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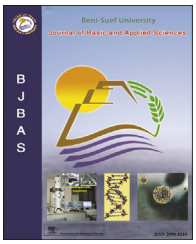

Full Length Article

Description of *Acleotrema maculatus* sp. nov. (Monogenea: Diplectanidae) infecting the spotted coral grouper *Plectropomus maculatus* (F:Serranidea) from the Red Sea and its histopathological impact

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ABSTRACT

Acleotrema maculatus sp. nov. (Monogenea: Diplectanidae) was described from the gills of The spotted coral grouper *Plectropomus maculatus* (F:Serranidea, Forsskal, 1775). Fish were collected from boot landing sites and fishermen at different water locations along the Red Sea at Hurghada City, Egypt. The morphology and morphometric characterization of the recovered worms were described by means of light microscopy. Eight (53.3%) out 15 specimens of *P. maculatus* were infected. Most of the infected fish had very pale gills. Morphologically, the adult worm of *A. maculatus* sp. nov., possessed a body which was elongated, fusiform with a total length 0.86–0.90 (0.88 ± 0.02) mm, and a maximum width 0.09–0.13 (0.11 ± 0.02) mm at the level of ovary. Haptor, broad, differentiated from the rest of the body, measured 0.04–0.08 (0.06 ± 0.02) mm and provided with continuous rows of squamodiscs. Two pairs of lateral hamuli, three bars and 14 marginal hooklets were also observed. Lateral (dorsal) bars two, stout, dumbbell-shaped, measured 0.052–0.056 (0.054 ± 0.002) mm in length. Ventral bar slender, with transverse groove, measured 0.09 –0.11 (0.10 ± 0.01) mm in length. Ventral hamuli measured 0.02–0.06 (0.04 ± 0.02) mm in total length with an outer root that was very long measured 0.016–0.02 (0.018 ± 0.002), stout, slightly notched at broad proximal end; inner root was conical and measured 0.013 –0.017 (0.015 ± 0.002) mm, with shaft measured 0.025–0.029 (0.027 ± 0.002) mm and point length 0.007–0.009 (0.008 ± 0.002) mm. Dorsal hamuli measured 0.034–0.038 (0.036 ± 0.002) in total length; base large, stout, with only lateral rudiment of roots; blade and point were long and curved with shaft length measured 0.024–0.028 (0.026 ± 0.002) mm and 0.02–0.06 (0.04 ± 0.02) mm point length. The worm is hermaphrodite, male copulatory organ measured 0.058–0.062 (0.060 ± 0.002) mm in length with a sclerotized part composed of a well-defined, sclerotized anterior sheath. The new species was compared with those

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described previously from the same genus, it was shown that there were significant morphological and morphometric, which was a strong criteria for the placement these monogenean parasites as new species with new host and locality records in Egypt.

1. Introduction

Fish are important members of aquatic ecosystems and an important source of human food. Increased interest in fish culture has also increased awareness of and experience with parasites that affect fish health, growth and survival. The gills of fish represent one of the biotope mostly exploited by different fish ectoparasites (Rhode, 1982). Among these ectoparasites, monogenetic trematodes that cause severe destructions of the gills as well as severe losses too (Morsy et al., 2012). Gills of infested fish were congested or pale haemorrhagic with hypersecretion of mucus. These signs may be due to severe irritation caused by movement, feeding activity, fixation and attachment of monogenean worms. Also, the presence of thick mucus secretion leads to respiratory failure and osmotic stress ending in the fish death. Like all ectoparasites, monogeneans have well developed attachment structures, the anterior attachment organ and the posterior haptor which is associated with hard (sclerotized) structures in the form of hooks, anchors, clamps. The disease caused by monogenean parasites, causes serious problems in aquaculture (Okamoto, 1963; Ogawa and Inouye, 1997; Yoshinaga et al., 2000, 2001, 2009; Mushiake et al., 2001; Nakayasu et al., 2002) with an obvious pathogenicity. Immature worms of these parasites attach to the gill filaments of their hosts and migrate to the buccal cavity wall for maturation, as the worms ingest the blood from the gills of host fish, heavily infected wild and cultured fish become anaemic (Nakayasu et al., 2002; Anshary et al., 2001).

The genus *Diplectanum* (Diesing, 1858) represents one of the genera of the family Diplectanidae Monticelli, 1903 which are gill parasites of marine perciformes (Oliver, 1982, 1993). Family Diplectanidae includes about 22 genera and more than 218 valid species. The first species which detected as a member of Diplectanidae was proposed by Wagener (1857) named as *Dactylogyrus aequans*. Later, Diesing (1858) proposed the genus *Diplectanum* as a new genus when he transferred *Dactylogyrus aequans* to *Diplectanum aequans* and considered it as a type species of this new genus. Recently, Diesing (1858) mentioned that the genus *Diplectanum* is restricted to species that have male copulatory organ with nested tubes, accessory copulatory organ, prostatic reservoir separated into three zones, ventral and dorsal squamodiscs.

Diplectanids often show strict host-specificity (Oliver, 1992); thus, it is a safe prediction that many other species of Diplectanids are still undescribed.

During a survey of gill monogenean parasites of marine fish, The spotted coral grouper *Plectropomus maculatus* (F:Serranidea) from the Red Sea, Egypt was found infested by one species of Diplectanids. Herein, descriptions of this new species were carried out using light microscopy. Also, the histopathological impact of this parasite on fish gills was studied.

2. Materials and methods

A total of fifteen specimens of *P. maculatus* (F:Serranidea Forsskal (1775)) (size range: 14–28 cm, mean 18.5 ± 7.15 cm; body weight 100–250 g, mean 205 ± 20 g) were caught from the coasts of Hurghada City of the Red Sea in Egypt. Samples were obtained at irregular intervals in 2013. Captured fish were kept alive in aquaria filled with the same water source and examined within few hours. Skin surface, fins and gills were firstly examined by naked eyes and with the help of a dissecting microscope for any attached parasites, lesions or external changes. After removing opercula and exposing gill arches, each gill was removed carefully from the fish, immersed in normal saline to remove any excess gill mucus. Monogenean parasites were recovered with a Pasteur pipette using a dissecting binocular microscope. Worms were fixed in 4% formalin and then washed with distilled water to remove excess fixative. Worm identification was confirmed by mounting specimens on slides in drops of ammonium picrate glycerine under cover slips, and examining hard parts using light microscopy. For each monogenean parasite, the sclerotized parts of the haptor were measured using an ocular micrometer calibrated against a stage micrometer slide (Gussev, 1985). Ten specimens were measured for the range and the mean ± standard deviation (SD). Prevalence, mean abundance and measurements followed the guidelines of Bush et al. (1997).

2.1. *Histopathological studies*

Small portions of gills from naturally infected fish were fixed in 10% formalin, dehydrated in series of alcohols, cleared in xylol, embedded in paraffin wax and sectioned by a microtome at 6 μm thick. The tissue sections were stained with Haematoxylin and Eosin, the stained sections were examined and photographed by a Zeiss research photomicroscope (Carleton, 1967).

3. Results

Eight (53.3%) out of 15 specimens of *P. maculatus* were infected with an *Acleotrema* sp., most of the infected fish had very pale gills and showed symptoms of anaemia.

3.1. Acleotrema maculatus *sp. nov.* (*Figs. 1 and 2*)

Body was fusiform, measured 0.86–0.90 (0.88 ± 0.02) in length, 0.09–0.13 (0.11 ± 0.02) in width at the level of ovary (Fig. 1). Tegumental scales not seen. Cephalic lobes well developed; 3 pairs of head organs and small cephalic glands were observed at the level of the pharynx. Two pairs of eye-spots anterior to the pharynx. The pharynx was subglobular measured 0.02–0.06 (0.04 ± 0.02) in length, 0.030–0.034 (0.032 ± 0.002) in width. Oesophagus short. Intestinal caeca not confluent, end posteriorly close to peduncle. Haptor (Fig. 1) short, wide, width of 0.04–0.08 (0.06 ± 0.02). Squamodiscs were 0.03–0.07 (mean

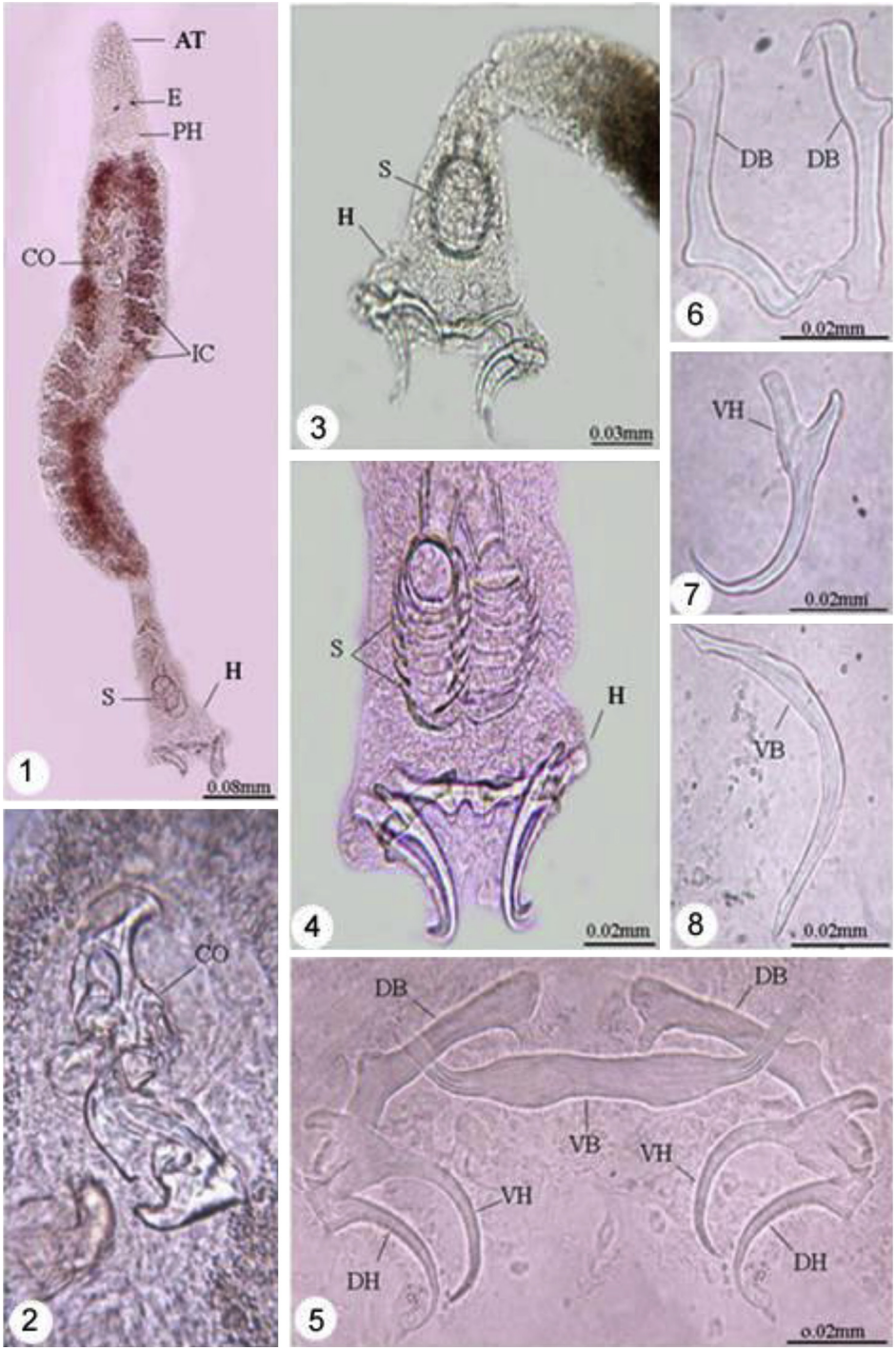

Fig. 1 – Photomicrographs of the adult *Acleotrema maculatus* sp. nov. infecting the gills and skin of roving coral grouper *Plectropomus maculatus*: 1 Whole mount preparation of the adult worm. Observe the anterior attachment area (AT), the two pairs of eyes (E), copulatory organ (CO), intestinal caeca (IC), squamodiscs (S) and the posterior haptor (H). 2–8 High magnifications of: 2 Copulatory organ (CO). 3, 4 Haptor (H) with its squamodiscs (S). 5 Haptor (H) consisting of two pairs of hamuli dorsal hamuli (DH) and ventral hamuli (VH), ventral bar (VB), dorsal bars (DB). 6 Dorsal bars (DB). 7 Ventral hamulus (VH). 8 Ventral bar (VB).

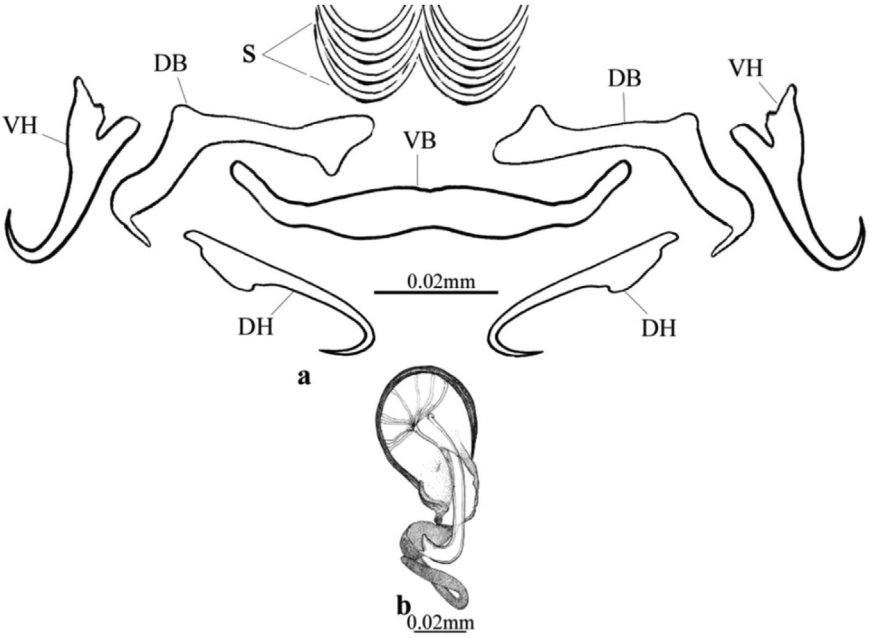

Fig. 2 – Schematic drawing of: a, The haptor of the adult monogenean worm *Acleotrema maculatus* sp. nov. consisting of ventral bars (VB) with two ventral hamuli (VH), a dorsal bar (DB) with two lateral dorsal hamuli (DH) and squamodiscs (S). b, Copulatory organ.

0.05 ± 0.02) mm in length and 0.01–0.05 (0.03 ± 0.02) in width with radial rows of contiguous dumbbell-shaped rodlets (Fig. 1); internal rows of rodlets form 'V'-shape. Lateral (dorsal) bars two, stout, dumbbell-shaped (Fig. 1), measured 0.052–0.056 (0.054 ± 0.002) in length and 0.007–0.011 (0.009 ± 0.002) in width. Ventral bar slender, with transverse groove (Fig. 1), measured 0.09–0.11 (0.10 ± 0.01) in length and 0.008–0.012 (0.01 ± 0.002) in width. Seven pairs of hooklet were observed. Ventral hamuli measured 0.02–0.06 (0.04 ± 0.02) in total length with an outer root that was very long measured 0.016–0.02 (0.018 ± 0.002), stout, slightly notched at broad proximal end; inner root was conical and measured 0.013–0.017 (0.015 ± 0.002) (Fig. 1), with shaft measured 0.025–0.029 (0.027 ± 0.002) and point length 0.007–0.009 (0.008 ± 0.002). Dorsal hamuli measured 0.034–0.038 (0.036 ± 0.002) in total length; base large, stout, with only lateral rudiment of roots; blade and point were long and curved (Fig. 1) with shaft length measured 0.024–0.028 (0.026 ± 0.002) and 0.02–0.06 (0.04 ± 0.02) point length. Male copulatory organ (Fig. 1) measured 0.058–0.062 (0.060 ± 0.002) in length with a sclerotized part composed of a well-defined, sclerotized anterior sheath.

A line diagram showing the different parts of the parasite haptor and its copulatory organ was shown at (Fig. 2).

Taxonomic summary

Family: Diplectanidae Yoshinaga et al. (2000)
Host: The spotted coral grouper *P. maculatus* (F:Serranidea Fowler, 1904).
Infestation site: Gills and skin.
Locality: Coasts of Hurghada City, Red Sea, Egypt.
Etymology: The specific name was derived from the specific name of the fish from which the parasite was discovered and described for the first time.

3.2. *Histopathological studies*

The histopathological alterations of the infected gills were compared to the control sections. The normal gills (Fig. 3) of fish consisted of horizontal flat filaments, which were supported by bony gill arches. On these filaments, the secondary lamellae were found. These lamellae composed of a thin epithelial cell layer covering pillar cells, which in turn surround the blood sinusoids. Gills of fish which were infected with monogenean parasites showed fusion of the secondary gill lamellae (Fig. 3). On the other hand, adult specimens of this parasites were seen attached between the secondary gill lamella, the parasite penetrates deeply with its opisthohaptor to the primary gill lamella causing damage and degeneration of epithelial cells leading to formation of a cup-shaped depression. The damage of secondary gill lamellae was represented by rupture of the basement membrane and breakdown of the coating layer of the epithelial cells by their haptors to the top surface of gill lamella. At the site of attachment of the worm, damage of the gill lamellae was represented by breakdown of the outermost layer of the coating epithelial cells. Moreover, an alteration of the normal position of the gill lamellae mostly occurred. Nevertheless, the major host response to monogenean infestation was represented by hyperplasia of the gill filament.

4. Discussion

Species of diplectanid of genus *Acleotrema* (Johnston and Tiegs, 1922) are characterized by possessing squamodiscs with articulated rodlets forming divergent rows, the male copulatory organ comprised two nested tubes surrounded proximally by a slightly sclerotized sac, the genital atrium was

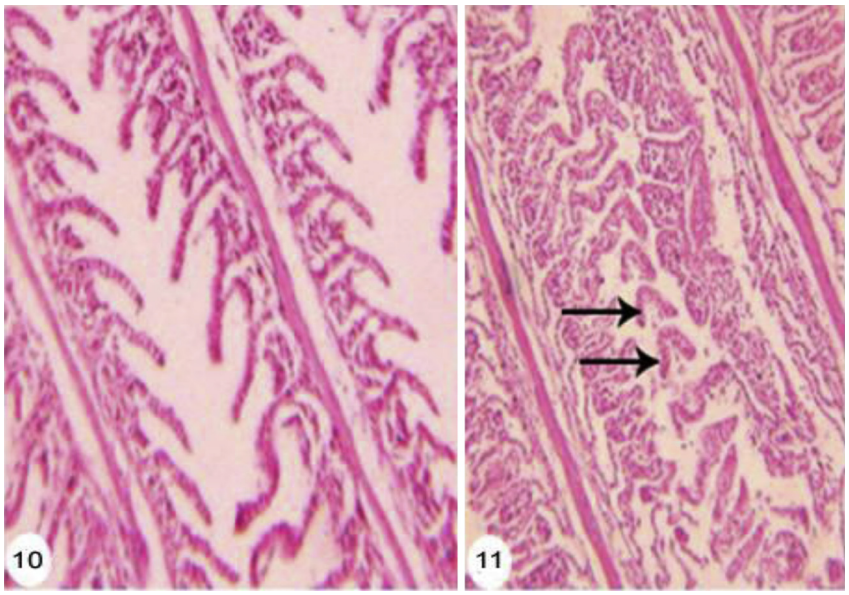

Fig. 3 – Photomicrographs showing a comparison between the structure of gills in normal and infected fishes. 10 Normal gills. 11: infected gills, observe the monogenean worms attached to the gill lamellae (arrows). The pathogenic effects due to this parasitic infestation result in gill hyperplasia and destruction of gill filaments and/or fusion of gill lamellae.

heavily sclerotized which was in accordance with (Domingues and Boeger, 2007). They parasitized perciform fishes, mainly kyphosids, throughout much of the warmer regions of the world oceans. There have, however, been no reports of their occurrence in South American waters (Kohn and Cohn, 1998; Santos et al., 2008).

In the present study *Acleotrema* sp. was isolated from gills of the roving coral grouper *Plectropomus maculates* (F:Serranidea) from the Gulf of Suez and Hurghada City, Red Sea, Egypt. It had been previously proposed by Johnston and Tiegs (1922) for *Acleotrema girellae* isolated from *Girellatricus pidata* (Quoy and Gimard) in Australian waters, *Acleotrema serrulopenis* was originally described from two different sparid fishes, *Rhabdosargus sarba* (Forsskal, 1775) and *Polyamblyodon gibbosum* (Pellegrin, 1914), from off Madagascar (Indian ocean), considered it a junior synonym of *Diplectanum* (Diesing, 1858), most authors have retained it as a valid genus (Yamaguti, 1934, 1938, 1953, 1963). A second species, *Acleotrema kyphosi* (Yamaguti, 1968), was added by from *Kyphosus cinerascens* (Yamaguti, 1968) of Hawaii. Oliver and Lambert (1987) isolated and described *Heteroplectanum raktofiringa* from sparid and carangid fishes of Madagascar. However, Recently Domingues and Boeger (2007) have revised this group and considered *Heteroplectanuma* junior synonym of *Acleotrema* to which they attributed ten species. Lim (2006) described another species of *Heteroplectanum*, *Heteroplectanum labeeliforme*, from the toxotid (archer fish) *Toxotes jaculatrix* of the western coast of the Malaysian peninsula. As pointed out by Lim, the generic allocution of this species is somewhat problematical. *Acleotrema diplobulbus* (Yamaguti, 1968), *A. kyphosi* (Yamaguti, 1968), *Acleotrema* (Oliver, 1983) and *Acleotrema oliveri* (Léon-Régagnon et al., 1997). Léon-Régagnon et al. (1997) described *A. oliveri*, *A. Kyphosi* and *Acleotrema nenue* from *Kyphosus elegans* (paters) off the pacific coast of Mexico. In their revision of the genus, Domingues and Boeger (2007) reported *A. kyphosi* as a synonym of the type-species, *A. girellae*, even though the latter was originally described from different subfamily of kyphosid in Australian waters.

Intraspecific variations between the measurements of *Acleotrema* sp. in the present work and those registered before were shown in (Table 1).

The present *Acleotrema* sp. was characterized by presence of larger body length and a smaller dimension of the ventral bar than previously recorded species. The ventral hamuli length were longer apart from *Acleotrema lamothei* in relation to the dorsal hamuli, a very long stout outer root, which is slightly notched proximately, the outer root was longer than the length of the inner root. They also differ from most of these species in that; the lateral (dorsal) bars were smaller; the dorsal hamuli were also smaller. The shape of the ventral hamuli was nearly equal in size to the dorsal humuli. The male copulatory organ differed significantly from the described species. The present new species appeared morphogically closest to those species with a spined male copulatory organ, i.e. *A. serrulopenis* Oliver and Lambert (1987). but with different dimensions so we should consider this species as a new one in Egypt.

4.1. *Histopathological studies*

The present study showed severe damages to the gill filaments, epithelial cell hyperplasia and fusion of the secondary lamellae together to form one unit, losing their architecture, dilation, and excessive mucus secretion. These results agreed with those recorded before (Khan and Thulin, 1991), they concluded that the destruction of the normal gill structure may be due to the heavy infestation due to monogenean infestation. In addition, these results agreed

Table 1 – Comparison between measurements (mm) of *Acleotrema maculates* sp. nov. recorded from the current study and *A. lamothei* (Santos et al., 2008).

Aspect	*A. lamothei*	Present study
Total body length	0.01 (0.93–0.01)	0.88 (0.86–0.90)
Maximum body width	0.18 (0.17–0.18)	0.11 (0.09–0.13)
Pharnyx length	0.06 (0.05–0.06)	0.04 (0.02–0.06)
Pharnyx width	0.05 (0.04–0.06)	0.032 (0.030–0.034)
Opithohaptor width	0.10 (0.09–0.01)	0.06 (0.04–0.08)
Ventral humuli		
- Total length	0.07 (0.05–0.08)	0.04 (0.02–0.06)
- Shaft length	–	0.027 (0.025–0.029)
- Inner root length	0.03 (0.02–0.03)	0.015 (0.013–0.017)
- Outer root length	0.06 (0.05–0.06)	0.018 (0.016–0.02)
- Point length	–	0.04 (0.02–0.06)
Ventral bar		
- Total length	0.03 (0.06–0.03)	0.10 (0.09–0.11)
- Width	–	0.01 (0.008–0.012)
Dorsal humuli		
- Total length	0.08 (0.07–0.08)	0.036 (0.034–0.038)
- Shaft length	–	0.026 (0.024–0.028)
- Point length	–	0.04 (0.02–0.06)
Dorsal bar		
- Total length	0.08 (0.08–0.09)	0.054 (0.052–0.056)
- Width	–	0.009 (0.007–0.011)
Copulatory organ length	0.16 (0.15–0.19)	0.060 (0.058–0.062)

with those observed on the gill filament of the grass carp infected with *Dactylogyrus lamellatus* (Molnar, 1972; Abdel-Megiuid, 1989; Ramadan et al., 1995; Hawkes, 1977). Moreover, excessive mucus secretion, capillary dilation, lamellar hyperplasia and fusion of gill filaments occur in fish exposed to monogenean infestation (Hawkes, 1977; Haensly et al., 1982; Khan and Kiceniuk, 1988). On the other hand, water-soluble oil fractions in the environment of the fish can affect the fish defence mechanism, and induce mucus cell hyperplasia, created a habitat conductive to parasitic infestation and parasite reproduction (Khan and Thulin, 1991). The exposure to pollutants acted synergistically as a stressor causing histopathological and physiological changes which altered host resistance to the parasite (Khan and Thulin, 1991).

Also these results agreed with (Paperna, 1980a) who stated that *Dactylogyrus vastator* presence on the gills, even in small numbers, induced extensive hyperplasia of the gill epithelium. These cellular changes eventually interfere with the respiratory function of the gills and may be the direct cause of death of the fish (Paperna, 1980b).

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