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Histopathological changes on the gills of asp (*Aspius aspius*) and European catfish (*Silurus glanis*) caused by *Lamproglena pulchella* and a *Lamproglena* sp. (Copepoda: Lernaeidae), respectively

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Abstract

In a parasitology survey of Hungarian fishes, heavy infections of parasitic copepods *Lamproglena pulchella* and a *Lamproglena* sp. were found in the gills of the asp and the European catfish, respectively. Individuals of both fish species were emaciated and infected with hundreds of *Lamproglena*. Copepods located close to the tip of gill filaments and formed a depression at the attachment sites. In histological sections, cell degenerations and local haemorrhages were present adjacent to the maxillipeds and where the maxillary claws pierced the gill tissue. Around maxillae and in the midgut of the *Lamproglena*, damaged piscine blood cells and remains of the gill tissue were observed. Host reaction was expressed by proliferation of epithelioid cells, increase in both number and size of goblet and mast cells and formation of giant cells.

KEYWORDS
Aspius, gills, histopathology, *Lamproglena*, *Silurus*

1 | INTRODUCTION

Currently, only a single species of the *Lamproglena* genus, *Lamproglena pulchella* Nordmann, 1832, has been reported from Hungary (Ponyi & Molnár, 1969). This species was regarded as a common but relatively rarely occurring parasite of cyprinid fishes. In 2016, however, two cases with highly intensive infection were recorded on an asp (*Aspius aspius* (L.)) from the Danube River and on some European catfish (*Silurus glanis* L.) from a water reservoir in the Pannonia Region of Hungary. In Europe, the occurrence of a single species, *L. pulchella*, is known. Markevich (1951) detected only *L. pulchella* in the Ukraine although earlier he found two other *Lamproglena* species, *Lamproglena orientalis* Markewitsch, 1936 and *L. compacta* Markewitsch, 1936, in the Amur Basin and in the Caspian Sea, respectively. *Lamproglena pulchella* is common also in several neighbouring countries of Hungary. Lucky and Dyk (1964) described it in Czechoslovakia from creeks and fishponds in the water basin of the Odera and Dyje rivers. Angelescu (1974) found it in the Danube River in Romania, while Cakic, Petrovic, Kataranovski, and Fister (1998) recorded it from some freshwater fishes in Yugoslavia. Galli et al. (2001) studied the effect of pollution to it in Italy. Recently, it was detected in Austria, as well (Jirsa, Zitek, & Schachner, 2006). The occurrence of a *Lamproglena* species on the gills of the European catfish is less known. Kurbanova, Urazbaev, and Yusupov (2002) reported a *Lamproglena* infection on European catfish and identified these copepods as *L. pulchella*. Gusev, Poddubnaya, and Avdeeva (1987), who surveyed the occurrence of *Lamproglena* spp. in the Palaearctic region of Eurasia, recorded seven different species, and the *L. pulchella* was found in the gills of most cyprinids fishes. Piasecki (2008) reported that there are 41 described species worldwide.

Diseases caused by parasitic copepods, including *Lamproglena* spp., have been studied by Shulman (1961), Lester and Roubal (1995) and Piasecki and Avenant-Oldewage (2008). Pathological changes in the *Lamproglena* infections were described in detail by Tsotetsi, Avenant-Oldewage, and Mashego (2005) studying

Lamproglena clariae Fryer, 1956, the parasite of *Clarias gariepinus* (Burchell), in South Africa; and by Ibraheem (2008) examining *Lamproglena monodi* Capart, 1944, the parasite of *Oreochromis niloticus* (L.), in Egypt. In addition, Öktener, Egribas, and Basusta (2008) investigated a case of fish mortalities in Turkey and reported pathological deformations on two cyprinid fishes, *Cyprinus carpio* (L.) and *Capoeta trutta* (Heckel) caused by *L. pulchella*.

In this study, we report on histological changes on the gills of asp and of European catfish caused by *L. pulchella* and a closely related non-identified *Lamproglena* sp., respectively.

2 | MATERIALS AND METHODS

Fish used in the study originated from two sources. Firstly, in May 2016, during a routine parasitology survey 50-cm-long asp specimens in poor health condition with remarkably meagre musculature (Figure 1) was collected from the Danube River at Szentendre City (47°39′51.1″N 19°04′51.9″E) located north of Budapest. Secondly, ten specimens of large (80–120 cm long) European catfish because of stunted bodies were sent to the laboratory from a water reservoir located in the Pannonia Region of Hungary (46°24′52.3″N 17°59′31.3″E) in November 2016. The fish arrived alive in oxygenated water and they were held in concrete basins in flowing water at the laboratory for 3 days until examination. In both cases, a complete parasitological necropsy was carried out.

The fish were sedated with 20 ppm clove oil (Javahery, Nekoubin, & Moradlu, 2012) added to the water and thereafter killed by a blow to the head. The excised organ tissues were observed with a Zeiss stereo microscope and followed by a more detailed study with a compound microscope. In both cases, heavy *Lamproglena* infection was diagnosed. Hemibranchia infected with parasites were fixed in Bouin's solution, embedded in paraffin wax, cut to 4- to 5-μm-thick sections, and stained with haematoxylin and eosin (H & E). Sections were studied using Nomarski differential interference contrast with an Olympus BH2 microscope and they were photographed with an Olympus DP 20 digital camera.

COLOR

FIGURE 1 Severely emaciated asp specimen before dissection. Bar = 10 cm

3 | RESULTS

Gills of the asp were heavily infected by *Lamproglena* copepods (Figure 2). Altogether 240 specimens were counted, they attached close to the distal end of gill filaments. From their size and shape these specimens (Figure 3) corresponded to data and figures of *L. pulchella* Nordmann, 1832 given by Gusev et al. (1987), Piasecki (2008) and Öktener et al. (2008). At the attachment point of the

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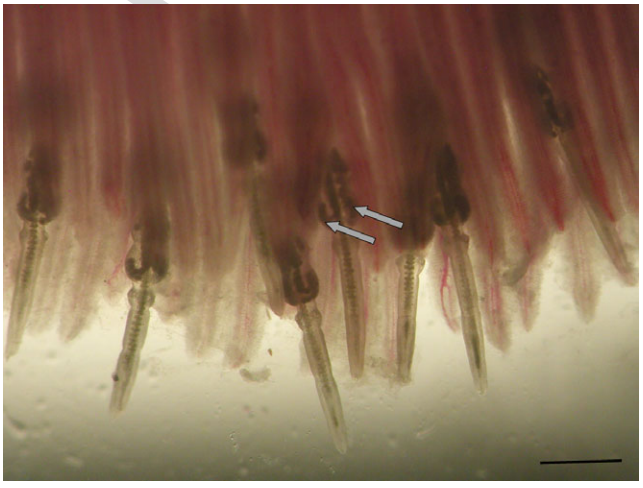

FIGURE 2 *Lamproglena pulchella* specimens attach to gill filaments of an asp. Well-developed ovaries are visible (arrows). Bar = 0.5 mm

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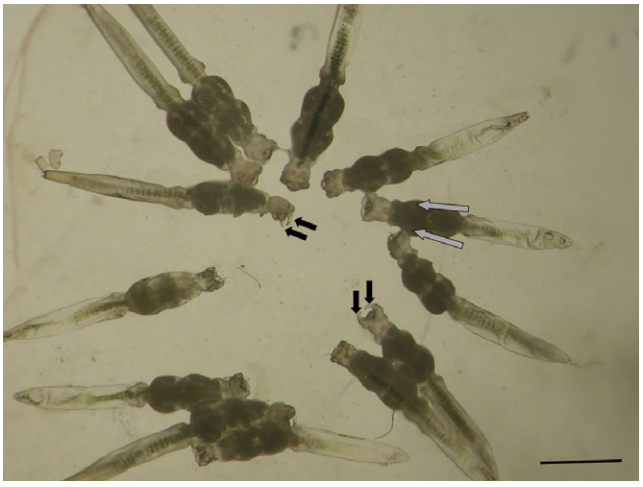

FIGURE 3 *Lamproglena pulchella* specimens detached from the gills of an asp. Females with well-developed ovaries—one on each side of the body. Ovaries indicated by white arrows in one individual, black arrows show the maxillae used for attachment of the gill filament. Bar = 0.5 cm

copepods, close to the tip of gill filaments, a depression appeared that was probably developed by the countereffect of the feeding activity of the copepod and regeneration process of the host tissue. Copepods plunged their claws deeply into the filament tissue. They attached to the gills with their maxillae and their bodies oriented along the length of the filament so that all appendages (including the maxillipeds and four pairs of biramous swimming legs) were in touch with it. When the copepods were mechanically detached from the gills, some rags of the host tissue frequently still adhered to their ventral surface. Although some *Dactylogyrus* (Monogenea) and *Trichodina* (Ciliata) specimens were also found their number was negligible. European catfish specimens arrived at the laboratory with a preliminary diagnosis of a cestode infection. Following the dissection of fish, 5–20 specimens of *Proteocephalus osculatus* Goeze, 1782 (Cestoda, Proteocephalidae) were indeed found in the intestine but the gills were heavily infected with a *Lamprolegna* sp. in all fish. The number of copepods varied from 200 to 450. In shape and size, these specimens corresponded to those found in asp; however, the claws of their maxillae proved to be much larger than those of *L. pulchella*. As far as gross pathology is concerned, a similar picture was observed in this case, as well. Crustaceans attached to the distal ends of gill filaments and their heads were driven far into the depressions. Their thorax and abdomen situated along the length of the gill filaments. Surprisingly, none of these female crustaceans carried egg sacs; however, the fully developed ovaries could be recognized easily in their thorax. In some fish, every third filament was invaded by the copepods. The tips of the infected filaments were thickened and whitish in colour. However, major bleeding, clubbing and fusion of the filaments were not recorded.

3.1 | Histopathological picture upon infection of the asp

In histological preparations made in longitudinal sections of the gill filaments, a thickening caused by cell proliferation at the site of the copepods was recorded (Figure 4). Lamproglena specimens attached close to the tip of the filaments, their heads penetrating deeply into the host tissue forming a cavity there (Figure 5). In their midguts among diffuse digested tissues, the remains of some cellular elements were seen (Figure 5 inset). Close to the head, the claws of the maxillae pierced the gill tissue from both sides of the cartilaginous gill rays. The claws often showed a double contour in section due to their recurving tips (Figure 5). At the attachment point of the maxillae to gill tissues, compressed cells of the multilayered epithelium appeared. Between the maxillae and the damaged epithelium, an amorphous material accumulated which was composed of red blood cells and fragments of epithelial cells (Figure 6). At the site where the claws pierced through the lamellar tissue of the gill (Figure 7), it became deformed and injured, and free red blood cells were released from the damaged capillaries. At these sites of the gill filaments, the original structure of the lamellae could not be recognized. The filaments were thickened, lamellae disappeared and only their remains were observed

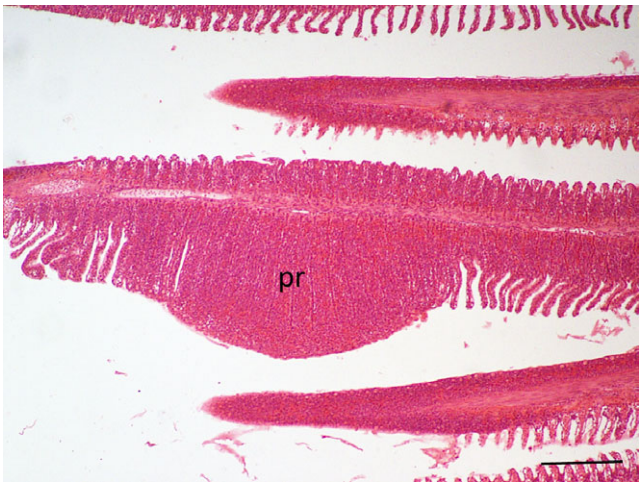

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FIGURE 4 Cell proliferation (pr) in the gill filament of the asp at the attachment site of Lamproglena copepods. Histological section, H & E. Bar = 250 μm

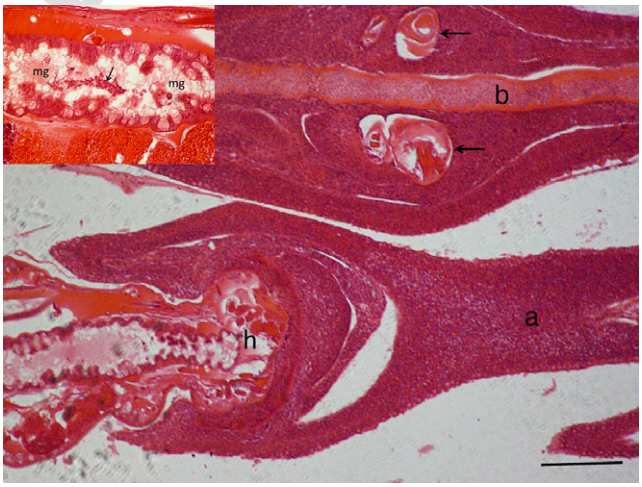

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FIGURE 5 Infection of two neighbouring gill filaments of an asp with *Lamproglena pulchella* specimens. (a) Gill filament in which the cephalothorax (h) of Lamproglena forms a hole. (b) Gill filament pierced through by the claws (black arrows) of the copepods. Inset: midgut (mg) of a *L. pulchella* specimen with semidigested host tissues (small arrow) inside. Histological section, H & E. Bar = 250 μm

deeply in the regenerative tissue. Remains of the capillaries were interspersed with the epithelial cells of the interlamellar epithelium. At other places, where the claws pierced the multilayered epithelium of the non-lamellated part of the filament (Figure 8) proliferation of the multilayered epithelium, formed from epithelioid cells, was recorded, but haemorrhage was seen only in the vicinity of the claws. Other parts of this regeneration tissue were composed of epithelioid cells, and mast cells occurred in large numbers between the remains of capillaries (Figure 9). In the vicinity of the attachment sites of *Lamproglena* specimen fusion of gill lamellae, hypertrophy of the lamellar epithelium and hyperplasia of goblet cells were recorded (Figure 10).

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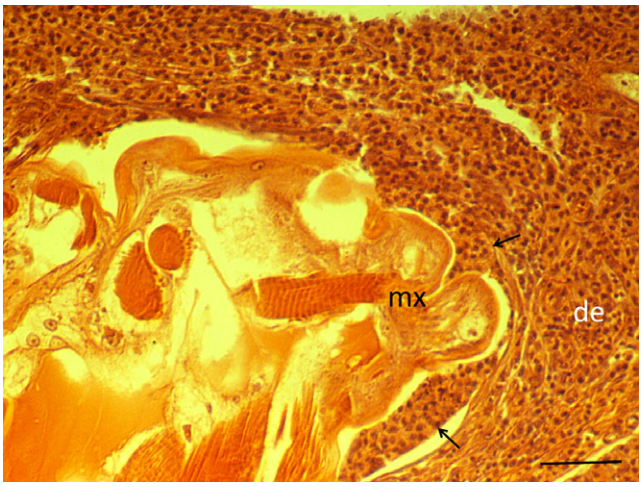

FIGURE 6 *Lamproglena pulchella* in the gills of asp. An accumulation of amorphous material (arrows) is seen between the maxillae (mx) and the damaged epithelium (de). This material consists of red blood cells and fragments of epithelial cells. Bar = 100 μm

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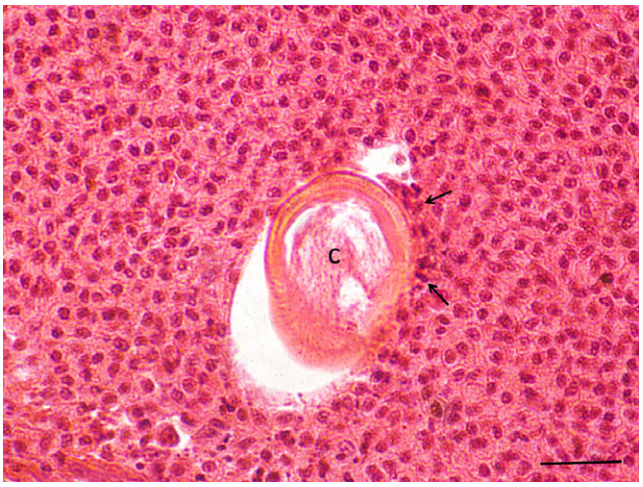

FIGURE 8 Cross section of a claw (c) pierced into the multilayered epithelium of the gill filament of an asp. Only minor haemorrhage (arrows) is seen at one side of the claw. Histological section, H & E. Bar = 50 μm

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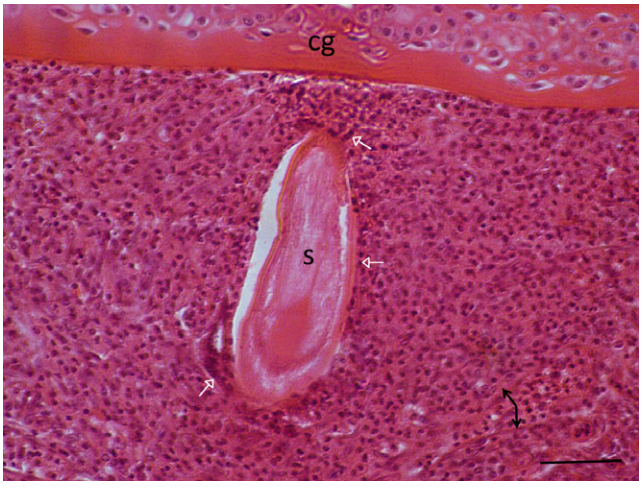

FIGURE 7 A claw(s) of a *Lamproglena pulchella* specimens pierced into the tissue of the gill filament close to the cartilaginous gill ray (cg). Around the claw, especially at the tip of the claw (open arrow) haemorrhage is seen. Inside regeneration tissue epithelioid cells and damaged capillaries are seen (black arrows). Histological section, H & E. Bar = 100 μm

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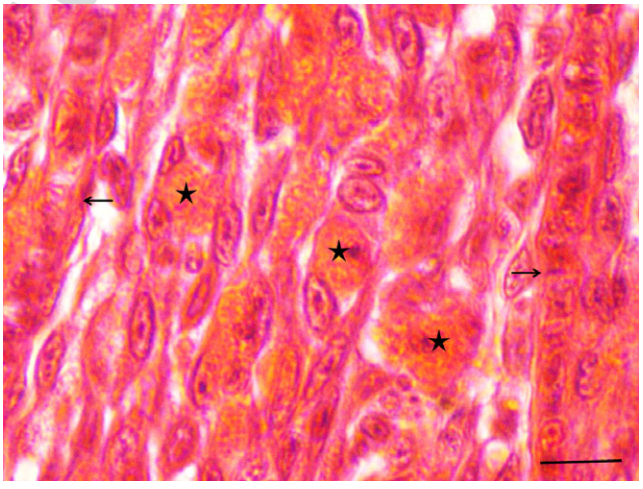

FIGURE 9 Proliferation tissue between two capillaries of the damaged gill lamellae of an asp (arrows). Among epithelial cells, several mast cells (stars) are located. Histological section, H & E. Bar = 15 μm

3.2 | Histopathological picture upon infected European catfish

Pathological changes of infection with a *Lamproglena* sp. were similar to those recorded in asp. Copepods also located close to the tip of the filament inserting their maxillary claws deeply into the tissue and causing hard depressions in the gill (Figure 11). Very often in these copepods the maxillae with buccal cavity, the suboesophageal ganglion, the midgut, the biramous legs and the posterior midgut with digested host tissues could be clearly identified even in histological slides. In host tissues around buccal cavity and especially at places where claws pierced the epithelium of the filament, agglomerations of Langhans-type giant cells and some mast cells appeared (Figure 11 and Figure 12). Goblet cells were present mostly in the superficial layers of the multilayered epithelium but close to the attachment sites their number significantly increased (Figures 11 and 12). Very often both claws were introduced into the gill filament from opposite sides of the cartilaginous gill ray damaging both the multilayered epithelium and the lamellar region of the filaments (Figure 13).

4 | DISCUSSION

Both the gross morphology and pathological picture caused by the two *Lamproglena* species in the asp and the European catfish were

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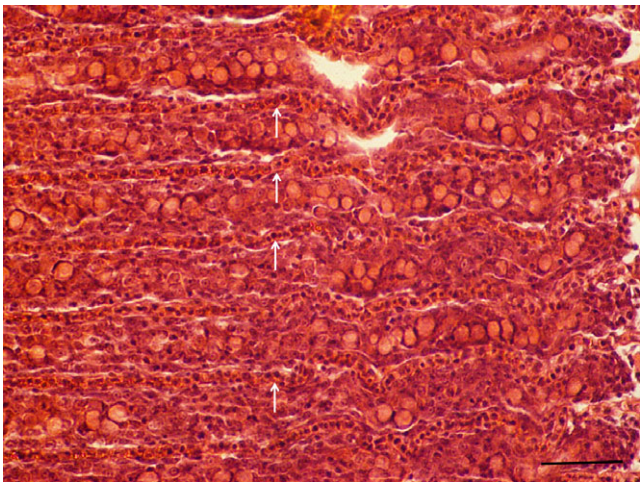

FIGURE 10 Goblet cells in the disarranged gill lamellae of a European catfish. The remainder of the capillary network (white arrows) is still observable. Histological section, H & E. Bar = 100 μm

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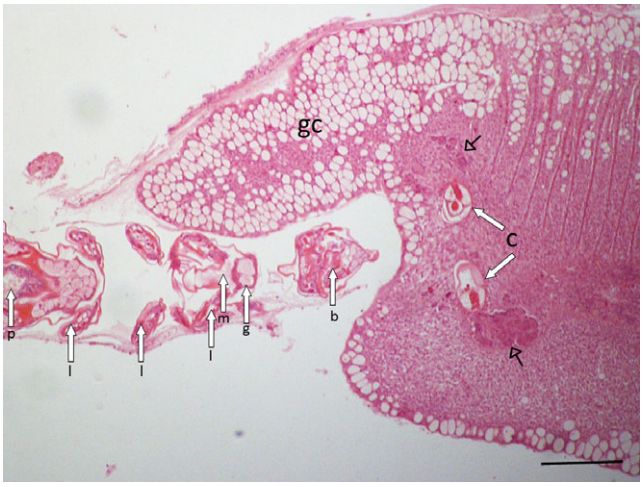

FIGURE 11 Attachment of a *Lamproglena* sp specimen to the gill filament of the European catfish. The thoracal part with buccal cavity (b) forms a hole in the filament. Inside the cross-sectioned parasite, the suboesophageal ganglion (g), a part of the midgut (m), the biramous legs (l) and a part of the posterior midgut (p) can be observed. The claws of the parasite's maxillae (c) penetrate into the gill tissue. Around the claws epithelioid tissue is formed, in which the goblet cells (gc) increase. Close to the claws, areas of giant cells (open arrows) are located in the granulation tissue. Histological section, H & E. Bar = 300 μm

similar. Although detailed morphological studies have not been performed, due to the significantly larger size of claws the species infecting the European catfish could not be confirmed to be *L. pulchella*. For studying and describing the species infecting the European catfish, the collection of further specimens of different age groups, a detailed morphological investigation and as far as possible molecular genetic studies are necessary. Currently, data on the DNA sequence of only two species, *L. orientalis* Markewitsch, 1936 and *L. chinensis* Yu, 1937, are available (Song, Wang, Yao, Gao, & Nie, 2008).

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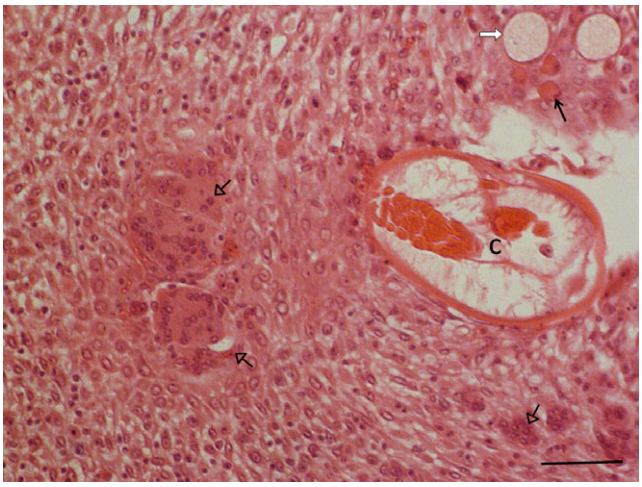

FIGURE 12 Degenerations in the multilayered epithelial tissue of the gill filament of the European catfish. Close to the piercing claws (c) of the *Lamproglena* sp, formations of giant cells (open arrows) appear. In the upper right corner, mast cells (dark arrow) and goblet cells (light arrow) are seen. Histological section, H & E. Bar = 150 μm

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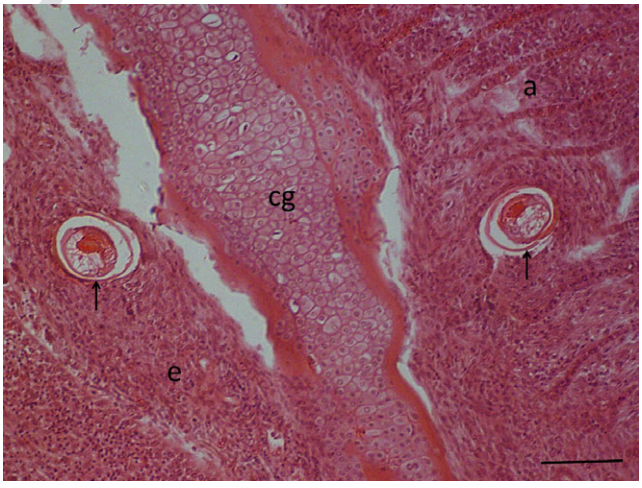

FIGURE 13 Gill filament pierced around the cartilaginous gill ray (cg) of a European catfish by the claws (arrows) of a *Lamproglena* sp in the lamellar region (a) and in the epithelial region (e). Histological section, H & E. Bar = 100 μm

Although the low number of examined fish and the lack of controls do not allow drawing general conclusions on the pathogenicity of *Lamproglena* spp., the great number of parasitic copepods found on fish in poor condition suggests a correlation between the emaciation of fish and degenerative changes in the gills. Specimens of both *L. pulchella* and *Lamproglena* sp. attached to the gills consistently close to the tips of the filaments forming a deep depression in the filament tissues. Damages were caused both by their feeding structures and by the claws on the maxillae introduced sharply into the lamellar and epithelial part of the filaments. At these sites proliferation of epithelioid cells, haemorrhages and degeneration of host tissues were the major signs. The pathogenic effect was enhanced by the maxillipeds and swimming

legs on the thorax which removed the superficial tissue through aberration. Host tissue, including blood was consumed.

The pathogenic effect of parasitic copepods is well known particularly through two species of salmon lice, *Lepeophtheirus salmonis* Kroeyer, 1837 and *Caligus elongatus* Nordmann, 1832, the skin parasites of the Atlantic salmon that cause heavy losses in cultured salmon stocks (Pike, 1989; Wootten, Smith, & Needham, 1982). Of the parasitic copepods infecting the gills of the freshwater fishes *Ergasilus* spp. are the best known pathogens. Einszporn (1964), Kabata (1970) and Kilian and Avenant-Oldewage (2013) described that these copepods, while feeding on epithelial cells, stimulate hypertrophy and cause coalescence of secondary lamellae. The tip of the damaged lamellae often shows clubbing close to the feeding sites of parasites due to the extensive proliferation of epithelioid cells. At infection of the see bream with *Ergasilus sieboldi* Nordmann, 1832. Dezfuli, Squerzanti, Fabbri, Castaldelli, and Giari (2010) and Dezfuli, Giari, Lui, Lorenzoni, and Noga (2011) besides hypertrophy of epithelioid cells observed haemorrhage, increase of rodlet cells, mast cells and mucous cells adjacent to the attachment site of copepods. Similar changes were caused by *Sinergasilus lieni* Yin, 1949 on silver carp in Hungary when besides the above mention signs, clubbing, fusion of neighbouring filaments were recorded (Molnár & Székely, 2004). In the present study, Lamproglena infections exhibited pathogenic changes on the tip of gill filaments. Although Tsotetsi et al. (2005) in infection with *L. clariae* on the African catfish described fusion and clubbing of gill filaments in our case neither of them were found. We regularly observed proliferation of gill tissues but instead of typical clubbing of the tip of the filaments only epithelial swellings on the spot of the claws was recorded. The pathogenic picture was dominated by degenerative changes. Most of changes described by Tsotetsi et al. (2005) were also recorded in both *L. pulchella* and *Lamproglena* sp. infections. The host tissue around the parasite head eroded, presumable by the scraping and rolling movement applied by maxillae and maxillipeds of the parasite. We observed proliferation of epithelial cells, and an increase in goblet and mast cell densities associated with the parasite. Goblet cells are normal components of the skin and the external layers of the gill epithelium. In the European catfish, their abundance characterizes the uninfected tissues, as well. In *Lamproglena* infections, however, we observed an increase in their number, and besides superficial regions of the epidermis, they were also found among the damaged lamellae. Due to the effect of claws, local haemorrhages were observed, and in the damaged lamellar region, mast cell proliferation was also recorded. A similar mechanical effect is supposed for damages observed around the maxillae of the copepods in tissue depressions. The presence of cell debris between the host tissue and the maxillae suggest the feeding of copepods on them. Although the applied staining method does not allow the identification of the damaged cell types, we suppose that the digested material and cell debris in the midgut comes for consumed host tissues and blood cells in a similar way as Tsotetsi et al. (2005) proved in *L. clariae* infection. In mammals and birds, degeneration processes are often followed by formation of giant cells, and in fish, their presence is only rarely mentioned mostly with nematode infections. In these rare instances, Molnár, Szakolczai, and Vetési (1995) observed them in the swim bladder with *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 infection due to invasion of second stage larvae, while Bakenhaster, Lowerre-Barbieri, Kiryu, Walters, and Fajer-Avila (2014) found giant cell formations with *Philometra* infections of fish. No information is available about giant cell formation at infection of fish in parasitic copepods. In our study following *Lamproglena* infection of the European catfish, typical Langhans-type giant cells developed in the vicinity of destructions caused by claws. The high intensity with some hundreds of copepods on the asp and the European catfish seems to indicate the poor condition of the fish, but we are sceptic about data of Öktener et al. (2008) that an infection with an average of 7–8 copepods might play a role in fish mortality.

Both the asp and European catfish are precious sport fishes. Their fingerlings are regularly resupplied to natural water, more over efforts are made with intensive and cage cultures of the European catfish Havasi et al. (2015). The future of these efforts will show whether an intensive lamproglenosis might play similar pathogenic role in these stocks as seen in lice infection in marine cage cultures.

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