult *S. japonicum,* using (SEM). They indicated that it is possible that minor differences exist in the shape and length of spines and precise distribution of other surface structures such as sensory bulbs of the surfaces of the four strains studied. Senft and Gibler (1977) described five basic types of sensory organs in the adult *S. mansoni*, three of which are similar to those found in *S. japonicum* described by Sobhon *et al.* (1986). They are the ciliated hemispherical papillae, the only non-ciliated fungiform papillae, the only non-ciliated crated papillae. Banaja *et al.* (1989) described the *in vivo* developed worms of *S. mansoni*, using (SEM). They demonstrated that, there are no differences between the tegumental surfaces of immature male and female worms, before the formation of the gynaecophoral canal. The surface of immature male and female worms is completely different from that of the adult worm by having many tegumental folds. The characteristic features of the tegumental surfaces of the adult male worms developed gradually. Similar work has been done by Voge *et al.* (1978a) on Puerto Rican strain of *S. mansoni*. Mansour and Voge (1981) have done similar work on *S. haematobium.* Awad and Probert (1990) described the reproductive system and tegument of *S. margrebowiei*, using (SEM) and (TEM). They stated that, the uterus of female possesses the same ultrastructure as that of the tegument but lacks spines and sense organs. The gonopore is seen to be composed of numerous leaf-like protrusions as seen by (SEM). Awadalla *et al.* (1991) using (SEM), reported similar description of the Egyptian strain of *S. haematobium*, to that of Kuntz *et al.* (1979) and Leitch *et al.* (1984), who studied different strains of the same species. Shalaby *et al.* (1991) described the tegumental surface of the Saudi Arabian strain of *S. mansoni,* untreated and *in vivo* treated with praziquantel and oxamniquine, using (SEM). They concluded that the tegumental surfaces of worms are shown to be different from other studied strains of the same species. Such differences include: the devoidness of the female tegument of several sensory appendages; the number of sensory bulbs per papillae is high and the interpapillar setae are absent on the dorsal and dorsolateral surfaces of the male worms. They discussed their results with those on different strains of *S. mansoni* to confirm the reported differences (Senft and Gibler, 1977; Senft *et al.,* 1978; Voge *et al.,* 1978a). Staudt *et al.* (1992) described the

normal adult Puerto Rican strain of *S. mansoni* by (LM) and (SEM). The dorsal surface of male specimens forms spined bosses' tubercles; end the surface inside the gynaecophoral canal exhibits very short spines. The tegument of both sexes is characterized by a labyrinthine arrangement of depressions (so-called tegumental surface pits) forming interconnected channels lined with receptors of sensory cells. Shalaby *et al.* (1993) described the male and female *S. mansoni*, (Egyptian strain) using (SEM). They compared their specimens with other previously described other strains of the same species. They identified the differences from other strains to include: first the presence of spines in the inter-tubercular space of dorsolateral and lateral areas of the male tegument. These spines are absent from the corresponding areas in the Saudi Arabian strain of *S. mansoni* (Shalaby *et al.*, 1991) and Puerto Rican strain of *S. mansoni* (Voge *et al.*, 1978a). Second difference is: the presence of spines in the tail region of the male. Such spines are absent in the Saudi Arabian strain of *S. mansoni* (Shalaby *et al.*, 1991) and present in small numbers in Puerto Rican strain of the same species (Voge *et al.*, 1978a). Third difference is: the presence of frills on the left side of the gynaecophoral canal of males. Such frills are reported by Voge and Mansour (1980). They are absent in both Saudi Arabian strain (Shalaby *et al.*, 1991) and Puerto Rican strain (Voge *et al.*, 1978a). Zahid *et al.* (1996) described the Saudi Arabian strain of *S. mansoni*, grown in experimentally infected Baboons. They recognized the following the differences from the same strain grown in infected mice: 1-the presence of spines in the intertubercular space of dorsolateral and lateral areas near the tail region, 2-the absence of intertubercular spines in anterior and middle parts of the lateral area, 3-the presence of spines on the tail region of both male and female worms, 4-the absence of tubercles on the tail region and on the lateral area near the gynecophoral canal. Moreover, the suckers in the specimens examined in our study are more developed and tend to be of equal size. It is suggested that there are inter-strain variations in terms of the host from which the worms are recovered. Neves *et al.* (2004) described the classical schemes of the adult *Schistosoma mansoni* reproductive system. In their study, whole adult worms derived from unisexual or mixed infections and stained with carmine chlorine were virtually and tomographically analyzed under confocal laser scanning microscopy. They found that: (1) there were morphological differences in the ovary, vitteline glands and testicular lobes between specimens derived from unisexual or mixed infections; (2) there was always a single lobed ovary (three or four lobes), presenting differentiation from the anterior to the posterior lobes, where the most mature oocytes were located; (3) the proximal segment of oviduct was connected to an ampullary dilatation, full of tailed spermatozoa, characterizing a seminal receptacle; (4) there was no long vitelline duct, but a short one that begins at the end of the proximal region of the vitelline gland; (5) long cells of Mehlis' gland placed radially around the ootype were not observed. Otherwise, the ootype was only lined by thick cuboidal epithelial cells with plaited bases and nuclei with flabby chromatin, making a clear distinction from the uterine epithelium. This morphological feature suggests that each cell represents a gland. (6) In coupled males, the specimens located inside the gynaecophoric canal had smaller testicular lobes, suckers and body length and width when compared to their partners. Their results show that the reproductive system does not follow a unique pattern within flatworms. Due to its better resolution, confocal laser scanning microscopy, using a reflected mode with tomographic sections, allows new interpretations, modifying the adopted and current descriptions of the internal morphological structures of *S. mansoni* adult worms. Soliman *et al.* (2005) studied the influence of long-term administration of lipid lowering agents (atorvastatin; AV) and the action of combined treatment with injectable contraceptive (medroxyprogesterone acetate; MPA) on tegumental ultrastrucutre and survival of *Schistosoma* worms, AV (0.9 mg kgᴳ¹) was administered orally for 49 successive days to *Schistosoma heamatobium*-infected hamster starting from day 35 post-infection (pi). Another group of infected hamster was administrated MPA intramuscularly (0.1 ml kgᴳ¹) at days 7 and 35 pi followed by AV treatment regimen. Both treatment regimens significantly affected the surface ultrastructure of the male worms more pronouncedly than the female ones. Combined treatment was more severe in action compared to single one. The combined treatment was characterized by losing of spines and damaging of tubercles throughout the tegument, severe erosion and peeling and appearance of deep crakes in different parts of the tegument. Moreover, mild to sever destruction to the oral suckers of both female and male worms was noticed. On the other hand, both treatment regimens significantly reduced numbers of recovered *S. haematobium* worms and tissue egg load. Oogram pattern was affected only in case of combined treatment

with high percentage of dead eggs. These promising results may encourage further investigation with the intention of their possible application on treatment of schistosomiasis as a complement strategy to praziquantel chemotherapy.

Jiraungkoorskul *et al.* (2006) investigated the efficacy and tolerance of 80 microg/ml praziquantel (PZQ) and 40 microg/ml artesunate (ATS) against adult stage *Schistosoma mekongi in vitro* after 3, 6, 12 and 24 h incubation by monitoring worm motility and compared tegumental changes using scanning electron microscopy (SEM). Contraction and decreased motor activity were observed after as little as 3 h' incubation with PZQ and ATS. Some of the worms were immobile 12 h after exposure and died within 24 h. The tegument of *S. mekongi* showed severe swelling, vacuolization and disruption, fusion of the tegumental ridges, collapse and peeling. After 12-24 h incubation, PZQ induced similar but they less severe, tegumental changes to those observed after exposure to ATS. The direct observation of the fluke motility and SEM study suggested that ATS is more effective than PZQ in causing tegumental damage in adult *S. mekongi* and provides a basis for subsequent clinical trials. Shaohong *et al.* (2006) analyzed the therapeutic effects of artesunate against experimental *Schistosoma mansoni* infection in mice. Electron microscopy revealed that artesunate caused morphological damage, especially on the worm tegument. Artesunate was also very effective in iron-deficient mice. Furthermore, the efficacy of artesunate was equal to or better than that of artemether against *S. japonicum* infection. Considering that artemether is more toxic, artesunate is currently one of the most efficient drugs against immature *S. mansoni*.

The ultrastructure studies on digeneans other than schistosomes: Hoole and Mitchell (1981) suggested that the arrangement of papillae on the adult of *Gorgodorina vitelliloba*, as seen by (SEM) may help to provide a basis for diagnosing closely allied species. Bakke (1982) could conclude that several species of the digenean genus *Leucochloridium* can be considered conspecific, based on observations of experimentally reared adults by Light Microscope (LM) and (SEM). The main tegumental features of this genus complex as revealed by (SEM) are: spines on the ventral side except for the regions of both sucker rims and their immediate surroundings and the median area between the suckers. The spines are evenly spaced, directed backwards posteriorly and to the side anteriorly. The dorsal side is unspined. There is a distinct

spined area forming a sharp border with the dorsal surface on the lateral margins subdorsally. This area of spines is composed of multipointed spines. The unarmed oral and ventral suckers are characterized by the presence of domed aciliated papillae. The tegument of the suckers is papillated. Radially arranged corrugations on the suckers may result from muscular activity. The unarmed surface of the body is covered by scattered papillae and relatively large transverse ridges. Smales and Blankespoor (1984) studied the tegumental surfaces of *Echinostoma revolutum* and *Isthmiophora melis* by (SEM). The surfaces of such species have a cobblestone-like appearance with interspersed pits. The presence of possible secretory granules is noticed. The collar spines on the anterior end are retractable. They also claimed that, the distribution and morphology of spines and papillae may be very useful in the taxonomy of echinostomes. Liou *et al.* (1985) described the tegumental surface of metacercaria and adult of *Clonorchis sinensis* by (SEM). They noticed that, the anterior tegument of metacercaria is encircled by scale-like structures with 6-8 pointed ends. Posterior half of the tegument consists of spine-shaped structures. The tegument of adults possesses characteristic ridges and protuberances with filiform projections. Two types of sensory structures, ciliated papilla and mushroom-like papillae without cilia were observed on the surface of metacercaria. These sensory structures were found to be aggregated anteriorly surrounding the oral sucker and distributed sparsely on the other parts of the body surface. They stated that results from (SEM) may be beneficial to the detailed differentiation for the parasites. They also stated that, Fujino *et al.* (1979) described *Clonorchis sinensis*, using SEM. The results of Fujino *et al.* (1979) revealed several differences from those of Liou *et al.* (1985). The divergence between these two observations may be resulted from stage or strain differences of the parasite (Liou *et al.*, 1985). Bakke and Bailey (1987) described *Phyllodistomum umblae* from salmonids using (LM) and (SEM). They concluded that after (SEM) examination of the specimens of three different species, they can be regarded as synonyms. The two other species are *P. lachancei* and *P. limnosa.* While *P. coregoni* and *P. superbum,* are regarded as different from *P. umblae.* They also stated that the arrangement of tegumental papillae (tegumental microtopography) of those species is a valuable taxonomic characteristic, when examined by (SEM). Sharma and Hanna (1988) made a detail (TEM) description for *Orthocoelium scoliocoelium* and

Paramphistomum cervi, (Trematoda: Digenea). They stated that, the tegument of such digenean trematodes comprises an outer surface syncytium underlain by a thick subsyncytial zone and musculature. Deeper still occur the nucleated "tegumental cells". The latter are in cytoplasmic continuity with the surface syncytium via vacuolated cytoplasmic trabeculae which traverse the muscle layers and the subsyncytial zone. Three types of tegumental cells each lacking mitochondria are observed. The surface syncytium lacks mitochondria and is traversed by infoldings of the basal plasma membrane. Beneath the surface syncytium the subsyncytial zone is largely comprised of fibrous interstitial material. This zone, which is particularly thick in amphistomes, is traversed by trabeculae and extensions of underlying parenchymal cells which usually contain mitochondria and lysosomes. The subsyncytial zone overlies numerous circular and longitudinal muscle fibers. The above description of the tegumental syncytium of amphistomes exhibits different features from those of other trematodes (*Fasciola hepatica*; Bjorkman and Thorsell, 1964, *Schistosoma mansoni*; Silk *et al.*, 1969, *Megalodiscus temperatus*; Nollen and Nadakavukaren, 1974). Bogitsh (1968) suggested the absence of active transport in the tegument of *M. temperatus* due to the lack of mitochondria. The same findings are reached by Shannon and Bogitsh (1971) and Dunn *et al.* (1987). Sharma and Hanna (1988) studied the surface of *Paramphistomum cervi* and *Orthocoelium scoliocoelium* differentiated from that of liver and blood flukes in possessing: i-a thick subsyncytial zone of interstitial tissue and well developed body wall musculature and ii-a surface syncytium and tegumental cells devoid of mitochondria. They suggested that the main function of such a tegument may be protection against chemical and mechanical damage in the rumen environment. Brennan *et al.* (1991) described the tegument of the paramphistome, *Gastrodiscoides hominis,* using (SEM) and (TEM). They stated that, it is basically similar to the other digeneans. It is folded into concentrically arranged furrows and ridges bearing numerous tightly packed tubercles and extends into the oral cavity. There is an area of specialized tegument on the ventral surface, anterior to the disc region. Ciliated and non-ciliated sensory papillae are present, particularly around the oral opening. TEM studies revealed that mitochondria are absent from the tegumental syncytium and underlying tegumental cells. Numerous lymph channels are present in the sub-tegument and may be involved in osmoregulation. The same and other related species have been investigated before, using (SEM) (Morris, 1973a; Nollen and Nadakavukaren, 1974; Edurado, 1980; Tandon and Maitra, 1981, 1982, 1983). and by (TEM) (Bogitsh, 1968; Shannon and Bogitsh, 1971; Morris, 1973b). Hong *et al.* (1991) described the tegument of juvenile and adult *Heterophyopsis continua* using (SEM). On the surface posterior to the ventral sucker, the tegumental processes are band-like in metacercariae, cobblestone-like in flukes 2 days postinfection (PI) and velvety at 3 days PI. The anterior surface between the oral and ventral suckers of the metacercariae is packed densely with tegumental spines having 10 to 14 pointed tip. In flukes 6 days PI, the number of points increased to 15-17. The tegumental spines immediately behind the ventral sucker on the metcercariae surface possessed 5-7 points; posteriorly the points are reduced in size and number. Ciliated sensory papillae (type I) as single or clumped papillae appeared bilaterally symmetrical on the dorsal and ventral surfaces. On the lip of the ventral sucker, 6-7 aciliated domed papillae (type II) are arranged in an equidistant manner. At 2 days PI each type II papilla becomes a clumped form having 2 or 3 papillae. They used SEM to describe several characteristic features of the tegumental surfaces of digenean and monogenean trematodes. These features are spines, ridges, ciliated and non-ciliated papillae. The arrangement and distribution of such structures are used as taxonomic criteria of monogenean and digenean trematodes. Dunn *et al.* (1987) reported similar findings for other species of paramphistomes using (TEM) technique. The same species has been described briefly by Coggins and Mcdaniel (1972) using (SEM). Irwin *et al.* (1991) made (SEM) and (TEM) observations on the tegument of excysted metacercariae and adults of the paramphistome, *Zygocotyle lunata* (Digenea: Trematoda). In accord with other paramphistomes studied, this species lacks spines and mitochondria in the tegumentary syncytium and associated cytons. The newly excysted metacercariae possess relatively few tegumental papillae, while adults have large numbers of tegumental papillae in the region of the oral sucker and acetabulum (ventral sucker). De Fatima *et al.* (1992) described the surface topography of *Balanorchis anastrophus* using (SEM). They reported that, there are dome-shaped multiciliated papillae surrounding the oral sucker. Edurado (1982) described the same species in addition to other paramphistomids. He has not reported such papillae. He reported different morphological types of tegumental papillae on the anterior extremity. Different papillae also have been reported from

other trematodes by several authors (Nollen and Nadakavukaren, 1974; Bennett, 1975; Nadakavukaren and Nollen, 1975; Bakke, 1976; Bakke and Lien, 1978). Koie (1992) described the tegumental surfaces of the larval and adult stages of

Pygidiopsis ardaea, using (SEM). He stated that, the tegumental surfaces of excysted metacercariae do differ from those of the adult stage. He also stated that, the surface of adult stages obtained from experimentally infected chickens does not differ from that of the adult specimens obtained from the natural host *Ardea cinerea* (Koie, 1990). The general external surface of adult heterophyids is apparently always covered with scale-like, multipointed spines that decrease in point number and size towards the posterior end, as in *Cryptocotyle lingua* (Koie, 1977), *Heterophys spp.* (Taraschewski, 1984), *Haplorchis spp.* (Fujino *et al.*, 1989; Srisawangwonk *et al.*, 1989), *Metagonimus spp.* (Fujino *et al.*, 1989) and *Heterophyopsis continua* (Hong *et al.*, 1991). Different types of papillae with and without cilium-like structures have reported anteriorly and between the spines (Koie, 1977; Taraschewski, 1984; Fujino *et al.*, 1989; Srisawangwonk *et al.*, 1989; Hong *et al.*, 1991). El-Naggar *et al.* (1993a) described the tegument of the digenean *Orientocreadium batrachoides* Tubangui, 1931*,* using (SEM) and (TEM). The genral body tegument is composed as in other digeneans, of a syncytial cytoplasmic layer connected, via cytoplasmic strands, to nucleated cell bodies (Cytons) lying beneath the tegumental muscle fibres. The pads are not glandular in nature but represent elevations of the ventral surface of the body. There are regional variations in the distribution of of spines all over the body. The surface papillae are of two kinds, ciliated and aciliated. The papillae are arranged in a bilateral symmetrical pattern on both oral and ventral suckers. El-Naggar *et al.* (1993b) described the tegumental ultrastructure of the digenean *Astiotrema reniferum* (Plagiorchiidae), using (SEM). The posterior region of the worm forms a slender stalk terminating with two ventrolateral lobes. The lobes are armed with numerous large spine that are sharply pointed and orientated in all directions. There are regional variations in the distribution of spines all-over the body. The spines are abundant on the general body surface of the head region, the rim of the oral sucker, the inner lip of the ventral sucker and on the posterior ventrolateral lobes. Yu *et al.* (1994) described the surface ultrastructure of *Parvatrema timondavidi* (Digenea: Gymnophallidae) according to its developmental stages, using (SEM). The

metacercariae have a large oral sucker and characteristic lateral projections. Around the lip of the oral sucker many type I and type II sensory papillae are observed. Type III papillae are located symmetrically on the medial side of the lateral projection. Numerous type I papillae are grouped around the genital pore. The tegumental spines are distributed over the worm surface except the lip of the suckers and genital pore. The one day old worm has a well developed ventral sucker, with 6 type II sensory papillae on its outer surface and another 6 type I papillae on the inner side. Two small type I papillae are seen on the anterior side of the ventral sucker. The genital pore is small and opened separately from the ventral sucker and 15 type I papillae are grouped around it. The two, three and seven day old worms reveal that as they grow to be adults, the spine tips become multipointed, the genital pore forms a genital atrium and the cytoplasmic process becomes well differentiated. In two and three days old worms 10 type II papillae encircling the lip of the oral sucker and additional 4 papillae at the dorsal side of 4 dorsal type II papillae are a characteristic feature. The distribution pattern of the sensory papillae around the oral sucker and genital pore and 2 type I papillae on the anterior side of the ventral sucker, were used for taxonomic differentiation from other gymnophallids. Cohen *et al.* (1995) studied the digenean *Prosorhynchoides arcuatus* (Linton, 1990) (Bucephalidae), using (SEM). They concluded that this species may be a synonym to *Bucephalopsis callicotyle* Kohn, 1962. The tegument of this species presents scale like and serrated spines and unciliated sensory papillae, distributed over the body surface. Choi *et al.* (1995) described the surface ultrastructure of the metacercariae and adults of *Gymnophalloides seoi*, the only known gymnophallid infecting humans. They observed the presence of two sizes of type I papillae, small and large, encircling the lip of the oral sucker of metacercaria. Type I papillae are also arranged in a row on both sides of the body. The ventral pit has several type I papillae on its inner surface. The ventral sucker is covered with cobble-stone like cytoplasmic processes and has 6 type I papillae on its lip. The surface of the body is covered with the tegumental spines except for the ventromedian area between the two suckers. The spines at anterior body are digitated into 3-5 points and their size decreases at posterior one third of the body. The tegument of the adult worms is covered with tegumental spines, except for the ventromedian area. The shape and distribution of the tegumental spines and sensory papillae are similar

to those of metacercariae. However, sensory papillae arranged in a row on the ventral surface of metacercariae are not observed in the adults. The ventral pit becomes larger and more prominent as the fluke grows. Ferrer *et al.* (1996) made ultrastructural observations of the tegument of *Postorchigenes gymnesicus* (Digenea: Lecithodendriidae) using (SEM) and (TEM). They showed that, the general body tegument is spinous and contains mitochondria, biconcave disc-shaped vesicles bounded by an unitary membrane and displaying a protein content and scarce spherical bodies. The tegument covering specialized body regions is aspinous. Few vesicles are evident in the tegument covering the suckers and oesophagus, being more abundant in the metraterm and cirrus where the tegument is thicker. Laurer's canal has a thick tegument with sparse vesicles, mostly arranged close to the apical membrane. A direct association is evident between the basal lamina underlying the spines and the muscular subtegumental fibers, suggesting a motile character for the spine. Shalaby and Hassanine (1996a) described the tegumental surface of *Neoerilepturus aegyptensis,* (new genus, new species), recovered from the Red Sea fish *Lutjanus ehrenbergi*, using (LM) and (SEM). They have reported villi-like appendages on the ventral side of the worm, around the acetabulum. They have suggested that these villi-like appendages are probably the erudite of the numerous gland cells found between the acetabulum and the postacetabular pits. They stated that the tegument of that region is highly wrinkled and scattered pores are seen on it, which are probably some gland pores without secretion. They have found one preacetabular and two postacetabular pits on the ventral side of the worm. The tegument of the worm is devoid of any tegumental appendages on both dorsal and ventral sides. The characteristic ecsoma (retractile tail) has a tegument similar to some (body proper). The tegument of both oral and ventral sucker is similar. They lack any tegumental papillae of any sort. The tegument in the area around the acetabulum is slightly elevated from the rest of the ventral side tegument. Shalaby and Hassanine (1996b) have described the tegumental surface of the body and rhynchus of three digenean trematodes namely; *Rhipicotyle angusticolle, Telorhynchus arripidis* and *Bucephalopsis belonae* using (LM) and (SEM). The three species belong to family Bucephalidae. The study revealed that the body surface of the first species is neither smooth nor spined as figured in the previous description. The tegument of this species is found to have

separate oval cuticular scale-like appendages, which are arranged regularly with their narrow edges embedded in the body, while the opposite edges appear to be free. The body surface of the second species is not spined as mentioned the previous description. Instead, the tegument forms slightly separated transverse bands and the space between each two bands is occupied by small separate semi-conical scale-like appendages. The latter is arranged transversely and their narrow anterior edges are attached to the upper band, while their wider posterior edges appear to be free. The body surface of the third species is not spined. Instead, the body is covered entirely with rounded tegumental scale-like appendages arranged in transverse rows overlapping over each other like those of bony fishes. The description of the three studied species was revised accordingly. They were quite different from the two bucephalids, namely, *Prosorhynchoides arcuatus* and *Bucephalopsis callicotyle*, described by Cohen *et al*. (1995). Shalaby and Hassanine (1997a) described a new genus and a new species named *Progyliauchen sigani,* (Trematoda: Digenea: Gliauchenidae) from a Red Sea fish. They based their conclusion on the bases that this parasite has several Gyliauchenid characteristics, as seen by both (LM) and (SEM). However, it differs from the latter in five characteristics namely; the reduction of the postero-dorsal protuberance-like papilla; the oral sucker is ventro-terminal; the acetabulum is definitely ventral, situated at the beginning of the posterior third of the body; the cirrus is present and strongly developed and having a peculiar enlarged free end and the ovary is tetralobed. The tegumental surface as seen by (SEM) is smooth and unspined. The texture of the oral and ventral suckers is not different from the rest of the body. The internal lining of the genital pore has several folds perpendicular on the rim of the pore. None of the members of that family is described by (SEM). Shalaby and Hassanine (1997b) also identified another new genus and a new species named *Cryptacetabulum aegyptensis,* (Trematoda: Digenea: Opitstholebetidae) from a Red Sea fish, based on observations by (LM) and (SEM). The tegument of the worms is smooth and devoid of any characteristic external structures, except for the presence of some faint circum-body annulations. There is a circum-acetabular body fold extending to cover most of the acetabulum. The anterior half of the fold is enlarged and forms a irregular or a puckered-fleshy mass, while the posterior half is less developed and provided with a transverse band composed of minute muscle fibers. The acetabulum is

fairly large, lying anteriorly in the middle third of the body, partially hidden behind the circum-acetabular body fold that pushes it back into the body. None of the members of that family was described previously by (SEM). And in 1999 Leon-Regagnon and others differentiated the species of *Haematoloecus* on molecular and morphological evidence. They used electron microscopy and molecular techniques, using internal transcribed spacers' 1 and 2 and 28S ribosomal genes. They sequenced the genes for 11 isolates. Phylogenetic analysis of separate partitions and combined databases was conducted. They analyzed results in the light of morphological evidence. The electron microscopy supports the molecular evidence they claimed. Leon-Regagnon *et al.* (2001) described new species from genus *Haematoloecus* which inhabits the lung of *Rana vaillanti*. The new species shows almost 5% sequence divergence from *H. medioplexus* in the variable D1 region of the 28s rDNA, complementing the morphological differences. Leon-Regagnon *et al.* (2002) described another new species of *Haematoloecus*, which lives in the lungs of *Rana vaillanti* based on scanning electron microscopy. The new species shows 1.2% sequence divergence in partial 28S sequence with respect to *H. medioplexus*, 1.1% with *H. parviplexus* and 2.5% with *H. meridionalis*, sequence divergences complementing the morphological differences. Abdou Nel (2001) studied the tegumental ultrastructure of adult *Erilepturus hamati* by scanning electron microscopy (SEM). Adult worms were recovered from intestine of Red Sea fishes, *Pherapon jarbua* and *Lethrinus mahsena* collected at Hurghada, Red Sea Governorate Egypt. The study revealed the presence of three types of sensory papillae: type I button papillae around suckers, type II ciliated dome papillae scattered on tegumental surface of soma and type III domed papillae distributed on tegument of posterior extremity of soma. High magnification of tegumental surface of the pit in which the ecsoma is retracted showed that it has a number of small dermal outgrowths. Many pores are scattered on the tegumental surface. Hong *et al.* (2004) studied the developmental surface ultrastructure of *Macroorchis spinulosus* by scanning electron microscopy. One-day-old juvenile fluke was leaf-shaped and bent ventrally. Body surface was covered densely with peg-like spines and with cobblestone-like cytoplasmic processes. Ciliated sensory papillae were concentrated around oral sucker. Several unciliated sensory papillae occurred equidistantly on oral sucker and acetabulum. The ciliated papillae appeared in two

longitudinal lines symmetric bilaterally on dorsal surface. On adult flukes, tegumantal spines became wider in middle of the body surface. The cytoplasmic processes differentiated into more fine velvety form. It is likely that the differentiated fine cytoplasmic processes are an increased absorptive surface to adult *M. spinulosus*. It is suggested that single pointed tegumental spines on anterior half of the body may be supportive for this fluke to migration. Sripa *et al.* (2004) described the prevalence and pathogenesis of these stones in *Opisthorchis viverrini* eggs, calcium and bilirubin. The stones were also processed for scanning electron microscopic (SEM). The SEM study demonstrated the presence of the parasite eggs in the stones. Numerous crystals, morphologically consistent with calcium derivatives and cholesterol precipitates, were seen. Podvyaznaya *et al.* (2004a) described the ultrastructure of the developing excretory system of *Prosorhynchoides gracilescens* and *Prosorhynchus squamatus* cercariae. The development pattern was similar in both species. In early embryos the two main collecting tubes were composed of a layer of cells which were wrapped around the lumen. Later, the tubes fused and the excretory epithelium of the fusion zone and that of the lateral caudal ducts became a syncytium. The collecting tubes in the cercarial body retained their cellular organization. As the tails grew, additional excretory pores were formed in the tail stem where thickened portions of the caudal duct epithelium contacted the surface tegument. Following this, the distal portions of the lateral caudal ducts lost contact with the primary excretory pores and progressively degenerated. Excretory atrium development started with differentiation of secretory active cytons peripheral to the fusion zone. These cells gave rise to cytoplasmic extensions that penetrated the fusion zone wall to eventually form a continuous cytoplasmic layer. This layer eventually replaced some of the fusion zone excretory epithelium and became the lining of the excretory atrium. The anterior end of the fusion zone differentiated into an excretory bladder and a short posterior portion gave rise to the caudal vesicle. Podvyaznaya *et al.* (2004b) studied the ultrastructure of the flame cells, capillaries, collecting tubes, excretory bladder, excretory atrium, caudal vesicle, lateral caudal ducts and excretory pores of cercariae of *Bucephaloides gracilescens* (Rudolphi, 1819) Hopkins, 1954 and *Prosorhynchus squamatus* Odhner, 1905 (Digenea: Bucephalidae). Both species are essentially similar except for some details. The terminal parts of the protonephridia have all the structural features that are

typical of trematodes. The collecting tubes in the cercarial body are composed of cells that are wrapped around the lumen. The main collecting tubes are joined to the excretory bladder syncytium by septate junctions. Features of *P. squamatus* excretory bladder epithelium indicate that it is involved in secretory activity, but this is not the case in *B. gracilescens*. In both species the luminal surface of the excretory bladder epithelium is increased by lamellae and the basal plasma membrane forms invaginations. In the bladder syncytium of *P. squamatus* both apical lamellae and basal invaginations are more developed and mitochondria are also more numerous. The excretory atrium is lined by a syncytium with nucleated cytons located in the surrounding parenchyma. The atrium lining is not continuous with the body tegument and possesses specific secretory inclusions and a thick glycocalyx. Septate junctions connect the atrium syncytium to the excretory bladder epithelium at its anterior end and to the syncytial excretory epithelium lining the caudal vesicle and the lateral caudal ducts at its posterior. In the excretory pores the caudal duct syncytium is joined to the tegument by septate desmosomes. Zd'arska *et al.* (2004) detected five types of presumed ciliate sensory receptors were detected in the forebody papillae of the adult fish trematode, *Crepidostomum metoecus* (Braun, 1900). The cilia are short and submerged in a tegumental pit. The apical bulb part of all types of receptors observed is supported by a dense collar and connected to the tegument basal plasma membrane by a circular septate junction. In sensory receptors types I and III no rootlet is present; the bulbs of sensory receptors types III and IV contain an electron-dense formation. Meaney *et al.* (2004) examined using transmission electron microscopy (TEM), both the tegument and gut of *Fasciola hepatica* in an effort to identify and characterise the ultrastructural changes induced following treatment with the flukicidal drug clorsulon. Male Sprague-Dawley rats infected with *F. hepatica* were dosed orally at 8-8.5 weeks post-infection with clorsulon at a concentration of 12.5 mg kgG[1] body weight. After 24, 48 and 72 h, rats were sacrificed by cervical dislocation and mature flukes recovered from the bile ducts. After 24 h treatment *in vivo*, disruption of the tegumental syncytium was concentrated at the apex of the syncytium where a dark band consisting of numerous secretory bodies was present. Some blebbing of the apex had also occurred, "open" bodies were present in this region and the mitochondria were slightly swollen. In the cell bodies, swelling of the mitochondria and their cristae

had also occurred and the Golgi complexes appeared to be smaller than normal. The disruption seen after 48 h treatment *in vivo* was similar but more severe: the frequency of blebbing had increased, as had the number of "open" bodies and the swelling of the mitochondria. Vacuoles had begun to appear in the syncytium-both autophagic and electron-lucent-and swelling of the mucopolysaccharide masses around the basal infolds had occurred. Lipid droplets were observed occasionally. In the cell bodies, autophagic vacuoles had begun to appear and swelling of the mitochondria had increased in severity. After 72 h treatment *in vivo*, more severe disruption was seen in the tegumental syncytium in which widespread swelling and blebbing of the apex was apparent. The basal infolds had become very badly swollen in a number of specimens and damage to the spines was evident. The mitochondria remained swollen, as did the mucopolysaccharide masses around the basal infolds. Lipid droplets were more frequently observed in the syncytium. In the tegumental cells, swelling of the mitochondria was greater and an increase in the number of autophagic vacuoles was apparent. The gut showed signs of disruption after 24 h treatment *in vivo*, in that the surface lamellae were disrupted and a build-up of autophagic vacuoles at the apex of the cells had taken place. Swelling of the mitochondria and the cisternae of granular endoplasmic reticulum (gER) was evident. There was a decrease in the number of secretory bodies. After 48 h treatment *in vivo*, the number of autophagic vacuoles in the gastrodermal cells had increased, the mitochondria and gER remained swollen and the disruption seen to the lamellae was still evident. In the 72 h-treated specimens, the disruption seen in the gastrodermal cells had increased significantly, with severe vacuolation of the apical cytoplasm. An increase in the number of autophagic vacuoles was evident, the mitochondria and the gER remained swollen and lipid droplets were present in the cells. Pinheiro *et al.* (2005) studied the morphology of *Echinostoma paraensei* using transmission electron microscopy. The terebratorium region has many electrondense secretory granules and many folds on the surface. The epidermal cells that cover the larval body have unique nuclear morphology, many mitochondria and vesicles being attached to the interepidermal ridges by a septate junction. The cilia present the organization 9+2 and a typical structure with a shaft, axosome, basal body and rootlet. Below the epidermal cells there is a layer of circular muscle and adjacent to it, a layer of longitudinal muscle fibers. The

excretory system has two flame cells, with internal and external ribs and leptotriches at the barrel region, an excretory vesicle and an excretory pore. Fathy *et al.* (2005) tried Mirazid (MZ), an oleo-resin extract derived from Myrrh on heterophyids (*Pygidiopsis genata*). This experimental work aimed at investigating the possible efficacy of MZ against the mentioned species, using praziquantel as a therapeutic control. Results showed that MZ in emulsion form is a promising drug for the treatment of heterophyidiasis, as proved by significant reduction of worm count, overt surface tegumental changes like deformity and erosion of tegumental spines observed by scanning electron microscope (SEM). The proved efficacy of the drug, together with reported low toxicity, relative to praziquantel, favours its use as a natural new alternative therapy for the treatment of human heterophyidiasis. Agostini *et al.* (2005) described the first ultrastructural results on spermiogenesis and on the mature spermatozoon of *Dicrocoelium hospes* (Trematoda, Digenea) collected in *Bos indicus* from Senegal (Africa). Examination of this species was processed by TEM. Spermiogenesis follows the general pattern found in the digenean, but reveals a particularity consisting of the appearance of glycogen granules in the late spermatids within the testes. The mature spermatozoon possesses five distinct regions and presents all features found in Digenea gametes: two axonemes, mitochondria, nucleus and parallel cortical microtubules. However, several characters distinguished *D. hospes* from other digenetic trematodes within the Dicrocoeliidae family. They observed several structures that are absent in the other species of Dicrocoeliidae studied, such as: a cytoplasmic expansion, extramembranar ornamentation, spine-like bodies and two parallel mitochondria in the mature sperm. Moreover, additional particular characteristics were observed in this species in both extremities of the spermatozoon. This work produced new data on the ultrastructure of this trematode family which might be useful for phylogenetic purposes. Rivera *et al.* (2005) evaluated the damage to the tegument of 3-week-old *Fasciola hepatica* was evaluated by scanning electron microscopy (SEM) following treatment with the 5-chloro-2-methylthio-6-(1-naphtyloxy)-1H-benzimidazole (called compound alpha) in its natural host. At 6h of treatment, the flukes showed some lesions on the ventral surface of the anterior region, such as a swollen tegument and blebs. At 12 h after treatment, the specimens showed structural disorganization and spine loss in the ventral anterior region. The tegument of the flukes treated for 24 h was completely lost in some areas of the ventral surface, leaving an exposed basal lamina. The tegument of immature *F. hepatica* might be a target organ for compound alpha to exert its fasciolicide effect. Meaney *et al.* (2005a) carried out three experiments *in vitro* to determine the effect of oral and trans-tegumental uptake of clorsulon on the fine structure of the tegument and gut of *Fasciola hepatica*. Changes were assessed by transmission electron microscopy. In the first experiment, the flukes were ligatured to prevent the oral ingestion of drug and treated for 24 h in clorsulon (10 mg ml⁻¹). Limited swelling of the basal infolds was observed in the tegumental syncytium. Swollen mitochondria were present in the syncytium, the underlying tegumental cells and in the gastrodermal cells. Swelling and vesiculation of the cisternae of the granular endoplasmic reticulum (ger) was evident in the gastrodermal cells, together with a reduction in secretory activity. In the second experiment, flukes were fed for 24 h on red blood cells isolated from rats dosed with clorsulon at 12.5 mg kg⁻¹ body weight; this experiment was designed to prevent the exposure of the tegumental surface to the drug. There was severe swelling of the basal infolds in the tegumental syncytium and swelling of mitochondria in the syncytium, tegumental cells and gastrodermal cells. In the tegumental cells there was a decrease in the number of Golgi complexes as well. A number of changes were evident in the gastrodermal cells: swelling of the ger cisternae, an increase in the number of autophagic vacuoles, a reduction in the number of secretory bodies and disruption of the lamellae projecting from the surface of the cells. In the third experiment, flukes were incubated for 24 h in clorsulon (10 microg/ml), with both absorptive surfaces being available for drug uptake. There was severe swelling of the basal infolds in the tegumental syncytium and large autophagic vacuoles were present. Swollen mitochondria were a feature of the tegument, tegumental cells and gastrodermal cells, as were swollen cisternae of ger in the tegumental and gastrodermal cells. Fewer Golgi complexes were observed in the tegumental cells and in the gastrodermal cells there were fewer secretory bodies and an increased number of autophagic vacuoles. Overall, the gastrodermal cells were more severely affected than the tegument. Greater disruption of the tegument occurred when the oral route of uptake was available. The results obtained support those of previous studies which point to oral uptake of clorsulon being the major route of entry into the fluke. Meaney *et al.* (2005b) carried out

three experiments were carried out *in vitro* to determine the roles of the tegument and gut of *Fasciola hepatica* in the uptake of the flukicidal drug, clorsulon. Changes to the two surfaces were assessed by scanning electron microscopy. In the first experiment, the flukes were ligatured to prevent the oral ingestion of drug and treated for 24 h in clorsulon (10 microg./ml.). The gastrodermal surface remained normal and few changes to the tegumental surface were observed. In the second experiment, flukes were fed for 24 h on red blood cells isolated from rats dosed with clorsulon at 12.5 mg kgG[1] body weight; this experiment was designed to prevent the exposure of the tegumental surface to the drug. The gastrodermal surface was severely disrupted and the gut lamellae were disorganised and necrotic. Swelling of the tegument and blebbing on the tegumental surface were evident, but the changes were not severe. More severe swelling of the tegument was observed in the third experiment, in which flukes were incubated for 24 h in clorsulon (10 microg/ml), with both absorptive surfaces being available for drug uptake. The gastrodermal surface was badly disrupted and the gut lamellae were disorganised and necrotic. Taking the results of the three experiments together, the gastrodermal surface was more affected than the tegument and the greatest disruption to the two surfaces was seen when both routes of entry were available to the fluke. The data supported a previous study which indicated that entry of clorsulon into the fluke *in vivo* is principally by the oral ingestion of drug bound to the red blood cells. McConville *et al.* (2006) studied the effect of sulphoxide metabolite of compound alpha [5-chloro-2-methylthio-6-(1-naphthyloxy)-H-benzimidazole]; the metabolite will be referred to as alpha. So, on mature *Fasciola hepatica* of the triclabendazole-resistant Sligo isolate. Changes resulting from drug treatment were examined by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and tubulin immunocytochemistry (ICC). SEM revealed that disruption to the tegumental surface mainly took the form of swelling and blebbing. Extensive spine loss occurred on the ventral surface of the oral cone and sloughing of the tegument was observed along the lateral margins of the fluke. Examination of sections from the anterior mid-body region at the TEM level revealed that treatment with alpha. SO led to swelling of the basal infolds and mitochondria within the tegumental syncytium; also, accumulations of secretory bodies beneath the apical plasma membrane. The tegumental cell bodies contained swollen mitochondria and cisternae

of granular endoplasmic reticulum, but few Golgi complexes were observed. An increase in T2 secretory bodies was observed, whilst in the T1 tegumental cells, the T1 secretory bodies had decreased in number. Immunocytochemical (ICC) studies showed that incubation with alpha.SO, ABZ.SO and TCBZ.SO did not cause significant changes to the distribution of tubulin within the tegumental syncytium of the Sligo isolate. In contrast, alpha.SO, ABZ.SO and TCBZ.SO caused severe disruption to tubulin organization within the syncytial layer of the TCBZ-susceptible Cullompton isolate. The EM results confirmed that compound alpha is a fasciolicide capable of disrupting the tegument of mature TCBZ-resistant *F. hepatica*; however, this was not accompanied by any change in tubulin immunoreactivity. Keiser *et al.* (2006) used scanning electron microscopic observations on adult *Echinostoma caproni* recovered from mice given a single dose of 150 mg kgG[1] tribendimidine intragastrically 2, 4 and 8 hr post-treatment. Severe damage of the tegument, including extensive peeling, formation of blebs and structural loss of the definition of collar and tegumentary spines already occurred within 2 hr after drug administration. Desclaux *et al.* (2006) described a new Himasthlinae species, *Curtuteria arguinae*, as metacercariae from the cockle *Cerastoderma edule* (L.), collected at Banc d'Arguin (southwestern France). Encysted and chemically excysted metacercariae were studied by light microscopy and scanning electron microscopy, respectively. Simoes *et al.* (2006) redescribed *Pygidiopsis pindoramensis* Travassos, 1928, from the holotype and specimens obtained from experimental infections of hamsters with metacercariae of the naturally infected poeciliids *Poecilia vivipara* and *Phalloptychus januarius* from Rio de Janeiro, Brazil. *Ascocotyle (Phagicola) pindoramensis,* Travassos 1928 n. comb. is proposed, based on the presence of a solid, tapering muscular posterior prolongation of the oral sucker and *Ascocotyle (Phagicola) mollienisicola* (Sogandares-Bernal and Bridgman, 1960) is synonymized with *A. (P.) pindoramensis* because of the identical morphology and measurements of adults and metacercariae. Choi *et al.* (2006) investigated the cercaria morphology of *Echinochasmus japonicus* using light and scanning electron microscopy. Cercariae, liberated from naturally infected snails (*Parafossarulus manchouricus*), had ovoid bodies and diminutive tails. The cercaria tegument was covered with minute spines. Four type II sensory papillae were observed on the dorsal side of the oral sucker and type I papillae were distributed on the dorsal tegument surfaces.

It can be obviously concluded from the above review that the contribution of electron microscopy to the taxonomy and biology of trematodes is of great value to parasitology in general.

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