The Thesis of the PhD Dissertation

WAN MUHAMMAD HAZIM BIN WAN SAJIRI

Gödöllő

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HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

PARASITIC INFESTATION AND CONTROL IN RECIRCULATING AQUACULTURE SYSTEMS (RAS): FOCUSING ON EUROPEAN CATFISH *Silurus glanis* Linnaeus, 1758 INFESTED WITH *Thaparocleidus vistulensis* (SIWAK, 1932)

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The PhD School

Name: Doctoral School of Animal Biotechnology and Animal Sciences, Hungarian University of Agriculture and Animal Sciences, Gödöllő, Hungary

Discipline: Animal Husbandry

Head: **Professor Dr. Mézes Miklós**
Head of the Doctoral School,
Member of the Hungarian Academy of Sciences,
Hungarian University of Agriculture and Life Sciences,
Institute of Physiology and Nutrition, Department of Feed Safety, Gödöllő, Hungary

Supervisors: **Honorary Professor Dr. Székely Csaba**
Scientific Advisor,
Fish Pathology and Parasitology Research Team Leader,
HUN-REN Veterinary Medical Research Institute,
Budapest, Hungary

Dr. Sellyei Boglárka
Senior Researcher,
Fish Pathology and Parasitology,
HUN-REN Veterinary Medical Research Institute,
Budapest, Hungary

Consultant: **Professor Dr. Kurt Buchmann**
Department of Veterinary and Animal Sciences,
Faculty of Health and Medical Sciences,
Section for Parasitology and Aquatic Pathobiology,
University of Copenhagen, Frederiksberg C, Denmark

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Approval of the Head of Doctoral School

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Approval of the Supervisors

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1. INTRODUCTION

1.1. Research background

This project is part of the European Union funded 'Safeguarding Future Production of Fish in Aquaculture Systems with Water Recirculation' (RASOPTA) project under the Horizon 2020 Marie Skłodowska-Curie Actions**,** which addresses challenges in recirculating aquaculture systems (RAS). The study focuses on ectoparasitic infections in European catfish (*Silurus glanis*), providing insights into parasite-host dynamics to develop mitigation strategies for intensive RAS production.

In 2022, Hungary's total fisheries and aquaculture production was 23,545 tonnes**,** with 18,948 tonnes from aquaculture and 4,597 tonnes from capture fisheries (FAO, 2024). European catfish ranked sixth in aquaculture production, underscoring its economic and ecological importance in Hungary (FAO, 2024). The rising demand for European catfish has led to intensive farming, increasing the risk of *Thaparocleidus vistulensis* outbreaks, a major gill parasite threatening intensive, which can cause fatal infections, particularly in catfish fry (Molnár, 1980; Molnár et al., 2019).

Understanding the epidemiological patterns and biological parameters, as well as exploring suitable treatment options, it is essential to effectively controlling infections caused by this ectoparasite. The use of chemical-based treatments has been expanding for decades, particularly in the field of aquaculture (Buchmann & Bresciani, 2006). Furthermore, environmental factors that can help control monopisthocotylean infestations in their hosts should also be explored. This study examines practical options for European catfish farmers, aiming to improve disease management and boost aquaculture productivity more sustainably and effectively.

1.2. Problem statement and significance of study

Thaparocleidus vistulensis has only been described based on morphological details, with limited molecular characterization, except for Šimková et al. (2003), and only a few sequences are available in the INSDC (International Nucleotide Sequence Database Collaboration). This study reassesses morphometric data, focusing on the sclerotized parts of haptor and copulatory organs, and adds new molecular and SEM (Scanning electron microscopy) findings to enhance knowledge of the species.

Though Molnár (1980) thoroughly investigated the histopathological effects of *T. vistulensis* infection, subsequent studies (e.g., Paladini et al., 2008; Reading et al., 2012) ignored these findings and simply focused on the parasite itself, creating uncertainty about the parasite's pathogenicity. The resulting knowledge gap prompted further investigations to elucidate the real impacts on hosts. Understanding these effects is key to better disease diagnosis and mitigation strategies in aquaculture populations. This study aimed to clarify the contradictions in available scientific literature and provide a clearer view of *T. vistulensis* infection.

However, the life cycle of *T. vistulensis* was studied (Molnár, 1968); its full infection dynamics and impact on European catfish aquaculture have already been underexplored. Investigating its reproductive strategies is crucial for understanding how the parasite spreads and persists in farmed environments, providing valuable data for improving infection control and disease prevention in aquaculture systems.

No studies have explored how environmental factors influence of the physiology *T. vistulensis* at different life stages, leaving theirs impact on host remained unclear. This study examined how the lack of light and water temperature affects the fecundity, hatching rate, and survival of *T. vistulensis*

in controlled conditions. The findings provide useful information for managing *T. vistulensis* infections in European catfish farms.

Previous treatments for *T. vistulensis* have been limited, failing to completely eradicate parasites from the gills (Antalfi, 1958; Székely & Molnár, 1990), and no studies have investigated the potential efficacy of herbal treatments. This study explored conventional drugs, Praziquantel (PZQ) and Mebendazole (MBZ), in both *in vitro* and *in vivo* settings, while assessing the novel drug Biokos (BIO) *in vitro*. Additionally, alternative therapeutic agents, including garlic (GAR), ginger (GIN), and neem bark (NMB), were tested *in vitro*. It presents the first comprehensive evaluation of these treatments, offering essential insights to improve therapeutic strategies in aquaculture.

1.3. Research aim and objectives

The main aim of this work was to investigate options for controlling monopisthocotylean *T. vistulensis* infection in European catfish.

Specifically, the objectives of this study

1. To perform the molecular characterization of *T. vistulensis* – provide for the first time, longer molecular characterization data including small subunit (SSU) ribosomal ribonucleic acid (rRNA) gene, partial sequence; internal transcribed spacer (ITS) 1, 5.8S rRNA gene, and ITS2, complete sequence; and large subunit (LSU) rRNA gene, partial sequence.
2. To study the morphology of adult *T. vistulensis* using light microscopy and SEM – provide morphometric measurements for the sclerotized structures including anchors and male copulatory organ.
3. To evaluate the pathological effects of *T. vistulensis* infection on the gills of European catfish – provide the condition of infected gills using histopathological and SEM studies.

4. To investigate the reproduction strategies of *T. vistulensis* infecting European catfish – provide the infection dynamics and detail life cycle parameters *in vitro* including oviposition, egg development, hatching, and survival of oncomiracidia, developing juvenile and adult parasites.
5. To study the influence of the light-dark cycle and various water temperatures on *in vitro* fecundity, hatching and survival rate of the *T. vistulensis* – provide for the first time, insights into the reproductive ability of *T. vistulensis* under different controlled environments.
6. To explore *in vitro* and *in vivo* efficacy of antiparasitic treatments against *T. vistulensis* infection in European catfish – provide for the first time, the use of bacterial-derived lipopeptide *Pseudomonas* H6 Biokos and the use of herbal therapeutic antiparasitic agents against *T. vistulensis*.

2. MATERIALS AND METHODS

2.1. Source of fish and parasites

European catfish, naturally infected with *Thaparocleidus vistulensis* were obtained from a local commercial fish farm, and transported to the Veterinary Medical Research Institute (HUN-REN VMRI), Budapest. Fish were kept in a 60-L flow-through tank system at 23 ± 1 °C. Upon arrival, they were anaesthetized with clove oil, and small gill samples were taken from the first gill arch to confirm the presence of *T. vistulensis* and estimate parasite abundance (Gussev, 1983; Bognár et al., 2024). Some monopisthocotyleans were isolated and studied alive. Parasites were identified as *T. vistulensis* based on morphological criteria described by Siwak (1932) and Bychowsky and Nagibina (1957). A few infected fish were sacrificed, and gill arches were preserved in 80% ethanol and 5% formalin for further analysis.

To ensure a continuous supply of *T. vistulensis* in the laboratory, they were maintained using: 1) Co-habitation, 2) Egg collection by Petri dishes, and 3) Frame-stretched mesh.

2.2. Molecular characterization of *T. vistulensis*

Molecular investigations, including PCR amplification and sequencing were used to confirm the morphological identification of *T. vistulensis*. DNA from adult parasites preserved in 80% ethanol was extracted with commercial kit (QIAamp® DNA Mini Kit) and quantified with a NanoDrop spectrophotometer. The ITS rDNA region was amplified with primers PDG_18S_F5 (in-house) and NLR1270 (Bartošová et al., 2009). The sequences were analyzed, confirmed with BLAST (Basic Local Alignment Search Tool), and submitted to GenBank. For the phylogenetic analysis, rDNA sequences of *Thaparocleidus* species were selected based on their

availability in the INSDC. The phylogenetic tree was constructed using MEGA 12, including 25 related sequences, and rooted with *Ligophorus* spp.

2.3. Morphological analysis of *T. vistulensis*

Morphological analysis was conducted by measuring morphometrics (μm) under a light microscope and observing external morphology using SEM. Monopisthocotyleans were softened with proteinase K treatment, modified from Harris and Cable (2000), and mounted in glycerine-ammonium-picrate (Malmberg, 1957). Some specimens were stained with Harris' modified hematoxylin, mounted with AQUA-TEX®, and photographed using a Leica MC170 HD camera on a Leica DM5000B microscope. Line drawings of key parasite structures (e.g., sclerotized haptor and male copulatory complex) were based on photomicrographs. Measurements were taken using ImageJ 1.53t software and compared with previous descriptions by Siwak (1932), Bychowsky and Nagibina (1957), and Paladini et al. (2008).

Parasites preserved in 5% formalin were transferred to Karnovsky's fixative (4% paraformaldehyde, 2% glutaraldehyde in 0.1 M sodium cacodylate buffer) for 15 min, rinsed in the buffer twice for 20 min, then immersed in demineralized water twice for 15 min. Samples were dehydrated in increasing ethanol concentrations (20-100%) for 15 min per concentration, and dried in 100% hexamethyldisilazane (HMDS) for 30 min. After drying, samples were mounted on carbon tape attached to SEM stubs, sputter-coated with gold, and examined using a FEI Quanta 200 SEM at 3–8 kV.

2.4. Pathological effects of *T. vistulensis* on gills

Pathological effects were observed by examining *T. vistulensis* attachment on European catfish gills using SEM and documenting its histopathological effects on the gill tissues. Formalin-fixed gill arches were

processed using standard histology techniques: dehydrated in ethanol (70, 96, 100 %), cleared in xylene, embedded in paraffin, and sectioned at 3–5 µm with a Leica RM 2135 microtome. Sections were placed on glass slides, dried, deparaffinized, rehydrated, and stained with hematoxylin–eosin (H&E) and Masson–Goldner trichrome, before mounted in DPX. Stained sections were examined under an Olympus BX53 light microscope and photographed with an Olympus DP74 camera.

2.5. Reproductive strategies of *T. vistulensis*

Reproductive strategies were investigated by monitoring infection dynamics, such as egg development, hatching, and *in vitro* survival rates of oncomiracidia, developing juvenile and adult *T. vistulensis*. Infection dynamics were assessed using parasite-free European catfish fingerlings. Infection trials (23 ± 1 °C) involved 30 naïve fish (10/trial) cohabiting with infected donor fish carrying <100, <200, and >500 gill flukes in total (roughly estimated following Gussev, 1983 method). Two artificially infected fish were sacrificed every two days over 10 days, and all gill flukes were counted under a stereo microscope, with images captured using an Olympus DP74 camera.

Egg development was observed on the released eggs, which were laid by in filtered tank water by gravid *T. vistulensis* after being gently shaken. Egg size (n = 30) was measured, and eggs were placed in groups (n = 5–10) on concave slides with 200 µL filtered water, covered, and stored at 23 ± 1 °C in a humid chamber. Oviposition time was set as 'Time 0', and eggs were monitored daily under a microscope until hatching, with morphological changes documented and photographed.

For studying hatching rates, eggs of *T. vistulensis* were collected overnight from tanks with heavily infected fish and transferred to a 96-well microtiter plate (100 µL filtered water/well, n = 445). Only viable eggs were

used. Hatching rates were recorded daily by counting empty eggshells with an open operculum. The assay ended 24 h after the last hatch.

For *in vitro* survival trials, oncomiracidia (n = 135), developing juveniles (4–6 day post infection (dpi), n = 204) and adults (>10 dpi, n = 153) isolated from gills, were kept in filtered water on a 96-well plate (100 µL filtered water) at 23 ± 1 °C. Only active individuals were selected. Survival was monitored daily based on movement and response to stimulation, with immobile or swollen parasites considered dead.

2.6. Effects of environmental conditions on *T. vistulensis*

The impact of environmental factors on *T. vistulensis* was assessed by evaluating fecundity, egg hatching rates, and survival of oncomiracidia and adults under different light-dark cycles and water temperatures.

The effects of light and darkness on *T. vistulensis* were appraised at 23 °C. Adult parasites (n = 20) were placed individually in 24-well plates with filtered tank water and monitored for egg production at 1, 2, 3, 4, 5, 6, and 24 hours, keeping them under light (16:8 [L:D]) and dark (0:24 [L:D]) conditions. Survival of adult was recorded daily until all individuals perished. To evaluate hatching success, eggs (n = 300) were distributed into 96-well plates under the same light-dark conditions, with daily observations of hatched eggs. Oncomiracidia survival was similarly assessed in 96-well plates (n = 90) until all individuals died. Death was determined by immobility and lack of response to stimuli.

The effects of water temperature on *T. vistulensis* fecundity, egg hatching, and oncomiracidia survival were estimated at 5, 10, 15, 20, 25, 30, and 35 °C. Adult parasites (n = 70) were placed individually in 24-well plates with filtered water and monitored for egg production at intervals up to 24 hours, while survival was recorded daily until all individuals died. For

hatching rates, eggs (n = 525) were distributed into 96-well plates and observed daily until no further hatching occurred. Oncomiracidia survival (n = 210) was similarly monitored at 24-hour intervals until all larvae perished.

2.7. Treatment trials against *T. vistulensis*

The *in vitro* and *in vivo* treatment trials against *T. vistulensis* involved the collection of all parasite life stages (egg, oncomiracidium, developing juvenile, and adult).

Six antiparasitic agents were tested *in vitro*; Herbs – Garlic (GAR), Ginger (GIN), Neem bark (NMB); and Drugs – Biokos (BIO), Praziquantel (PZQ), and Mebendazole (MBZ). Only PZQ and MBZ were used for *in vivo* trials.

Herbal stock solutions were prepared following Goswami (2021) and diluted at 1:10, 1:50, and 1:100. Drug stock solutions were prepared according to the applied treatment concentrations (0, 1, 5, 10, 20, 40, 60, 80, and 100 mg/L). *In vitro* assays were conducted on 900 eggs for herbal treatments and 2025 eggs for drug treatments, which were exposed to the treatment solutions in 96-well plates (200 µL final volume). The isolated oncomiracidia, juveniles, and adults were also exposed to the treatment solutions, and observations were made at various intervals (every 15 minutes – 24 hours post-treatment (hpt)) to monitor the effects.

The toxicity of MBZ and PZQ was evaluated using juvenile European catfish (n = 60). Experimental concentrations of 10 and 20 mg/L were tested. The catfish were transferred to a new tank after a 24-hour bath treatment. Toxicity signs were monitored throughout the experiment, and the study halted if adverse behavioural signs, such as lethargy, loss of equilibrium, abnormal respiratory function and skin pigmentation, were observed in the fish.

In the *in vivo* efficacy assay, juvenile European catfish (n = 144) were infected with oncomiracidia and treated with MBZ and PZQ. The fish were subjected to a 24-hour bath treatment before being transferred to a new tank. Efficacy was determined by assessing the parasite count in the gills of sacrificed fish under a stereo microscope at 1, 7, and 14 days post-treatment (dpt). The efficacy percentage was calculated using the equation (Onaka et al., 2003): EF (%) = [(MNPCG – MNPTG) / MNPCG] × 100, where MNPCG represents the mean number of parasites in the control group, and MNPTG represents the mean number in the treated group.

2.8. Statistical analysis

Biological parameters such as egg production, hatching, and survival rates were analyzed under different light and water temperature conditions using the Mann–Whitney test (for light vs. dark) and the Kruskal–Wallis test (for varying water temperatures). Kaplan–Meier survival analysis estimated cumulative survival probabilities, and the log-rank test compared survival curves. For *in vitro* and *in vivo* treatment trials against *T. vistulensis*, survival analysis in R (version 4.1.2) compared drug and herb treatments, using censoring for unhatched eggs. Significant differences between treatments were analyzed with pairwise comparisons using Benjamini-Hochberg correction. *In vivo* analysis included three stages: 1) overall treatment efficacy, 2) concentration effects over time, and 3) parasite counts at different intervals. Normality tests determined whether parametric (ANOVA, t-test) or non-parametric (Kruskal–Wallis, Mann–Whitney) tests were applied, and post-hoc comparisons were made using Tukey HSD or Dunn's test with Bonferroni correction. A P value of < 0.05 was considered statistically significant, with all analyses performed using SPSS (version 29.0) or R (version 4.1.2).

3. RESULTS

3.1. Molecular analysis and phylogenetic tree

In this study, two identical 2694 bp sequence of *Thaparocleidus vistulensis* was obtained, covering partial 18S, ITS1, 5.8S, ITS2, and partial 28S rDNA regions, and one of them were submitted to GenBank (OR916383). Phylogenetic analysis using related sequences from INSDC showed 96.04% similarity with *T. vistulensis* from European catfish in Czechia (AJ490165) and 94.27% with *T. siluri* from the same host and location (AJ490164) (Šimková et al., 2003). The Maximum Likelihood tree clearly grouped the new sequences with the known *T. vistulensis* (**Figure 1**). It formed a sister group with *T. siluri*, and both clustered with *T. varicus* and *T. mutabilis* in a well-supported branch. The sequence variabilities within genus/species group with *T. vistulensis* were 86.1–99.8%. This result confirm that the species observed in this study is *T. vistulensis*.

3.2. Morphological description

3.2.1. *External morphology*

The body (691.2 ± 163.0 µm in length and 155.2 ± 27.2 µm in width) is elongated and cylindrical, narrowing posteriorly and ending in a slightly wider, non-segregated caudal disc. Its anterior region is flattened, with two pairs of dorsal eyespots and four pairs of head organs with cephalic glands. The eyes consist of dispersed pigment spots arranged in a trapezoid, with nearby reflective corpuscles bodies. A subterminal mouth leads to a short oval pharynx located ventrally behind the eyes. The haptor contains two pairs of robust dorsal and ventral anchors connected by dorsal and V-shaped ventral bars, respectively, along with 14 marginal hooks and a small dorsal cuneus. All haptoral sclerites are chitinous and adapted for attachment to gill lamellae. Oncomiracidia (167.3 ± 7.6 µm in length and 72.5 ± 4.0 µm in width) possess

14 marginal hooks similar in size to adults and an underdeveloped pair of ventral anchors in the attachment disc.

3.2.2. Internal morphology

Vitellaria are densely distributed throughout the trunk, except around the reproductive organs. The testicle is posterior and connects to a single seminal vesicle via the vas deferens. The male copulatory organ includes a flask-shaped bulb and a long, 5–7 looped sclerotized penis at the midsection connected to a V-shaped accessory piece. The germarium lies anteroventral to the testis and contains large ovules anteriorly. The sinistral vaginal opening is above the germarium, leading to a coiled vaginal duct ending in a muscular chamber with a small chitinous plaque.

3.3. Pathological effects of *T. vistulensis* infection on the gills

3.3.1. Attachment of T. vistulensis on gills

The parasite primarily uses its opisthaptor for attachment, with dorsal and ventral anchors penetrating between gill lamellae in opposite directions, supported by marginal hooks. The prohaptor aids in temporary attachment. The opisthaptor attaches both superficially and basally to gill filaments, often causing deep, and cup-like depressions on the lamellar surface.

3.3.2. Histopathological effects of T. vistulensis infection on gills

Histopathological analysis showed epithelial hyperplasia, lamellar fusion, and erythrocyte leakage in the gills of *T. vistulensis* - infected fish. Severe infections caused epithelial disintegration, excess mucus with extravasated erythrocytes, and filament clubbing. Eosinophilic granular cells were frequently found at anchor penetration sites. The parasite's anchors and hooks caused tissue rupture, pressure damage, and loss of lamellar structure. Feeding activity increased the tissue disruption, with debris found in the parasite's gut. In some cases, anchor penetration extended to the cartilaginous matrix, affecting chondrocytes and distorting the gill ray structure.

3.4. Reproductive strategies of *T. vistulensis*

3.4.1. Infection dynamics

Experimental exposure of European catfish to *T. vistulensis* resulted in a marked increase in gill monopisthocotyleans over 10 days, with infection intensity linked to the donor fish's parasite load. In the highest infection group (Third Trial), fish showed severe behavioural and pathological signs, including equilibrium loss and up to 18,000 parasites on the gills, along with structural damage, lamellar fusion, and colour changes. In contrast, fish from lower infection trials showed mild symptoms. Mature parasites with eggs were observed from 8 to 10 days post-infection in all groups.

3.4.2. Egg development

Eggs of *T. vistulensis* are spheroidal (~72 × 56 μm) with a short, hooked polar filament for attachment. Eggs are laid individually, with viable ones appearing light brown. Embryonic development follows a consistent pattern, with embryos becoming visible around 24 hours post-oviposition (hpo) and developing progressively. By 48–72 hpo, eyespots, hamulus primordia, and ciliated cells form. Sclerotized structures and active larval movement appear after 72 hpo, leading to hatching through the operculum. Empty egg shells and free-swimming oncomiracidia were observed between 72–96 hpo.

3.4.3. In vitro hatching rates

Eggs of *T. vistulensis* hatched on average within 3 days, with a total hatching success of 89.7%. Most hatching occurred between 3 and 4 dpo, peaking at 84% by day 5. No hatching occurred after 5 dpo, and unhatched eggs turned dark brown, indicating arrested development.

3.4.4. In vitro survival rates

In vitro survival of *T. vistulensis* varied by life stages. Oncomiracidia survived up to 5 days (7.4%), while juveniles and adults survived up to 3 days

(0.9% and 1.6%, respectively). Mobility declined over time, and death was indicated by lack of response to stimuli, often accompanied by body swelling or opacity.

3.5. Influences of environmental conditions against different life stages

3.5.1. Fecundity of T. vistulensis in relation to light and darkness

Light significantly increased egg production in *T. vistulensis*, with adults laying three times more eggs under light (96 eggs / 10 flukes) than in constant darkness (30 eggs / 10 flukes) ($P < 0.001$).

3.5.2. *Hatching rates of T. vistulensis in relation to light and darkness*

Eggs of *T. vistulensis* hatched between 3 and 5 dpo under both normal light and constant darkness, with similar total hatching success (88.0% and 88.7%). No significant difference was found between the groups ($P > 0.926$).

3.5.3. Survival rates of T. vistulensis adults and oncomiracidia in relation to light and darkness

The survival of adult *T. vistulensis* was similar under normal light and darkness, with no significant difference ($P > 0.142$), and all individuals died within 3 days post-isolation (pi). Oncomiracidia survived up to day 5 pi in both conditions, but their survival curves differed significantly ($P < 0.001$), with earlier mortality observed under darkness. No clear phototactic behavior was detected.

3.5.4. Fecundity of T. vistulensis at different water temperatures

Egg production by *T. vistulensis* was greatest at 15 °C, with significantly fewer eggs produced at 20–30 °C ($P < 0.001$). No significant difference was found between 25 °C and 30 °C ($P > 0.583$), or between 10 °C and 35 °C ($P > 0.051$), both of which showed low fecundity. No egg production occurred at 5 °C, which was significantly different from all other temperatures ($P < 0.001$).

3.5.5. *Hatching rates of T. vistulensis at different water temperatures*

The hatching rates of *T. vistulensis* have changed with temperature, the highest at 20–25 °C (89.3%) and the lowest at 10 °C (80%). No hatching occurred at 5 °C and 35 °C ($P = 1.000$). Hatching was fastest at 30 °C (day 2–3 post-oviposition (pop)), gradually slowing at lower temperatures, with the longest period at 10 °C (day 12–19 pop). Hatching at extreme temperatures (5 °C and 35 °C) was significantly different from the other groups ($P < 0.001$).

3.5.6. Survival rates of T. vistulensis adults and oncomiracidia at different water temperatures

The survival of *T. vistulensis* adults and oncomiracidia have changed significantly with water temperature. Higher temperatures (35 °C) led to rapid mortality, each adult died within 24 hours, and oncomiracidia survived for less than one day. As the temperature decreased, survival duration increased, peaking at 12 days for adults at 5 °C and at 9 days for larvae at 10 °C. Log-rank tests confirmed significant differences in survival across all temperatures ($P < 0.001$).

3.6. Treatment trial against *T. vistulensis*

3.6.1. In vitro herbal treatment against T. vistulensis

3.6.1.1. Egg hatching

Egg hatching rates of *T. vistulensis* differed significantly among herbal treatments ($P < 0.0001$). Garlic (GAR) extract was the most effective, fully inhibiting egg development at all dilutions, with 0% hatching success. Ginger (GIN) and neem bark (NMB) extracts showed complete suppression only at the highest concentration (1:10). At higher dilutions (1:50 and 1:100), GIN and NMB had moderate hatching rates (GIN: ~71%, NMB: ~65%), with no significant differences between the two dilutions in either treatment.

3.6.1.2. Oncomiracidia longevity

Survival analysis revealed significant differences in the efficacy of herbal treatments against *T. vistulensis* oncomiracidia ($P < 0.0001$). NMB was the most effective, eliminating 100% of oncomiracidia within 75 minutes, even at the highest dilution (1:100). GAR was moderately effective, killing all oncomiracidia within 4 hours at 1:100 dilution, while GIN was the least effective, requiring up to 24 hours to achieve complete mortality only at the highest concentration.

3.6.1.3. Lifespan of developing juvenile and adult flukes

The survival analysis revealed significant differences in the effects of herbal treatments on juvenile and adult *T. vistulensis*. GAR was the most effective, killing all juvenile parasites within 3 hours, even at the highest dilution (1:100), and all adults within 24 hours across all tested dilutions. NMB was highly effective at the lowest dilution (1:10), eradicating juveniles and adults within 15 minutes, while higher dilutions took longer but remained effective within 24 hours. GIN was the least effective, showing delayed action even at lower dilutions, and no clear impact at 1:100 dilution for adult parasites. All treatments demonstrated statistically significant effects ($P < 0.0001$).

3.6.2. In vitro drug treatments against T. vistulensis

3.6.2.1. Egg hatching

The egg hatching rates of *T. vistulensis* differed significantly among treatments ($P < 0.0001$). Mebendazole (MBZ) was the most effective, fully inhibiting egg development at all concentrations, with 0% hatching and visible egg deterioration. In contrast, Biokos (BIO) showed no significant effect, maintaining over 85% hatching success similar to the control. Praziquantel (PZQ) reduced hatching rates only at higher concentrations (80–100 mg/L),

with success dropping to about ~6%. Hatching in BIO and PZQ groups started on day 3 and was mostly completed by days 4–5.

3.6.2.2. Oncomiracidia longevity

The survival analysis revealed significant differences in oncomiracidia survival across treatments ($P < 0.0001$). BIO was the most effective treatment, especially at concentrations ≥40 mg/L, killing all oncomiracidia within 3 hpt. Lower BIO concentrations (1–10 mg/L) were not significantly different from the control. PZQ showed moderate efficacy, with over 50% mortality at the lowest concentration by 72 hours, while MBZ required higher concentrations (≥40 mg/L) to achieve full mortality within the same period.

3.6.2.3. Lifespan of developing juvenile and adult flukes

The survival analysis showed significant differences in juvenile and adult *T. vistulensis* survival across treatments ($P < 0.0001$). For juveniles, BIO and PZQ were most effective at ≥60 mg/L, with BIO demonstrating faster (first death at 75 minutes vs 3 hpt for PZQ), while MBZ had limited effect except at 100 mg/L. For adults, BIO achieved full mortality within 24–72 hpt, depending on the concentration. PZQ was effective at ≥60 mg/L within 24 hpt, while MBZ eliminated all adults by 48 hpt at ≥20 mg/L and by 72 hpt at lower concentrations.

3.6.3. In vivo drug treatments against T. vistulensis

3.6.3.1. Toxicity test

A toxicity test on juvenile European catfish showed that all fish survived 24-h exposure except those exposed to 20 mg/L of MBZ, which caused immediate toxicity symptoms, leading to test termination within 10 minutes. PZQ at 20 mg/L caused mild, temporary body paling but no mortality, and fish recovered after a full water change.

3.6.3.2. In vivo treatment efficacy assessment

Antiparasitic treatments with PZQ and MBZ against *T. vistulensis* showed significant differences compared to controls ($P < 0.001$). While 10 mg/L PZQ maintained complete efficacy through 14 dpt, parasite reemergence was observed in the lower concentration groups (1 and 5 mg/L) by 14 dpt, with both adult and juvenile parasites detected. Lower PZQ doses showed reduced and variable effectiveness. MBZ had minimal effects at 1 dpt but showed a delayed response, with >98% parasite reduction observed by 14 dpt at all concentrations. Overall, PZQ demonstrated rapid-, while MBZ required more time to achieve powerful efficacy.

4. DISCUSSION

4.1. Morphological characterization and molecular analysis

Three congeneric *Thaparocleidus* species; *T. siluri* (Zandt, 1924), *T. vistulensis* (Siwak, 1932), and *T. magnus* (Bychowsky & Nagibina, 1957), have been reported from *Silurus glanis*, distinguished primarily by differences in sclerotized structures, especially the haptoral and copulatory organs (Gussev, 1985; Wu et al., 2005; Pouyaud et al., 2006; Mendlová et al., 2012; Řehulková et al., 2013; Khang et al., 2016).

In the present study, *T. vistulensis* specimens exhibited a broader body size range than literature data, which was probably influenced by early-stage maturity and ethanol preservation. The male copulatory organ, featuring a coiled, thread-like sclerotized penis and a distinctive accessory piece, closely resembles that of *T. magnus* but differs in morphometrics (Bychowsky & Nagibina, 1957). Given the morphological overlap among species, molecular tools were applied for accurate taxonomic identification. A 2694 bp rDNA fragment (including partial 18S, ITS, 5.8S, and partial 28S) was sequenced (GenBank: OR916383), and molecular analysis clustered the isolate with a previously published *T. vistulensis* sequence (AJ490165), confirming species identity. Phylogenetic analysis placed *T. vistulensis* in a well-supported clade with *T. siluri*, reinforcing its preliminary morphometric taxonomic classification.

4.2. Pathological effects of *T. vistulensis* infection on the gills

This study expanded the morphological characterization of *T. vistulensis*, particularly highlighting early sclerotized anchor development and damage to European catfish gills through histopathology and SEM. Consistent with observations of Molnár (1980), *T. vistulensis* attaches deeply between the gill lamellae using dorsal and ventral anchors, often penetrating

the cartilaginous matrix and causing mechanical damage. SEM revealed opisthaptor-induced compression and deformation of gill structures, potentially impairing respiration. Histopathological effects observed, such as epithelial hyperplasia, capillary rupture, and filament clubbing, were consistent with those reported in infections by other gill-infecting monopisthocotyleans (e.g., Arafa et al., 2009; Buchmann, 2012; Igeh & Avenant-Oldewage, 2020). While increased mucous cells are often noted in parasite infections (Reda & El-Naggar, 2003; Arya & Singh, 2020; Vankara et al., 2022), this study found goblet-like mucous cells to be largely absent, aligning with Molnár (1980), possibly due to parasite-induced inhibition or immune-mediated depletion (Xian et al., 1999; Bergstrom et al., 2008). Eosinophilic granular cells were observed in high numbers at anchor sites, differing from previous findings by Molnár (1980). Together, these observations provide new insights into the pathological impact and host responses to *T. vistulensis* infection.

4.3. Reproductive strategies of *T. vistulensis*

This study expands on previous work by Molnár (1968, 1980) by examining the life cycle parameters of *T. vistulensis*, including embryonic development, hatching rate, and survival without a host. The parasite demonstrated high propagation potential, with sexual maturity reached as early as 8 dpi, and the entire life cycle completed within 13–15 days at 23 ± 1 °C, closely aligning with Molnár's earlier findings. Environmental (e.g., temperature, light, salinity) and biotic factors (e.g., host species and its microbiota) can significantly influence the parasite reproductive dynamics (Bauer et al., 1973; Gannicott & Tinsley, 1997; Buchmann & Bresciani, 2006). The parasite's eggs adhered to surfaces and developed similarly to other monogeneans such as *Sparicotyle chrysophrii* and *Dawestrema cycloancistrium*, showing clear embryonic stages and spontaneous hatching

(Repullés-Albelda et al., 2012; Maciel et al., 2017). Unhatched eggs darkened over time, and hatching success was influenced by environmental conditions and possibly predation or biofilm interference (Buchmann, 1988a; Whittington & Kearn, 1988).

Oncomiracidia survived up to 5 days post-hatching, facilitating potential reinfection, especially problematic given the sedentary nature and high site fidelity of *Silurus glanis* (Carol et al., 2007; Copp et al., 2009). Both juvenile and adult *T. vistulensis* exhibited vermiform crawling, enabling movement toward preferred gill attachment sites (Reed et al., 2012). Adult parasites could survive briefly post-detachment and continue egg production, though fecundity declines under starvation (Whittington, 1997; Mooney et al., 2008). Importantly, egg deposition was observed in this study, and the fecundity data of *T. vistulensis* are discussed in detail in subsection 4.4, complementing earlier approaches used in *Pseudodactylogyrus* research (Buchmann, 1988b, 1990)

4.4. Influences of environmental conditions against different life stages

Understanding parasite ecology and infection dynamics is essential for developing effective control strategies in aquaculture, particularly regarding how environmental factors influence their life cycles (Villar-Torres et al., 2018; Huston et al., 2020). Although the present *in vitro* study provides insight into the reproductive biology of *T. vistulensis*, including egg production, hatching, and survival, such approaches may not perfectly reflect natural conditions, as parasite viability tends to decline when separated from the host due to starvation and stress (Whittington, 1997; Mooney et al., 2008). Nevertheless, these findings offer foundational biological and ecological knowledge necessary for future field-based research and effective parasite management.

4.4.1. The influence of light-dark cycle against T. vistulensis

The influence of photoperiod on the biology of *T. vistulensis* was assessed by exposing them at different life stages to natural light conditions and continuous darkness, revealing that darkness reduced fecundity, while egg hatching and adult survival rates remained largely unaffected. These results suggest that oviposition may be influenced by host behavior, as the European catfish is predominantly nocturnal (Boujard, 1995; Slavík et al., 2007), potentially limiting egg attachment due to increased host activity and water movement at night. In contrast, other studies found that many monogeneans release eggs in darkness to synchronize with host behavior (Macdonald & Jones, 1978; Mooney et al., 2006, 2008; Hoai & Hutson, 2014; Woo et al., 2024). *Zeuxapta seriolae*, for example, accumulates eggs and releases them at dusk to align with host activity (Mooney et al., 2006). While *T. vistulensis* egg hatching appeared unaffected by light conditions, suggesting reliance on internal developmental cues, the survival of oncomiracidia was significantly longer under natural light, with nearly half surviving up to 5 days. This extended lifespan, longer than most monopisthocotyleans at 20–25 °C, may increase host-finding success (Kearn, 1963, 1973, 1982; Ernst & Whittington, 1996; Whittington & Ernst, 2002). The findings highlight the importance of further exploring parasite adaptation to host diel activity and environmental factors for improved understanding and control strategies.

4.4.2. The influence of temperature against T. vistulensis

Water temperature significantly influences the reproductive biology and survival of *T. vistulensis*. This study found that 15 °C was optimal for egg production, while egg-laying occurred across a broader range (10–30 °C) but ceased entirely at 5 °C, aligning with trends in other monopisthocotyleans (Cone & Burt, 1981; Chan & Wu, 1984; Buchmann, 1988b; Zhang et al.,

2022). At extreme temperatures (10 °C and 35 °C), egg output was minimal, likely due to reduced metabolic activity (Woo et al., 2024).

Hatching success remained high (>80%) between 10–30 °C, but development was slower at cooler temperatures, consistent with the previous findings (Kearn, 1986; Reantaso et al., 1995; Yoshinaga et al., 2000; Tubbs et al., 2005; Turgut, 2012; Zhang et al., 2015). The fastest hatching occurred on day 2 pop at 25–30 °C, while at 10 °C it extended up to 12–19 days, highlighting the species' adaptability to varying thermal conditions (Molnár, 1968; Perry, 1989; Thompson, 2020; Marcus et al., 2023).

The survival of *T. vistulensis* adults and oncomiracidia decreased with rising water temperatures, showing greater longevity at lower temperatures, similar to trends reported for other monopisthocotyleans (Brazenor & Hutson, 2015; Valles-Vega et al., 2019). In this study, all *T. vistulensis* stages died within 24 hours at 35 °C. By contrast, *Dactylogyrus vastator* oncomiracidia survived up to 42 hours at the same temperature (Zhang et al., 2015), and *in vivo*, *Pseudodactylogyrus bini* and *P. anguillae* survived up to 14 and 17 days at 34 °C, respectively (Buchmann, 1988b, 1990).

Furthermore, synchrony between parasite reproduction and host behavior, particularly European catfish activity during warmer months and their use of shallow waters, may increase infection risk (Říha et al., 2022). Understanding thermal thresholds can therefore improve the timing and frequency of anthelmintic treatments, especially since such treatments may leave detached but viable parasites capable of continued reproduction (Whittington, 1997; Burka et al., 1997; Watson, 2009; Woo et al., 2024).

4.5. Treatment trial against *T. vistulensis*

Effective management of monogenean infestations in aquaculture is essential for ensuring fish health and welfare. Due to the direct life cycle, high fecundity, and short generation time of monopisthocotyleans, especially under

high-density farming conditions, these parasites can rapidly proliferate (Buchmann & Bresciani, 2006). Anthelmintic treatments may be required where pathogen-free systems are not feasible, though treatment outcomes are often unpredictable due to varying drug sensitivities across life stages (Morales-Serna et al., 2018). *In vitro* testing offers a practical approach to assess parasite susceptibility to antiparasitic agents and simulate *in vivo* bath treatment conditions (Reimschuessel et al., 2011). This study evaluated the efficacy of herbal treatments (garlic: GAR, ginger: GIN, neem bark: NMB) and antiparasitic drugs (Biokos: BIO, praziquantel: PZQ, mebendazole: MBZ) against all life stages of *T. vistulensis in vitro*. In view of the *in vitro* results, toxicity tests (MBZ, PZQ) were performed on juvenile European catfish before conducting *in vivo* trials.

4.5.1. In vitro herbal treatment against T. vistulensis

This study is the first to report the antiparasitic efficacy of herbal treatments against *T. vistulensis*. Among the tested herbs, GAR showed the strongest activity across all life stages. It completely inhibited egg hatching even at 1:100 dilution, an exceptional result given the high resistance of monopisthocotylean eggs (Whittington, 2012). GAR also reduced the survival of oncomiracidia and juveniles to under 4 and 3 hours, respectively, supporting its known broad-spectrum bioactivity, including antibacterial, antiviral, and immunostimulant properties (Lee & Gao, 2012; Valenzuela-Gutiérrez et al., 2021). This aligns with previous findings on other monopisthocotyleans (Militz et al., 2013).

Neem bark (NMB) was highly effective against oncomiracidia, killing them within 75 minutes at all tested dilutions. It showed moderate activity against juveniles and adults. Its efficacy is likely due to triterpenoids such as azadirachtin, which can shorten parasite survival via oxygen depletion

(Mordue & Nisbet, 2000), consistent with earlier reports on *Diplectanum* and *Dactylogyrus* species (Aly et al., 2022; Suryani & Arya, 2017).

GIN exhibited the weakest antiparasitic effect; it had limited activity at the lowest dilution and with longer exposure times. Its reduced efficacy may stem from instability or low concentration of bioactive compounds in aqueous extracts, as noted in prior comparisons between water and ethanol-based preparations (Levy et al., 2015). Despite this, GIN has shown some activity against other monopisthocotyleans (Fu et al., 2017; Trasviña-Moreno et al., 2019).

4.5.2. In vitro and in vivo drug treatments against T. vistulensis

This study is the first to assess the efficacy of the Biokos (BIO), a biological surfactant, against a platyhelminth parasite. While BIO (from *Pseudomonas H6*) had no effect on *T. vistulensis* eggs, it caused >90% mortality of oncomiracidia within 24 hours at ≥20 mg/L. This larvicidal effect likely results from the disruption of ciliated membranes, as seen in protozoans like *I. multifiliis* and *C. irritans* (Al-Jubury et al., 2018; Marana et al., 2023; Watanabe et al., 2023). Juveniles and adults were less sensitive, with visible effects only at ≥60 mg/L, possibly due to surfactant-induced membrane destabilization (Vandyke et al., 1991).

PZQ showed stage-dependent activity. *In vitro*, concentrations of 80–100 mg/L reduced egg hatching and caused moderate mortality in larvae and juveniles. However, the concentrations ≥20 mg/L proved to be intolerable for catfish. Thus, *in vivo*, 10 mg/L PZQ was applied and led to complete parasite detachment within 1 day and sustained efficacy for 14 days, outperforming previous studies (Székely & Molnár, 1990; Sitjà-Bobadilla et al., 2006). Lower doses (1 and 5 mg/L) caused only temporary effects, with parasites surviving and re-infecting the host.

MBZ exhibited strong ovicidal activity even at 1 mg/L, likely by blocking the cell transport via binding tubulin in the cytoskeleton (Lacey et al., 1987; Buchmann & Bjerregaard, 1990). It shortened oncomiracidia lifespan at ≥40 mg/L but had minimal effects on juveniles and adults. *In vivo*, 10 mg/L MBZ gradually reduced parasite loads over 7–14 days, aligning it with a delayed mode of action via glucose metabolism inhibition (Ahmad & Nizami, 1987; Zhou et al., 2023). Toxicity appeared at 20 mg/L, with 10 mg/L the safe threshold for catfish.

5. CONCLUSION AND RECOMMENDATIONS

This study presents a thorough characterization of *Thaparocleidus vistulensis*, a gill monogenean parasite of European catfish (*Silurus glanis*), and using morphological and molecular approaches. A preliminary classification, mainly based on the male copulatory organ, was validated by genetic identification, and re-description of the species was provided. Despite previous descriptions by Molnár (1980), new histopathological insights were revealed, including the presence of eosinophilic granular cells at attachment sites and damage to the gill's extracellular cartilaginous matrix. Additionally, SEM imaging for pathological changes in infected gills was presented for the first time.

Life cycle studies highlighted key developmental parameters of *T. vistulensis* and how temperature and photoperiod affect reproduction. Egg production was temperature-dependent but not significantly influenced by photoperiod. The findings emphasize the need for forecasting parasite outbreaks and tailoring life cycle-based interventions in intensive aquaculture. However, as this study was conducted *in vitro*, future *in vivo* studies are necessary to fully understand the parasite's reproductive dynamics in real-world aquaculture environments.

Antiparasitic potential of three herbal extracts was evaluated for the first time against *T. vistulensis*. Garlic extract (GAR) demonstrated the strongest efficacy, eliminating parasites across life stages and fully inhibiting egg hatching even at high dilutions. Neem bark (NMB) extract was effective primarily against oncomiracidia, while ginger (GIN) showed minimal activity. These findings suggest GAR and NMB as promising herbal alternatives for monogenean control, but further *in vivo* validation is required before application in aquaculture systems.

In parallel, three drug treatments, Biokos (BIO), praziquantel (PZQ), and mebendazole (MBZ), were assessed. BIO, a biological lipopeptide, proved highly effective against oncomiracidia and juveniles, but not against eggs or adults. PZQ showed only moderate efficacy *in vitro* but demonstrated high effectiveness *in vivo*, with complete parasite elimination following a 24-hour bath at 10 mg/L. MBZ had strong ovicidal effects even at low concentrations, reducing egg viability and delaying parasite development, although its *in vivo* efficacy appeared only after several days. Importantly, drug effects were stage-specific; BIO targeted larvae, PZQ affected juveniles, and MBZ was most effective against eggs. This highlights the need for a combination treatment strategy to destroy all life stages of the parasite.

While *in vitro* data indicated variable efficacy, *in vivo* experiments confirmed the potential of prolonged treatments with lower, safer doses. Notably, both PZQ and MBZ achieved significant parasite reduction or eradication, with MBZ demonstrating a persistent antiparasitic effect at sublethal concentrations.

As no single compound could completely eradicate all parasite stages, additional management strategies are recommended. Quarantine protocols with elevated temperatures and fish transfers could help to eliminate residual eggs and larvae. However, such methods harbour risks in relation to host thermal tolerance and require careful validation *in vivo*.

Overall, this study contributes significantly to understanding of *T. vistulensis* biology, pathology, and control. The findings support integrated management using thermoregulation, herbal alternatives and targeted chemotherapeutics, emphasizing eco-friendly practices in closed systems such as RAS, where water treatment and fish health are closely linked. Future work should validate these treatments *in vivo* to develop sustainable and effective parasite control strategies for European catfish farming.

6. NEW SCIENTIFIC RESULTS

1. For the first time, the present study provides extensive molecular characterization data of *Thaparocleidus vistulensis* (2964 bp), including partial sequences of the SSU rRNA gene, complete sequences of the ITS1, 5.8S rRNA gene, ITS2, and partial sequences of the LSU rRNA gene.
2. This study presents, for the first time, the external morphology and pathological effects of *T. vistulensis* using scanning electron microscopy (SEM) images.
3. This study demonstrates new insights into previously undescribed aspects of the life cycle and reproductive strategies of *T. vistulensis.*
4. This research delivers, for the first time, insights into the influence of environmental factors, including the light-dark cycle and various water temperatures, on different life stages of *T. vistulensis*.
5. This study introduces, for the first time, the use of bacterial-derived lipopeptide *Pseudomonas* H6 Biokos (BIO) and the use of herbal therapeutic antiparasitic agents against *T. vistulensis*.

7. LIST OF PUBLICATIONS

7.1. Peer-reviewed journal articles

1. **Wan Sajiri, W.M.H.**, Székely, C., Molnár, K., Buchmann, K., & Sellyei, B. (2023). Reproductive strategies of the parasitic flatworm *Thaparocleidus vistulensis* (Siwak, 1932) (Platyhelminthes, Monogenea) infecting the European catfish *Silurus glanis* Linnaeus, 1758. *International Journal for Parasitology: Parasites and Wildlife, 22,* 113–120. https://doi.org/10.1016/j.ijppaw.2023.09.010
2. **Wan Sajiri, W.M.H.**, Székely, C., Molnár, K., Kjeldgaard-Nintemann, S., Kania, P. W., Buchmann, K., & Sellyei, B. (2024). Molecular and SEM studies on *Thaparocleidus vistulensis* (Siwak, 1932) (Monopisthocotyla, Ancylodiscoididae). *Scientific Reports*, *14*(1), 10292. https://doi.org/10.1038/s41598-024-61032-3
3. **Wan Sajiri, W.M.H.**, Székely, C., Molnár, K., Buchmann, K., & Sellyei, B. (2025). Pathological effects of *Thaparocleidus vistulensis* (Siwak, 1932) infection on the gills of *Silurus glanis* Linnaeus, 1758. *Acta Veterinaria Hungarica*, *73*(1), 56–63. https://doi.org/10.1556/004.2025.01121
4. **Wan Sajiri, W.M.H.**, Székely, C., Sellyei, B. (2025). Influences of light-dark cycle and water temperature on *in vitro* egg laying, hatching and survival rate of the *Thaparocleidus vistulensis* (Dactylogyridea: Ancylodiscoididae). *Parasitology Research*. *124*(1), 4. https://doi.org/10.1007/s00436-024-08430-8
5. **Wan Sajiri, W.M.H.**, Székely, C., Czeglédi, I., Buchmann, K., & Sellyei, B. (2025). Comparative *in vitro* effects of novel and conventional parasiticides on *Thaparocleidus vistulensis* (Siwak, 1932) (Monopisthocotyla) parasitizing European Catfish (*Silurus glanis*). *Aquaculture Reports*, *43*, 102952. https://doi.org/10.1016/j.aqrep.2025.102952

7.2. Publications in progress

1. **Wan Sajiri, W.M.H.**, Székely, C., Buchmann, K., & Sellyei, B. *In vivo* efficacies of conventional parasiticides on *Thaparocleidus vistulensis* (Siwak, 1932) (Monopisthocotyla) parasitizing European Catfish (*Silurus glanis*). *Aquaculture Reports.*

2. **Wan Sajiri, W.M.H.**, Székely, C., Buchmann, K., & Sellyei, B. *In vitro* efficacy of herbal treatments targeting *Thaparocleidus vistulensis* (Siwak, 1932) in European Catfish (*Silurus glanis*). *Aquaculture Reports.*

7.3. Other related publication

1. **Wan Sajiri, W.M.H.**, Székely, C., & Sellyei, B. (2023). Survey of ectoparasite diversity in different rearing systems (fish ponds and RAS) at a fish farm. In: Hungarian Journal of Aquaculture and Fisheries Science, ISSN 3003-9797. (Vol. 9), No. 2, pp. 22 – 29. https://www.agrarlapok.hu/halaszat-tudomany-20232

7.4. Conference abstracts/ papers/ oral and poster presentations

1. **Wan Sajiri, W.M.H.**, Székely, C., & Sellyei, B. (2022). Advancement of my PhD work at the field and the laboratory: Parasitic infestation and feasible control against them in RAS. [Oral presentation]. In: Proceedings of Young Researchers' Day 2022, Budapest, Hungary, October 12, 2022.
2. **Wan Sajiri, W.M.H.**, Sellyei, B., & Székely, C. (2023). Reproductive strategies of the parasitic flatworm *Thaparocleidus vistulensis* (Platyhelminthes, Monogenea) infecting the European catfish (*Silurus glanis*). [Oral presentation]. In: Proceeding of Academical Days / Parasitology-Zoology-Fish Pathology Session, Budapest, Hungary, January 20–23, 2023.
3. **Wan Sajiri, W.M.H.**, Székely, C., & Sellyei, B. (2023). Reproductive strategies of the parasitic flatworm *Thaparocleidus vistulensis* (Monogenea) infecting the European catfish (*Silurus glanis*). [Oral presentation]. In: Proceedings of the 47th Hungarian Scientific Conference on Fisheries & Aquaculture (A XLVII. Halászati Tudományos Tanácskozás), Szarvas, Hungary, June 7–8, 2023.
4. **Wan Sajiri, W.M.H.**, Székely, C., Buchmann, K., & Sellyei, B. (2023). Reproductive strategies of the parasitic flatworm *Thaparocleidus vistulensis* (Platyhelminthes, Monogenea) infecting the European catfish

(*Silurus glanis*). [Oral presentation]. In: Proceeding of the 21st International EAFP Conference on Diseases of Fish and Shellfish, Aberdeen, Scotland, September 11–14, 2023.

5. **Wan Sajiri, W.M.H.**, Székely, C., Molnár, K., Buchmann, K., & Sellyei, B. (2023). Reproductive strategies of the parasitic flatworm *Thaparocleidus vistulensis* (Platyhelminthes, Monogenea) infecting the European catfish *Silurus glanis* Linnaeus, 1758. [Poster presentation]. In: Proceeding of the 9th International Symposium on Monogenea, Lucknow, India, October 8–11, 2023.
6. **Wan Sajiri, W.M.H.**, Székely, C., & Sellyei, B. (2024). Introducing RASOPTA: Safeguarding future production of fish in aquaculture systems with water recirculation. [Oral presentation]. In: Scientific Meeting of the Hungarian Parasitological Society on Fish Parasitology, April 10, 2024.
7. **Wan Sajiri, W.M.H.**, Székely, C., & Sellyei, B. (2024). Introducing RASOPTA: Safeguarding future production of fish in aquaculture systems with water recirculation. [Oral presentation]. In: Proceedings of the 48th Scientific Conference on Fisheries & Aquaculture (A XLVIII. Halászati Tudományos Tanácskozás), Szarvas, Hungary, June 5–6, 2024.
8. **Wan Sajiri, W.M.H.**, Székely, C., Molnár, K., Kjeldgaard-Nintemann, S., Kania, P.W., Buchmann, K., & Sellyei, B. (2024). Molecular and SEM studies on *Thaparocleidus vistulensis* (Siwak, 1932) (Monopisthocotyla, Ancylodiscoididae). [Poster presentation]. In: Proceedings of the 48th Scientific Conference on Fisheries & Aquaculture (A XLVIII. Halászati Tudományos Tanácskozás), Szarvas, Hungary, June 5–6, 2024.