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The gill monogenean *Sciadicleithrum variabilum* induces histomorphological alterations in the gill tissues of the discus *Symphysodon aequifasciatus*

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ABSTRACT: High mortality is among the most serious problems and challenges in the ornamental fish trade. Examination of the discus *Symphysodon aequifasciatus* from ornamental fish hatchery revealed infestation with the monogenean *Sciadicleithrum variabilum*. Gill infestation with this monogenean induced serious damage to the gill lamellae, including clavate lamellae, vascular congestion in the peripheral blood vessels, lamellar blood sinus dilation, and other structural anomalies. Light and transmission electron microscopy showed that in all infested hosts the interlamellar cell mass (ILCM) completely filled the interlamellar space. The monogenean-associated damage combined with the ILCM led to severe impairment of respiratory efficiency of the gill. Anti-parasitic treatment was applied during breeding (hatchery), which was followed by almost complete regression of the ILCM seen in the fish. A single point of ILCM hyperplasia was observed in only one specimen at the site of parasite attachment to the gill filament. The ILCM covering the gill lamellae protected the discus against infestation with this monogenean, but considerable reduction in the gaseous exchange surface and serious damage to the gill lamellae contributed to the increased mortality of the fish in the hatchery, which reached 90%.

KEY WORDS: Discus · *Sciadicleithrum variabilum* infestation · Gill lesions · Fish transportation · Ornamental fish trade

1. INTRODUCTION

It is estimated that the annual trade in ornamental fish is worth approximately US$30 billion (Raghavan et al. 2013). The ever-increasing popularity of household aquaria worldwide favors further development of ornamental trade (Olivier 2003). Unfortunately, high mortality is observed in the ornamental fish trade (Olivier 2003, Masud et al. 2019). The above situation results in huge economic losses and depletion of ornamental fish populations in the wild (Olivier 2003). The trade of ornamental fish has rarely been reliably monitored (Biondo & Burki 2020), and careful estimation of the causes of mortality requires detailed research. More than 1 billion fish are traded annually throughout the world (Tru-

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jillo-González et al. 2018). The transportation of fish is associated with multiple stressors causing increased disease susceptibility (Trujillo-González et al. 2018, Masud et al. 2019). Parasitic infestations have a strong negative influence on aquaculture (Shinn et al. 2015). The accurate assessment of the risk related to the occurrence of parasitic infestations enables the development of more appropriate biosecurity practices (Shinn et al. 2015). Although anti-parasitic chemicals are routinely used to reduce disease outbreaks in the ornamental fish trade, infestations caused by monogeneans are a serious problem, in addition to which there is only a short list of approved treatments (Trujillo-González et al. 2018, Masud et al. 2019). Deterioration in water quality associated with the accumulation of metabolic waste products and potentially other pollutants are additional risk factors that may adversely affect the health of farmed fish, either directly or by increasing their susceptibility to infectious diseases and ectoparasites (Abdel-Latif et al. 2020).

In fish culture, ectoparasite infestations are prevalent and can impair gill function, leading to respiratory and osmoregulatory failure (Ojha & Hughes 2001), and this is unsurprising considering gills are among some of the most complex and sensitive structures (Evans et al. 2005, Wegner 2011, Gilmour & Perry 2018). In environmental conditions that impair physiological function, the gill epithelia can become eroded and inflamed (Mallatt 1985). The gill lamellae may also be infested by monogeneans — small hermaphroditic flatworms with an unsegmented body and direct life cycle. Most monogeneans are ectoparasites equipped with 2 attachment organs: the prohaptor (anterior) has 1 or 2 suckers, while the opisthaptor (posterior) has a differentiated structure. Some species (*Dactylogyridae*) have 2 pairs of eyespots (Kritsky et al. 1986, 1989, Aguinaga et al. 2015).

The discus fish genus *Symphysodon*, has a natural distribution in the Amazon basin. Discus fish of any species have always been highly rated by aquarists (Onal et al. 2011, Aquaro et al. 2012).

Cultures of the discus *Symphysodon aequifasciatus* Pellegrin, 1904 exist in Europe, South America, and Asia. Their propagation is an important source of income for some Asian countries (Aquaro et al. 2012, Wen et al. 2017). High mortality of discus in fish facilities can cause large economic losses for fish breeders, and such situations have been reported in many countries, including Poland (Onal et al. 2011, Aquaro et al. 2012, Sobecka et al. 2012). Discus breeders in Poland suspect that the high mortality observed in their cultures can be caused by gill parasites (communications from the breeders). The aim of this work was to examine the histomorphology of the gill apparatus in *S. aequifasciatus* in a culture with an outbreak of a severe respiratory disease and a high mortality rate of up to 90%.

2. MATERIALS AND METHODS

2.1. Hatchery

The discus farm examined in our study consisted of 18 pairs of discus fish, and approximately 100 young individuals were obtained from each pair. Infestation appeared in the progeny of the 18 initial breeder fish. Symptoms of infestation appeared 1 mo post-hatch, and the fish had rapid breathing (movements of the gill operculum). Visual inspection provided by the breeder showed that the slowest growing individuals died between 2 and 3 mo after hatching. Only the largest individuals (the fastest growing) survived. The highest observed mortality (up to 90%) occurred between 80 and 90 d after hatching. Infestation with monogeneans was also found in adults, though none of them died during the period of observation.

2.2. Materials and treatment

On the fish farm, the discus were kept in recirculating tanks (50 fish per 300 l) under a 12 h light:12 h dark cycle. Water quality was managed by an aquarium sump filtration system (Argus) eliminating ammonia (ammonia level below 1 ppm). The other water parameters were as follows: temperature 27 ± 2°C; pH 5.7–6.7; and dissolved oxygen 9 mg l^{-1}. The animals were fed an artificial diet (Tropical Discus Gran Wild) at a daily ratio of 5% of wet body weight.

The 1st group (Group 1, untreated), consisted of 17 specimens aged 3 to 6 mo (weight 0.81–9.6 g) and was characterized by a very high mortality of up to 90%. The 2nd group (Group 2, anti-parasitic treatment), included 17 specimens age 3 to 6 mo (weight 0.59–11 g) after a 3 mo anti-parasitic treatment.

2.2.1. Treatment procedure. Group 2 was treated with Tetra Medica Lifeguard tablets (diflubenzuron): 1 tablet d^{-1} for every 19 l of aquarium water for 5 consecutive days at 24 h intervals. After 3 wk, Tetra Medica TremaEx (1 ml per 20 l water) was used for 7 d. Then, after an additional 3 wk, Zoolek Capiforte

treatment (10 ml per 100 l water) was performed for 7 d with repetition (the same dose as earlier) after a 1 wk break. Then, a daily partial water change was applied after carefully collecting all contaminants from the entire bottom surface, thus eliminating possible eggs and larvae of monogeneans.

2.2.2. Breathing frequency. The breeder measured the breathing frequency (operculum movements min^{-1}) 1 h after feeding 10 randomly selected individuals (5 from each group).

2.3. Sampling procedure

The study involves the retrospective analysis of animals under standard care in a breeder farm (Statistics Poland, Permission No. 5521397155). All activities were conducted as part of routine care on the farm and were independent of any experimental research. The fish sample, *Symphysodon aequifasciatus* (n = 34), was obtained from a commercial breeder, a fish exporter to EU countries. The sampling procedure and euthanasia of the fish were carried out by the breeder at the discus farm.

Fish were quickly removed from the breeding aquaria and anesthetized using an overdose of buffered MS 222 (pH 8.0, ethyl 3-aminobenzoate methane-sulfonic acid, 1 g l^{-1}, Acros Organics) (Sinha et al. 2014). All gill arches were removed from each fish under a stereomicroscope (Carl Zeiss Discovery V12) in phosphate-buffered saline (PBS). The gills from the left side of the fish were subjected to H&E staining (34 specimens), and those from the right side were used for parasite counting and identification (7 specimens), mitochondria-rich cell (MRC) analysis (4 specimens), transmission electron microscopy (TEM) studies (5 specimens), and histomorphometry (8 specimens).

2.4. H&E staining

Gills were fixed in 4% formaldehyde (FA) in phosphate buffer (pH 7.4) for 12 h. Next, the tissues were dehydrated in ethanol, treated with xylene, embedded in paraplast wax, and sectioned using a Rotary Microtome HM 310. The dewaxed sections (2.5 µm) were treated with Mayer's H&E Y (Diapath). Subsequently, the sections were dehydrated in a graded ethanol series and mounted under coverslips in DPX medium (Distyrene Plasticizer and Xylene). Photographs were taken with a Nikon 2000 SE optical microscope with a Zeiss camera.

2.5. Mitochondria-rich cell (MRC) test

Four specimens were used in the MRC test (n = 2 from Groups 1 and 2). Gill arches were sectioned at 1.5–3 mm and stained using the test according to the procedure described by Kaneko & Shiraishi (2001) with minor modifications. Slides were analyzed under a Nikon 2000 SE light microscope (LM) with a Zeiss camera and NIS-Elements Br software. Note that chloride cells are visible with standard H&E staining, but for confirmation, specific staining was used (Kanenko & Shiraishi 2001 method).

2.6. Electron microscopic (EM) study

The gill filaments (n = 5 from Group 1) were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 4 h at 4°C. Following washing in phosphate buffer, the samples were postfixed in 1% osmium tetroxide (Polysciences) for 2 h at 4°C, dehydrated (ethanol: 10–100%), infiltrated (propylene oxide-resin mixtures), and embedded in epoxy resin (Epon). Ultrathin sections (approximately 100 nm thick) were cut with a diamond knife and a Leica Ultracut UTC ultramicrotome. The sections were examined using a TEM (model JEM 1400; JEOL) equipped with a camera (MORADA G2, EMSIS) in the Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology, PAS, Warsaw (Poland).

2.7. Parasite identification

The gill arches from the left side (Group 1) were removed and placed in vials containing formalin (4% solution). The contents of each vial were examined under a stereomicroscope, and the monogeneans were collected with a pipette. The monogeneans were identified from wet mounts on microscopic slides and examined under an Olympus BX 50 microscope with a differential (Nomarski) interference contrast (DIC). The monogeneans and their sclerotized parts were measured using a Nikon Eclipse TE 2000-S microscope, and their species were determined according to Kritsky et al. (1986, 1989).

2.8. Histomorphometry

The second gill arch of 8 fish from Group 1 (n = 5) and Group 2 (n = 3) were used for morphometric

study. Three gill filaments were removed from each second gill arch, and the average number of secondary lamellae per 1 mm of filament length was determined. Then, the gill filaments were dehydrated in alcohol, cleared in xylene, and fixed in DPX medium on microscope slides. Seven well-separated gill lamellae (from each filament) were measured using a Nikon 2000 SE with a Zeiss camera and NIS-Elements Br software. The following parameters were measured to estimate the changes: protruding lamellae (PL), embedded lamellae (EL), surface area of interlamellar cell mass (ILCM), mean lamella height, PL height, PL width, water-blood diffusion distance, width of capillary, ILCM height, and surface area of lamella.

2.9. Statistical analysis

Statistical analysis involved the correlation between the mean number of gill lamellae per 1 mm length of gill filament and the body mass (W), as well as between the mean surface area of gill lamellae and W. The results were compared with analogous results available in the literature (Jakubowski 1992, Satora & Romek 2010, Satora & Wegner 2012). Spearman correlations among the tested traits were calculated on the means using cor.test function in R programming. The mean values were compared using Student's t-test.

Note that the discus second gill measurements followed the latest techniques used at Jagiellonian University; Jakubowski (1992) took gill area measurements for the following teleost species: pike *Esox lucius*, ruffe *Gymnocephalus cernuus* and perchpike *Sander lucioperca* (Jakubowski 1992, Satora & Romek 2010, Satora & Wegner 2012).

3. RESULTS

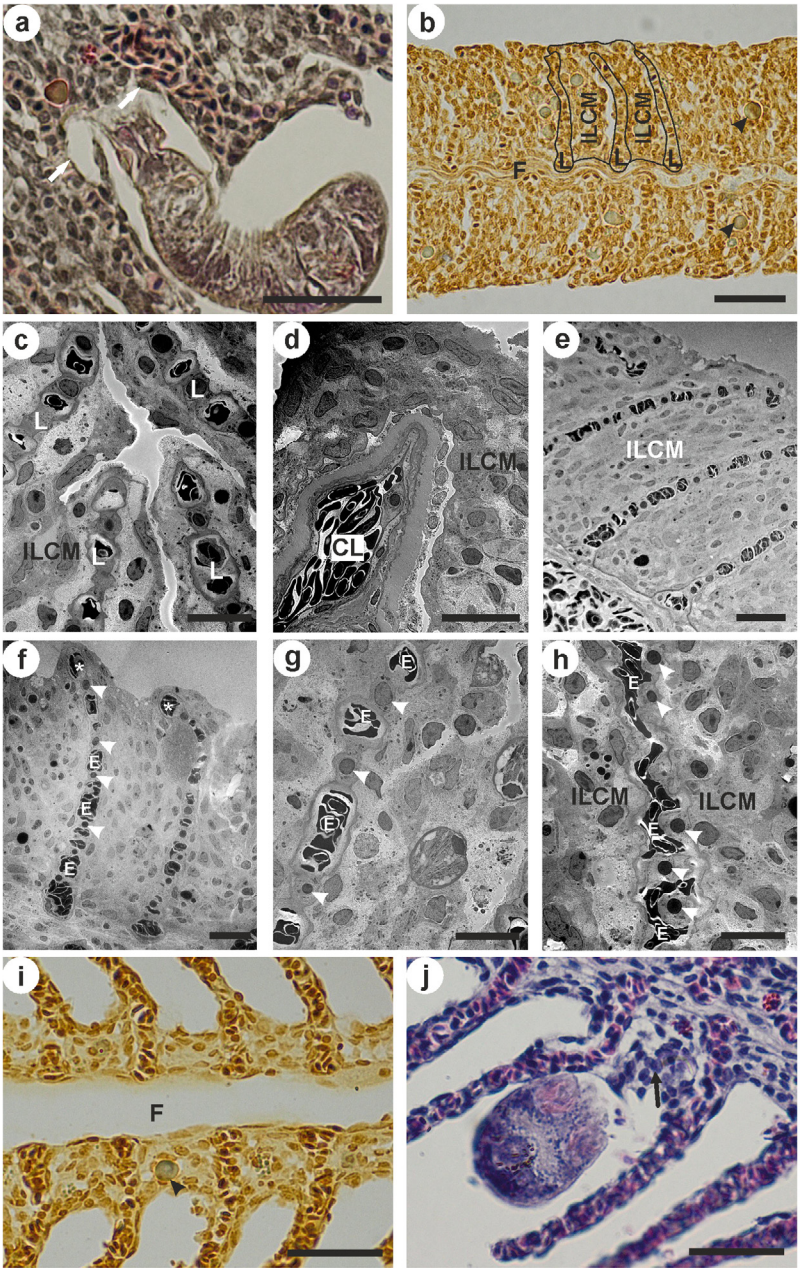
3.1. Light microscopic observations

3.1.1. Group 1 (infested). The LM observations showed that all the gill filaments of the discus were infested with the ectoparasitic monogenean *Sciadicleithrum variabilum* (Mizelli & Kritsky, 1969) (Figs. 1a & 2a–c). The parasites were distributed throughout the length of all gill arches. The intensity of infestation was very high (from 236 to 1572 parasites per specimen), and it was the highest in the specimens with the smallest body mass (Table 1). The prevalence of *S. variabilum* in this study was 100%. Spearman correlations showed a negative correlation (−0.82; p = 0.03) between body weight and the number of parasites (Table 1). Gill lesions were evident at the sites of contact with the monogeneans, as indicated by the distorted lamellar structure in the vicinity of the haptor (Fig. 1a). Light micrographs of the gills showed lamellae largely embedded in the ILCM. Exposure to parasites triggered a striking change in gill morphology. The gill filaments resembled 'sausage-like' forms, completely lacking PL (Fig. 1b). A few PL were visible only on single gill filaments in this group (n = 4) (Table 2). The gill filaments of all the remaining specimens (n = 13) were completely embedded in the ILCM, without PL (Fig. 1a,b).

3.1.2. Group 2 (anti-parasitic treatment). LM studies showed the presence of typical, ILCM-free gill lamellae in all the examined fish (n = 17) (Fig. 1i). No damage to the gill lamellae was noted. The MRC test showed an occasional appearance of MRC on the gill filaments (Fig. 1i). Only 1 fish was observed carrying a single monogenean; it had an elevated ILCM at the site of parasite attachment in the first gill filament (Fig. 1j). The rest of the filament was ILCM-free. No gill lesions were observed in this group.

3.2. Histomorphometry

3.2.1. Group 1 (infested). The histomorphometric characteristics of the second gill arches in infested discus (Group 1) showed no correlation between the body weight and the mean number of gill lamellae per 1 mm gill filament length (Tables 2 & 3). The bilateral surface area of an average secondary lamella increases with body weight (Table 2), but in this group, there was no correlation between the body weight and the mean bilateral surface of lamella (Table 3). In selected representatives of Perciformes and *E. lucius*, body weight showed a strong negative correlation with the average number of gill lamellae per 1 mm gill filament length (Table 3). In these species, body weight correlated positively with the mean bilateral surface of lamella (Table 3). In the 1st group of discus, the mean capillary diameter in the gill lamellae and the water-blood distance increased with body mass (Table 4); likewise, the mean diameter of the ILCM-embedded capillary increased with body mass (Table 4). The mean water-blood distance in the protruding and EL in the larger specimen was the same as that in the smaller specimen (Table 4). In the smaller specimen,



the mean water-blood distance was shorter in the PL than in the ILCM-EL (Table 4).

3.2.2. Group 2 (anti-parasitic treatment). The histomorphometric characteristics of the second gill arches in the discus after treatment showed that the bilateral surface area of the average secondary lamellae increased with body weight (Table 2). Additionally, the mean capillary diameter in the gill lamellae and the water-blood distance increased with body weight (Table 4). In this group, there was no correlation between the body weight and the mean number of gill lamellae per 1 mm gill filament length or between the body weight and the mean bilateral surface of lamella (Table 3).

The discus from Group 1 made 65 ± 5 SD operculum movements min^{-1}. The discus from Group 2 made 50 ± 6 SD operculum movements min^{-1}. There was a significant difference in the parameter operculum movements min^{-1} between Groups 1 and 2 ($p < 0.05$, df = 4, $t = 2.776$).

3.3. Mitochondria-rich cells

In Group 1 (infested), numerous MRCs were visible as embedded in the ILCM between the gill lamellae. The MRC was ILCM-covered on the water-facing surface (Fig. 1b). In Group 2 (anti-parasitic treatment), MRCs were visible in the gill filament (Fig. 1i).

Fig. 1. (a,b,i,j) Light and (c–h) electron micrographs of the middle parts of gills of *Symphysodon aequifasciata*. Tissue from (a–h) Group 1 (infested gills) and from (i,j) Group 2 (anti-parasite-treated group). (a) Tissue infested by *Sciadicleithrum variabilum*; white arrows indicate damage to gill lamellae. (b) Gill filament (F) of *S. aequifasciatus* infested by *S. variabilum* stained by Kanenko & Shiraishi method (2001). Three lamellae (L) and associated interlamellar cell mass (ILCM) are outlined. Note extensive epithelial cell hyperplasia causing total obstruction of respiratory surface area. Note mitochondria-rich cells (MRC) (arrowheads) located in the ILCM. (c) Ends of adjacent ILCM-embedded gill lamellae (L). (d) Clavate lamella (CL), lamellar aneurism, ILCM-embedded. (e) Undifferentiated cells fill most of the ILCM in *S. aequifasciatus*; ILCM fills the whole space between gill lamellae. Lack of protruding lamellae. Nerves and blood vessels are absent from ILCM. Capillary with erythrocytes and pillar cells visible in gill lamella. (f) Gill lamella embedded in the ILCM, vascular congestion in peripheral blood vessels (white asterisks), lamellar blood sinus dilation with erythrocytes (E), and pillar cells (white arrowheads) are visible. (g) Gill lamella embedded in the ILCM, lamellar blood sinus dilation with erythrocytes (E), pillar cells (white arrowheads). (h) Gill lamella embedded in the ILCM, with some of the pillar cell bodies displaced (white arrowheads) in relation to the lamellar blood sinus with erythrocytes (E). (i) Tissue of parasite-free fish stained using the Kanenko & Shiraishi method (2001), MRC (arrowhead) in gill filament (F). Note the complete regression of ILCM and protruding lamellae. (j) Gill of *S. aequifasciatus* after treatment. Single parasite located on gill filament; growing ILCM (arrow) visible at parasite's site of attachment. Scale bars = (a,b,e,f,i,j) 20 µm, (c,d,g,h) 10 µm

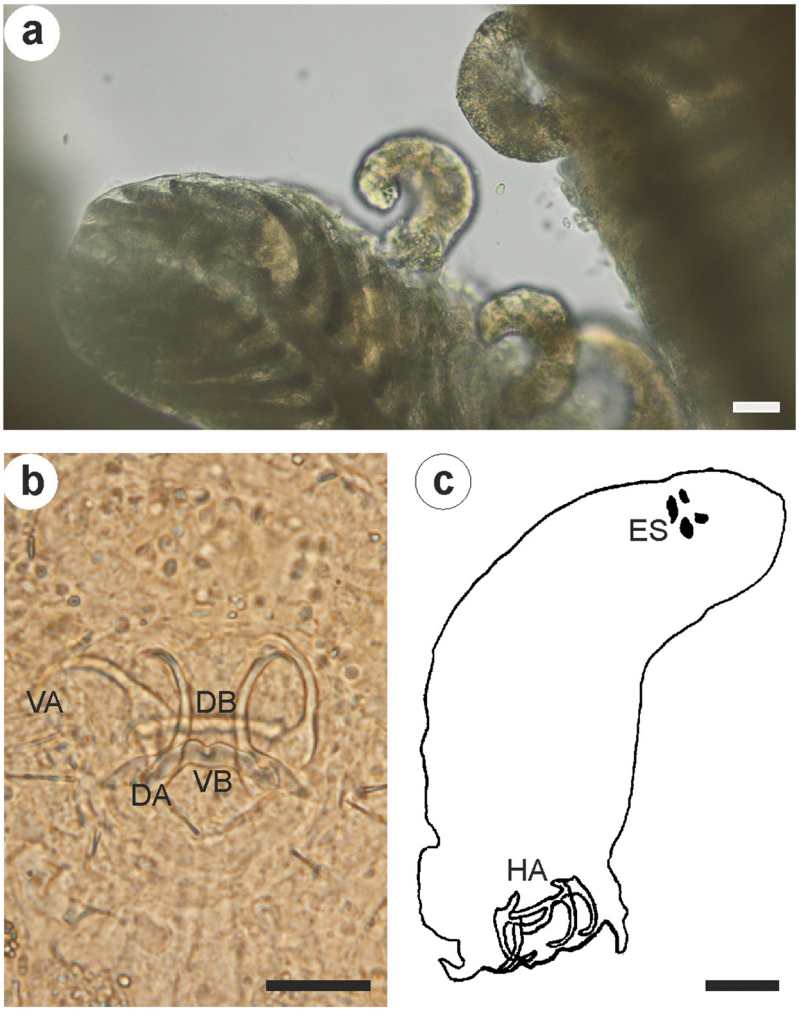


Fig. 2. (a,b) Light microscopic image and (c) drawing of *Sciadicleithrum variabilum*. (a) *S. variabilum* attached to gill lamella. (b) Detail of the sclerotized structures of the haptor: dorsal bar (DB), ventral bar (VB), 2 dorsal anchors (DA), 2 ventral anchors (VA), and 14 marginal hooks. (c) Fluke structure, diagrammatic. ES: eye spots; HA: haptor. Scale bars = 20 µm

Table 1. Example of monogenean infestation levels in the 3 to 6 mo old Group 1 *Symphysodon aequifasciatus*

Fish body weight (g)	No. of parasites fish^{-1}
3.45	236
3.16	322
2.77	316
1.78	296
1.24	383
0.87	1572
0.81	756

3.4. Transmission electron microscopy

Cross-sections through the gills from Group 1 (infested) showed ILCM-EL (Fig. 1c–h). Undifferentiated cells appeared to make up most of the ILCM in the gill apparatus. Capillaries with erythrocytes, pillar cells (PCs) with collagen bundles, and epithelial cells were visible in the gill lamellae (Fig. 1f–h). Nerves and blood vessels were absent from the ILCM (Fig. 1c, e–h). TEM examination of the gill filaments showed various kinds of lesions: vascular congestion in peripheral blood vessels (Fig. 1f) and numerous lamellar blood sinus dilations (Fig. 1f,g). In some gill lamellae, atypical lesions were observed: displacement of the body of PCs to the lower part of the lamella in relation to the lamellar blood sinus (Fig. 1h). In the middle part of the lamella, the blood sinus seemed to merge into one entity (Fig. 1h). The abovementioned lesions were not numerous. In the case of lamellar blood sinus dilation and vascular congestion, the structure of the lamella in cross-section remained typical of teleosts (Mallatt 1985); no displacement of the PC body in relation to the lamellar blood sinus was observed (Fig. 1f,g). In cross-section, the lamella resembled a string of beads — lamellar blood sinus dilations filled with numerous erythrocytes (E) and spool-shaped PCs (Fig. 1f,g). Clavate lamellae (lamellar aneurism), completely embedded in the ILCM, were also observed (Fig. 1d).

3.5. Parasite identification

The body is dorso-ventrally flattened and symmetrical on both sides. Head present with 2 pairs of eyespots; opisthaptor readily observed. For species identification, the most important part of the body is the copulatory organ sclerite and the opisthaptor with its sclerotized structures. These are the pairs of dorsal and ventral anchors, dorsal and ventral bars, and 7 pairs of marginal hooks (Fig. 2b). Their shape (Fig. 2b,c) and size were consistent with the description of the species *S. variabilum* (Mizelle & Kritsky 1969, Kritsky et al. 1989). The host species was also helpful in determining the species of parasite found.

Table 2. Morphometric characteristics of the discus second gill arch in Group 1 (infested) and Group 2 (anti-parasite-treated, tr) discus. ILCM: interlamellar cell mass; EL: embedded lamella

Body weight (g)	Mean lamellar width (µm)	ILCM height (µm)	Mean distance between adjacent lamella (µm)	Mean lamella height (µm)	Mean bilateral surface of lamella (µm^2)	EL height (% total lamella height)
9.6^{tr}	6.6	0	15.3	64.7	11260	0
4.9	5.0	25.4	9.8	56.1	6227	45.3
4.5^{tr}	3.7	0	11.9	42.0	5383	0
3.7	3.7	79.8	18.3	79.8	5140	100
3.5	6.1	54.9	17.5	78.9	6220 (52%)[a]	69.6
2.8	8.5	78.2	14.9	82.0	5990	95.4
1.8^{tr}	5.3	0	15.8	56.0	4087	0
1.2	4.6	32.3	15.0	51.0	3266 ◊51%	63.3

[a]Percent of lamella surface area occupied by ILCM

Table 3. Spearman correlation coefficients for analyzed traits in the following fish species: discus (with monogenean and after the anti-parasite treatment), *Esox lucius, Gymnocephalus cernuus* and *Stizostedion lucioperca.* Significant correlations indicated by *p < 0.001; ns: not significant

Species	Body weight Avg. no. gill lamellae per 1 mm gill filament	Body weight Mean bilateralsurface of lamella
Discus with monogenean	ns	ns
Discus after treatment	ns	ns
Esox lucius (Jakubowski 1992)	−0.95*	1.00*
Gymnocephalus cernuus (Satora & Romek 2010)	−0.95*	0.95*
Stizostedion lucioperca (Satora & Wegner 2012)	−1.00*	1.00*

Table 4. Morphometric characteristic of the capillary and water-blood distance in the second gill arch in Group 1 (infested) (n = 2) and Group 2 (anti-parasite treated, tr) (n = 2) discus. PL: protruding lamella; ILCM: interlamellar cell mass; WBD: water-blood distance

Body weight (g)	Mean diameter of capillary in PL (µm)	Mean diameter of capillary embedded in ILCM (µm)	WBD in PL (µm)	WBD in lamella embedded ILCM (µm)
9.6^{tr}	2.60	0	1.3	0
4.9	2.20	3.90	1.1	1.1
4.5^{tr}	2.28	0	0.8	0
2.8	1.42	1.48	0.7	1.1

4. DISCUSSION

In most fish, the primary site for oxygen uptake is the gill lamellae, which are mainly composed of 2 epithelial sheets held apart by pillar cells. The space around epithelial layers is perfused with blood. The epithelium provides only a thin barrier between the fish's blood and the surrounding water (Evans et al. 2005, Wegner 2011). The large lamellar surface area may increase the susceptibility of gills to parasitic infestations (Ojha & Hughes 2001, Nilsson et al. 2012, Abdel-Latif et al. 2020). In our study, all *Symphysodon aequifasciatus* from the first group showed severe infestation of the gill tissues. Numerous specimens of *Sciadicleithrum variabilum* (Fig. 2a–c) caused damage to the gill lamellae (Fig. 1a). The intensity of infestation was very high, the highest parasite burden being 1572 worms fish^{-1} (Table 1). LM and TEM studies revealed that the lamellae were embedded in an undifferentiated mass of cells, without blood vessels and nerves, which completely filled up the interlamellar space in the infested specimens (Fig. 1b–h). Furthermore, TEM investigations of the Group 1 discus showed lesions of the gill lamellae, such as vascular congestion in peripheral blood vessels (Fig. 1f), lamellar blood sinus dilation (Fig. 1g), and clavate lamellae (Fig. 1d). Such gill lesions, especially hyperplasia of the lamellar epithelium, have been interpreted as immune responses of the fish (Mallatt 1985, Nilsson et al. 2012). Single structural anomalies of the gill lamella were also observed, with displacement of the PC in relation to the lamellar blood sinus (Fig. 1h). However, they can hardly be regarded as lethal. It seems that the observed lesions of the lamellae and the single structural anomalies were

not the reasons for the high mortality in the Group 1 discus. A significant difference in the frequency of operculum movements between Groups 1 and 2 was noted, indicating respiratory distress in infested fish. The significant reduction in the gill respiratory surface and the numerous gill lesions was probably the reason for the high mortality in the first group.

During normal development of the gill apparatus, the distance between adjacent lamellae increases gradually with increasing body mass (Jakubowski 1992, Satora & Romek 2010, Satora &Wegner 2012). The observed relationship between body weight and mean distance between lamellae (Tables 3 & 4) seem to indicate parasite-induced disturbances. It should be noted that in this study, the measurements were taken only for the second arch. Considering the monogenean infestation in young discus fish (3 to 6 mo), an attempt was made (pilot study) to assess the effect of early infestation on the development of the gill apparatus. Statistical analysis showed no correlation between the body weight and the mean number of gill lamellae per 1 mm gill filament length or between the body weight and the mean bilateral surface of lamella (Table 3) in the 2 groups of discus: infested and after treatment. At the same time, there was a strong negative correlation between the mean number of gill lamellae per 1 mm gill filament length and body mass (W) and a strong positive correlation between the mean bilateral surface of lamella and W in ruffe, pike, and perchpike (Table 3). These preliminary results indicate an effect of the gill parasite on gill development in the discus fish. It was logistically difficult to measure gill respiratory surface area precisely because of the lesions and ILCM, and therefore for this study it was not possible to perform any further analysis.

The identified *S. variabilum* has been reported in the wild in 3 closely related host species, *Symphysodon discus* Heckel, 1840, *S. aequifasciatus*, and *Geophagus surinamensis* (Bloch, 1791), and occurs only in fish from the Amazon River basin (Kritsky et al. 1986, Thatcher 2006). Along with the import of aquarium fish, monogeneans were introduced to other European countries (Czech Republic, Ergens & Prouza 1984; Poland, Sobecka et al. 2012; Turkey, Onal et al. 2011; Italy, Aquaro et al. 2012). Apart from serious tissue damage, monogeneans can facilitate microparasite infections; therefore, interregional trade in live fishes offers a vehicle for bacterial or viral spread (Sobecka et al. 2012).

Bacterial gill disease (flavobacterial infection) characterized by epithelial hypertrophy and hyperplasia has been observed in the rainbow trout *Salmo gairdneri* (Richardson). It is suggested that unspecialized epithelial cells are the main type of cell involved in the hyperplastic process in that species (Morgan & Tovell 1973, Daoust & Ferguson 1983). According to Morgan & Tovell (1973), lamellar epithelial cells may retain their potential to proliferate, and they are responsible for epithelial hyperplasia. According to Mallatt (1985), hyperplasia of the lamellar epithelium could have a defensive function through the increase in the distance across which waterborne irritants must diffuse to reach the bloodstream.

In *S. aequifasciatus* Group 2 (treated against parasites), we found only a single specimen on the first gill arch in 1 specimen (Fig. 1j). At the parasite's site of attachment, ILCM growth was observed, suggesting an early stage of infestation. The proliferation confined to the site of attachment may suggest a local defensive reaction. The adjacent gill lamellae were not filled with the ILCM (Fig. 1j). The remaining discus in this group (after treatment) showed no signs of ILCM (Fig. 1i), and the mortality was close to zero.

In the case of *S. aequifasciatus*, larvae infested with *S. variabilum* showed gill damage at the sites of parasite attachment, but there was no proliferative cell response in the gill (Onal et al. 2011). In Onal et al. (2011), they found the first occurrence of *S. variabilum* in the discus was observed when gill differentiation was completed and before complete resorption of the yolk sac. Different responses of the gill apparatus to parasite infestation occurring both in the larval stages during parental care (Onal et al. 2011) and in discus aged 3 to 6 mo (present study) need further investigation, especially regarding the physiological changes in the gill apparatus during development.

Ornamental fish in hatcheries are subject to constant observation and health monitoring. Additionally, breeders periodically use prophylaxis against bacteria and viruses. However, threats from monogeneans in the case of ornamental fish constitute a serious, poorly recognised problem worldwide (Aquaro et al. 2012, Trujillo-González et al. 2018).

The observation suggests that crucian carp *Carassius carassius* use their ILCM in defense against gill flukes, covering up the gill surface, even if it has significant physiological consequences for the fish when they are exposed to hypoxia (Nilsson et al. 2012). In our study, *S. aequifasciatus* also used ILCM in defense against parasites. The gill remodeling ability has been described in freshwater and marine fish species (Sollid et al. 2003, Nilsson 2007, Ong et al. 2007, Perry et al. 2010, Nilsson et al. 2012, Sinha et al. 2014, Blair et al. 2017, Gilmour & Perry 2018);

however, the physiological effects of gill remodeling on transepithelial gas or ion transfer are not fully understood (Perry et al. 2010, Gilmour & Perry 2018).

The transport of fish is associated with increased stressors that can decrease resistance to disease. Masud et al. (2019) revealed that the impact of mechanical disturbances during fish transport could significantly increase susceptibility to parasitic infections. As ornamental fish are widely transported pre and post sale, increased susceptibility to parasitic infections may be common in such fish. The combination of hypoxia, transport-related stress (Harmon 2009, Sampaio & Freire 2016), and gill parasite infections in species with the ability to build gill plates by the ILCM can have a very negative impact on the condition of the fish. However, the abovementioned environmental conditions require investigation under stimulated conditions in relation to their impacts on host–parasite interactions. The results of our observations also indicate that juveniles are the most vulnerable to parasite infections (Table 1).

5. CONCLUSIONS

The main response to the *Sciadicleithrum variabilum* infestation recorded in Group 1 discus was the reorganization of the gill structure, also referred to as gill remodeling, manifested as enlargement of the ILCM. The parasite-induced damage to the gill lamellae and the significant reduction in the surface area for gaseous exchange were probably the reasons for the high mortality. The massive infestation of very young individuals may have affected the development of their gill apparatus. Our observations highlight the importance of monogenean-caused gill diseases, which could potentially cause significant losses in the ornamental trade. The physiological changes that take place during adaptive hyperplasia in *Symphysodon aequifasciatus* need further research. Gill adaptive hyperplasia may serve as an adaptation to changing oxygenation and as an antiparasite defense mechanism.

As shown in this work, severe infestations with *S. variabilum* and other monogeneans in *S. aequifasciatus* should be monitored on a regular basis, both to prevent severe losses, and to prevent the emergence of resistant strains of gill and skin worms by unnecessary repeated prophylactic treatments. Both situations can cause massive losses in intensively run ornamental fish breeding facilities and can be easily prevented.

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