

Infection of Atlantic tripletail *Lobotes surinamensis* (Teleostei: Lobotidae) by brain metacercariae *Cardiocephaloides medioconiger* (Digenea: Strigeidae)

Isaure de Buron ^{Corresp., 1}, Kristina M. Hill-Spanik ¹, Tiffany Baker ², Gabrielle Fignar ³, Jason Broach ³

¹ Department of Biology, College of Charleston, Charleston, South Carolina, United States

² Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, South Carolina, United States

³ Marine Resources Research Institute, South Carolina Department of Natural Resources, Charleston, South Carolina, United States

Corresponding Author: Isaure de Buron

Email address: deburoni@cofc.edu

Three juvenile Atlantic tripletail *Lobotes surinamensis* caught opportunistically in Charleston Harbor (South Carolina, USA) and maintained in captivity for over three months displayed an altered swimming behavior. While no direct causation can be demonstrated herein, fish were infected in their brain by strigeid trematode larvae (metacercariae) of *Cardiocephaloides medioconiger*, which were identified via ITS2 and 28S ribosomal RNA gene sequencing. Histology showed nonencysted metacercariae within the brain ventricle between the optic tectum and tegmentum, causing distortion of tegmental parenchyma. Aggregates of mononuclear inflammatory cells were in the ventricle adjacent to metacercariae. Metacercarial infection by *Cardiocephaloides medioconiger* has been reported from the brain and eyes of only two other fish species from the northern US Atlantic coast: the grey mullet *Mugil cephalus* and silverside *Menidia menidia*, but this identification is problematic and needs molecular verification. Atlantic tripletail is a new report as a second intermediate host for *C. medioconiger* and South Carolina is a new locality. *Cardiocephaloides* species in general have a low host specificity and infection by *C. medioconiger* could propagate to other fishes and affect neighboring natural ecosystems.

Infection of Atlantic tripletail *Lobotes surinamensis* (Teleostei: Lobotidae) by brain
metacercariae *Cardiocephaloides medioconiger* (Digenea: Strigeidae)

Running head: Brain parasite in tripletail fish

Isaure de Buron^{1*}, Kristina M. Hill-Spanik¹, Tiffany Baker², Gabrielle Fignar³, Jason Broach³

¹Department of Biology, College of Charleston, Charleston, South Carolina 29412, USA

²Department of Pathology and Laboratory Medicine, Medical University of South Carolina,
Charleston, South Carolina 29425, USA

³South Carolina Department of Natural Resources, Marine Resources Research Institute, 217 Ft
Johnson Rd, Charleston, South Carolina 29412, USA

*Author for correspondence: Isaure de Buron, E-mail: deburoni@cofc.edu

Abstract: Three juvenile Atlantic tripletail *Lobotes surinamensis* caught opportunistically in Charleston Harbor (South Carolina, USA) and maintained in captivity for over three months displayed an altered swimming behavior. While no direct causation can be demonstrated herein, fish were infected in their brain by strigeid trematode larvae (metacercariae) of *Cardiocephaloides medioconiger*, which were identified via ITS2 and 28S ribosomal RNA gene sequencing. Histology showed nonencysted metacercariae within the brain ventricle between the optic tectum and tegmentum, causing distortion of tegmental parenchyma. Aggregates of mononuclear inflammatory cells were in the ventricle adjacent to metacercariae. Metacercarial infection by *Cardiocephaloides medioconiger* has been reported from the brain and eyes of only two other fish species from the northern US Atlantic coast: the grey mullet *Mugil cephalus* and silverside *Menidia menidia*, but this identification is problematic and needs molecular verification. Atlantic tripletail is a new report as a second intermediate host for *C. medioconiger* and South Carolina is a new locality. *Cardiocephaloides* species in general have a low host specificity and infection by *C. medioconiger* could propagate to other fishes and affect neighboring natural ecosystems.

Introduction

Digeneans are parasites with complex life cycles that involve definitive hosts, in which adults reproduce, and one to three intermediate hosts in which various larval stages (including metacercariae) develop. Most cycles involve a total of three hosts with the second intermediate host harboring metacercariae, which are trophically transmitted to their definitive hosts (Poulin & Cribb, 2002). In the aquatic environment, metacercariae that infect organs associated with the nervous system impact their fish host's metabolism (Nadler et al., 2020) and can modify their behavior, often making them more prone to predation by their bird definitive hosts (Lafferty & Morris, 1996; Seppälä, Karvonen, & Valtonen, 2004; Fredensborg & Longoria, 2012). These brain parasites fully integrate within food webs (Bartoli & Boudouresque, 2007; Lafferty, 2008), and all hosts involved in their life cycles can overlap, especially in confined habitats such as shallow waters, which consequently favor encounters and enhance transmission (Combes, 2001; Osset et al., 2005). Transmission can also be intensified in the wild because brain metacercariae typically display low specificity for their fish hosts (Born-Torrijos et al., 2016) and infect fishes with ecological similarities (Hernandez & Fredensburg, 2015). These brain parasites are therefore important to monitor as they can serve as bioindicators of alteration in food web dynamics and environmental disturbance (Lafferty, 2008; Born-Torrijos et al., 2016) and can negatively impact fishery and aquaculture industries (Rosser et al., 2016; Palacios-Abella et al., 2018).

The geographic range of the Atlantic tripletail *Lobotes surinamensis* (Bloch, 1790) encompasses subtropical and tropical waters of all oceans, and the fish is found in estuaries as well as offshore (Stretlcheck et al., 2004; Froese & Pauly, 2021). In the US, its range extends along the Atlantic coast from New England to the Gulf of Mexico, where they are often

associated with flotsam and structures such as pilings (Stretlcheck et al., 2004). In January 2020, juvenile tripletail that had been opportunistically collected from Charleston Harbor, South Carolina (SC), USA, displayed an altered swimming behavior that was indicative of possible parasitic infection in their nervous system. While healthy tripletail exhibit a unique swimming behavior where they float on their side, mimicking floating leaves or flotsam (Breder, 1949), our specimens had difficulty maintaining their orientation in the water column, their tail sank below the plane of their body, and they often failed to reach the surface (video S1). Although no causation can be certain without experimental infection, this alteration led us to suspect infection by neurotropic parasites.

Despite its broad distribution, to our knowledge only a few helminths have been reported in the Atlantic tripletail (e.g., in Baughman, 1943; Moravec, Walter, & Yuniar, 2012; Dewi & Palm, 2017) and none are metacercariae in their brain or eyes. The high quality of its flesh, the growing interest from anglers, and preliminary successful larval culture make this fish a promising candidate for mariculture in the US (Saillant et al., 2021). Thus, the objective of this study was to examine these fish for the presence of neurotropic parasites that could potentially explain their altered swimming behavior. Herein, we report infection by a little-known brain parasite that has the potential to impact both the natural communities that include *L. surinamensis* and the culture endeavors of this fish species.

Materials and Methods

Fish collection and maintenance and parasite collection

Three juvenile Atlantic tripletail (average 12 cm total length; range 10.7-14.2 cm) were opportunistically collected in Charleston Harbor from the Fort Johnson boat slip (32°74'27" N,

79°87'24" W) in October 2019. Fish were maintained in recirculating chlorinated/dechlorinated settled seawater in individual substrate-less glass aquaria for three months, at which time alterations in swimming behavior were observed. Fish were euthanized via an overdose of tricaine methanesulfonate, MS-222 (Sigma-Aldrich, St Louis, MO) and immediately necropsied. Fish were collected, raised and euthanized by authorized staff under official permits or scientific exemptions of U.S. state government agencies. Brains and eyes were resected and examined under a dissecting microscope. The infected brain of one individual was fixed in 10% neutral buffered formalin (NBF) and processed using standard histological techniques. Parasites were isolated from each of the two other individuals and fixed either in 95% ethanol or in sarcosyl urea for molecular identification or in 5% NBF for voucher preparation (deposited at the Museum National d'Histoire Naturelle, Paris, France under the number MNHN-HEL1889).

Molecular identification

DNA of one metacercaria was isolated using Sera-Mag™ Carboxylate-modified SpeedBeads (Global Life Sciences Solutions, Marlborough, MA, USA) as in O'Donnell et al. (2016). DNA from a second metacercaria was extracted using a QIAGEN DNeasy blood and tissue kit (Valencia, CA, USA) following the manufacturer's protocol. Primers GA1 (5'-AGAACATCGACATCTTGAAC-3'; Anderson & Barker, 1998) and ITS2.2 (5'-CCTGGTTAGTTTCTTTTCCTCCGC-3', Bowles et al., 1993) were used to amplify the second internal transcribed spacer (ITS2) region of the ribosomal RNA (rRNA) gene of the parasite. A portion of the large subunit (28S) rRNA gene was also amplified from the first specimen using primers LSU5 (5'-TAGGTCGACCCGCTGAAYTTAAGCA-3'; Jensen & Bullard, 2010) and ECD2 (5'-CTTGGTCCGTGTTTCAAGACGGG-3'; Tkach et al., 2003). PCR reagent concentrations and cycling followed Hill-Spanik et al. (2021), as did product visualization,

purification, sequencing, and sequence editing. We used the Basic Local Alignment Search Tool (BLASTN; Altschul et al., 1990) to compare our sequences to those in the NCBI GenBank database.

Results

All three fish examined were infected by metacercariae in the cerebellum (two in two specimens (used as voucher and for molecular identification) and one in the third fish used for histology. Microscopic examination of the serially sectioned whole brain showed two additional free (nonencysted) metacercariae within the ventricle between the cerebellum, optic tectum, and tegmentum, both of which distorted the tegmental parenchyma. One metacercaria was more deeply invaginating the brain tissue with some sections showing ependyma-lined brain tissue present nearly circumferentially around the parasite. No definitive necrosis, gliosis, nor inflammatory infiltrate was seen within the brain tissue. However, aggregates of single cells resembling mononuclear inflammation were present adjacent to these metacercariae within the ventricle (Fig. 1). No infection occurred in the optic nerves nor within the eyes. The resulting ITS2 rRNA gene sequences (each 344 base pairs (bps)) were identical to one another and were 100% similar to a sequence of *C. medioconiger* (Dubois & Viguera, 1949) in GenBank (accession number MN820664) collected from royal tern *Thalasseus maximus* in Mississippi, USA. The one partial 28S rRNA gene sequence (903 bp) was also 100% similar to a sequence of *C. medioconiger* in GenBank (MH521247), which was also collected from *T. maximus*, but in the Florida Keys, Florida, USA.

Discussion

Cardiocephaloides Sudarikov, 1959 is a genus of strigeid trematodes whose adults infect the intestine of marine fish-eating birds (Dubois 1970). Based on the life cycle of *C. longicollis* (Rudolphi, 1819), which is the only one of seven currently accepted species of *Cardiocephaloides* whose life cycle has been fully unraveled (Prevost & Bartoli, 1980; Born-Torrijos et al., 2016), worm eggs are released in the water with the bird host feces; miracidia hatch and actively infect a gastropod first intermediate host, which releases free-living cercariae that swim, penetrate, and encyst as tetracotyle metacercariae into a fish second intermediate host, most often in the brain or eyes. The cycle is completed when birds eat infected fish. Our knowledge of the *C. medioconiger* life cycle is mostly limited to its definitive hosts: adult worms infect a variety of larid birds in Massachusetts on the northeast US coast, the Florida Keys, and the Gulf of Mexico, including the royal tern *T. maximus* (see Dronen et al., 2007) and several species of gulls *Larus* (see Stunkard, 1973; Hernández-Mena et al., 2014; Locke et al., 2018; Achatz et al., 2020). It was also reported in the herring gull *L. argentatus* from the Republic of Korea (Lee, Seo, & Chai, 2020).

While a few studies refer to the possibility of the nassariid eastern mudsnail *Ilyanassa obsoleta* as intermediate host of *Cardiocephaloides* in the eastern USA, they appear to be based on misidentifications of *Diplostomum nassa* (Martin, 1945) Stunkard, 1973 (formerly *Cercaria nassa* Martin, 1945). Hunter & Vernberg (1960) claimed that they were successful at infecting young mullet *Mugil cephalus* with cercariae *Cercaria nassa* shed by mudsnails, and Prevost & Bartoli (1980) noted that these were morphologically similar to cercariae of *C. longicollis* from *Nassa* (now *Tritia*) *corniculum* in the Mediterranean. However, while Hunter & Vernberg (1960) identified their specimens as *Cardiocephalus brandesi* (which later was synonymized as *C. medioconiger* (Dubois & Vigueras, 1949) Baer, 1969 - see below), these specimens were from

birds fed metacercariae from naturally infected individuals of *M. cephalus* and *Menidia menidia* and not from their experimentally infected fish. Stunkard (1973) expressed skepticism at the validity of these authors' experimental infections and emphasized that "there is no evidence that *Cercaria nassa* is the larval stage of *C. medioconiger*" (p. 528). Lastly, DeCoursey & Vernberg (1974) designated cercariae also shed by mudsnails in a nearby area as *Cardiocephalus brandesii* Szidat, 1928, which were later re-identified as *D. nassa* by Sullivan et al. (1985). Therefore, the gastropod first intermediate host of *C. medioconiger* is not known, and studies of the natural history of congeneric species do not allow for accurate targeting of particular gastropods in field sampling in our area. In effect, Donald & Spencer (2016) reported infection by an unidentified *Cardiocephaloides* species in buccinoid whelks *Cominella* in New Zealand, while Born-Torrijos et al. (2016) suggested that *C. longicollis* has a narrow specificity for nassariid gastropods as first intermediate hosts.

Atlantic tripletail is a new report of a fish intermediate host for *C. medioconiger*, and this finding is particularly significant because *L. surinamensis* is being considered for extensive aquaculture in the USA (Saillant et al., 2021). Because the populations of intermediate hosts (and consequently their parasites' life cycles) can be amplified in aquaculture settings that may create favorable habitats (e.g., Rosser et al., 2016), finding the gastropod host of *C. medioconiger* would allow for mitigation of the parasite in such an environment, and possibly limit infection in the wild. In effect, the lack of specificity of *Cardiocephaloides* species for their fish hosts could also be detrimental to the natural communities. For instance, Vidal-Martinez et al. (2012) reported *Cardiocephaloides* sp. from the Eastern Indo Pacific infecting 9 fish species belonging to 7 families, and *C. longicollis* was reported from 31 species of 9 families (in Born-Torrijos et al., 2016). On the US Atlantic coast, unidentified strigeids have been reported from the brain of

mummichog *Fundulus heteroclitus* along the northeastern coast (Abbott, 1968; Stunkard, 1973) and from the brain of the red grouper *Epinephelus morio* in the Gulf of Mexico (Moravec et al., 1997). To our knowledge the only report of a putative occurrence of metacercariae of *Cardiocephaloides* (as *Cardiocephalus brandesi*) in fish were, as mentioned above, by Hunter & Vernberg (1960) in the brain and eyes of grey mullet *Mugil cephalus* and silverside *Menidia menidia*. However, these authors reported their specimens as “*Cardiocephalus brandesi* Szidat, 1928”, although this species was originally described by Vigueras (1944) and not, as they noted, by Szidat (1928), who described *Cardiocephalus brandesii* (now *C. brandesii* (Szidat, 1928) Sudarikov, 1959), which is a valid species that also occurs in the USA (Lumsden & Zischke, 1963; Dronen et al., 2007). Because of zoological nomenclature rules, *Cardiocephalus brandesi* is an invalid species, synonymized as *C. medioconiger*. The close spelling of the two epithets may explain the inaccuracy of the descriptive authority associated with the parasite species in the report of Hunter and Vernberg (1960). Stunkard (1973) indicated that worms collected from experimental infection of birds by these latter authors were later “submitted to Dr. Dubois and identified as *Cardiocephalus medioconiger* [= *Cardiocephaloides medioconiger*]” (see p. 528), but the report by Hunter & Vernberg (1960) is confusing in several aspects, making the information on the natural history of *C. medioconiger* unreliable at best and demonstrates the need for further investigation of this parasite in the USA.

Pathological effects of metacercariae in a fish brain vary from severe (e.g., Dezfuli et al., 2007) to mild (Siegmund, Franjola, & Torres, 1997; Grobbelaar et al., 2015). In the tripletail infection, inflammation was limited to the ventricular space, and metacercariae appeared unaffected, as seen in other studies (Dezfuli et al., 2007; Grobbelaar et al., 2015). The mass effect exerted by the metacercariae on potentially critical tegmental regions along with the

observed inflammation may at least partially explain the fish's altered swimming behavior as the proximity of the unencysted worms and inflammation to the optic tectum may have affected the processing of visual and non-visual stimuli typical of that brain region (Northmore, 2011). While only experimental infection would allow us to determine causation by the metacercariae of our specimens' altered swimming behavior, fish have been shown to be more predated upon when infected in their brain by various metacercariae (Lafferty & Morris, 1996; Fredensborg & Longoria, 2012; Osset et al., 2005). This, added to the lack of specificity of *Cardiocephaloides* for their hosts (whether definitive or intermediate), can further enhance their transmission in habitats where all hosts cohabitate such as shallow water and in areas with fisheries activity where snails and birds often congregate (Osset et al., 2005; Born-Torrijos et al., 2016). The discard of carcasses in the environment is one particular anthropogenic activity that increases transmission of this parasite (Osset et al., 2005; Born-Torrijos et al., 2016), and because it is common in Charleston Harbor, it further emphasizes the need for angler education efforts to limit this and other parasite infections in our area (de Buron et al., 2017).

In conclusion, the little-known pathogenic parasite *C. medioconiger* was found to infect Atlantic tripletail *L. surinamensis*, a fish with ecological and economic relevance. The low specificity of *Cardiocephaloides* for its hosts, the fact that the infection could induce an impediment in the ability of the fish to escape predators, and discard of carcasses are factors that could lead to the amplification of parasite transmission in the wild. It is important to fully unravel and describe this parasite life cycle and a survey of wild Atlantic tripletail would provide a better understanding of its geographic distribution.

Acknowledgements

Thanks to Matt Walker at SC-DNR for assistance with DNA isolations and Mary Ann Taylor for bringing these sick fish to our attention. Fish were euthanized by authorized SC Department of Natural Resources staff under scientific exemptions of U.S. state government agencies.

References

Abbott FS (1968) Metacercariae of a trematode in the brain of *Fundulus heteroclitus* L. Canadian Journal of Zoology 46: 1205–1206

Achatz TJ, Pulis E, González-Acuña D, Tkach VV (2020) Phylogenetic relationships of *Cardiocephaloides* spp. (Digenea, Diplostomoidea) and the genetic characterization of *Cardiocephaloides physalis* from Magellanic Penguin, *Spheniscus magellanicus*, in Chile. Acta Parasitologica 65: 525–534. <https://doi.org/10.2478/s11686-019-00162-5>

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. doi: 10.1016/S0022-2836(05)80360-2

Anderson GR, Barker SC (1998) Inference of phylogeny and taxonomy within the Didymozoidae (Digenea) from the second internal transcribed spacer (ITS) of ribosomal DNA. Systematic Parasitology 41: 87–94

Bartoli P, Boudouresque CF (2007) Effect of the digenean parasites of fish on the fauna of Mediterranean lagoons. Parassitologia 49: 111–117

Baughman JL (1943) Additional notes on the occurrence and natural history of the Triple Tail, *Lobotes surinamensis*. American Midland Naturalist 29: 365–370

Born-Torrijos A, Poulin R, Pérez-del-Olmo A, Culurgioni J, Raga JA, Holzer AS (2016) An optimised multi-host trematode life cycle: fishery discards enhance trophic parasite transmission

233 to scavenging birds. *International Journal for Parasitology* 46: 745–753.
 234 <http://dx.doi.org/10.1016/j.ijpara.2016.06.005> 0020-7519/ 2016

235 Bowles J, Hope M, Tiu WU, Liu X, McManus DP (1993) Nuclear and mitochondrial genetic
 236 markers highly conserved between Chinese and Philippine *Schistosoma japonicum*. *Acta Tropica*
 237 55: 217–229. [https://doi.org/10.1016/0001-706X\(93\)90079-Q](https://doi.org/10.1016/0001-706X(93)90079-Q)

238 Breder CM (1949) On the behavior of young *Lobotes surinamensis*. *Copeia* 1949: 237–242.
 239 <https://doi.org/10.2307/1438372>

240 Combes C (2001) Parasitism: The ecology and evolution of intimate interactions. University of
 241 Chicago Press, Chicago, IL

242 de Buron I, Hill-Spanik KM, Haselden L, Atkinson SD, Hallett SL, Arnott SA (2017) Infection
 243 dynamics of *Kudoa inornata* (Cnidaria: Myxosporea) in spotted seatrout *Cynoscion nebulosus*
 244 (Teleostei: Sciaenidae). *Diseases of Aquatic Organisms* 127: 29–40
 245 <https://doi.org/10.3354/dao03174>

246 DeCoursey PJ, Vernberg WB (1974) Double infections of larval trematodes: competitive
 247 interactions. In: Vernberg WB (ed) *Symbiosis in the Sea*. University of South Carolina Press,
 248 Columbia, SC

249 Dezfuli AS, Capuano S, Simoni E, Giari L, Shinn AP (2017) Histopathological and
 250 ultrastructural observations of metacercarial infections of *Diplostomum phoxini* (Digenea) in the
 251 brain of minnows *Phoxinus phoxinus*. *Diseases of Aquatic Organisms* 75: 51–59. doi:
 252 10.3354/dao075051.

253 Dewi K, Palm HW (2017) Philometrid nematodes (Philometridae) of marine teleosts from
 254 Balinese waters, Indonesia, including the description of *Philometra damriyasai* sp. nov. *Zootaxa*
 255 434: 577–584. doi: 10.11646/Zootaxa.4341.4.10

256 Donald K, Spencer H (2016) Host and ecology both play a role in shaping distribution of
 257 digenean parasites of New Zealand whelks (Gastropoda: Buccinidae: *Cominella*). *Parasitology*
 258 143: 1143–1156. doi: 10.1017/S0031182016000494.

259 Dronen NO, Blend CK, Gardner SL, Jiménez FA (2007) *Stictodora cablei* n. sp. (Digenea:
 260 Heterophyidae) from the royal tern, *Sterna maxima* (Laridae: Sterninae) from Puerto Rico and
 261 the Brazos County area of the Texas Gulf coast, U.S.A., with a list of other endohelminths
 262 recovered in Texas. *Zootaxa* 1432: 35–36. doi: 10.11646/zootaxa.1432.1.3

263 Dubois G (1970) Synopsis des Strigeidae et des Diplostomatidae (Trematoda). Part II. Memoires
 264 de la Societé Neuchâteloise des Sciences Naturelles 10: 259–728

265 Fredensborg BL, Longoria AN (2012) Increased surfacing behavior in Longnose Killifish
 266 infected by brain-encysting trematode. *Journal of Parasitology* 98: 899–903. doi: 10.1645/GE-
 267 3170.1.

268 Froese R, Pauly D (2021): FishBase. www.fishbase.org (accessed June 22, 2022)

269 Grobbelaar A, van As LL, Van As JG, Butler HJB (2005) Pathology of eyes and brain of fish
 270 infected with diplostomids, southern Africa. *African Zoology* 50: 181–186.
 271 <http://dx.doi.org/10.1080/15627020.2015.1055701>

272 Hernandez RN, Fredensborg BL (2015) Experimental test of host specificity in a behaviour-
 273 modifying trematode. *Parasitology* 142: 1631–1639. doi:10.1017/S0031182015001171

274 Hernández-Mena DI, García-Prieto L, García-Varela M (2014) Morphological and molecular
275 differentiation of *Parastrigea* (Trematoda: Strigeidae) from Mexico, with the description of a
276 new species. *Parasitology International* 63: 315–323. doi: 10.1016/j.parint.2013.11.012.

277 Hill-Spanik KM, Sams C, Connors VA, Bricker T, de Buron I (2021) Molecular data reshape our
278 understanding of the life cycles of three digeneans (Monorchhiidae and Gymnophallidae)
279 infecting the bivalve, *Donax variabilis*: it's just a facultative host! *Parasite* 28: 34.
280 <https://doi.org/10.1051/parasite/2021027>

281 Hunter WS, Vernberg WB (1960) Preliminary studies on the life history of *Cardiocephalus*
282 *brandesi* Szidat, 1928 (Trematoda: Strigeidae). *Journal of Parasitology* 46: 797–799

283 Jensen K, Bullard SA (2010) Characterization of a diversity of tetraphyllidean and
284 rhinebothriidean cestode larval types, with comments on host associations and life-cycles.
285 *International Journal for Parasitology* 40: 889–910. doi: 10.1016/j.ijpara.2009.11.015

286 Lafferty KD (2008) Ecosystem consequences of fish parasites. *Journal of Fish Biology* 73:
287 2083–2093. doi:10.1111/j.1095-8649.2008.02059.x

288 Lafferty KD, Morris AK (1996) Altered behavior of parasitized killifish increases susceptibility
289 to predation by bird final hosts. *Ecology* 77: 1390–1397. <https://doi.org/10.2307/2265536>

290 Lee Y-I, Seo M, Chai J-Y (2020) Intestinal flukes recovered from a Herring Gull, *Larus*
291 *argentatus*, in the Republic of Korea. *Korean Journal of Parasitology* 58: 81–86.
292 <https://doi.org/10.3347/kjp.2020.58.1.81>

293 Locke SE, Van Dama A, Caffara M, Alves Pinto H, López-Hernández D, Blonar CA (2018)
294 Validity of the Diplostomoidea and Diplostomida (Digenea, Platyhelminthes) upheld in

295 phylogenomic analysis. *International Journal for Parasitology* 48: 1043–1059. doi:
 296 10.1016/j.ijpara.2018.07.001

297 Martin WE (1945) Two new species of marine cercariae. *Transactions of the American*
 298 *Microscopical Society* 64: 203–212

299 Moravec F, Vidal-Martinez VM, Vargas-Vasquez J, Vivas-Rodriguez C, Gonzales-Solis C,
 300 Mendoza-Franco E, Sima-Alvarez R, Guemez-Ricalde I (1997) Helminth parasites of
 301 *Epinephelus morio* (Pisces: Serranidae) of the Yucatan Peninsula, southeastern Mexico. *Folia*
 302 *Parasitologica* 44: 255–266

303 Moravec F, Walter T, Yuniar AT (2012) Five new species of philometrid nematodes
 304 (Philometridae) from marine fishes off Java, Indonesia. *Folia Parasitologica* 59: 115–130. doi:
 305 10.14411/fp.2012.017

306 Nadler LE, Bengston E, Eliason EJ, Hassibi C, Helland-Riise SH, Johansen IB, Kwan GT,
 307 Tresguerres M, Turner AV, Weinersmith KL, Øverli Ø, Hechinger RF (2020) A brain-infecting
 308 parasite impacts host metabolism both during exposure and after infection is established.
 309 *Functional Ecology* 35: 105–116. doi:10.1111/1365-2435.13695

310 Northmore DPM (2011) The Optic Tectum. In: Farrell AP (ed) *Encyclopedia of fish physiology:*
 311 *from genome to environment*. Elsevier, Amsterdam

312 O'Donnell TP, Arnott SA, Denson MR, Darden TL (2016) Effects of cold winters on the genetic
 313 diversity of an estuarine fish, the spotted seatrout. *Marine and Coastal Fisheries* 8: 263–276. doi:
 314 10.1080/19425120.2016.1152333

315 Osset EA, Fernandez M, Raga JA, Kostadinova A (2005) Mediterranean *Diplodus annularis*
 316 (Teleostei: Sparidae) and its brain parasite: Unforeseen outcome. *Parasitology International* 54:
 317 201–206 doi:10.1016/j.parint.2005.05.002

318 Palacios-Abella JF, Raga JA, Mele S, Montero FE (2018) Blood fluke diseases in *Pagellus* spp.
 319 (Sparidae): Pathology and description of a new species of *Skoulekia* in the blackspot seabream *P.*
 320 *bogaraveo* (Brünnich). *Aquaculture* 495: 472–483.

321 Poulin R, Cribb TH (2002). Trematode life cycles: short is sweet? *Trends in Parasitology* 18 :
 322 176–183. [https://doi.org/10.1016/s1471-4922\(02\)02262-6](https://doi.org/10.1016/s1471-4922(02)02262-6).

323 Prevot G, Bartoli P (1980) Démonstration de l'existence d'un cycle marin chez les Strigéides:
 324 *Cardiocephalus longicollis* Szidat, 1928 (Trematoda: Strigeidae). *Annales de Parasitologie*
 325 Humaine et Comparée 55: 407–425. doi: 10.1051/parasite/1980554407

326 Rosser TG, Alberson NR, Khoo LH, Woodyard ET, Pote LM, Griffin MJ (2016)
 327 Characterization of the life cycle of a fish eye fluke, *Austrodiplostomum ostrowskiae* (Digenea:
 328 Diplostomidae), with notes on two other diplostomids infecting *Biomphalaria havanensis*
 329 (Mollusca: Planorbidae) from catfish aquaculture ponds in Mississippi, USA. *Journal of*
 330 *Parasitology* 102: 260–274. doi: 10.1645/15-850

331 Saillant E, Adams N, Lemus JT, Franks JS, Zohar Y, Stubblefield J, Manley C (2021) First data
 332 on aquaculture of the Tripletail, *Lobotes surinamensis*, a promising candidate species for U.S.
 333 marine aquaculture. *Journal of World Aquaculture Society*. 52: 582–594. doi:
 334 10.1111/jwas.12807

- 335 Seppälä O, Karvonen A, Valtonen T (2004) Parasite-induced change in host behavior and
336 susceptibility to predation in an eye fluke-fish interaction. *Animal Behavior* 68: 257–263.
337 <https://doi.org/10.1016/j.anbehav.2003.10.021>
- 338 Siegmund I, Franjola R, Torres P (1997) Diplostomatid metacercariae in the brain of Silversides
339 from Lake Rinihue, Chile. *Journal of Wildlife Diseases* 33: 362–364. doi: 10.7589/0090-3558-
340 33.2.362
- 341 Strelcheck AJ, Jackson JB, Cowan JH Jr, Shipp RL (2004) Age, growth, diet, and reproductive
342 biology of the Tripletail, *Lobotes surinamensis*, from the North-Central Gulf of Mexico. *Gulf of*
343 *Mexico Science* 22 (1) <https://doi.org/10.18785/goms.2201.04>
- 344 Stunkard, HW (1973) Studies on larvae of strigeoid trematodes from the Woods Hole,
345 Massachusetts region. *Biological Bulletin* 144: 525–540
- 346 Sullivan JT, Cheng TC, Howland KH (1985) Studies on parasitic castration: Castration of
347 *Ilyanassa obsoleta* (Mollusca: Gastropoda) by several marine trematodes. *Transactions of the*
348 *American Microscopical Society* 104: 154–171.
- 349 Szidat L (1928) Zur Revision der Trematodengattung Strigea Abildgaard. *Zentralblatt für*
350 *Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* 105: 204–215.
- 351 Tkach VV, Littlewood DTJ, Olson PD, Kinsella JM, Swiderski Z (2003) Molecular phylogenetic
352 analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology* 56:
353 1–15. doi: 10.1023/a:1025546001611
- 354 Vidal-Martinez VM, Aguirre-Macedo M.L, McLaughlin JP, Hechinger RF, Jaramillo AG, Shaw
355 JC, James AK, Kuris AM, Lafferty KD (2012) Digenean metacercariae of fishes from the lagoon

356 flats of Palmyra Atoll, Eastern Indo-Pacific. *Journal of Helminthology* 86: 493–509. doi:
 357 10.1017/S0022149X11000526

358 Viguera IP (1944) Trematodes de la Super-Familia Strigeoidea; descripción de un género y siete
 359 especies nuevas. *Revista de la Universidad de la Habana* 52: 294–314

Figure 1

Histological sections of brain of Atlantic tripletail *Lobotes surinamensis* infected with metacercariae of *Cardiocephaloides medioconiger* (hematoxylin and eosin)

A. Low magnification showing two metacercariae (arrows) within ventricle between the cerebellum, optic tectum, and midbrain tegmentum (star). **B.** Brain tissue is displaced and distorted around metacercariae (arrows) with no definitive necrosis, gliosis, nor inflammatory reaction within the brain parenchyma. **C.** Non-encysted metacercaria (arrow) invaginating brain tissue with ependymal lining intact around the parasite. **D.** Non-encysted metacercariae within the ventricle in direct contact with brain parenchyma (arrow). Aggregates of single cells closely resembling mononuclear inflammation were within the ventricle adjacent to the metacercariae (star). **E.** High magnification of aggregate of mononuclear cells adjacent to metacercariae (star indicates adjacent brain parenchyma).

