Ultrastructural observations on the tegumental surface of *Polyonchobothrium clarias* Woodland, 1925 (Cestoda: Bothriocephalidae), infecting the cat fish *Clarias gariepinus* in Egypt

S.E. Ahmed¹, A.A. Taeleb¹, S. Z. Arafa¹, S. S. Syam¹, A. B. Darwish²
¹Department of Zoology, Faculty of Science, Zagazig University, Egypt.
²Department of Zoology, Faculty of Science, Suez University, Egypt.

**ARTICLE HISTORY**
Received: August/2020
Revised: October/2020
Accepted: January/2021

**KEY WORDS**
Microtriches, Bothriocephalidae, Tegumental cell, TEM

**ABSTRACT:** The fine structure of the tegument of mature proglottids in *Polyonchobothrium clarias*, Woodland, 1925, family: Bothriocephalidae, a cestode parasite of the cat fish *Clarias gariepinus* was studied using transmission electron microscopy. The ultrastructural observations of the tegument revealed that it consists of distal cytoplasm connected with underlying perikarya by cytoplasmic bridges. The distal cytoplasm differs in thickness between the different regions of *P. clarias* and lies on the basal lamina below which there are two layers of muscles, circular and longitudinal bundles. The main characteristic inclusion in the distal cytoplasm is the presence of electron-dense bodies and vesicles of variable size, shape and density. The tegument perikarya lies beneath the surface musculature. Three different types of microtriches varying in shape and size have been observed on the tegumental surface of the mature proglottids of *P. clarias*, filiform, blade-like spiniform and digitiform microtriches. Multiple functions of such structures; the amplification of the surface area for digestion, absorption, excretion, movement, attachment to the intestinal mucosa of their hosts and external protection. The types and distribution of microtriches are considered to be of systematic and phylogenetic importance.

**1. Introduction**
Bothiocephalidean cestodes are widely distributed parasites of marine and fresh water fishes with a few species parasitising amphibians (Schmidt, 1986; Bray et al., 1994; Kuchta & Scholz, 2007).
Cestodes with paired attachment organs called bothria, were traditionally placed in the order Pseudophyllidea. However, recent molecular studies have shown that the order is paraphyletic and consists of two unrelated assemblages (orders): Bothriocephalidea and Diphyllobothriidea (Brabec et al. 2006; Kuchta 2007; Kuchta et al. 2008). The existence of these two groups raises the question as to their differentiation based on morphological and
ultrastructural characters. (levron et al.,2008). The most remarkable morphological characteristic of the tegument surface of cestodes is the presence of tegumental apical structures; the microtriches that are supposed to play different functions, such as amplification of the surface area for absorption, excretion, movement, attachment to the intestinal mucosa of their host sand external protection (Halton, 2004; Žďárská and Nebesárová, 2005; Poddubnaya et al.,2007; Levron et al.,2008; Radwanet al.,2012 and Świderski et al., 2018.

The use of transmission electron microscopy (TEM) has provided another means for the investigation of the details of tegument. These studies have revealed that all the cestode species examined possess microtriches in larval and adult stages, and that they are probably of universal occurrence (Palm et al., 2000; Žďárská and Nebesárová, 2005; Poddubnaya et al., 2007 and Levron et al.,2008). Microtriches represent one of the synapomorphies of all tapeworms, and they are absent in closely related groups of parasitic flatworms (Trematoda and Monogenea) that together with Cestoda form the Neodermata (Ehlers 1985).

Numerous studies have shown that microthriches patterns may be of taxonomic importance and may help in phylogenetic studies. The most intensively studied wereon tapeworms from various orders of elasmobranchs and onchoproteocephalideans from Neotropical catfishes (Caira et al. 2001, 2014; Gil de Pertierra 2005; Caira & Jensen 2014 and Yoneva et al., 2018).

The morphology and distribution of microtriches of the scolex is considered to be of phylogenetic importance and represent a potentially significant taxonomic character (Richmond & Caira 1991; Caira et al. 1999; Palm 2004; Agust´ et al. 2005; Gil de Pertierra 2005). The present study aimed to provide data on the ultrastructure of the tegument of the mature proglottids of Polyonchobothrium clarias displaying the characteristic features and its comparison with the previous studies carried on other cestodes.

2. Materials and Methods
Specimen collection
Adult specimens ofPolyonchobothrium clarias were collected alive from the intestine of the fish Clarias gariepinus, Burchell, 1822, that were sampled from different localities; fresh water systemin Egypt; in Al-Qaliobiah province and in some fish farms in Ash Sharqiyyah province. The worms were washed several times in 0.9 % NaCl solution and immediately prepared for transmission electron microscopy (TEM) observations according to the methods described by Anuracpreeda et al., (2013a, b) and Panyarachun et al., (2013)

TEM preparation
Preparation of the parasite specimens for TEM observation was performed as described by Anuracpreeda et al., (2006, 2015). Specimens were fixed in cold 2.5% glutaraldehyde for 1-2 hours, then washed in two changes of sodium cacodylate buffer (pH 7.4) for 15 minutes, they were then post-fixed in cold 1% osmium tetroxide (OsO4) for one hour and washed again in sodium-cacodylate buffer. All the steps of fixation were then dehydrated in ascending series of ethanol, then passed in two changes of propylene oxide for 15 minutes each, and embedded in epoxy resin (Epon 812) and finally placed in gelatin capsules with pure resin and polymerized by heating in an oven at 60-80ºC for 24-48 hours. The capsules were cut on JEOL (Jum-7) ultramicrotome by glass knives into semi thin sections (about 1.0 µ thick) for observation under light microscopy, and ultrathin sections about (50-200 nm) for electron microscopy. They were carried on copper grids and stained by uranyl acetate followed by lead citrate according to Reynolds (1963). Finally, the grids were examined using a (JOEL- JEM-1010 Transmission Electron Microscope) operating at 80 kV. This was in Al-Azhar University, (electron microscopic unit)

3. Results
The ultrastructure observation revealed that the tegument of Polyonchobothrium clarias mature proglottids composed of an external a
nucleate cytoplasmic layer (distal cytoplasm), covered with polymorphic microtriches and connected with underlying perikarya by cytoplasmic bridges (Figs. 1 and 2). The distal cytoplasm lies on the basal lamina which consists of two layers, an outer most dense homogeneous layer with closely spaced fibrils and an inner layer of a fibrillary extracellular lamina (Fig. 3). The outer layer of the basal lamina is folded and form finger-like extensions into the distal cytoplasm (Fig. 4). Below the basal lamina, there are two layers of muscle arranged in bundles of variable thickness (Fig. 5).

The distal cytoplasm of *P. clarias* contains two types of inclusion bodies that are distributed throughout the distal tegumental cytoplasm. Electron-lucent vesicles variable in shape and size are present in large numbers in the syncytial layer of the tegument (Fig. 6). The second type of vesicular inclusions is represented by electron-dense bodies that are less numerous than electron-lucent vesicles (Fig. 7). The distal cytoplasm bears three different types of microtriches; filiform, blade-like spiniform and digitiform microtriches, which differ markedly in their form, size and density (Figs. 8 and 9). All of them possess a shaft with an electron lucent core surrounded by a dense walled cylindrical tube and an electron–dense spine separated from the shaft by a baseplate (Figs. 10 and 11).

The external surface of microtriches is covered with a thick layer of surface coat (Fig. 12). The plasma membrane covering the microtriches is continuous with the membrane limiting the distal cytoplasm. The spines of microtriches seem to be directed posteriorly (Figs. 8-12).
Filiform microtriches have a slender long shaft or base and a short spine separated by a base plate and they are covered with a thick layer of filamentous coat at the surface of them (Figs. 9-11). The shaft has a core marked by a dense-walled tube. These microtriches are uniform in shape and they represent the most type in spreading on the surface of mature proglottids (Fig. 12).

Fig. (9): T.S.in the tegument of the mature proglottids of *P. clarias* showing the microtriches with different lengths and shapes; digitiform microtriches (arrowhead), filiform microtriches (arrow). Scale bar=500nm.

Figs. (10,11): - T.S.in the tegument of the mature proglottids of *P. clarias* showing the filiform microtriches (arrow) which are covered with tube (t) and they contain core (c), sheath (sh), base (b) which is long, basal plate (bp) and spine (sp), also there are digitiform microtriches (arrow head) which consists of core (c), basal plate (bp) and very short and rounded spine. Note the cortex (ct) surrounding the spine which consists of electron dense medulla (md). Scale bars: (10,11) =100nm.

Fig. (12): - T.S.in the tegument of mature proglottids of *P. clarias* showing the blade-like spiniform microtriches (arrows). Scale bar=500nm.

Digitiform microtriches, TEM observations revealed that these microtriches are observed as small microtriches with very short and rounded spine on the dorsal surface of each proglottids (Figs. 11, 12 and 14), they have long shaft and a short round spine, thus resembling filiform microtriches except for the presence of a very short and round spine. The spine consists of an electron-dense medulla and is surrounded by an electron lucent cortex (Fig. 12).

The third type observed of microtriches is blade-like spiniform microtriches which is interspersed with filiform microtriches (Figs. 8-12), it consists of an enlarged long shaft and a short electron-dense spine of a tusk shape or cylindrical shape (Figs. 12-15) and possess a short base that contains the granular matrix of the tegumental distal cytoplasm. (Figs. 13 and 14). The lining of the base is slightly thickened (Figs. 13 and 14). The electron dense cap is more slender towards the apex of microtriches. The membranous cap is separated from the base by basal plate at which the membranous cap begins to be slim towards the top (Figs. 13 and 14).

The tegumental perikarya located beneath the musculature layers (Figs. 15 and 16). The perikarya have large irregular nuclei with nuclear heterochromatin materials adjacent to the nuclear membrane (Figs. 17 and 18) and the perinuclear cytoplasm is characterized by the presence of densely distributed lipid droplets, glycogen granules, mitochondria, rough endoplasmic reticulum and associated tegumentary bodies and vesicles (Figs. 19 and 20).
**Figs. (13,14):** T.S. in the tegument of mature proglottids of *P. clarias* showing the blade-like spiniform microtriches (arrows) which consist of the membranous cap (c), base (b) and basal plate (bp) separating the distal part about the proximal part of microtriches. Note the filiform microtriches indicated by (arrow head). Scale bars: (13) = 500nm; (14) = 100nm. **Fig. (15):** T.S. in the tegument of the mature proglottids of *P. clarias* showing many of tegumental cells (tc) with irregular nuclei (n) through the intra level of tegumental cytoplasm which is situated beneath the muscle layers. Note the longitudinal muscle (m) and some lipid droplets (l). Scale bar = 2µm. **Fig. (16):** T.S. in the tegument of mature proglottids of *P. clarias* showing the tegumentary cells (tc) with irregular nuclei which have heterochromatin patches (h) deposited near from nuclear membrane. These cells possess glycogen (g) Note the muscle layers (m) and large lipid droplets (l). Scale bar = 2µm.

**Fig. (17):** T.S. in the tegument of the mature proglottids of *P. clarias* showing one of tegumentary cells having an irregular nucleus (n) and patches of heterochromatin (h) deposited near from nuclear membrane. Note the glycogen particles (g) filling the surrounding cytoplasm. Scale bar = 500nm. **Fig. (18):** T.S. in the tegument of mature proglottids of *P. clarias* showing one of tegumentary cells with an irregular nucleus (n) and heterochromatin patches (h). Note presence of the granular endoplasmic reticulum (GER) around the nucleus and presence of glycogen particles (g) filling the surrounding cytoplasm. Scale bar = 500nm. **Fig. (19):** T.S. in the tegument of mature proglottids of *P. clarias* showing two tegumentary cells with irregular large nuclei (n) surrounded by glycogen particles (g) with presence of mitochondria (M). Scale bar = 500nm. **Fig. (20):** T.S. in the internal level of the tegument showing magnification of one of tegumentary cells containing mitochondria (M) with low cristae, glycogen (g) and a peripheral nucleus (n). Scale bars = 500nm.

**4. Discussion**

The basic tegumental morphology of *P. clarias* Woodland, 1925, observed using transmission electron microscopy does not differ substantially from that described in other cestodes in which the tegument is composed of an outer syncytial cytoplasmic layer (distal cytoplasm) that contains different types of inclusions and underlying perikarya (Chervy, 2009). However, apart from the basic similarity of organization of the syncytium, some differences have been observed, especially with regard to the electron-dense bodies. Folded basement membrane contributes in active transport as osmoregulation or secretion (Pease, 1956) and (Braten, T., 1968 a). Increase of mitochondria numbers lead to increase the respiratory activity, (although existence of few cristae inside them which is, however, a contra-indication, as respiratory enzymes are known to be associated with mitochondrial cristae.) (Braten, T., 1968 a). The distal cytoplasm contains electron-dense inclusions represented by discoidal or spherical electron-dense bodies. These dense discoidal bodies tend to be oriented close to the apical plasma membrane of the distal cytoplasm. They are evenly distributed throughout the distal cytoplasm of *P. clarias* similar to *Dibothriocephalus latus, Schistocephalus solidus, Dibothriocephalus fimbriata, and Bothridium pithonis* and they appear similar to those commonly found in most cestode teguments. (Yoneva et al., 2018).

Inclusions with similar structure have several different terms throughout the literature including “rhabditiform organelles” “discoidal-like bodies” and “rod-shaped bodies” (Rothman 1963; Beguin 1966; Braten1968a, b; Morris & Finnegan 1969; Threadgold, 1984; Osaki 1990). According to Osaki (1990), these bodies are associated with the replacement of the tegumental surface membrane and, hence, of microtriches. Although microtriches described to date have a characteristic basic structure (electron-lucent base, electron-dense cap, and a base plate
separating the base from the cap), it has been found that they vary among species, lifecycle stages, and body regions (Thompson et al. 1980; MacKinnon & Burt 1983). The present study by using transmission electron microscopy investigation of the tegumental ultrastructure of *P. clarias* revealed the presence of three markedly different types of microtriches: filiform, blade-like spiniform and digitiform microtriches on the mature proglottids. The distal cytoplasm also differs in thickness between the different regions of the tapeworm body (Levron et al., 2008).

During the present study, the ultrastructure of filiform microtriches are similar with those described among species of all known cestodes and appear to be the most abundant cestodes (Holy & Oaks 1986; Kuperman1988; Jones 1998; Palm et al. 2000; Halton 2004; Agust et al. 2005). Filiform microtriches are considered to increase the absorption area and thus facilitate uptake of nutrients, in accordance with (Poddubnaya et al., 2007). These microtriches are flexible and may agitate the microenvironment adjacent to the tapeworm and enhance nutrient flux across the tegument surface (Halton, 2004; Poddubnaya et al., 2007). In *P. clarias* as in *Paraechinophallus japonicus*, the filiform microtriches are present on the strobilar surface, as observed in other bothriocephalideans, eg *Bothriocephalus acheilognathi, Echinophallus wageneri, Galaxitaieniatoloi, and Triaeenophorus nodulosus* (Timofeev & Kuperman 1972; Andersen 1979; Hamilton-Attwell et al.1980; Kuperman 1988; Diaz-Castaneda et al. 1995; Gil de Perttierra & Semenas 2005; Poddubnaya et al. 2007). Most authors have suggested a nutritional function for this microtrich type (Thompson et al. 1980; MacKinnon & Burt 1983; Kuperman 1988; Jones 1998; Palm et al. 2000; Caira & Littlewood 2001; Halton 2004). However, morphological variability exists for filiform microtriches, indicating the possibility of multiple functions (Palm et al. 2000). The spiniform microtriches are considered to have attachment function in the host intestine, as reported by Jones (1998); Halton (2004) palm (2004) and Poddubnaya et al. (2007). All distal parts (spines) of microtriches point posteriorly which aid the cestode in maintaining its position against the posterior flow of the intestinal content of the host. The posterior margin of each proglottid in *P. clarias* covered with a band of spiniform microtriches, which are apparently not identical to *Echinophallus wageneri, Neobothriocephalus, Paratelemerus, Parabothriocephalus, Parabothriocephaloides* (Kuchta 2007, Poddubnaya et al. 2007) and *Paraechinophallus japonicus* (Levron et al., 2008). Digitiform microtriches of *P. clarias* with an extremely short, rounded spine covered with the extensively developed coat and they were observed using TEM on the dorsal side of *P. clarias* similar to those of mature proglottids and as in *Echinophallus wageneri* (Poddubnaya et al., 2007). Microtriches with a short but pointed spine have also been reported in the Gyrocoytiidea (Xylander 2001; Poddubnaya et al. 2006). Microtriches found in *P. clarias* may differ from those observed using TEM in other cestodes, but Hayunga and Mackiewicz (1975) reported an apical structure (named as microtriches without electron-dense spine) with a rounded to blunt end of the shaft on the posterior third of the caryophylidean Hunterella nodulosa. Short digitiform microtriches of *P. clarias* with low density may indicate that they play an important role in the worm protection against the host immune response. It is supposed that the tegument of intestinal helminthes is involved in evasion of the host immunity. (Halton, 2004; Poddubnaya et al., 2007). Some of these structures were shown to possess a darker area in their distal ends (Hayunga and Mackiewicz 1975).

In *P. clarias*, small microtriches contain a shaft, rounded short spine, and baseplate between them as typical microtriches components. The present study describes for the first time the tegumental ultrastructure of a mature proglottids of *P. clarias* which is covered with filiform, digitiform and blade-like spiniform microtriches. Bands of shaped microtriches on the border of the proglottids appear to be characteristic of *P. clarias*. Tegumental vesicles and bodies appear to be involved with
the different structural and metabolic activities of the tegument. Both the bodies and vesicles were observed at the base of the microtriches. The electron-dense bodies confirm their role in microtriches synthesis (Poddubnaya et al., 2007).

5. References
Andersen, K.(1975): Ultrastructural studies on Diphyllobothrium ditremum and D. dendriticum (Cestoda, Pseudophyllidea), with emphasis on the scolex tegument and the tegument in the area around the genital atrium. Z. Parasitenkd., 46: 253–264.


Pease, D. C. (1956): Infolded basal plasma membranes found in epithelia noted for their
Ahmed et al./ BFSZU 1 (2021)