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Lamproglena Monodi Capart, 1944, Attachment Scheme and Associated Pathology on the Gills of Oreochromis Niloticus Niloticus, with a Special Reference to Thoracic Appendages

نظام تشبث لامبروجلينا مونودى كابارت 1944 والضرر الباثولوجى المصاحب له على خياشيم أوريوكروميس نايلوتيكاس نايلوتيكاس مع مرجعية خاصة للزوائد الصدرية

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ABSTRACT: This work comprises a parasitological and a histopathological examination of the Nile tilapia, Oreochromis niloticus niloticus, naturally infected with Lamproglena monodi (Crustacea: Lernaeidae). Fish specimens were collected weekly from El-Minia Nile basin, Egypt (between April 2006 to March 2008). From a total of 420 fish examined, 96 (22.86%) were found infected. Attachment of L monodi was mainly enhanced by the armed maxillae that were seen deeply introduced into the underlying tissues reaching the axial cartilage of the gill filament. The maxillipeds were not involved in the attachment to the gill epithelium. Histological changes were restricted only to the free ends of gill filaments, where copepods were found attached; the central and basal parts appeared normal and their gill lamellae remained intact. Deep and shallow lesions associated sometimes with compressed or exfoliated hyperplastic epithelium were encountered in front of the cephalothorax and around oral apparatus of the parasite. In slight and moderate infections gill lamellae showed partial fusion. In many cases of heavy infection, the attacked area of gill filaments was eroded through. The cephalothorax was sometimes found in a deep cavity of the proliferated epithelium that was infiltrated by granular cells and lymphocytes.

Keywords: *Attachment, freshwater fish, gills, Lamproglena monodi, Lernaeidae, Oreochromis niloticus niloticus, pathology.*

المستخلص: يتضمن هذا البحث دراسة طفيلية عن التغيرات النسيجية المرضية لخياشيم أسماك البلطى النيلى أوريوكروميس نايلوتيكاس نايلوتيكاس كنتيجة للعدوى الطبيعية بالطفيلى القشرى مجدافى الأرجل لامبروجلينا مونودى، كما يتضمن أيضا وصفا للزوائد الصدرية للطفيلى بالإستعانة بالميكروسكوب الإليكترونى الماسح. تم تجميع 420 عينة سمكية بصفة أسبوعية فى الفترة من ابريل 2006 حتى مارس 2008 من حوض النيل المجاور لمدينة المنيا – جمهورية مصر العربية ووجد منها 96 سمكة مصابة، أى

أن معدل النسبة المئوية للإصابة بهذا الطفيلى هى 22.86%. وجد أيضا من خلال الفحص المعملى أن وسيلة التشبث الأساسية للامبروجلينا مونودى هما الفكان المسلحان اللذان كانا دوما يلاحظان منغرسين فى أنسجة الخيوط الخيشومية حتى مستوى الغضروف المحورى. لم يكن هناك أى دلائل على اشتراك الرجلان الفكيتان فى عملية التشبث. اقتصرت التغيرات النسيجية على صفائح الأطراف الحرة للخيوط الخيشومية، وهى الأماكن المفضلة لتشبث لامبروجلينا مونودى، بينما ظلت الصفائح الخيشومية على الأجزاء الوسطى والقاعدية للخيوط الخيشومية بحالتها الطبيعية. تمثلت التغيرات النسيجية فى انضغاط الطبقة الطلائية أو تقشر خلاياها لتكون قرحا ضحلة أو عميقة فى مواجهة الرأس صدر أو جهاز الفم الخاص بالطفيلى. تلتحم الصفائح الخيشومية جزئيا فى حالات الإصابة الخفيفة والمتوسطة بينما فى حالات الإصابة الشديدة تختفى الصفائح الخيشومية تماما مما يؤدى الى تعرية ما أسفلها من أنسجة. الخلاصة أنه على الرغم من أن التغيرات النسيجية المرضية الناتجة عن الإصابة بلامبروجلينا مونودى ربما لا تشكل خطرا كبيرا على حياة أسماك البلطى النيلى إلا أنه من المتوقع فى حالات زيادة كثافة الإصابة عن معدلاتها الطبيعية أن يؤثر ذلك سلبا على الوظيفة التنفسية للخياشم مما يقلل من فرصة تبادل الغازات خلال الأنسجة المعطوبة.

كلمات مدخلية : *طرق التشبث، أسماك المياه العذبة، الخياشيم، لامبروجلينا مونودي، عائلة ليرنيدي، البلطي النيلي، أوريوكروميس نايلوتيكاس نايلوتيكاس، الأثر الباثولوجي.*

INTRODUCTION

The cyclopoid family Lernaeidae comprises freshwater parasites that are highly adapted to parasitic way of life (Piasecki, 2004). Lernaeids have been responsible for serious disease resulting in mortality in several species of farmed fish and as such they may post serious health threats to the fish farm industry (Pavanelli, *et al.* 2000; Tsotetsi, *et al.* 2005). The lernaeid genus *Lamproglena* was established by Alexander von Nordmann in 1832 and currently comprises more than 40 nominal species with a cosmopolitan distribution (Piasecki, 1993). Copepods of this genus are typical gill dwellers of freshwater fish, except *L. lichiae* von Nordmann 1832, that has controversially been reported as a parasite of the double-spotted queen-fish (*Scomberoides lysan* Forskål) from the Red Sea. Members of the genus *Lamproglena* are distributed in Africa (Marx and Oldewage, 1996), Asia (Kuang and Qian 1985; Kumari, *et al.* 1989 and Yambot, and Lopez, 1997), Europe (Cakić, *et al.* 1998 and Galli, *et al.* 2001) and Southern America (Azevedo, *et al.* 2006). In Africa alone, 14 species of this genus have been reported (Dippenaar, *et al.* 2001; Van As and Van As, 2007), with one of them (*L. monodi* Capart, 1944) being reported from Egypt (Ibraheem and Izawa, 2000). The present work reports the frequent and severe infections of the Nile tilapia (*Oreochromis niloticus niloticus*) with *L. monodi*, and gives a description of the thoracic appendages, mode of attachment and the resultant tissue reaction as seen by light and scanning electron microscopoy (SEM).

MATERIALS AND METHODS

Six to ten specimens of *O. niloticus niloticus* (21.6 ± 5.4 cm total length; 300 ± 30 g mean body weight) were captured weekly by net in one locality at El-Minia (Lat. 28°04` - 28°06`N, Long. 30°45` - 30°46` E), Upper Egypt, from April 2006 through March 2008. Fish were brought to the laboratory alive using a portable aquarium fitted with an air pump. For parasitological examination the gills were removed and carefully washed with 0.75% physiological saline solution. Gill archs were then separated and carefully observed under a stereomicroscope for the presence of *L. monodi* on the gill filaments.

For routine histological analysis, infected gill filaments were fixed in Bouin's solution for 6 hours and embedded in paraffin after proper washing in 70 % ethanol, dehydration and clearing in xylene. Cross and longitudinal sections (6 μm) were stained with haematoxylin and eosin (H&E) and observed with a light microscope. For histochemical demonstration both Alcian blue (pH 2.8) and Toluidine blue were applied.

For scanning electron microscopy (SEM) studies, some infected gill filaments and few copepods, carefully picked off the infected gills, were fixed in a solution of 2.5% glutaraldehyde, in 0.1 M sodium cacodylate buffer at 4°C. Thereafter, they were post-fixed in 1% osmium

tetroxide. After washing several times in buffer, specimens were dehydrated through graded ethanol, followed by freeze drying, mounting on stubs then sputter coating with gold-palladium in an ion sputtering device (JEOL JFC - 1100 E). Examination was carried out with a stereoscan JEOL JSM - 5400 LV at 15 kv.

RESULTS

From a total of 420 fish examined, 96 (22.86%) were found infected. The number of copepods present on the gills of all the 96 infected fish varied between 2 and 14 specimens. Fish were found infected all year round though, the intensity of infection was generally higher in summer (2 – 11 /infected fish) and autumn (2 – 14 /infected fish) months. No male specimens of *L. monodi* have been detected; only females were found attached to the gill filaments of *O. niloticus niloticus*. Gut contents of *L. monodi* contained cellular debris, red blood cells and inflammatory cells. They also showed extensive mucy content indicated by high affinity to staining with Alcian blue and Toluidine blue.

Morphology

The body of *L. monodi* is divided into cephalothorax, free leg-bearing thoracic segments and abdomen. The cephalothorax is partially separated from the first leg-bearing thoracic segment that forms a distinct neck (Figure 1A). First antennae are bisegmented and each bears about 23 – 24 setae; antennules are small and hidden behind antennae. Oral apparatus is a fleshy bilobed structure. Maxillae are elongated; each is armed with an acute terminal chitinized claw. Maxillipeds are tipped with two chitinized recurved claws and a third atrophied one in the form of a small process (Figure 1B). Thoracic legs are indistinctly segmented. The legs 1 – 4 are biramous, with two-segmented exopod and two-segmented endopod. Exopod of legs 1 – 4 has a long seta on the outer margin of the basal segment, a smaller medial seta on the outer margin and a varying number of distal setae on the distal segment. Endopod of legs 1 – 4 are with unarmed basal and armed distal segments. The distal segment of exopod of leg 1 has four apical setae (two long setae medially and two short setae laterally); the distal segment of endopod has one large terminal seta and a smaller distoleral one. Leg 2 (Figure 1C) has two unequal setae and a conical process on the distal segment of exopod; the endopod has two apical conical processes on the distal segment. Two apical setae of equal size characterize the distal segment of exopod of leg 3 (Figure 1d); the distal segment of endopod has one apical conical process. The distal segment of exopod of leg 4 has two small, equal sized apical setae and a small median sub-apical one; the distal segment of endopod has a very small apical process. Leg 5 (Figure 1E) is reduced; exopod is a cylindrical protrusion with two equal-sized terminal setae on the distal segment; endopod is present as an elevated bump tipped with a small process.

Mode of Attachment

All parasites were located near to the free end of gill filaments, each of them grabbing only a single filament (Figure 1F). Attachment of *L. monodi* to the gill filaments is mainly enhanced by their maxillae which are elongated enough to let their robust claws deeply pierce the proliferated epithelium, reaching both sides of filament cartilage (Figure 2A), or contacting one another over the oral apparatus across the ventral surface of the cephalothorax (Figure 3B). On the other hand, maxillipeds, were in all cases, in a superficial position to filament epithelium showing no signs of penetration (Figure 2B). The well-developed oral apparatus appeared to be also deeply embedded in filament epithelium (Figure 2A). Below the oral apparatus the maxillae were frequently seen with their claws embedded (Figures 2A & 3B). In some cases the anterior end of the parasite was buried under exfoliated gill epithelium (Figure. 2C).

Tissue Reaction

No macroscopic lesions were observed around the site of attachment of *L. monodi*. Histopathological changes were confined about the anterior of the copepod's cephalothorax; no tissue reaction was noticed below the free thoracic appendages. As revealed by scanning electron microscopy of infected gill filaments, a hole may be formed on the gill filaments below

the copepod's cephalothorax. This sometimes was associated with a small mound or a mass of thickened and proliferated epithelium in front of the anterior end of the parasite (Figure 2D). Histological sections showed that the pathological changes are restricted mainly to the area of the filament tips. A consistent feature of attachment was exfoliation of gill lamellae in contact with the cephalothorax of the parasite; Gill lamellae, opposing the site of attachment on the same gill filament, showed varying degrees of epithelial hyperplastic reactions ranging from little to extensive proliferation associated with complete adhesion (Figures 3 B - D). Fusion of gill lamellae and epithelial proliferation were restricted to distal part of gill filaments, while the middle and basal parts close to gill arch remained intact. In slight infection (2 – 5 copepods / infected fish) gill lamellae opposite to the attachment site of copepods appeared proliferated and adhered (Figure 3B). A short distance above the attachment site gill lamellae kept a nearly normal appearance, compared with the normal histology of uninfected gill filaments (Figure 3A). In moderate cases of infection (6 – 9 copepods / infected fish) there was an increased proliferation associated with adhesion of gill lamellae (Figure 3C). In a few cases of heavy infection (10 – 14 copepods / infected fish) the surface area of filament, where the copepods were attached, was associated with deep lesions and eroded through resulting in a complete loss of lamellar structure (Figure 3D). Within the mass of proliferated epithelium of gill filaments there was a sub acute inflammatory response resembled in a hyperplastic infiltration with few granulocytes, and lymphocytes; some free erythrocytes were also seen. The lymphocytes appeared larger in number than normal. Infiltrating cells were occasionally seen in the connective tissue between the maxillae and the oral apparatus (Figure 3B). Generally, at the attachment sites, inflammatory cells replaced the normal architecture of gill filaments.

DISCUSSION

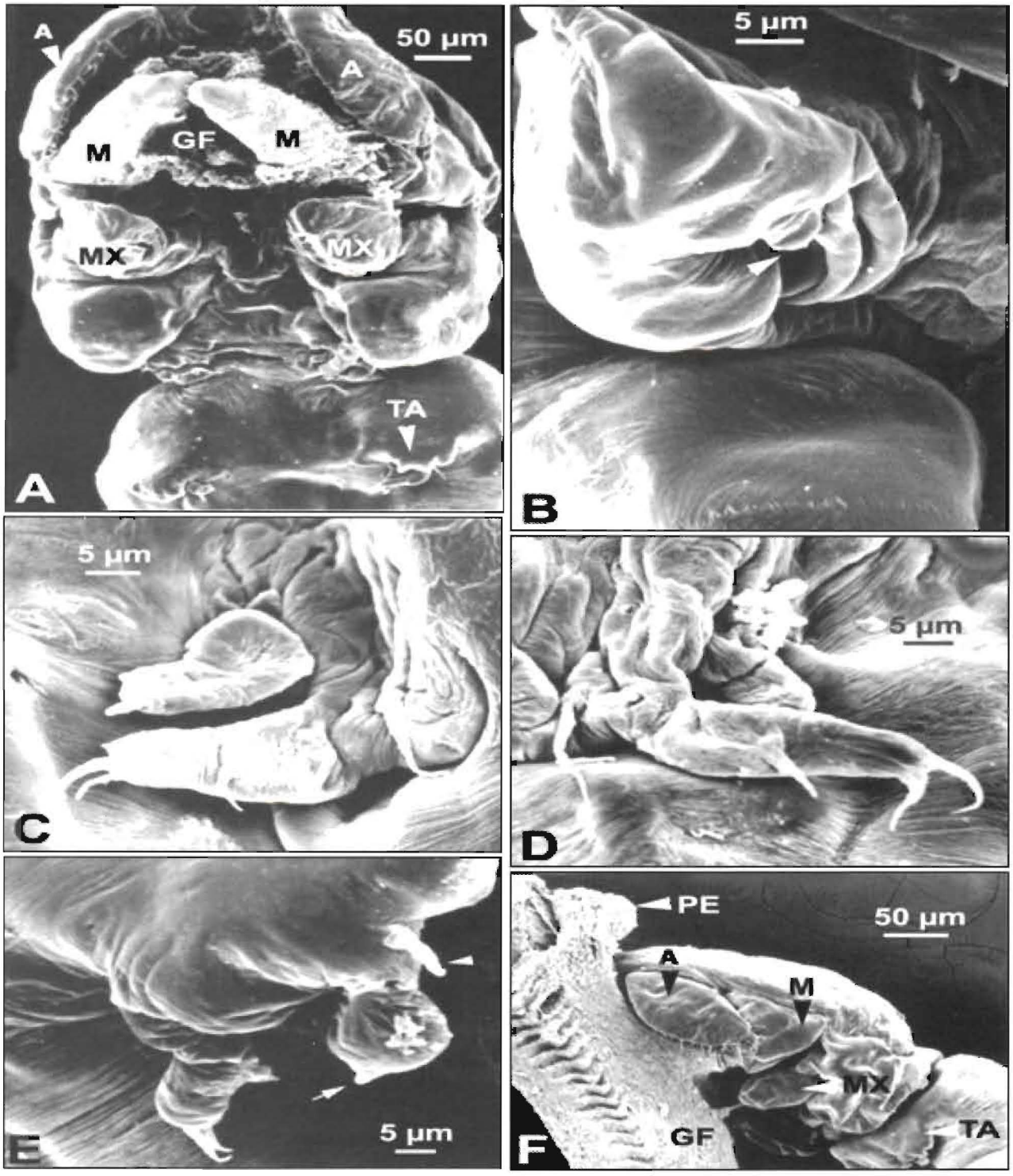
In the present investigation no male specimens of *L. monodi* have been encountered; only females were found attaching to the gill filaments of *O. niloticus niloticus*. Similar findings were observed by Ibraheem and Izawa (2000). Males were considered by Fryer (1956) as very small, free-swimming creatures, less than 1 mm in length. Fryer (1961) described only one adult male found associated with an adult female on the gills of *Haplochromis sp.* in Lake Victoria.

Mode of attachment

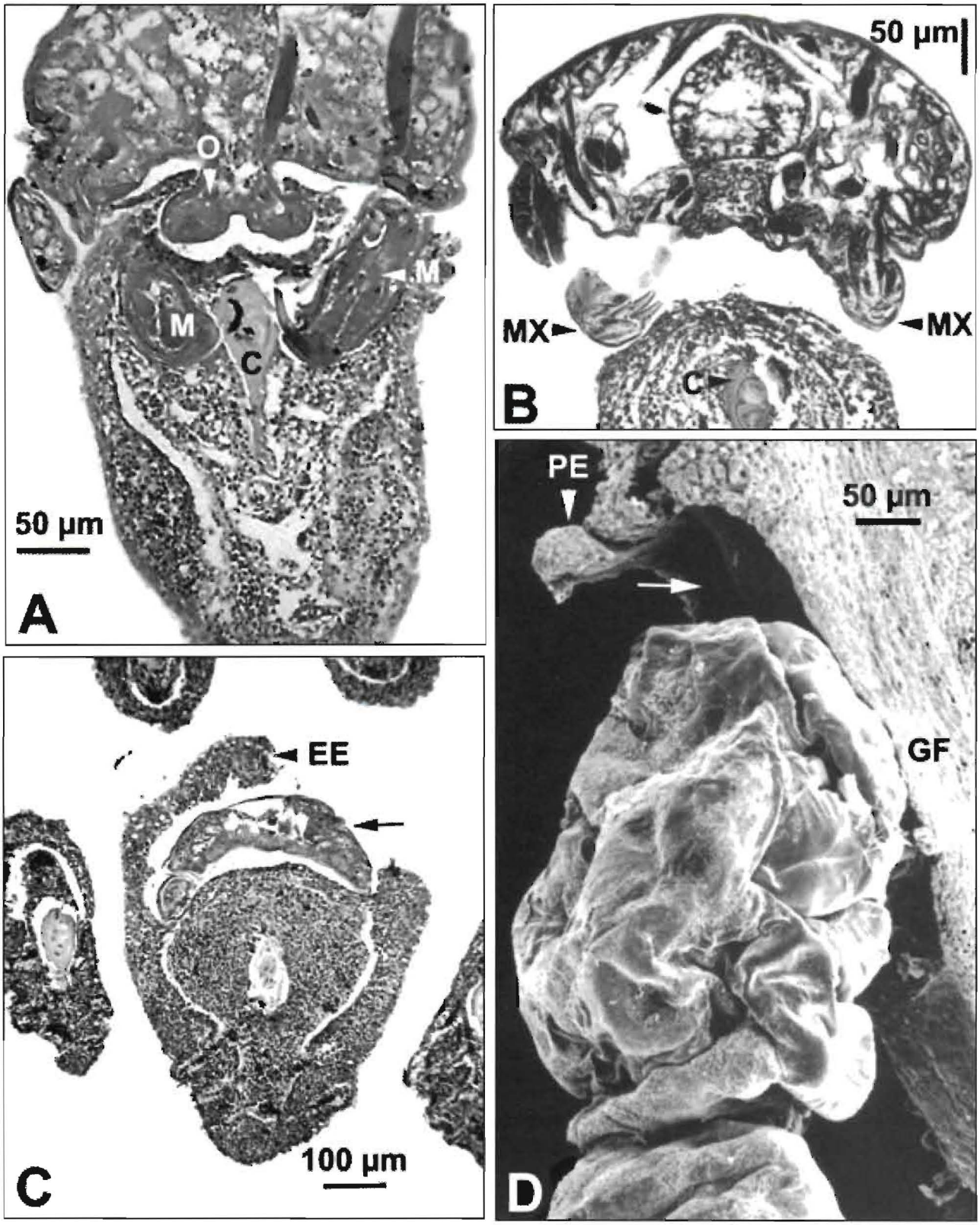
Kabata (1981) considered second antennae as a characteristic feature for primary attachment in all parasitic copepods, while on the other hand, according to Avenant and Van As (1985), adherence to the host is enhanced by maxillulae which are responsible for most of the host damage. The antennae of *L. monodi* have no function in attachment (Ibraheem and Izawa, 2000). In the present study the primary prehensile organs were the hook-like maxillae; they were seen grasping the gill filaments and deeply embedded in the underlying tissue reaching the axial cartilage. The maxillipeds of *L. monodi*, though possessing sharp recurved claws, did not appear grasping or hooking into filament epithelium. Instead, they were in a superficial position. This may confirm the suggestion of Ibraheem & Izawa, 2000 that the sharp claws of maxillipeds are used in scrapping off the filament epithelium for food.

Host response

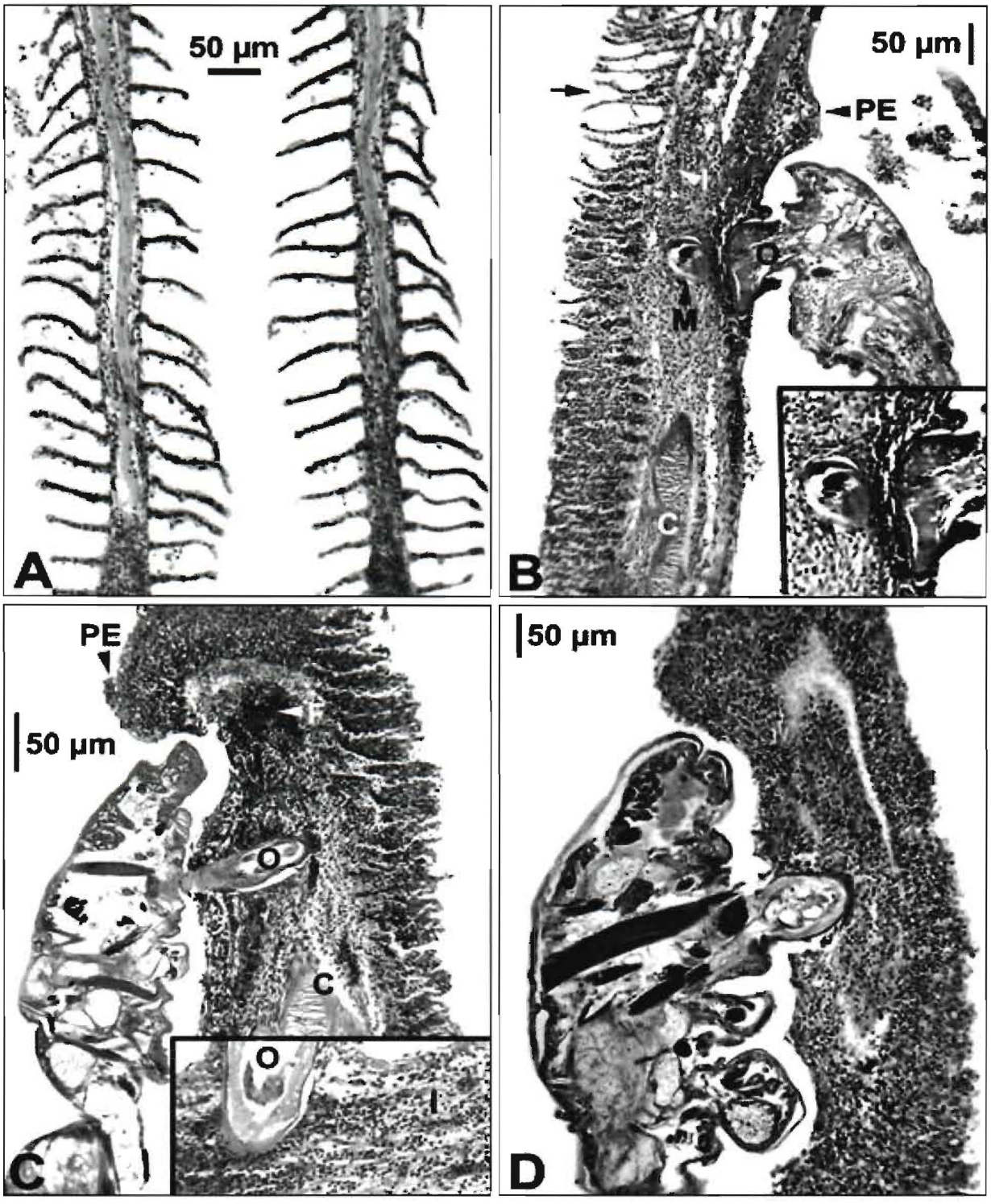
As has been indicated by Piasecki, 1993 and Pavanelli, *et al.* 2000, many species of lernaeid copepods may have the potential to cause damage and mortalities in several species of wild and farmed populations of fish. Changes induced by parasitic copepods on the gills of fish are mostly known from many other studies such as those of Kabata (1966), Bennett and Bennett (1994), Molnár and Székely (2004). In the present study attachment of *L monodi* onto the gill filaments of *O. niloticus niloticus* showed a range of responses. There were deep lesions, associated sometimes with cellular infiltration and epithelial hyperplasia, caused by the insertion of maxillae and oral apparatus into filament core. Pathological findings of *Dissonus manteri* on the gills of coral trout *Plectropomis leopardus* (Lacépède) were almost proliferative with little or no cellular infiltration; the host response was hyperplastic



Figs. 1. Scanning Electron Micrographs (SEM) of L. Monodi. (A. Ventral view of the cephalothorax showing first antennae (A), maxillipeds (MX) and the second thoracic appendage (TA). Mouth apparatus is obscured below a fragment of gill filament (GF) of the host grasped by maxillae (M); B. Right maxilliped tipped with two recurved claws and a third atrophied one (arrowhead); C. Left Leg 2 with two unequal setae on the distal segment of exopod; distal segment of endopod have two apical unequal conical processes, D. Right leg 3 with two apical setae of equal size on the distal segment of exopod; E. Leg 5 reduced, Exopod is a cylindrical protrusion tipped with two equal setae and the endopod is an elevated bump tipped with a small conical process (arrow) and a long seta lateral to the bump (arrowhead); and F. Lateral view of L. monodi attaching to gill filament (GF) of O. niloticus niloticus. Note mound of proliferated epithelium (PE) anterior to the tip of the cephalothorax.).



Figs. 2. (A – C). Photomicrographs of Transverse Sections of L. Monodi on the Gill Filaments of O. Niloticus Niloticus. (A. The maxillae (M) are deeply embedded in the gill filament reaching the axial cartilage (C). Note tissue debris between maxillae and oral apparatus (O); B. The maxillipeds (MX) are in a superficial position out of filament border; C. The anterior end of L. monodi (arrow) is buried under exfoliated epithelium (EE); and D. SEM showing a hole (arrow) on the gill filament (GF) below the cephalothorax of the parasite).



Figs. 3. A. Photomicrograph of a longitudinal section (LS) of gill Filaments of a non-infected O. niloticus niloticus showing normal array of gill lamellae, B – D L. S. of infected gill filaments of O. niloticus niloticus., B. A gill filament is completely eroded below the ventral surface of the copepod in a slightly infected fish. Many gill lamellae on the opposite side show adherence and congestion of blood vessels; few distal lamellae (arrow) are nearly normal. Note necrotic epithelium between maxilla (M) and oral apparatus (O). Inset shows infiltrating cells around the maxilla; C. In moderate infection there is a higher level of congested blood vessels and more fusion of lamellae on the opposing side of gill filament. Note fibrosis (F) in front of the anterior end of the parasite that is partially introduced into the proliferated epithelium (PE). Inset shows infiltrating cells (I) around the oral apparatus (O); and D. A gill filament of a heavily infected fish showing complete atrophy, associated with complete erosion of lamellae.

one associated with the formation of fibrotic cup around the parasite. Bennett and Bennett (1994) explained such findings as attempts of the host to seal off the parasite from the surrounding tissue. Hyperplasiasis of host tissues in the present investigation may initially be considered as an attempt to shed off the parasite. Fustish and Millemann (1978) and Kabata (1984) concluded that some parasites might be shed off from host by a well-developed hyperplasia. Boxshall (1977) noticed deep lesions on the pectoral fins extended to the dermis of the flounder, *Platichthys flesus* (L.) parasitized by chalimus larvae; such lesions have been attributed to feeding activity of the parasite. Bennett and Bennett 1994 considered epithelial proliferation as a renewable food resource for parasitic copepods. Analysis of gut contents of *L. monodi*, in the present study, showed cellular debris, mucous, red blood cells and inflammatory cells. It seems that the oral apparatus and the maxillipeds may scrape the filament epithelium. A result consistent with caligid copepods that are considered histophagous, feeding on mucus tissue and blood by scraping host surface with their tubular mouths (Kabata 1974, Brandal, *et al.* 1976 and Jones, *et al.* 1990). Wilson (1902) stated out that the insertion of powerful hooks is sufficient to cause a strong flow of blood. Fryer (1968) noted that piercing claws could cause a noticeable wound from which the parasite can draw blood, resulting in killing of young heavily infected individuals.

In the present study the host response was characterized by massive proliferation of lamellar epithelium associated with an infiltration of lymphocytes. In some parts on the infected gill filament lamellae were fused together due to intensive proliferation of epithelial cells. Schäperclaus (1954), Rogers (1969) and Alston and Lewis (1994) reported proliferation of gill epithelium and associated fusion of gill filaments in fish hosts infected with *Ergasilus spp.* Musselius (1967) observed degeneration and breakage at attachment sites on the gill filaments of fish parasitized by *Sinergasilus lieni.* He concluded that one copepod could cause fusion to 5 or 6 lamellae. Dezfuli, *et al.* (2003) noticed proliferation of mucous cells associated with an increase in the number of eosinophils in fish infected with copepod parasites. On the other hand, Rogers (1969) and Roubal (1986) reported infiltration of lymphocytes and granulocytes into the proliferated gill epithelium. The latter are commonly regarded as normal elements of healthy gill structure, but in the present study their numbers often increased than normal especially in heavy cases of infection. Reite (1997) reported that infiltration with leucocytes increases in persistent inflammations due to infection with helminthes or unknown causative agents.

Finally, depending on the above-mentioned findings, the pathology attributed to *L. monodi* probably does not present a serious threat to the host. However it is expected that if found in large numbers *L. monodi* may interfere with respiratory function of gills, reducing the rate of gas exchange through disrupted epithelium.

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