

## Surface Topography and Spermiogenesis of *Lamellogadus Diplodiscus* (Monogenea, Diplectanidae), A Parasite of *Gerres Oyena* (teleostei, Gerridae) from The Suez Gulf, Egypt

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**Abstract:** Out of 150 *Gerres oyena* fish examined only, 60 were found infected with the Diplectinid parasite, *Lamellogadus diplodiscus* with an infection rate of 40%. Scanning electron microscopy revealed that the worm body possesses an anterior prohaptor and a symmetrical posterior one. The opithaptor contains squamodisc of 19–20 concentric rings; each is fringed with enormous number of small spinose structures. The squamodisc is rich in small rounded-shaped presumed sensory structures. Tegumental corrugations are present on the dorsal and ventero-lateral surfaces of both the fore and hind bodies in the form of ridges running longitudinally forming folds. Between the folds, pit-like depressions are found, besides microvillus-like tegumental projections. Numerous tapering small sized unciliated structures are found around the region of the vaginal pore. Moreover, non-ciliated dome-shaped sensory papillae occur on the clamps and haptor vicinity, while on the ventral surface there is a pair of cone-like presumed protruded sensillae covered with large dome-like structures. Concerning spermiogenesis, transmission electron microscopy showed the occurrence of a common cytoplasmic mass of the spermatids. Each spermatid contains a large nucleus with scattered chromatin and numerous mitochondria. At the beginning of spermiogenesis, the common cytoplasmic mass shows protruberances; each with two distinct regions: a basal region (the pre-zone of differentiation) and a distal cone shaped region (zone of differentiation). The distal pointed region of the zone of differentiation contains two centrioles. On either side of the inter-centriolar body, three growing elements are attached at the pointed distal extremity of the differentiation zone; two free flagella originating from the two centrioles and a central element termed the median cytoplasmic process. After the flagellar elements have rejoined, the main central process, the nucleus and mitochondria have migrated into the projection. Then, the zone of differentiation constricts and each spermatozoon is released from the general cell mass. Mature spermatozoon, transverse section, is found to be surrounded with a membrane containing nucleus, mitochondrion, a single axoneme and an incomplete ring of peripheral microtubules that disappear gradually.

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### 1. Introduction

It is generally assumed that many fish hosts (agnathans, cartilaginous and bony fish) harbor at least one unique monogenean species and this presumption can be used to estimate the total number of species present (Woo, 2006). Monogeneans tend to select many specific microhabitats (skin, fins, gills, mouth cavity and nostrils) on their fish hosts (Euzet and Combes, 1998). Diplectanids are a group of monogeneans that mainly parasitize on the gills of serranid fish and have a worldwide distribution (Wu *et al.*, 2005). Members of Diplectanidae constitute a large family of small-sized dactylogyroid monogeneans infecting teleost fishes, especially marine perciforms. *Diplectanum* is the largest genus in the family, with approximately 80 species. This genus is characterized by the possession of two squamodiscs (one dorsal and one ventral, each comprised of continuous rows of rodlets), and two pairs of haptor hooks united by three transverse bars. *Lamellogadus* sp. Is currently

composed of 52 described species (Kritsky *et al.* 2000; Amine and Euzet, 2005; Amine *et al.*, 2007; Neifar 2008, Boudaya *et al.*, 2009; Justine & Briand, 2010 and Diamanka *et al.*, 2011). Diplectanids often show strict host-specificity (Oliver, 1992); thus, it is a safe prediction that many other species of *Lamellogadus* are still undescribed (Poulin and Mouillot, 2005 and Justine, 2007).

The parasite of the present article was detected before from the gills of two Red Sea fish species *Diplodus noct* and *Rhabdosargus haffara* (Bayoumy, 2003). In general, Diplectanids and specially, *Lamellogadus diplodiscus* was previously studied morphologically through the use of optical microscopy (Ogawa and Egusa, 1978; Bayoumy, 2003; Amine and Euzet, 2005 and Aquaro *et al.*, 2009). To our knowledge, no Scanning and Transmission electron microscopic studies have been made in *Lamellogadus diplodiscus*. Thus, we describe the surface topography and spermiogenesis features of the presented

parasite to through some light on the parasite adaptation to suit its microhabitat.

## 2. Materials and Methods

150 Egyptian common named fish, Gass, were captured alive from the Red Sea at Suez Gulf in a period extended from September, 2010 to February, 2011 in a purpose searching for presence of monogenean ectoparasites. The fish were identified according to Randall (1983). They belong to Family Gerridae under the name as *Gerres oyena*. The monogenean flukes collected from fish gills were washed thoroughly in natural seawater to remove any fish gill mucus and fixed for 24h at 4°C in 4% (w/v) glutaraldehyde buffered to pH 7.2 with M-sodium cacodylate-HCl containing sucrose (3%) and NaCl (0.5%). For scanning electron microscopy (SEM), the flukes were postfixed in 1% osmium tetroxide (OsO<sub>4</sub>) for one hour, then dehydrated through graded acetone and dried in a CPD 750 critical-point-dryer using liquid CO<sub>2</sub>. The specimens were whole-mounted on an aluminum stub and fixed by double-phase sticker. The specimens were then coated with goldpalladium in a sputter coating unit (Polaron E5000; Polaron Equipment, UK) and examined using a scanning electron microscope (JEOL SEM T330; JEOL, Japan) operating at 20 Kev.

For transmission electron microscopy (TEM), the fixed parasites were washed in several changes of cold buffer and then post-fixed for 30 min. in 1% osmium tetroxide in the same buffer at room temperature. The specimens were then processed either for semi-thin sectioning and staining with 1% toluidine blue in 1% borax or for transmission electron microscopy (TEM) as proposed by El-Naggar and Kearns (1983).

## 3. Results

During the parasitological investigations of *Gerres oyena*, one monogenean fluke was found parasitizing the gills. It was identified as *Lamellodiscus diploicus* (Bayoumy, 2003). Out of 150 fish examined only 60 were found infected with this parasite with an infection rate of 40%.

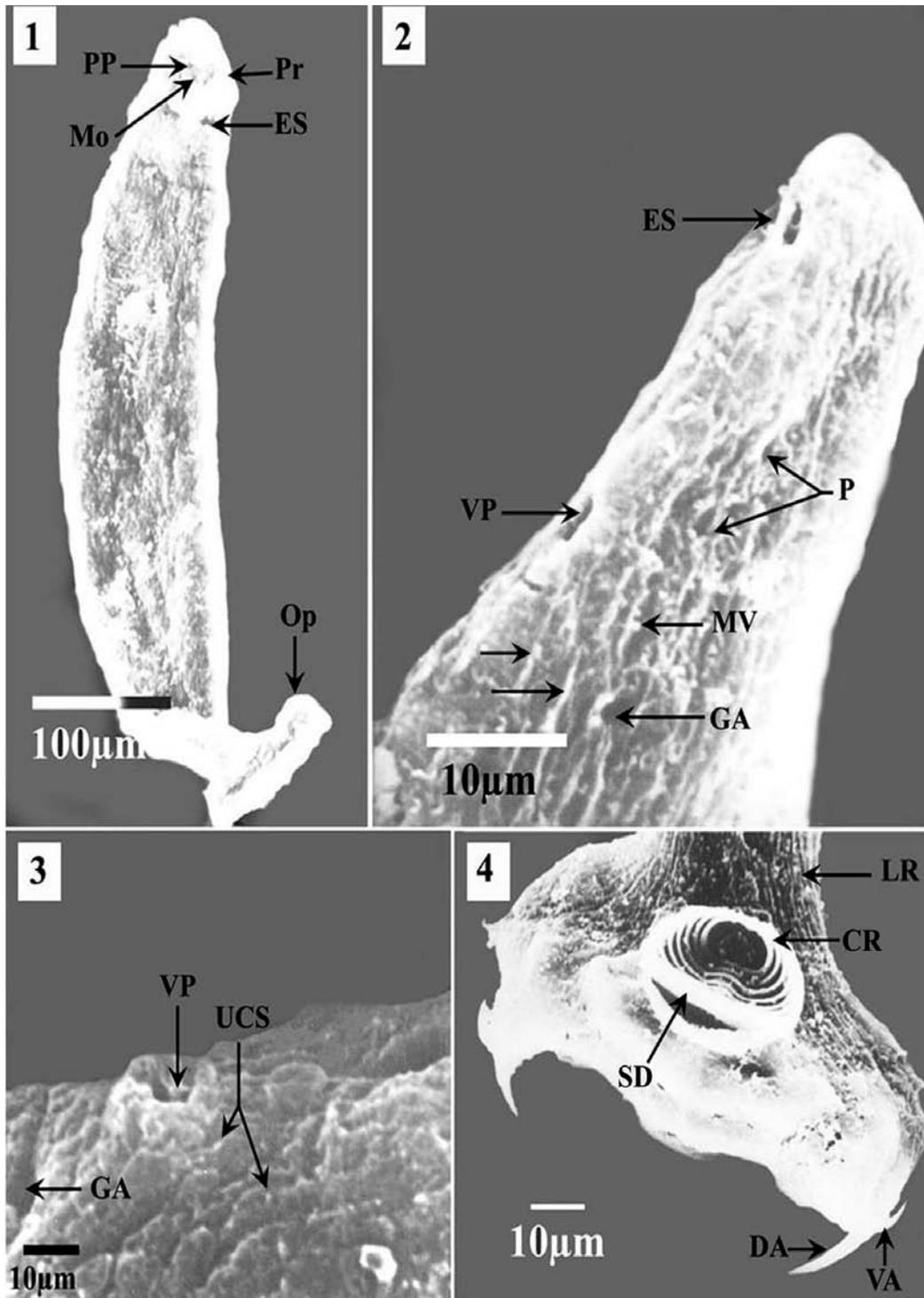
### 3.1 Scanning electron microscopy:

Scanning electron microscopy revealed that the body of the presented parasite (Figs. 1, 2 & 3) is

elongated, approximately 5 mm long and 1.15 mm broad with an anterior prohaptor (Pr) and a posterior opithaptor (Op). The mouth (Mo) opens subterminally on the ventral side of the body just behind the preoral pit (PP), midline between and anterior to the two eyespots (ES). Two genital apertures are noticed on the ventral surface a long a distance from the mouth (Mo); of these, a common genital atrium (GA) that opens midventrally, while the vaginal pore (VP) opens at the ventero-lateral margin. Numerous pores (P) were observed in the tegumental folds especially at the vicinity of the prohaptor.

The tegumental corrugations are present on the dorsal and ventero-lateral surfaces of both the fore and hind bodies (Figs. 2&3). The tegumental ridges (arrows) run longitudinally, at regular intervals forming folds in both the dorsal and the ventral surfaces along the whole body (Fig. 2). Between the folds, (Fig. 2), pit-like depressions (PD) are found, besides microvillus-like tegumental projections (MV). Also, longitudinal annulations were detected on the body surface specially at the region near to the opithaptor (Fig.4) which is folded into a complex anastomosing lamellae-like reticulum (LR) containing pit-like depressions (PD) as shown in (Fig. 5). Numerous tapering, small-sized unciliated structures (UCS) are found around the region of the vaginal pore (Fig. 3). Moreover, non-ciliated dome-shaped sensory papillae occur on the clamps and haptor vicinity, while on the ventral surface there is a pair of cone-like, presumed protruded sensillae (PS) covered with large dome-shaped papillae, DSP, (Fig. 5).

The opithaptor (Op) is symmetrical in shape (Figs. 4&6) containing squamodisc (SD) which is formed of 19 – 20 concentric rings and is located just before the terminal end of the parasite. Each concentric ring (CR) is fringed with enormous number of small spinose structures. The squamodisc, as shown in (Fig. 6), is rich in small rounded-shaped presumed sensory structures (SS). Diplectinid parasite of the present article has two pairs of anchors (Figs. 4 -7); each is formed of a dorsal internally directed large anchor (DA) and a small externally directed ventral one (VA). On the lateral sides of the opithaptor, just above the anchor, there are 14 directly upward small spine-like marginal hooklets (MH) embedded in the fleshy part of the parasite (Fig 8).



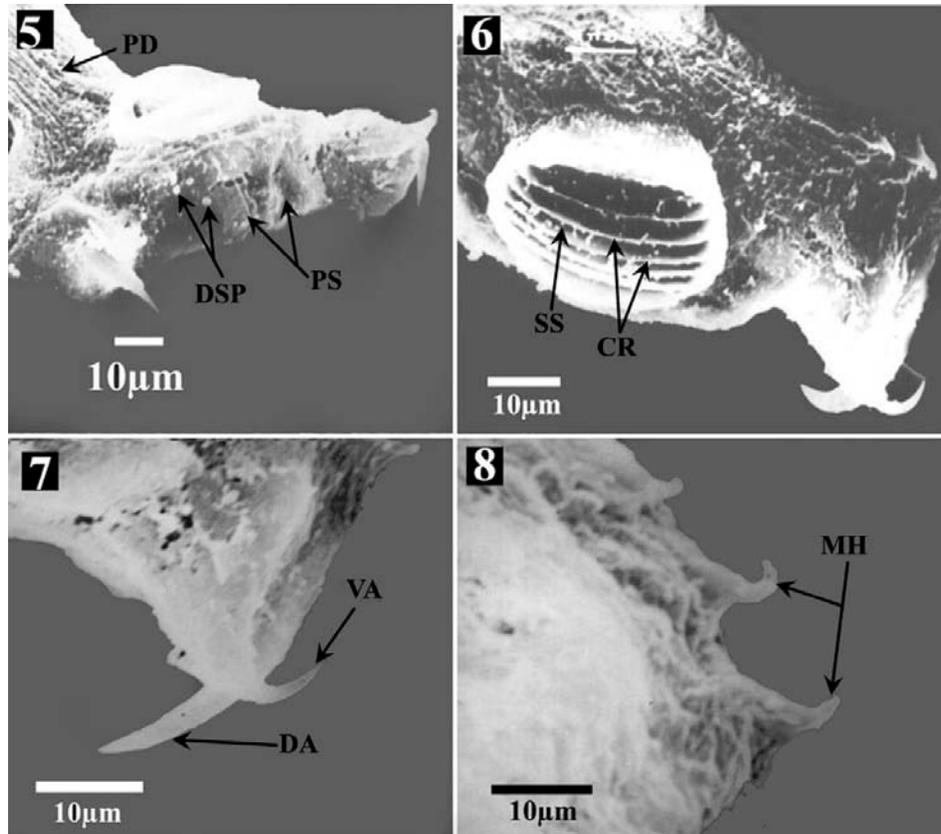
Figs. (1-9): Scanning electron photomicrograph of surface topography *Lamellogadus diploicus*

Fig. (1): The ventral surface showing the anterior prothaptor (Pr) and the posterior opithaptor (Op), the mouth (Mo), the preoral pit (PP) and the two eyespots (ES).

Fig. (2): The ventero-lateral side of the body showing the common genital atrium (GA) that opens midventrally, the vaginal pore (VP), pores (P), microvillus-like tegumental projections (MV) and the tegumental ridges (arrows).

Fig. (3): showing the vaginal pore (VP), uniciliated structures (UCS) and the common genital atrium (GA).

Fig. (4 & 7): The dorsal surface of the opithaptor showing the squamodisc (SD) formed of 19 – 20 concentric rings (CR), lamellae-like reticulum (LR), and the dorsal internally directed large anchor (DA) and the small externally directed ventral one (VA).



**Fig. (5):** The posterior extremity of the opithaptor showing the pit-like depressions (PD), pair of cone-like, presumed protruded sensillae (PS) covered with large dome-shaped papillae (DSP).

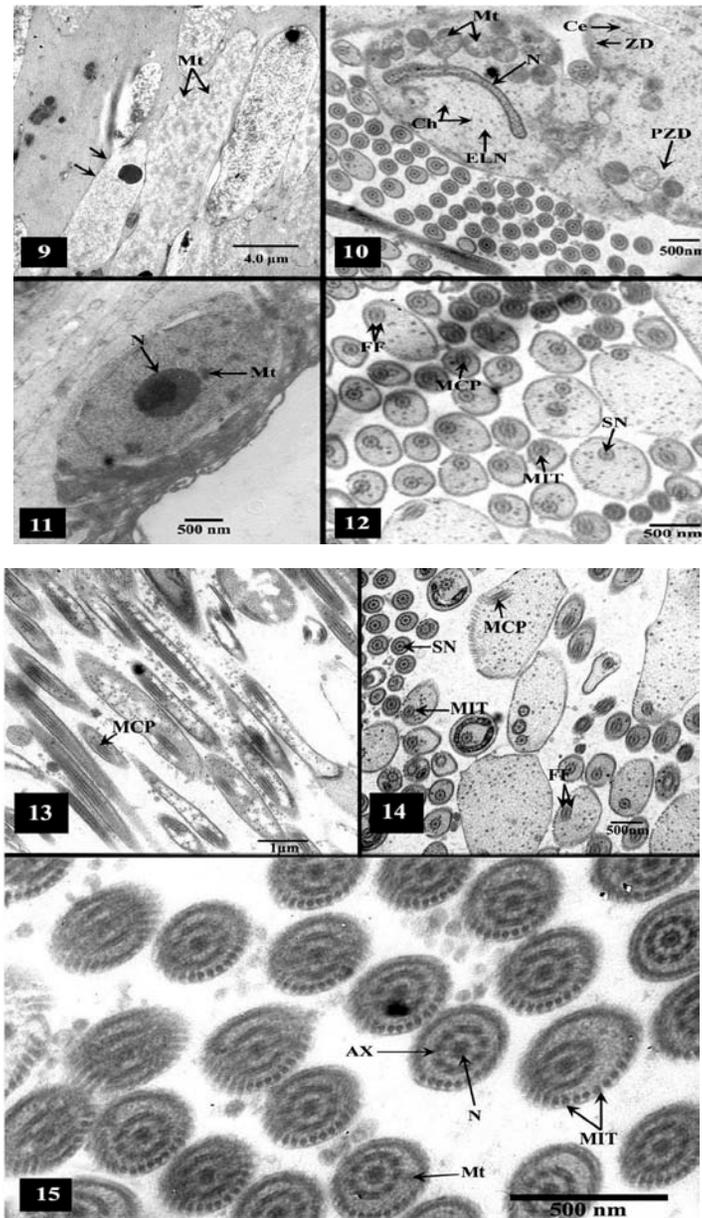
**Fig. (6):** The posterior part of the opithaptor showing the structure of the squamodisc with its concentric rings (CR) and the small rounded-shaped presumed sensory structures (SS).

**Fig. (8):** The posterior-lateral part of the opithaptor showing directly upwarped small spine-like marginal hooklets (MH) located just above the anchor

### 3. 2 Spermiogenesis:

Spermiogenesis is a developmental period through which the spermatids are differentiated into mature spermatozoa. Transmission electron microscopy revealed as shown in (Fig. 9) that at the beginning of spermiogenesis, the common cytoplasmic mass of the spermatids shows protruberances (arrows) where mitochondria (Mt) gather and fuse together. Each protruberance corresponds to a nucleus, and each will become a spermatozoon. After that (as in Fig. 10), the protruberance lengthens and its large nucleus (N) appears with relatively electron-lucent nucleoplasm (ELN). Each spermatid contains a large nucleus (N) with scattered chromatin (Ch) and numerous mitochondria (Mt) that are regularly spaced around it. Later during spermiogenesis, the nucleus shows a denser nucleoplasm with irregular clear patches (Fig 11). Each protruberance shows two distinct regions: a basal region (the pre-zone of differentiation, PZD) attached to the common cytoplasmic mass and a distal cone shaped region. This distal region is termed the

zone of differentiation (ZD) located at the periphery of each spermatid containing the nucleus and the mitochondria circling it (Fig. 10). The distal pointed region of the ZD (Fig. 11) contains two centriols (Ce), with associated striated roots, on either side of the inter-centriolar body (Fig. 12). Three growing elements are attached at the pointed distal extremity of the differentiation zone; two free flagella (FF), originating from the two centriols, and a central element termed the median cytoplasmic process, MCP (Figs. 12-14). The spermatid nuclei (SN) become elongated (Fig. 14), moving into the cytoplasmic projections, close to the plasma membrane which is supported by a single row of microtubules (MIT). After the flagellar elements have rejoined, the main central process, and the nucleus and mitochondria have migrated into the projection, the zone of differentiation constricts and each spermatozoon is released from the general cell mass (Figs. 13 & 14).



**Figs. 9-15: Transmission electron micrographs showing spermiogenesis of *Lamellodiscus diploicus***

**Fig. (9):** The beginning of spermiogenesis, the common cytoplasmic mass of the spermatids shows protrances (arrows) where mitochondria (Mt) gather and fuse together.

**Fig. (10 & 11):** The protrance lengthens and the micrograph shows the nucleus, with relatively electron-lucent nucleoplasm (ELN). Each spermatid contains a large nucleus (N) with scattered chromatin (Ch) and numerous mitochondria (Mt) that are regularly spaced around it. Each protrance shows two distinct regions: a basal region termed the pre-zone of differentiation, (PZD) and distal cone shaped region, zone of differentiation, (ZD). The distal pointed region of the ZD contains two centriols (Ce). Later during spermiogenesis, the nucleus shows a denser nucleoplasm with irregular clear patches.

**Fig. (12-14):** The distal pointed region of the zone of differentiation contains two centriols (Ce), with associated striated roots, on either side of the inter-centriolar body. Three growing elements are attached at the pointed distal extremity of the differentiation zone; two free flagella (FF), originating from the two centriols, and a central element termed the median cytoplasmic process (MCP). The spermatid nuclei (SN) become elongated moving into the cytoplasmic projections, close to the plasma membrane, which is supported by a single row of microtubules (MIT).

**Fig. (15):** Transverse section of the mature spermatozoon containing nucleus (N), mitochondrion (Mt), a single axoneme (AX) and an incomplete ring of peripheral microtubules (MIT).

Transverse section of the mature spermatozoon showed the surrounding of each one by a membrane containing nucleus, mitochondrion (Mt), a single axoneme (AX) and an incomplete ring of peripheral microtubules (Figs. 14 & 15) that disappear gradually. The axial units comprise nine sets of doublet tubules arranged in a circle around a central unit. These nine microtubules are connected to the central cored element by nine radial structures (Fig. 15).

#### 4. Discussion

Monogeneans are usually considered to be host and site specific parasites (Sasal *et al.*, 1999). The fore body of monogeneans plays a crucial role in attachment, movement, feeding and reproduction (Whittington *et al.*, 2000 and Woo, 2006). Although the tegument of many monogenean species is relatively smooth, it can be equipped with microvilli, especially in the adhesive regions of the parasite, such as cephalic openings in *Gyrodactylus* (Buchmann *et al.*, 2004). The tegumental corrugations including transverse annulations are observed on the body surface of the presented parasite, which is folded into a complex of lamellae-like projections. These with functional significance as they help in the uptake of nutrients and may serve in osmoregulation and excretory functions. The same structures were previously observed in *Acanthogyrus tilapiae*, *Paranella diplodae* and in *Gyrodactylus salaris* (Bayoumy *et al.*, 2006 & 2007) and Woo (2006), respectively. In our specimens, microvillous-like tegumental projections were located in anterior and middle regions of body and are characteristic of the tegument of the majority of the species studied, including *Diclidophora merlangi* (Halton, 1979); *Microcotyle labracis* (Oliver, 1981); *Heterapta chorinemi* (Ramasamy and Hanna, 1986). However, they are absent in others, such as *Pseudothoracocotyla indica* and *Bicotyle vellavoli* (Ramasamy and Hanna, 1986 & 1989). As suggested by Cohen *et al.* (2004), the folds may be originated due to contraction of longitudinal and circular muscle fibers of the body and their significance is to increase the efficiency of the tegumental surface in protecting the worm against the effect of the surrounding media (Khidr, 1997 and Santos and Lanfredi, 2000).

The ridges and microvillous-like projections present in the tegument increase the surface area suggesting metabolic exchange (Oliver, 1981; Brennan & Ramasamy, 1996 and Cohen *et al.*, 2004) and absorption of micromolecular nutrients from the surrounding environment (Ramasamy and Hanna, 1986). Cohen *et al.* (2004) postulated that the occurrence of microvilli and the absorptive function of the tegument provide evidence of a close phylogenetic

relationship between Monogenea and Cestoda. The pit-like depressions and the numerous pores shown in the tegument of the present fluke may increase the surface area of the region where pits are found. Pits are so far detected by Williams and McKenzie (1995) and Cohen *et al.* (2001) in case of *Polystoma integerrimum* and *Paranella luquei*, respectively and their presence could play an important role in the worm exocrine discharge (Williams and McKenzie, 1995).

Numerous tapering, small-sized unciliated structures are observed around the region of the vaginal pore of the parasite under discussion. These act as sensory structures (Ramasamy and Brennan, 2000). Nevertheless, different species of Monogenea may show ultrastructural variations in their tegument, like the presence of microvilli and secretory pores (Cohen *et al.*, 2001). Consequently, it is important to investigate the tegument of each individual species in order to elucidate questions on maintenance and survival of the parasite on their microhabitat.

Monogeneans may have papillae equipped with and penetrated by nerves, serving in the attachment to the host (Woo, 2006). It is evident that they respond actively to both mechanical and chemical stimuli. Unicellulated papillae and non-ciliated dome-shaped sensory papillae occur on the clamps and haptor vicinity are embedded in the tegument and can be seen projecting from the general worm surface and may register touch or flow in the water. Moreover, on the worm ventral surface, a pair of cone-like presumed protruded sensillae are localized and covered with large dome-like structures. This finding is similar to that previously reported by Ramasamy *et al.* (1995) on describing the surface topography of *Allodiscocotyla diacanthi*. These sensillae may serve in maintenance of the parasite's adhesive attitude between gills.

Ramasamy and Brennan (2000) reported ciliated and non-ciliated sensory receptors on the body surface of *Empleurosoma pyriforme*. The ciliated receptors may occur singly or in groups, located in the anterior region. The authors assumed that the length and flexibility of the cilia in each of the unciliated forms might be rheo-receptors/tangoreceptors involved in the orientation of the fluke's body in relation to the flow of the ventilation water currents. The dome-shaped, non-ciliated papillae may have a chemo-mechanoreceptor involvement in site selection for feeding and, possibly, in mating during cross-fertilization. The dome-shaped, non-ciliated papillae observed herein are similar to those previously recorded on the tegument of other species, such as *Bicotyle vellavoli* (Ramasamy and Hanna, 1986), *Allodiscocotyla diacanthi* (Ramasamy *et al.*, 1995) and in *Paranaella luquei* (Cohen *et al.*, 2001). Unciliated,

non-ciliated and various other types of presumed sensory receptors have been described on the body surface and haptor of monogeneans (Rohde and Watson 1995). In *Allodiscocotyla diacanthi*, four distinctly different types of presumed receptors have been mapped and are used for taxonomic significance (Ramasamy *et al.*, 1995). According to Rohde *et al.* (1986), little experimental evidence is available to suggest a function for the various types of receptor found in platyhelminths.

The fore part of the worm has attaching capacity (adhesive pads, cephalic openings) and is referred to as the prohaptor (Ramasamy and Brennan, 2000). In addition, the most important adhesive apparatus in adult monogenean parasite is the opisthaptor (Woo, 2006), it is often equipped with characteristic sclerotized structures (dorsal and ventral anchors) used for attachment to the host. They are able to penetrate host tissue in a firm contact due to their opposite position and concave-like direction. Squamodiscs of the present parasite are found in association with the opisthaptor. Marginal hooks found in the present parasite are located in the lateral margins of the opisthaptor. From our opinion, these may act as a secondary attachment organ, where during the parasite movement; the anchors become loosely attached to the host tissues.

Justine *et al.* (1985) distinguished four patterns of spermatozoa in the Monogenea. Their pattern I, characterized by two axonemes and a row of cortical microtubules, is presumed to be the ancestral one from which the remaining three patterns are derived: pattern 2 by the reduction of the cortical microtubules, pattern 3 through the loss of one of the two axonemes and most of the cortical microtubules and pattern 4 by means of the reduction of one axoneme and all cortical microtubules.

At the beginning of spermiogenesis, the common cytoplasmic mass of the spermatids shows protruberances where mitochondria gather and fuse together, which is considered as an exceptional characteristic of the polyopithocotylean gill parasite *Atristaster* sp. reported by Justine (1992), where he showed large abundance of mitochondria and presence of pre-zone of differentiation that is intercalated between the common cytoplasmic mass.

In Monogenea, as declared by Levron *et al.* (2004), the process of rejoining of flagella with the median cytoplasmic process is proximo-distal in position. This flagellar fusion of the parasite under discussion is followed by spermatid elongation, which is in agreement with the studies that have been previously reported in Aspidogastrea, Monogenea, Amphiliidea and Eucestoda (Justine, 1991; Foata *et al.*, 2007 and Bruňanská *et al.*, 2011).

Justine *et al.* (1985) found that sperm pattern 2 of a capsalid species is brought about by the loss of the cortical microtubules at the beginning of the Spermiogenesis and concluded that sperm pattern 2 is derived from pattern 1. This in turn indicates that the capsalids have an early type of sperm pattern 2, and likewise that the capsalids represent an early intermediate step in monogenean evolution. In our material, certain transverse sections of *Lamellodiscus diploicus* showed microtubules inside the sperm membrane. These sections represent developing spermatids and similar to the development of *Myxinidocotyle californica* (Malmberg and Afzelius, 1990), possibly indicate an early step in the evolution of sperm pattern 2. Similar findings were reported by Justine and Mattei (1985) in case of monopithocotylean parasite, *Loimosina wilsoni*; by Bayoumy *et al.* (2005) in case of *Pseudohaliotrema plectocirra* and by Levron *et al.* (2009) in case of the diphyllbothriidean worm, *Ligula intestinalis*. The cortical microtubules of the present parasite may be considered as reinforcement elements, which developed at the beginning of the transformation process as previously discussed by Baptista-Farias *et al.* (1995) on describing the ultrastructural features of spermiogenesis in *Metamicrocotyla macracantha*.

In conclusion, the fluke under discussion is highly adapted in its parasitizing site, where it is armored with four different attachment structures; prohaptor gland (for recognizing the host), squamodiscs, anchors and the marginal hooks. All the former structures help the parasite in facing the strong water current inside the fish gills. In addition, the study of spermiogenesis may help in the phylogenetic studies of Monogenea and its relation to other platyhelminth worms through two distinctive characteristics; the number of axonemes and the presence or absence of cortical microtubules.

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