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Transmission route used by parasitic lasidium larvae of the freshwater mussel *Anodontites trapesialis* on guppies *Poecilia reticulata* during short cohabitation

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Summary

We describe for the first time the transmission route employed by the parasitic larvae of the freshwater mussel *Anodontites trapesialis* (Lamarck, 1819) during cohabitation on the guppy *Poecilia reticulata* Peter, 1859. The freshwater mussel and fish-host were employed as a model system to investigate the infection and parasite establishment. Laboratory experiments included video recording and histopathological analysis of the infection. *In vivo* video observations demonstrated that lasidium larvae could be transmitted to guppies during direct contact. A series of histology samples and photography suggest that this larva attaches and colonizes possibly on-site on the outer surface of the fish, causing cellular inflammation in the epidermis layer with cellular hyperplasia in the zone of parasite attachment. An evident hyaline layer, cellular hypertrophy, and a large number of undifferentiated proliferating cells were observed. Hemorrhagic tissue and swelling were observed in the epidermis and dermic zone. The total number of larvae per male and female guppy was 525 ± 86 and 494 ± 167, respectively. No parasitic preference was detected for male versus female parasitized fish.
Keywords: Freshwater; bivalve; environmental interaction; lasidium larvae; parasitism; histology

Introduction

Bivalves are considered one of the most representative groups in the Phylum Mollusca, with more than 1,100 freshwater species worldwide according to Torres *et al.* (2018). The limnic mussel *Anodontites trapesialis* (Lamarck, 1819) has a lasidium larvae that require fish as a temporary host (Torres *et al.,* 2018). These larvae are ectoparasites of aquatic vertebrates, generally, fish, and the occurrence of the limnic mussel *A. trapesialis* (Mycetopodidae) has increased along with the fish aquaculture. In aquaculture facilities, fish are reared in ponds or land-based tanks and such systems provide an opportunity for diseases or chronic infections associated with the development of different pathogens (Kearn, 2004). In the last decade, with the increasing fish aquaculture in the South American river basins, especially in Brazil according to FAO (2012), aquaculture of the freshwater cichlid fish Nile tilapia *Oreochromis niloticus* Linnaeus, 1758 has been an appreciated sector of food production. These cichlids, in general, are the main hosts for parasites with direct cycle (García-Magaña *et al.,* 2019; Rodriguez-Santiago *et al.,* 2019; Valladão *et al.,* 2013, 2014, 2016; Grano-Maldonado *et al.,* 2018).

Currently, the best interest in aquaculture is to identify fish health problems that cause excessive economic losses and provide an accurate description of the larvae pathogen transmission and its effect on fish to suggest an effective treatment in a controlled fish farm. However, studies related to transmission and treatment are minimal. There are few studies concerning the limnic mussel *A. trapesialis* describing anatomic characters, histopathology, and

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embryonic development (Simone, 1994; Silva-Souza & Eiras, 2002). Although the major routes of transmission of these larvae have not been extensively studied, relatively few studies have examined some effects in fish (Silva-Souza *et al.,* 2011). More studies concerning and contributing to a better understanding of the transmission of these bivalves between other fish hosts are needed, and in particular, why lasidium larvae may abandon a suitable fish host and transfer to the water column or substrate or other fish since these questions are as yet unknown. Furthermore, knowledge about host-parasite interaction and its impact on animal health and production is still scarce. In this sense, the present work aims to describe the experimental infection of *A. trapesialis* larvae parasitizing the guppy *Poecilia reticulata* (Peter, 1859) in laboratory conditions. The histopathological damage caused by larvae as ectoparasites were analyzed.

Materials and Methods

Mussel and fish collection

Bivalves *Anodontites trapesialis* were collected with hand nets in fish ponds in Guapó, Goiás, Brazil (16°48'45.90"S, 49°32'8.74"W) and transferred in bags to the aquarium facility at the Federal University of Goiás (UFG). Upon arrival, the animals were acclimatized in 10-L aquaria tanks under laboratory conditions for 17 days, one animal *per* tank, and fed with commercial Spirulina algae (Source Naturals) (Ramli *et al*., 2021).

P. reticulata specimens were collected from aquaculture tanks at the Sanitation Company of Goiânia (SANEAGO) (16°37'59 "S, 49°15'44" W). Fish were naïve to the infection and were acclimatized in reconstituted water in 50-L tanks for 30 days and maintained at controlled temperature (27 ± 1 °C), oxygenation and pH (7 ± 0.5) and fed with commercial food (Cardume® 36 %; VB alimentos) with daily clean water exchange (30 %) to control ammonia levels. Feeding was stopped on the day before the start of all experiments to maintain water quality for video footage.

Fish cohabitation experiment

Acclimatized fish were distributed so that nine male (standard length = 1.62 ± 0.14 cm, total weight = 0.11 ± 0.01 g) and nine female (standard length = 2.09 ± 0.24 cm, total weight = 0.24 ± 0.09 g) guppies (n = 18) were cohabitated with three mature mussels *A. trapesialis* (length: 12.07 ± 0.42 cm; weight: 150.46 ± 12.95 g) at the same time in 50-L tanks. The experimental aquaria were maintained for 15 days under controlled temperature (26 ± 1.0 °C) and pH (7.6 ± 0.2). A control group of guppies was kept in another aquarium without the presence of mussels.

Video footage

Cohabitation between *P. reticulata* and *A. trapesialis* was video recorded during a series of three-minute video footages to investigate the bivalve infection process (i.e., larvae- fish attachment). A Cannon T3i camera was set up and focused on the interaction between fish-bivalve. Video editing was performed with ApowerEdit software for better resolution and photography. After exposure,

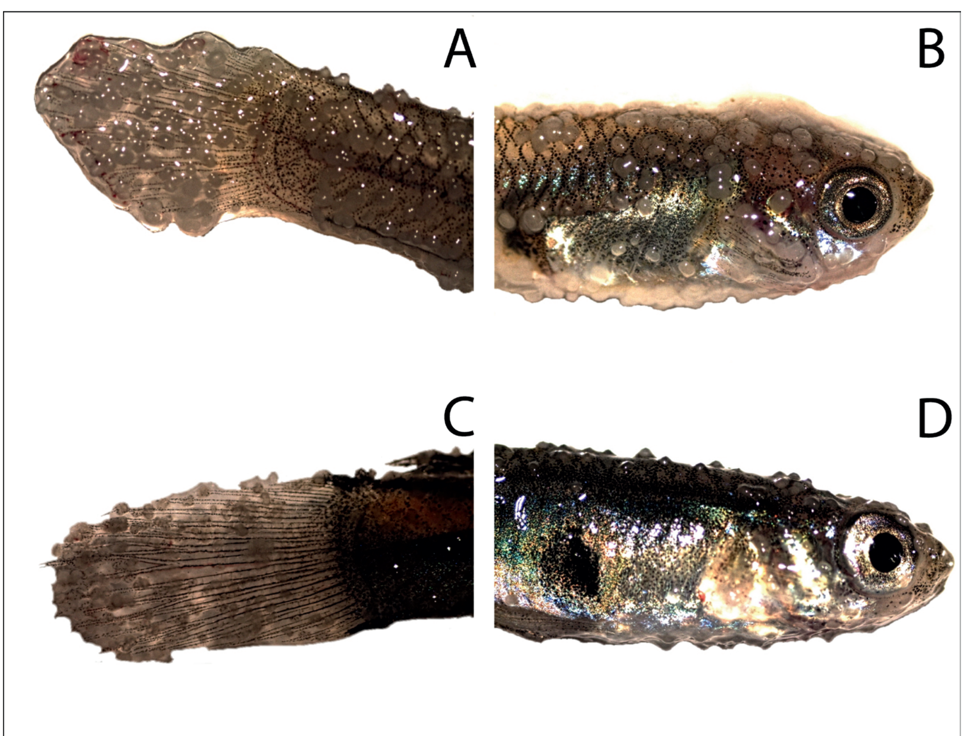

Fig. 1. Male (A – B) and female (C – D) guppy *Poecilia reticulata* infected with lasidium larvae of *Anodontites trapesialis.*

each fish was euthanized by hypothermia, fixed by immersion in 4 % buffered formaldehyde in 0.2 M phosphate buffer (PBS) at pH 7.2 for 4 h in constant agitation, washed in 0.2 M PBS buffer at pH 7.2, and stored in 70 % ethanol (Rocha *et al*., 2015). The skin surface, head, dorsum, venter, and fins (ventral, pectoral, dorsal, anal, caudal) of each fish were examined for larvae under a Zeiss stereomicroscope at total magnification of 40x. Some sections of the median region of the fish were processed for histological analysis. All experiments followed the Brazilian laws for animals and scientific procedures by the Biodiversity Authorization and Information System – SISBIO.

Histological analysis

Fish sections from the upper body area were embedded in glycol-methacrylate resin (Historesin, Leica, Germany) according to the manufacturer´s recommendations. Next, transverse sections were cut at 2.5 µm and stained with 1 % toluidine blue pH 8.5 (Rocha *et al*., 2015). Finally, the slides were microscopically examined under an optical microscope (Axio Lab.A1, Zeiss®) associated with an Axiocam 105 color (Zeiss®) camera and ZEN 2.6 software. Each photograph of the host-parasite interaction was subjected to analysis. The histopathology of the infection was analyzed qualitatively and quantitatively. After, for ten parasites attached to the skin, the parasite's area (μm^2), thickness (µm), and area of the hyaline layer (μm^2) were measured (average ± SD) using ImageJ software.

Statistical analysis

Statistical analyses were performed using R software, and graphs were prepared in Prism 7 GraphPad®. Normality and homoscedasticity were verified with Shapiro-Wilk and Levene tests, respectively. To determinate the parasitic preference between male versus female parasitized fish. *The student's t-test* was employed to assess an association between parasite infection and fish sex. Results were considered significant with $p < 0.05$.

Ethical Approval and/or Informed Consent

Animal care and handling were carried out by institutional guidelines according to Brazilian laws by a Permanent License for Collections of Zoological Material number 34144-1 by the Biodiversity Authorization and Information System – SISBIO.

Conflict of Interest

Authors have no potential conflict of interest about this submission to Helminthologia.

Data Availability Statement

We author authorize the availability of the research data presented.

Results

After cohabitation of guppies to mussels for 15 days, larvae of *A. trapesialis* on the host epidermis were visible to the naked eye (Fig. 1). The most affected regions were the dorsum, head, caudal fin, and venter, respectively (Fig. 2A). A similar total number of

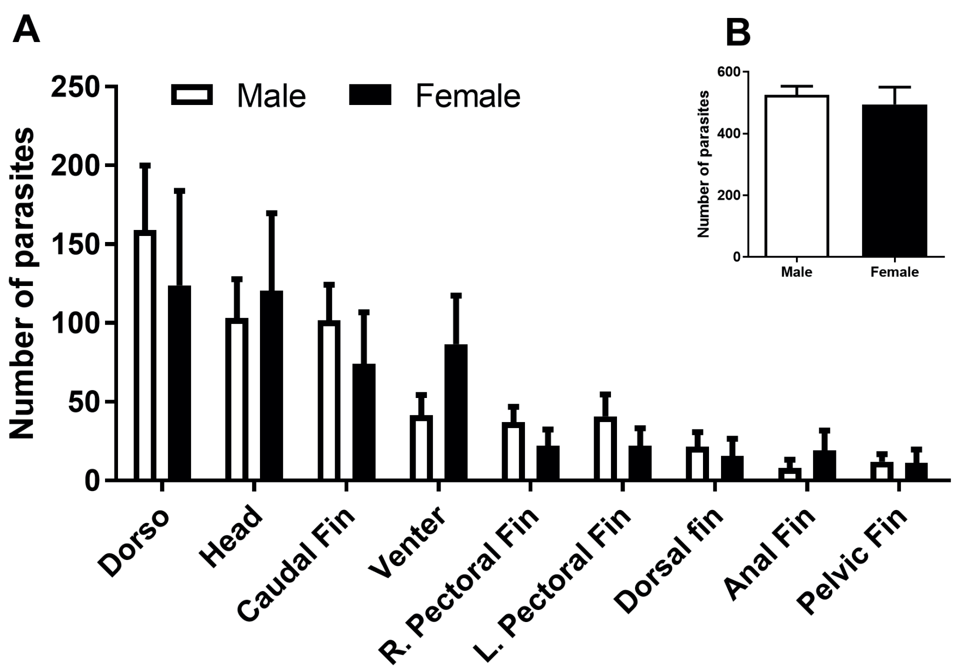

Fig. 2. The number of lasidium larvae on the skin of male and female *P. reticulata* per region (A).
The total number of larvae per sex (B). R. pectoral fins: right pectoral fins; L. pectoral fins: left pectoral fins. Values expressed on mean ± SD.

larvae per male and female guppy was observed (525 ± 86 and 494 ± 167, respectively; $p > 0.05$; Fig. 2B). During a total of 3 min of recorded video, the fish host took small bites from any surface of the bivalve foot, and in some parts of the video, it was possible to observe the fish diving towards the bivalve foot to make tactile stimulations. During this approach, the fish had direct contact, demonstrating physical contact/interaction between organisms. These contacts were recorded in this study in terms of photo and video editions (see the video in supplementary material).

Histological assessment

Histological sections of *P. reticulata* skin infected by lasidium larvae of freshwater mussel *A. trapesialis* from the cohabitation experiment showed several larvae attached to the fish epidermis. There was an evident extended hyaline layer (1.30 ± 0.48 µm wide) with a total area of 513.5 ±189.34 μm^2, and the parasite area was 2592.28 ± 931.89 μm^2. There was noticeable cellular inflammation in the epidermis with cellular hyperplasia in the zone of parasite attachment. The presence of cellular hypertrophy and a large number of undifferentiated proliferating cells were observed (Fig. 3). Haemorrhagic and evident cell enlargement and tissue swelling were observed in the epidermis and dermic zone (Fig. 3).

Discussion

This investigation represents the first study of guppies *P. reticulata* infected by lasidium larvae of the freshwater mussel *A. trapesialis* during a small cohabitation experiment and showed several larvae attached to the fish epidermis. Results indicate that the infection induces dermal alterations and may increase the susceptibility of fish to other parasites. A similar infection was reported in *Tilapia rendalli* (Boulenger, 1897) and *Hypostomus regani* (Ihering, 1905) (Silva-Souza & Eiras, 2002). Silva-Souza & Eiras (2002) reported similar histopathology skin damage caused by *A. trapesialis* larvae attached to the fish epidermis. In the present study, a hyaline layer formed by fish cells covering the parasite caused evident inflammation that was visible without a microscope and hemorrhagic zones after larval release from the skin. This investigation indicates that the guppy *P. reticulata* was an appropriate model system for the analysis of the host-parasite interaction of freshwater bivalves (Unionoida, Mycetopodidae). Several studies of a fish model- parasite have been demonstrated previously on the guppy *P. reticulata*, e.g. *Gyrodactylus turnbulli* Harris, 1986 (Cable *et al.,* 2002; Cable *et al.,* 2005; Harris, 1985, 1988, 1989; Harris & Cable, 2000). Gyrodactylids use a variety of different strategies to infect new hosts. Grano-Maldonado (2014) examined the transmission strategies employed by parasites to colonize new hosts during feeding, i.e. scavenging activities from dead parasitized hosts to predatory clean fish. The fish model was the three-spined sticklebacks, *Gasterosteus aculeatus* L., exposed to *Gyrodactylus gasterostei* Glaser, 1974 in a small tank. After 15 days of cohabitation, female fish mortality (n = 1) was observed; however, due to the observed infection, it is recommended that mortality after cohabitation be analyzed for long periods in future studies.

Colonization of new fish host was demonstrated using a live video recording. Similarly, in the present study, video recording was employed to demonstrate the live interaction between species, guppies approached the mussel and took small bites (direct contact), and this approaching behavior would increase the chances of parasites contacting a fish host, particularly if potential hosts are either scavengers or benthophagous feeders. Some guppies' feeding behaviour has been extensively studied and, whilst it is generally considered to be a surface feeder (Cable *et al*., 2002), which would decrease the possibility of infection, the video recording in this study shows the guppies approaching the mussel, which is known to live preferentially on the benthic zone's substrate. Other researchers, such as Meyers & Millemann (1977), employed the parasite/fish and mollusk infection mode and studied the susceptibility of salmonid fishes to infection with the glochidia of the freshwater mussel *Margaritifera margaritifera* under controlled

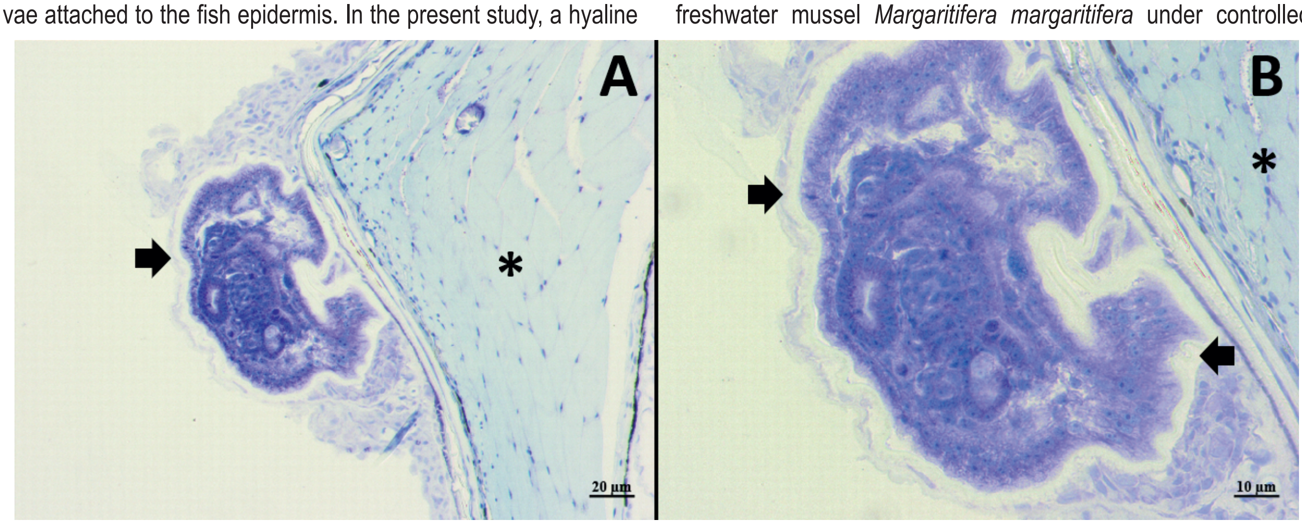

Fig. 3. Histological section of infected *P. reticulata* with lasidium larvae of *A. trapesialis.* Arrow indicates the hyaline barrier and asterisks the fish muscle. Technique: skin embedded in glycol-methacrylate resin and stained with 1 % toluidine blue pH 8.5. Scale bars: 20 µm (A) and 10 µm (B).

conditions. Other authors such as Meyers & Millemann (1977) and Howerth & Keller (2006), performed other experimental gill inoculation, inducing glochidiosis in fish as a model to evaluate the severe branchial lesions caused by the glochidia infection. The increase of parasitic infection may be promoted by the association between fish host and parasite in an environment with poor water quality, nutritional deficit, and other parasitic diseases in fish causing severe epidermal lesions (Khan, 2004). Freshwater mussels perform crucial ecosystem services, as they can restore habitat functionality by acting as "microhabitat engineers" which promote local biodiversity (Chowdhury *et al.,* 2015). Recent laboratory experiments have demonstrated the critical role of the host–parasite relationship of freshwater mussels for quantifying fish physiology and behaviour. For example, Douda *et al.,* (2018) analysed that stressed fish increased their susceptibility to these parasitic mussel larvae. Other studies suggested that different glochidia species interact with their fish host in distinct ways. Gendron *et al.* (2019) showed that glochidia retention and survival is enhanced in stressed and cortisol-injected fish hosts. The impact of glochidia infections on the host's physiology is scarce, and no information is available concerning energy consumption in parasitized fish. However, Methling *et al.* (2019) showed that tolerance is the predominant coping mechanism for fish *Rhodeus amarus* (Cyprinids) (exploit mussels by ovipositing into their gills) and mussels *Anodonta anatina* (develop on the epidermis and gills of fish) so this interaction may be considered *high* in terms on energy metabolism mainly for the fish. Other researchers such as Slavik *et al.* (2017) employed the model glochidia of the invasive unionid mussel *Sinanodonta (Anodonta) woodiana* and common carp, *Cyprinus carpio.* These authors studied parasite-induced increases in the energy costs of movement of host freshwater fish, since the larvae (glochidia) can cause damage to a fish's gills and, as a consequence, less effective respiration rate, and this may reduce the swimming activity by the host fish. Concerning the effect in fish blood parameters, Douda *et al.* (2017) employed the model parasitic larvae (glochidia) of the invasive freshwater mussel *Sinanodonta* (*Anodonta*) *woodiana* and the fish host *Squalius cephalus*. Results showed that the infection may cause an unpredicted decrease in the condition factor of parasitized native fish. Changes in host physiology and condition status were recorded measuring fish plasma. These authors concluded that fish hosts are an obligatory part of the reproductive cycle of the mussel and the main vector for spreading and it has great potential to affect fisheries and aquaculture sectors and to serve as a strong motivating factor for invasive species control. Horký *et al.,* (2014, 2019) focused their research on fish behaviour and thermal preferences studying the fish- parasite model *Squalius cephalus- Anodonta anatine.* These authors investigated alterations in host behaviour induced by the parasite, which revealed that infected fish were mostly less active in the laboratory and in the field, suggesting that behavioural changes in the fish that are induced by glochidia and may cause reductions in fish swimming activity. Concerning, thermal preferences, Horký *et al.* (2019) employed the model of *Salmo trutta-Margaritifera margaritifera* to reveal whether parasitisation alters fish behavioural thermoregulation. Their results showed that infested fish prefer temperatures either above or below the temperature preferred by uninfested individuals. With these findings, these authors revealed that thermoregulation plays a fundamental role in the relationship of mussels and their fish hosts. Supporting these findings, Slavik *et al.* (2017) demonstrated that parasites have a direct relationship in the energy costs of movement (mainly swimming) of host fish.

Among other researchers, such as Douda *et al.* (2017) mentioned that there is a direct impact of invasive bivalve parasitism on freshwater fish physiology, this result agrees with the latest research by Chowdhury *et al.* (2021) using the model *Salmo trutta- Margaritifera margaritifera.* Demonstrated that glochidiosis would impair the growth of fish. Infected fish gained less weight than the control parasite- free fish group throughout the experiment and no effect on host mortality was detected. In the present study, by the parasitic larvae of the freshwater mussel *A. trapesialis* during cohabitation on the guppy *P. reticulate,* no considerable host mortality was detected either. In agreement with Douda *et al*. (2017), fish hosts are an obligatory part of the reproductive cycle of the mussel and the key vector for spreading and affecting particularly the aquaculture sector, further studies of the biology of invasive species control are needed.

In Brazil, the molluscan research in the Cerrado biome has been focused on gastropods, mainly the genus *Biomphalaria* which was intensely studied due to its relation to schistosomiasis (Sampaio *et al.,* 2020) and bioindicator of contamination (Batista *et al.,* 2020). Studies of lasidium larvae in fish ponds in Brazil have been carried out on *Oreochromis niloticus* (L.) (Cichlidae) and it has been considered as an emerging pest in fish farm areas and tourist fishing places in the states of Paraná, Santa Catarina and the Rio Grande do Sul, Southern Brazil region (Agudo-Padrón & Lenhard, 2011; Agudo-Padrón, 2012, 2013, 2019) generating significant socioeconomic farming impact. The data show that the presence of bivalves in fish ponds may cause damage to fish health and possible fish production could be reduced and further studies should be implemented.

Our study examined the transmission strategies employed parasitic larvae of *A. trapesialis* parasites to colonize new fish hosts such as guppies *P. reticulata.* Larvae did not show specificity between female and male fish. Until now, fish *T. rendalli* (Cichlidae) and *H. regani* (Osteichthyes) were known as possible temporary hosts (Silva-Souza & Eiras, 2002). In this study, we employed for the first time the fish parasite model to guppies *P. reticulata* challenged with *A. trapesialis*. More studies should be conducted with other farmed fish exposed to this parasite to describe the possible effect during aquaculture activities. This study provides information to support that the lasidium larvae may be a cause of high mortality on farmed fish in Brazil. However, further transmission strategies, natural control remedies may be worth studying.

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References

Agudo-Padrón, A.I. (2019): The Giant Native Freshwater Mussel/ Naiad Mycetopodidae *Anodontites trapesialis* (Lamarck, 1819), an Emerging Invasive Plague in Fish Culture Farms of Santa Catarina State/ SC and Other Localities in Southern Brazil: New Geographical Records and Brief Revision. *Ellipsaria*, 21(2): 32 – 38

Agudo-Padrón, A.I. (2012): Proposed strategies for conservation of Neotropical endangered limnic mussels/ naiads (Unionoida, Mycetopodidae) in Southernmost Brazil. *Ellipsaria*, 14(4): 44 – 47

Agudo-Padrón, A.I. (2013): New occurrences of native freshwater mussels/ naiads in fish farms/ dams in the Southern Brazil region. *Ellipsaria*, 15(4): 40 – 41

Agudo-Padrón, A.I., Lenhard, P. (2011): Continental mollusc fauna of the Great Porto Alegre central region, RS, Southern Brazil. *Biodivers J*, 2(4): 163 – 170

Batista, M., Sampaio, P., Bastos, B., Da Silva, L., Grano-Maldonado, M., Lopes, R. (2020): Toxicity of engineered nanomaterials to aquatic and land snails: A scientometric and systematic review. *Chemosphere*, 260: 127654. DOI: 10.1016/j.chemosphere.2020.127654

Cable, J., Scott, E.C.G., Tinsley, R.C., Harris, P.D. (2002): Behaviour favoring transmission in the viviparous monogenean *Gyrodactylus turnbulli*. *J Parasitol*, 88: 183 – 184. DOI: 10.1645/0022-3395(2002)088[0183:BFTITV]2.0.CO;2

Cable, J., Van Oosterhout, C., Barson, N., Harris, P.D. (2005): *Gyrodactylus pictae* n. sp. from the Trinidadian swamp guppy *Poecilia picta* Regan, with a discussion on species of *Gyrodactylus* von Nordmann, 1832 and their poeciliid hosts. *Syst Parasitol*, 60: 159 – 164. DOI: 10.1007/s11230-004-6348-4

Chowdhury, G. W., Zieritz, A., Aldridge, D. C. (2015): Ecosystem engineering by mussels supports biodiversity and water clarity in a heavily polluted lake in Dhaka, Bangladesh. *Freshw Sci,* 35: 188 – 199. DOI: 10.1086/684169

Chowdhury, M.M.R., Marjomäki, T.J., Taskinen, J. (2021): Effect of glochidia infection on growth of fish: freshwater pearl mussel *Margaritifera margaritifera* and brown trout *Salmo trutta*. *Hydrobiologia* 848: 3179 – 3189. DOI: 10.1007/s10750-019-03994-4

Douda, K., Martin, M., Glidewell, E., Barnhart, C. (2018): Stress-induced variation in host susceptibility to parasitic freshwater mussel larvae. *Hydrobiologia*, 810(1): 265 – 272. DOI: 10.1007/s10750-016-2895-3

Douda, K., Velíšek, J., Kolářová, J., Rylková, K., Slavík, O., Horký, P., Langrová, I. (2017): Direct impact of invasive bivalve (*Sinanodonta woodiana*) parasitism on freshwater fish physiology: evidence and implications. *Biol Invasions*, 19(3): 989 – 999. DOI: 10.1007/s10530-016-1319-7

Food and Agriculture Organization (FAO) (2012): *El estado mundial de la pesca y la acuicultura 2012* [*Status of World Fisheries and Aquaculture 2012*]. Roma, 251 pp.

García-Magaña, L., Rodríguez-Santiago, M., Grano-Maldonado, M., Jiménez-Vasconcelos, L., Guerra-Santos, R. (2019): The effectiveness of sodium chloride and formalin in trichodiniasis of farmed freshwater tilapia *Oreochromis niloticus* (Linnaeus, 1758) in southeastern Mexico. *Lat Am J Aquat Res*, 47(1): 164 – 174. DOI: 10.3856/vol47-issue1-fulltext-18

Gendron, A. D., Sanchez, D., Douville, M., Houde, M. (2019): Stress-related gene transcription in fish exposed to parasitic larvae of two freshwater mussels with divergent infection strategies. *Dis Aquat Organ*, 132(3), 191 – 202. DOI: 10.3354/dao03319

Grano Maldonado, M. I., Rodríguez-Santiago, M.A., García-Vargas, F., Nieves-Soto, M., Soares, F. (2018): An emerging infection caused by *Gyrodactylus cichlidarum* (Monogenea) associated with mortality on farmed tilapias *Oreochromis niloticus* on the Mexican Pacific Coast. *Lat Am J Aquat Res,* 46(5): 961 – 968. DOI: 10.3856/vol46-issue5-fulltext-9

Grano-Maldonado, M.I. (2014): *Gyrodactylus gasterost*ei a difficult meal to swallow for the 3-spined sticklebacks, *Gasterosteus aculeatus* L. *Scanning*, 36: 614 – 621. DOI: 10.1002/sca.21162

Harris, P.D. (1985): Observations on the development of the male reproductive system in *Gyrodactylus gasterostei* Gläser, 1974 (Monogenea, Gyrodactylidae). *Parasitology,* 91: 519 – 529. DOI: 10.1017/S0031182000062764

Harris, P.D. (1988): Changes in the site-specificity of *Gyrodactylus turnbulli* Harris, 1986 (Monogenea) during infections of individual guppies (*Poecilia reticulata* Peters, 1859). *Can J Zool*, 66: 2854 – 2857. DOI: 10.1139/z88-414

Harris, P.D. (1989): Interactions between population growth and sexual reproduction in the viviparous monogenean *Gyrodactylus turnbulli* Harris, 1986 from the guppy *Poecilia reticulata* Peters. *Parasitology,* 98: 245 – 251. DOI: 10.1017/S0031182000062156

Harris, P.D., Cable, J. (2000): *Gyrodactylus* poeciliae n. sp. and *G. milleri* n. sp. (Monogenea: Gyrodactylidae) from *Poecilia caucana* (Steindachner) from Venezuela. *Syst Parasitol,* 47: 79 – 85. DOI: 10.1023/A:1006413804061

Horký, P., Douda, K., Maciak, M., Závorka, L., Slavik, O. (2014): Parasite-induced alterations of host behaviour in a riverine fish: the effects of glochidia on host dispersal. *Freshw Biol*, 59(7): 1452 – 1461. DOI: 10.1111/fwb.12357

Horký, P., Slavík, O., Douda, K. (2019): Altered thermoregulation as a driver of host behaviour in glochidia-parasitised fish. *J Exp Biol*, 222(1): jeb184903. DOI: 10.1242/jeb.184903

Howerth, E., Keller, A. (2006): Experimentally Induced Glochidiosis in Smallmouth Bass (*Micropterus dolomieu*). *Vet Path*, 43: 1004 – 1007. DOI:10.1354/vp.43-6-1004

Kearn, G. (2004): *Leeches, Lice, and Lampreys. A natural history of skin and gill parasites of fishes*. Springer, Netherlands, 230 pp.
Khan, R.A. (2004): Disease outbreaks and mass mortality in cultured Atlantic cod, *Gadus morhua* L., associated with *Trichodina murmanica* (Ciliophora). *J Fish Dis*, 27: 181 – 184. DOI: 10.1111/j.1365-2761.2004.00525.x
Methling, C., Douda, K., Reichard, M. (2019): Intensity-dependent energetic costs in a reciprocal parasitic relationship. *Oecologia*, 191(2): 285 – 294. DOI: 10.1007/s00442-019-04504-y
Meyers, T.R., Millemann, R.E. (1977): Glochidiosis of salmonid fishes. I. Comparative susceptibility to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). *J Parasitol*, 63(4): 728 – 733
Ramli, M.Z., Ibrahim, A., Yusoff, A., Rak, A.E., Wei, L.S. (2021): Effects of feeding treatments on growth and survival of Asian clam (*Corbicula fluminea*) in the hatchery. *J Agrobiotechnol*, 12: 58 – 65. DOI: 10.37231/jab.2021.12.1.244
Rocha, T.L., Santos, A.P.R. Dos, Yamada, Á.T., Soares, C.M.D.A., Borges, C.L., Bailão, A.M., Sabóia-Morais, S.M.T. (2015): Proteomic and histopathological response in the gills of *Poecilia reticulata* exposed to glyphosate-based herbicide. *Environ Toxicol Pharmacol*, 40: 175 – 186. DOI: 10.1016/j.etap.2015.04.016
Rodríguez-Santiago, M., García-Magaña, L., Grano-Maldonado, M., Silva-Martínez, E., Guerra-Santos, J., Gelabert, R. (2019): First record of *Trichodina centrostrigeata* Basson, Van As & Paperna, 1983 (Ciliophora: Trichodinidae) from *Oreochromis niloticus* (Linnaeus, 1758) cultured in southeastern Mexico. *Lat Am J Aquat Res*, 47(2): 367 – 370. DOI: 10.3856/vol47-issue2-fulltext-18
Sampaio, P., Batista, M., Da Silva, B., Bastos, B., Sueli, M., Celma De Oliveira, E., Da Silva, L., Barreto, J., Lopes, R. (2020): Molluscicidal activity of polyvinylpyrrolidone (PVP)-functionalized silver nanoparticles to *Biomphalaria glabrata*: Implications for control of intermediate host snail of *Schistosoma mansoni. Acta Trop,* 11: 105644. DOI: 10.1016/j.actatropica.2020.105644
Silva-Souza, Â.T., Eiras, J.C. (2002). The histopathology of the infection of *Tilapia rendalli* and *Hypostomus regani* (Osteichthyes) by Lasidium larvae of *Anodontites trapesialis* (Mollusca, Bivalvia). *Mem Inst Oswaldo Cruz*, 97(3): 431 – 433. DOI: 10.1590/S0074-02762002000300029
Silva-Souza, Â.T., Guardia-Felipi, P., Arrebola, N.R. (2011): Embryonic development of *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia: Mycetopodidae). *Braz J Med Biol Res*, 71(1): 139 – 144. DOI: 10.1590/S1519-69842011000100020.
Simone, L.R.L. (1994): Anatomical characters and systematics of *Anodontites trapesialis* (Lamarck,1819) from South America (Mollusca, Bivalvia, Unionoida, Muteloidea). *Stud Neotrop Fauna E.*, 29(3): 169 – 185. DOI: 10.1080/01650529409360929
Slavik, O., Horky, P., Douda, K., Velíšek, J., Kolářová, J., Lepič, P. (2017): Parasite-induced increases in the energy costs of movement of host freshwater fish. *Physiol Behav*, 171: 127 – 134. DOI: 10.1016/j.physbeh.2017.01.010
Torres, S., Cao, L., Gutiérrez, G., De Lucía, M., Brea, F, Darrigran, G. (2018): Distribution of the Unionida (Bivalvia, Paleoheterodonta) from Argentina and its conservation in the Southern Neotropical Region. *PLoS ONE*, 13(9): e0203616. DOI: 10.1371/journal.pone.0203616
Valladão, G.M.R., Alves, L.O., Pilarski, F. (2016): Trichodiniasis in Nile tilapia hatcheries: diagnosis, parasite: host-stage relationship and treatment. *Aquaculture*, 451: 444 – 450. DOI: 10.1016/j.aquaculture.2015.09.030
Valladão, G.M.R., Gallani, S.U., Pádua, S.B., Martins, M.L., Pilarski, F. (2014): *Trichodina heterodentata* (Ciliophora) infestation on *Prochilodus lineatus* larvae: a host-parasite relationship study. *Parasitology,* 141: 662 – 669. DOI: 10.1017/S0031182013001480
Valladão, G.M.R., Pádua, S.B., Gallani, S.U., Menezes-Filho, R.N., Dias-Neto, J., Martins, M.L., Pilarski, F. (2013): *Paratrichodina africana* (Ciliophora): a pathogenic gill parasite in farmed Nile tilápia. *Vet Parasitol*, 197: 705 – 710. DOI: 10.1016/j.vetpar.2013.04.043