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

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**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 thetegument revealed that itconsists 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.

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## 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; Žd'ãrská and Nebesárová, 2005; Poddubnaya *et al.*,2007; Levron *et al.*,2008; Radwan*et 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; Žd'ãrská 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 relatedgroups 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; Agustı´ *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 of*Polyonchobothrium 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 Sharqiyah 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)

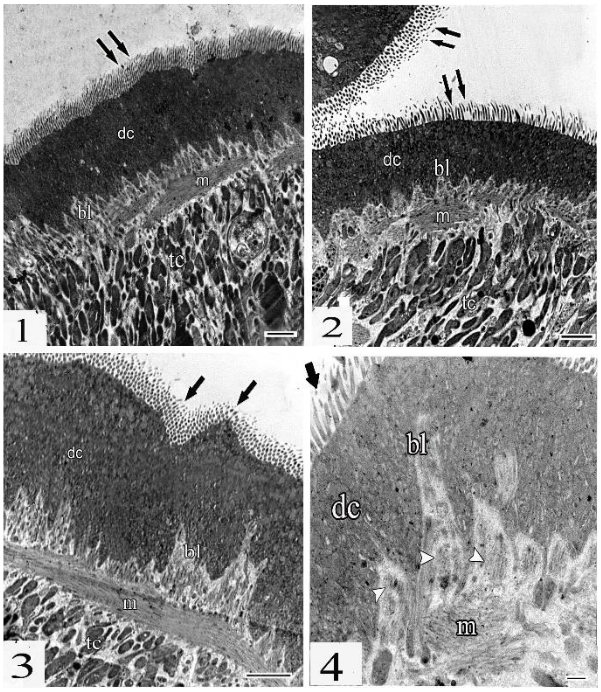
### TEM preparation

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 ($OsO_4$) for one hour and washed again in sodium-cacodyl ate 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) ultra-microtome 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).




**Figs. (1,2): -** T.S. in the tegument of the mature proglottids of *P. clarias* showing the general pattern of the tegument which has microtriches (arrow)along the surface area where they vary in length and shapes., distal cytoplasm(dc), basement layer (bl) which contains fibril layers, longitudinal muscle and circular muscles (m) and tegumental cells (tc). Scale bars**: (1,2)** = 2 µm. **Figs. (3,4):** T.S. in the tegument of the mature proglottids of *P. clarias* showing the basal lamina consisting of the outer dense homogeneous layer and the inner fibrillar extracellular layer(white arrow head). Note the microtriches indicated by (arrows). Scale bar (**3**) = 2 µm; (**4**) =500 nm.

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.8and9). 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 and11).

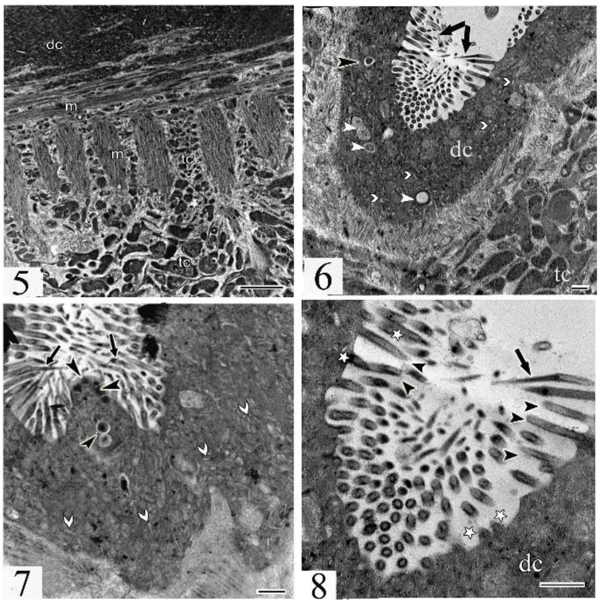



**Fig. (5)**: T.S. in the tegument of the mature proglottids of *P. clarias* showing the muscles(m) and different tegumentary cells(tc). Scale bar **=** 2µm. **Figs. (6,7)**: T.S. in the tegument of the matureproglottids of *P. clarias* showing the distal tegumental cytoplasm (dc) which contains two types of inclusion bodies that are distributed throughout cytoplasm. Electron-lucent vesicles (white arrow head), The second type of vesicular inclusions is represented by electron-dense bodies (black arrow head) that are less numerous than electron-lucent vesicles. Note the microtriches indicated by (black arrows) Scale bars: **(6,7**) = 500nm. **Fig. (8**): 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) and the blade like spiniform microtriches (star). Scale bars=500nm**.**

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).

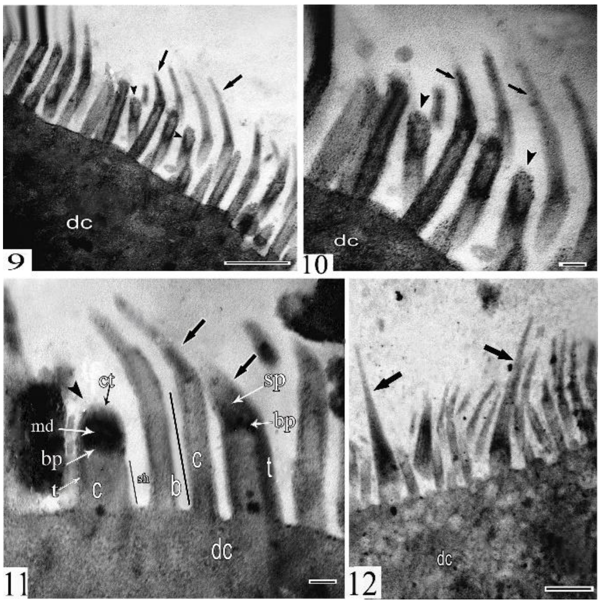
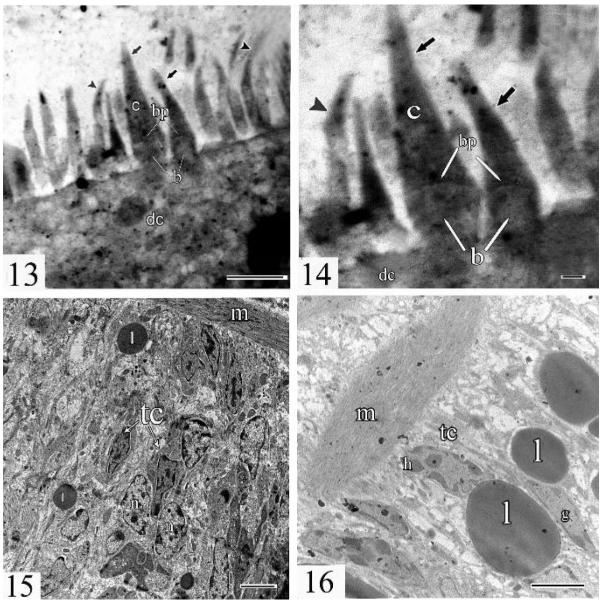



**Fig. (9)**: T.S.in the tegument of the mature proglottids of *P. clarias* showing the microtriches with different lengths and shapes; digitiform microtriches (arrowhead), filiform microtriches (arrow). Scale bar=500nm.**Figs. (10,11): -** T.S.in the tegument of the mature proglottids of *P. clarias* 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 and14). 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 and14). The lining of the base is slightly thickened (Figs.13and14). 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. 13and14).

The tegumental perikarya located beneath the musculature layers (Figs. 15and16). The perikarya have large irregular nucleiwith nuclear heterochromatin materials adjacent to the nuclear membrane(Figs. 17and18) 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 lipids droplets(l). Scale bar = 2µm. **Fig.**(**16**): T.S.in the tegument of the mature proglottids of *P. clarias* showing the tegumentary cells (tc) which have 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

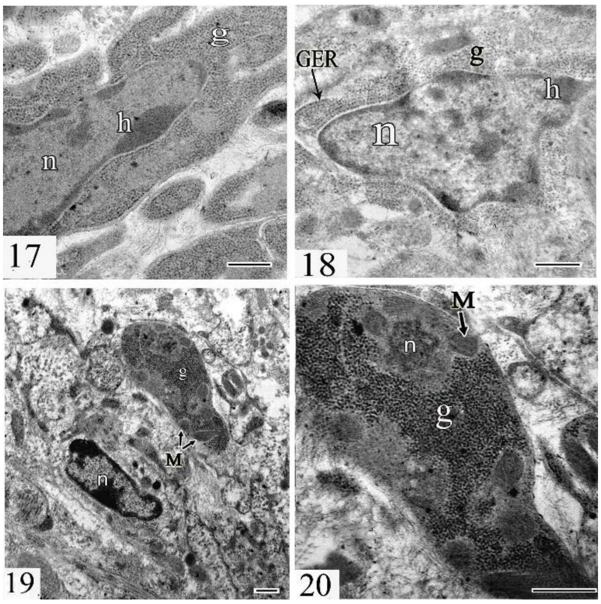



**Fig.(17):** T.S.in the tegument of the mature proglottids of *P. clarias* showing one of tegumentary cells having an irregular nucleus (n) and patches of heterochromatin (h) deposited near from nuclear membrane. Note the glycogen particles(g) filling the surrounding cytoplasm. Scale bar= 500nm. **Fig.** (**18**): T.S.in the tegument of matureproglottids of *P. clarias*showingone 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 presentstudy, the ultrastructure of filiform microtriches are similar with those describedamong 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; Agustı *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, Galaxitaeniatoloi, and Triaenophorus nodulosus* (Timofeev & Kuperman 1972; Andersen 1979; Hamilton-Attwell *et al*.1980; Kuperman 1988; Dıaz-Castan˜eda*et al.* 1995; Gil de Pertierra & Semenas 2005; Poddubnaya *et al.* 2007).Most authors have suggested a nutritional function for this microthrix 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 indentical to *Echinophalluswageneri, 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 ofmature proglottids *and as inEchinophallus wageneri* (Poddubnaya *et al.*,2007). Microtriches with a short but pointed spine have also been reported in the Gyrocotylidea (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 caryophyllidean *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 tegumentultrastructure 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).

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