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OCCURRENCE AND PATHOLOGY OF *SINERGASILUS LIENI* (COPEPODA: ERGASILIDAE), A PARASITE OF THE SILVER CARP AND BIGHEAD, IN HUNGARIAN PONDS

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Sinergasilus lieni Yin, 1949, a well-known and pathogenic parasitic copepod in China and Russia, has been detected in Hungarian carp farms for the first time. The parasite infected the third-year generation of silver carp (*Hypophthalmichthys molitrix*) and bighead (*Aristichthys nobilis*). The gills of the infected fish specimens showed severe pathological changes. At the attachment sites of female copepods clubbing and fusing of the gill filaments were observed and in some parts of the pale or whitish hemibranchia deep indentations were recorded in places where the tips of the damaged filaments had broken off. Silver carp and bighead were infected at a similar rate, having 8 to 27 copepods attached to the end of the clubbed filaments or the proliferated epithelium of 2 to 10 fused filaments. In histological sections the head part of the parasite was found in a deep cavity of the proliferated epithelium, piercing its antennae deep into the tissues. Only the end of the filaments showed changes. In this part the proliferated epithelium was infiltrated by eosinophilic granular cells. In the central and basal parts of the hemibranchia the original structure of the filaments was preserved with intact secondary lamellae.

Key words: *Sinergasilus*, parasitic copepod, pathogenicity, gills, silver carp, bighead

Fingerlings of silver carp *(Hypophthalmichthys molitrix)* and bighead *(Aristichthys nobilis)*, together with the grasscarp *(Ctenopharyngodon idellus)*, were introduced to Hungary from Chinese freshwaters in 1963. Fish introduced and placed into quarantine proved to be infected by a relatively rich parasite fauna (Szakolczai and Molnár, 1964, 1966). Further imports from Russia, which included also the age groups of spawners, enriched this parasite fauna with new pathogenic species such as *Bothriocephalus acheilognathi* (Molnár, 1970), and very soon severe diseases appeared among colonised fishes (Molnár, 1971*a, b*, 1976, 1979*a*). Most of the introduced parasites belonged to protozoans and

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monogeneans, while crustaceans were represented only by *Neoergasilus japonicus*. In addition to the latter species, *Lernaea cyprinacea,* a parasite that had been known from Europe for a long time, was commonly found on the colonised phytophagous fish species (Ponyi and Molnár, 1969). At the same time, from Central Asia and the European part of Russia several reports appeared on the pathogenic effects of introduced stocks of *Sinergasilus* spp. of Far Eastern origin. Bauer and Babaev (1964) found *Sinergasilus maior* (Markevitch, 1940) on grasscarp, while Musselius (1966) found the species *S. lieni* Yin, 1949 on the gills of silver carp and bighead. The developmental cycle and biology of *S. lieni* were studied by Mirzoeva (1972), while the pathological effect on the gills of the fish and the possible ways of control were investigated by Musselius (1967) and Bauer et al. (1981). Damage caused by this parasite is rather common in China (Nie and Yao, 2000; Wang et al., 2002). These latter authors, however, identified the parasite with the species *S. polycolpus* (Markevitch, 1946).

Until recently, 10 species of parasitic copepods *(Ergasilus sieboldi, E. nanus, E. gibbosus, Neoergasilus japonicus, Paraergasilus rylowi, Lamproglena pulchella, Lernaea cyprinacea, Achtheres percarum, Tracheliastes polycolpus* and *T. maculatus)* had been known from Hungary (Ponyi and Molnár, 1969). Two of these species, *Ergasilus sieboldi* and *L. cyprinacea,* proved to be pathogenic to fishes. *E. sieboldi* is a frequent parasite in natural waters, but the gills of pond-cultured fishes are only exceptionally infected by this parasite and there are no data on its occurrence on phytophagous fishes (Molnár and Székely, 1995, 1997). In the latter fishes, however, *L. cyprinacea* might cause heavy infections in fish farms (Molnár, 1979*b*; Molnár and Szakolczai, 1980).

The present paper reports frequent and severe infections of silver carp and bighead with *Sinergasilus lieni* Yin, 1949 affecting the gills of the older generations, and gives a detailed histopathological description of the changes caused by these copepods.

Materials and methods

Silver carp and bighead specimens of different age groups were obtained from two fish farms of Hungary. One of the farms was located in the eastern part of the country, while the other was close to Budapest. In 2001 and 2002 altogether 72 specimens of fish were examined. Of the three-year-old age group 7 silver carp and 6 bighead, of the two-year-old age group 23 bighead, while from the fingerlings 23 silver carp and 23 bighead specimens were dissected. Fingerlings and two-year-old fishes were examined in different seasons of the years, while three-year-old fishes were examined only in March and November. In addition to these fishes 6 two-years-old grasscarp and 20 grasscarp fingerlings were examined from the same fish farms. In addition to fish dissected in the laboratory, fish samples were examined at the ponds by opening the gill covers and

looking for macroscopic changes of the gill filaments. At the beginning of the examinations a complete parasitological dissection was carried out; after observing the first cases of *Sinergasilus* infection, however, only the gills were studied. In the case of *Sinergasilus* infection the hemibranchia of the gills were cut off and checked for the location of parasites. Some of the copepods were carefully picked off the gills and placed in 70% ethanol, while portions of the gills showing pathological changes, together with attaching copepods, were fixed in Bouin's solution for 4 h, washed several times in 80% ethanol, embedded in paraffin wax, cut into 4 to 6 μm thick sections and stained with haematoxylin and eosin. Low magnifications were photographed with an Olympus C4040 digital camera mounted on a stereomicroscope, while photos of the attaching copepods and the histological changes caused by them were taken with an Olympus DP-10 digital camera mounted on an Olympus BH2 microscope.

Results

Among the third-year stocks of silver carp and bighead *Sinergasilus* infection was well observable already by gross examination of the gills at the ponds. Most of the fish harvested showed macroscopic lesions on the gills. By opening the gill cover of the fish, gills showed a pale colour, the tips of the filaments grew together into a whitish mass, and in shorter or longer sections deep indentations of the hemibranchia were seen. No macroscopic alterations were found in fish of the first- and second-year age groups.

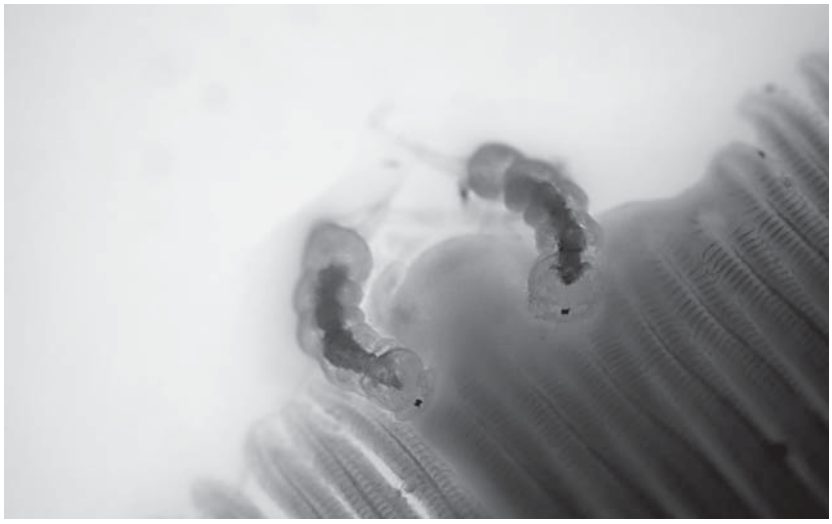
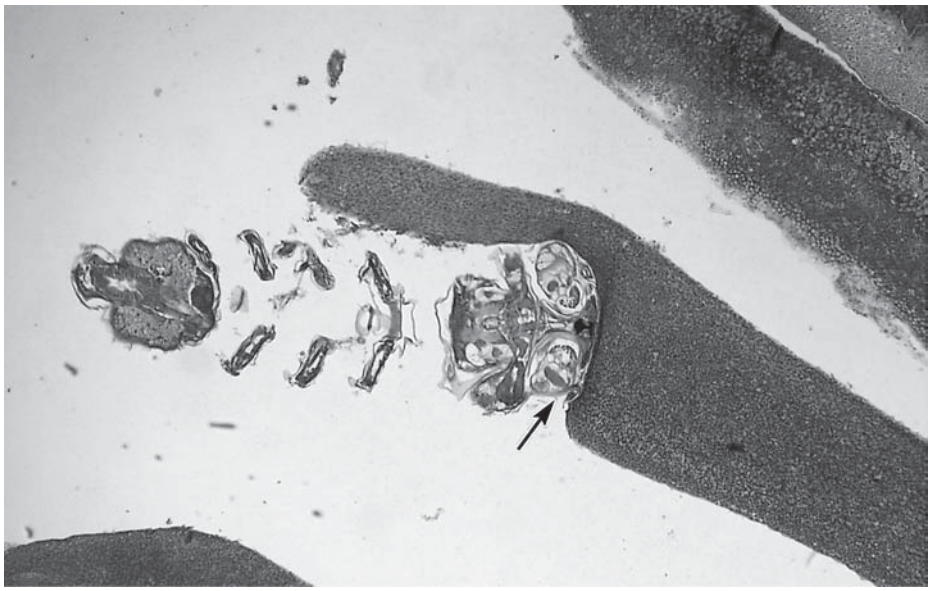


Fig. 1. Sinergasilus lieni females without eye sacs attach to the proliferated and fused gill filaments of a silver carp. Fresh preparation, × 17



Figs 2–8. Haematoxylin and eosin stained histological sections of the gills of silver carp infected by *Sinergasilus lieni*. *Fig. 2. S. lieni* female attaching to the clubbed end of a gill filament. The mouth part of the copepod (arrow) is located in a deep impression of the proliferated epithelium. × 53

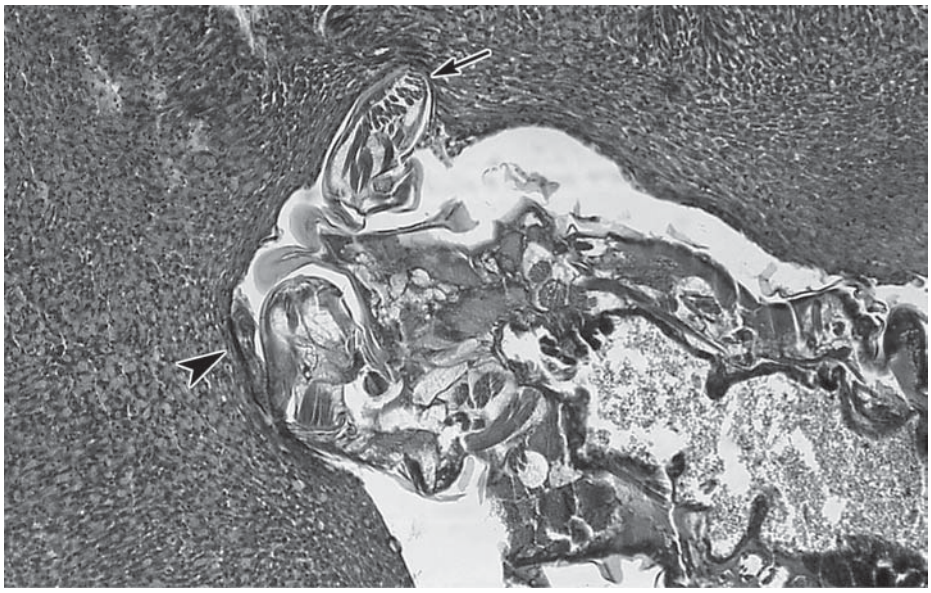


Fig. 3. A female specimen of *S. lieni* forms a hole in the epithelium of fused neighbouring lamellae. One of the large antennae of the parasite is pierced into the tissue (arrow). The epithelium around the head shows a cuticle formation (arrowhead). × 126

Among fish dissected in the laboratory, six out of the seven examined three-year-old silver carp and five out of the six three-year-old bighead specimens proved to be infected by *S. lieni* (Fig. 1), but signs of a passed-off infection were encountered also in fish specimens free from copepods. The number of copepods present on the gills of the 11 infected fish varied between 8 and 27 specimens. Infection was found both in the spring and the autumn months. Neither the fingerlings nor the small-sized two-year-old fishes harboured parasitic copepods. No parasitic copepods were found on grasscarp either.

All parasites were located at the edges of the gill filaments, some of them grabbing only a single filament (Fig. 2), while some others were attached to the proliferated epithelium of the fused gill filaments (Figs 1 and 3). Parasites, both in solitary filaments and in the fused ones, formed a hole at their attachment site in the proliferated epithelium. All parasites found proved to be imago-stage females, some of them having egg-sacs at the tail. The morphological features of the copepods, identified as *S. lieni*, corresponded to the species descriptions presented by Gussev et al. (1987).

In histological sections, pathological changes were restricted mainly to the area of the filament tips. In some parts fusion of several filaments due to intensive epithelial proliferation was seen (Figs 3 and 4) while other areas were characterised by clubbing of individual filaments (Fig. 2). Both in fused areas and in separated filament regions attaching *Sinergasilus* specimens could be observed fixed to the fish tissues by their antennae (Figs 3 and 5). The robust claws of the large antennae were deeply pierced into the proliferated epithelium of the filaments (Figs 5 and 6), reaching the connective tissue around the cartilage and the arteries running alongside it (Fig. 7). Inside the affected area proliferation and degeneration of the connective tissue were also recorded (Fig. 7). Around the feeding organs of the copepods there was a deep depression in the proliferated epithelium (Figs 2 and 3). The superficial layer of the epithelium in these parts lost its original structure and epithelial cells became flattened like those of the skin, with a cuticle-like formation above the flattened cells (Figs 2 and 8), and cell debris was also seen around the damaged area. The proliferation, fusion and clubbing always affected only the distal part of the gill filaments but the central parts and portions close to the cartilaginous gill arch preserved their original structure (Fig. 4). Proliferation tissue was mostly composed of proliferating epithelial cell of the multilayered epithelium of the non-lamellar part of the filament, but it contained a large number of eosinophilic granular cells (Figs 7 and 8), which are normal elements of the gill tissue, but in this case their number was greatly increased. In some areas of the proliferated zone remnants of the secondary lamellae were also seen with damaged capillaries (Figs 5, 6 and 7). Within the mass of epithelial cells and granular cells some free erythrocytes could also be recorded, but granulocytes or lymphocytes were not seen. In a similar way, there was a shortage in goblet cells which are normal elements of the healthy gill tissue.

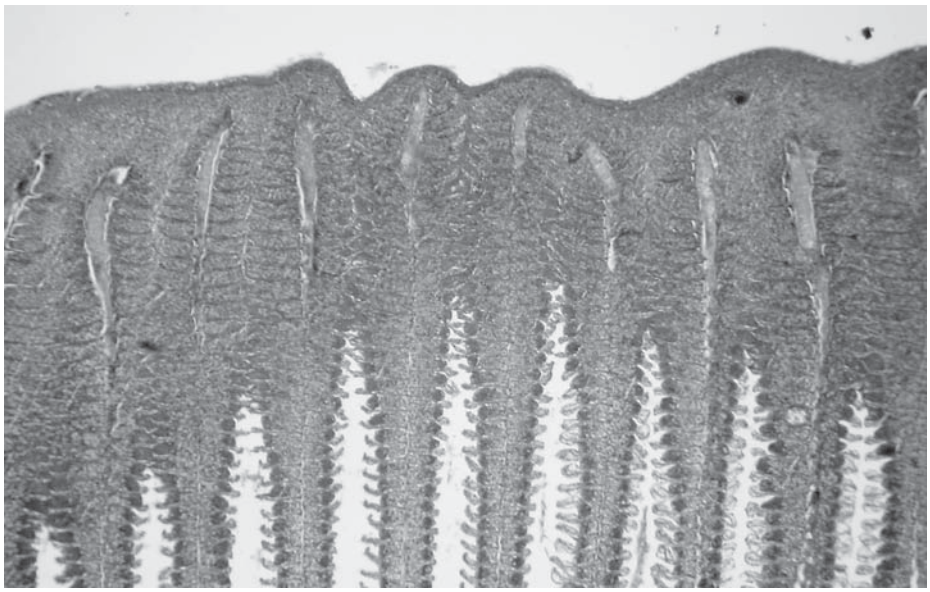


Fig. 4. Distal part of the fused filaments. The filament tips grow together due to epithelial proliferation; however, in the central and proximal region of the filaments the gill lamellae seem to be intact. × 39

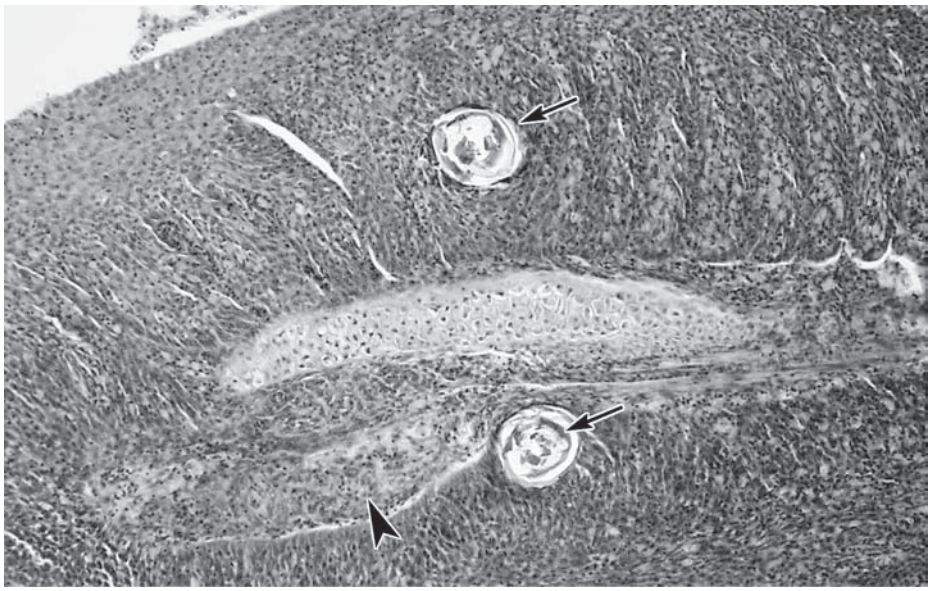


Fig. 5. The picture shows cross-sections of antennae (arrows) piercing through the gill filament. Inside the proliferated epithelium remnants of the capillary network of the lamellae are still recognisable. Close to the piercing robust antennae granulation tissue around the damaged gill arteries is seen (arrowhead). × 147

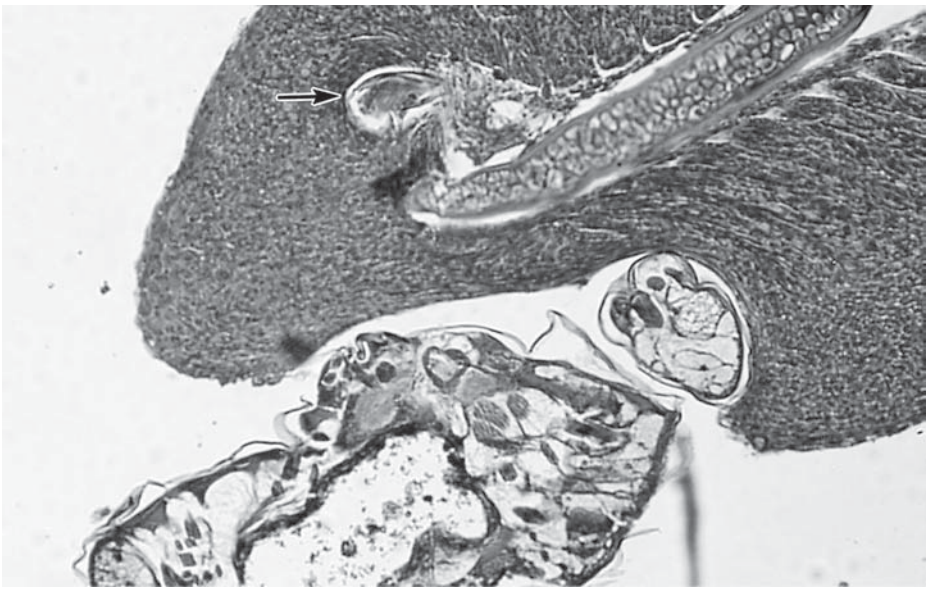


Fig. 6. Head part of a *S. lieni* inside impression of the filament tip. A cross-sectioned part of the antennae (arrow) is pierced into the connective tissue. × 142

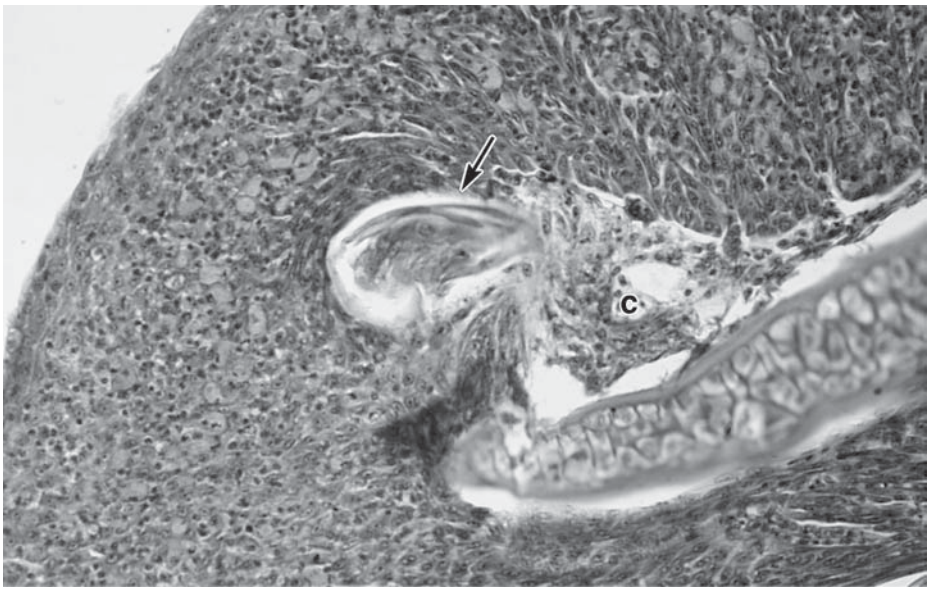


Fig. 7. Enlarged part of *Fig. 6.* The piercing antenna of the copepod (arrow) is introduced into the adventitia of the gill artery causing local proliferation of the connective tissue (c). × 270

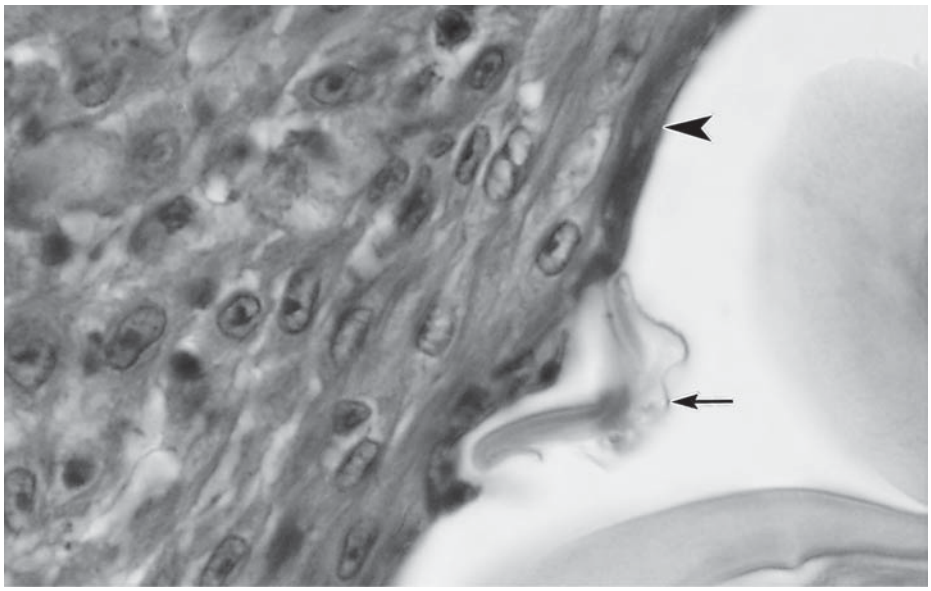


Fig. 8. Around the feeding antennae of the copepod (arrow) the epithelial cells are flattened and at the surface they show a cuticle-like formation (arrowhead). Inside the antennae degenerated cells and cell debris are located. × 1216

Discussion

Changes induced by parasitic copepods on the gills of fishes are mostly known from studies on *Ergasilus sieboldi*. Important data on changes caused by different *Ergasilus* spp. can be obtained from books written on fish diseases (Schäperclaus, 1954; Dogiel et al., 1961; Bauer et al., 1981), but histological works concerning changes on cellular level are rare. Destruction and clubbing of the tip of the filaments and proliferation of the gill epithelium at the attachment points of *E. sieboldi* were relatively well described by Schäperclaus (1954), Zmerzlaya (1972) and Alston and Lewis (1994), and useful data have been reported on the histopathological effects caused by *E. cyprinaceus, E. lizae* and *Pseudoergasilus zacconis* by Rogers (1969), Roubal (1986) and Nakajima et al. (1974), respectively. Schäperclaus (1954), Rogers (1969) and Alston and Lewis (1994) described the fusion of filaments by a solid mass of proliferated epithelial cells as well. Alston and Lewis (1994), who studied *E. sieboldi* infection of the tench, reported also haemorrhages and inflammation accompanied by granulocytosis, while Dezfuli et al. (2003) found mucous cell proliferation and an increase in the number of eosinophilic granular cells and rodlet cells. These latter authors regarded rodlet cells as cells representing an inflammatory cell type. Some of the authors (Rogers, 1969; Roubal, 1986) reported lymphocyte and granulocyte infiltration into some areas of proliferation. The histopathology of *S. lieni* infection was studied by Musselius

(1967), who observed degeneration and breakage of the gill filaments at the attachment points of the parasites. The latter author also noted that one copepod could affect 5 or 6 lamellae, causing their fusion.

In the present case neither lymphocyte infiltration nor increase in the number of granulocytes was found. The host response was characterised by a massive proliferation of the lamellar epithelium; the epithelial cells, however, were infiltrated by eosinophilic granular cells. These cells, which are commonly found among the epithelial cells of the non-lamellar tip of the gill filaments, are regarded as normal elements of the healthy gill structure, but their number increases very often. Reite (1997), who identified these cells with mast cells, stated that the number of eosinophilic granular cells increases in persistent inflammations due to helminths or unknown causative agents. In the cases studied by us we concluded that already a relatively low number of the parasites can cause severe histological changes, since in infections with 8 copepods and 27 copepods similar extensive changes were observed. We think that *S. lieni* is a new pest for Hungarian fish culture and its pathological effect warrants more attention in the future.

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