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A novel neurotropic microsporidium from the swamp guppy *Micropoecilia picta* from Grenada, West Indies

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ABSTRACT: A novel microsporidium was observed in wild swamp guppies *Micropoecilia picta* from Levera Pond within Levera National Park Grenada, West Indies. Initial observations indicated similarity with *Pseudoloma neurophilia*, an important pathogen in zebrafish *Danio rerio*. *P. neurophilia* exhibit broad host specifity, including members of the family Poecillidae, and both parasites infect the central nervous system. However, spore morphology and molecular phylogeny based on rDNA showed that the swamp guppy microsporidium (SGM) is distinct from *P. neurophilia* and related microsporidia (*Microsporidium cerebralis* and *M. luceopercae*). Spores of the SGM were smaller than others in the clade (3.6 µm long). Differences were also noted in histology; the SGM formed large aggregates of spores within neural tissues along with a high incidence of numerous smaller aggregates and single spores within the surface tissue along the ventricular spaces that extended submeninx, whereas *P. neurophilia* and *M. cerebralis* infect deep into the neuropile and cause associated lesions. Analysis of small subunit ribosomal DNA sequences showed that the SGM was <93% similar to these related microsporidia. Nevertheless, one of 2 commonly used PCR tests for *P. neurophilia* cross reacted with tissues infected with SGM. These data suggest that there could be other related microsporidia capable of infecting zebrafish and other laboratory fishes that are not being detected by these highly specific assays. Consequently, exclusive use of these PCR tests may not accurately diagnose other related microsporidia infecting animals in laboratory and ornamental fish facilities.

KEY WORDS: Microsporidium · Swamp guppy · *Pseudoloma neurophilia*

1. INTRODUCTION

Microsporidia are spore-forming obligate intracellular parasites infecting hosts of nearly all animal taxa, including over 120 species known to infect fishes, with some causing severe disease (Kent et al. 2014, Schuster et al. 2022a). Microsporidiosis was first reported in zebrafish *Danio rerio* collected from a pet store over 40 yr ago (de Kinkelin 1980), and 2 decades later it was observed in a large zebrafish facility, at which time it was assigned to a new genus and species, *Pseudoloma neurophilia* (Matthews et al. 2001). This microsporidium, which targets the central nervous system (CNS) is commonly found in zebrafish research laboratories, with the infection in zebrafish being primarily subclinical (Kent et al. 2020).

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However, a subset of infected populations may present with general clinical signs, such as emaciation and skeletal deformities. Microsporidian spores primarily invade the CNS and may cause associated gliosis; however, the parasite also infects other organ systems causing various forms of inflammation (myositis, menixitis, and encephalitis) (Sanders et al. 2014, Spagnoli et al. 2015a). While the parasite presents with non-pathognomonic signs of disease, it is also associated with significant alteration in behavior and has been reported to alter the brain transcriptome (Midttun et al. 2020). Infections with *P. neurophilia* are often associated with reduced fecundity and growth, while stress exacerbates the prevalence of the parasite (Ramsay et al. 2009).

Typical for microsporidia, the transmission of *P. neurophilia* is complex, as it is transmitted both vertically and horizontally (Sanders et al. 2013). The parasite has broad host specificity among freshwater fishes held in warmer temperatures, including members of the families Adianichthyidae, Cyrpinidae, Characidae, Osphornemidae, and Poeciliidae (Sanders et al. 2016). Additionally, *P. neurophilia* has not been described from wild-caught zebrafish populations collected from their native habitat, and hence the origin of *P. neurophilia* in laboratory and pet store zebrafish remains unknown. Another similar microsporidium that infects the CNS, *Microsporidium cerebralis,* was described from seawater reared Atlantic salmon *Salmo salar* (Brocklebank et al. 1995, Jones et al. 2017). A third microsporidium, *M. luciopercae* from pike-perch *Sander lucioperca,* is phylogenetically similar to *P. neurophilia* and *M. cerebralis* (Jones et al. 2017). This parasite was described from muscle, but tissues from the CNS were not examined.

Swamp guppies *Micropoecilia picta* are a tropical fish with native ranges described in fresh and mildly brackish waters in Trinidad and northern South America (Lucinda 2003). In Grenada, they are present in high density in Levera Pond (12° 13′ 10.1″ N, 61° 36′ 37.4″ W) located within the 450 acre Ramsar-protected Levera National Park in northeast Grenada. Their status as a native or introduced species is currently unknown. We conducted a parasite survey of the swamp guppy using tissue wet mounts, as this survey was initially focused on documenting helminths. Following an examination of various organs, we discovered aggregates of microsporidian spores associated with the cranial cavity. This promoted focused examination of the brain and anterior spinal cord in wet mounts to document spore morphology and histology to elucidate locations of infections and associated pathologic changes. We also obtained and evaluated small subunit ribosomal DNA (SSU rDNA) to resolve the relationship of this microsporidium to related species. The parasite was similar to *P. neurophilia* of zebrafish *Danio rerio* in that it infected the CNS of a tropical freshwater fish. Given these similarities between *P. neurophilia* and the novel microsporidium, and the similarity of their hosts, we include here a revaluation of spore morphology and SSU rDNA sequence of *P. neurophilia*. Our findings suggest that the newly identified microsporidium found in swamp guppy (hereafter referred as SGM) is related to *P. neurophilia, M. luciopercae* and *M. cerebralis* based on SSU rDNA sequence. However, differences in rDNA and the smaller spore size of SGM indicate that this is a novel species.

2. MATERIALS AND METHODS

2.1. Collection

Swamp guppies were collected in May 2022 with dip nets from Levera Pond. *Pseudoloma neurophilia* material from zebrafish came from a stock of infected zebrafish maintained in our vivarium at Oregon State University since 2021. The parasite was originally derived from infected fish from the Zebrafish International Resource Center (ZIRC), Eugene, Oregon.

2.2. Swamp guppy wet mount cytology and histology

Twenty-five swamp guppies were dissected for wet mount light microscopic examination. Brains were sharply dissected and placed on a glass slide with a small amount of sterile saline and observed under a coverslip. Spore measurements (n = 30) were obtained from 4 fish at 1000× with ImageJ (Schneider et al. 2012) at St. George's University School of Veterinary Medicine (SGU-SVM) using an Olympus BX51 microscope with attached Olympus DP73 camera.

Ten whole fish were preserved in 10% Dietrich's solution (30% ethanol, 10% formalin, 25 glacial acidic acid, 5% water) and processed for histology. Fish were sectioned throughout the body anterior to the tail fin with 5 to 8 stepwise transverse sections per fish. Sections were processed routinely, cut at 5 µm and stained with hematoxylin and eosin. Further Gram's and acid-fast staining were performed on sections where microsporidia were observed.

2.3. Wet mount microscopy

Spore morphology of the SGM was recorded from 30 spores at 1000× using ImageJ. Measurements of *P. neurophilia* spores were obtained from 3 fish (n = 113) using 2 different camera systems; a Zeiss Axiocam 105 and a Leica DMLB microscope using SPOT Advanced imaging software version 4.0.9 (Diagnostic Instruments).

2.4. Molecular biology

2.4.1. DNA extraction from SGM and zebrafish

The protocol described in Schuster et al. (2022b) for DNA extraction from whole-body samples was used with some modifications. Briefly, 11 swamp guppies and 1 zebrafish that had been preserved in 90% ethanol and then frozen at −80°C were prepared by targeting the head and spinal cord, excluding the posterior end of the fish and caudal fin from the sample. Individual fish were macerated with a sterile razor blade and forceps in a sterile petri dish and macerated tissue was split into 2 halves for subsequent DNA extraction. The samples were then added to individual 1.5 ml microcentrifuge tubes containing 500 µl phosphate buffered saline (PBS), and a micropestle was used to homogenize the tissues. For each sample, a new sterile pestle was used. Following homogenization and brief centrifugation at 8000 × *g* to pellet tissues, DNA extraction was completed using the Qiagen Blood and Tissue DNA kit (Qiagen) following the manufacturer's protocol.

2.4.2. PCR testing

Given the similarities of SGM to *P. neurophilia,* we assayed swamp guppies with 2 PCR assays that were designed to detect *P. neurophilia*. First, we tested the 11 fish using our quantitative PCR (qPCR) assay developed by Sanders & Kent (2011) at the Oregon Veterinary Diagnostic Laboratory, Corvallis, Oregon. This test amplifies a 113 bp target of the SSU rDNA gene from position 1175 to 1288. The protocol was followed without deviations using the recommended probe (6-carboxyfluorescein [FAM]-5'-ACA CAC CGC CCG TCG TTA TCG AA-3'-Black Hole Quencher 1 [BHQ1]), forward primer PN10F (5'-GTA ATC GCG GGC TCA CTA AG-3') and reverse primer PN10R (5'-GCT CGC TCA GCC AAA TAA AC-3').

We also tested the same 11 samples with the conventional PCR assay used at ZIRC (Murray et al. 2011). This assay is comprised of a forward primer PNA_03 (5'-TGA AAT GTG GTG ACC CGT TTA GG-3') and reverse primer PNA_04 (5'-TCC TTG ACC CAT CCT TCC TGT G-3'), which amplify a 441 bp portion of the *P. neurophilia* rRNA gene from position 605 to 1045. The PCR was executed in a 25 µl reaction volume using 5 µl template, 5 µl Taq buffer (5×), 1.50 µl $MgCl_2$, 3.75 dNTP (2 mM), 1 µl each of 10 µM primer, 8.48 µl nuclease-free water, and 0.27 µl Taq Polymerase (5 U μl^{-1}). PCR products were purified using QIAquick PCR purification kits (Qiagen).

2.5. Phylogenetic analysis

SGM and zebrafish samples identified as *P. neurophila*-positive using our conventional PCR assay were then used to target a ~1000 bp segment of the 5' region of the SSU rRNA gene using general microsporidia primers V1F (5'-CAC CAG GTT GAT TCT GCC-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). PCR was executed in 50 µl volumes using GoTaq Hot Start Colorless Master Mix (Promega, Cat. No. M5132), 0.5 µM of each primer, and 5 µl of DNA template. Amplification was performed using the following cycling parameters: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. The resulting PCR products were purified using the QIAquick PCR purification kit (Qiagen) and Sanger sequencing was performed using a 3730 capillary sequence machine (ThermoFisher Scientific). The ~1000 bp segment was only amplified from 2 of the PCR positive SGM samples, and only 1 sample resulted in sequence data of sufficient quality to conduct phylogenetic analysis.

2.6. Molecular phylogeny

The resultant reads of both SGM and *P. neurophilia* were assembled and trimmed using MegaX (Kumar et al. 2018). The SGM sequence was then aligned with SSU sequences from related microsporidia. Following the phylogenies of Bojko et al. (2022) and Lovy et al. (2021), we ran analyses using closely related microsporidia to the *Pseudoloma* clade. These are members of the Glugeida (Dub), represented by species in the genera *Glugea*, *Tuzetia* and *Pleistophora*, and others (Lovy et al. 2021, Bojko et al. 2022). Additional taxa were included that are closely related to *P. neurophilia*.

A total of 37 sequences with 1283 sites were aligned using MAFFT v7.520 (L-INS-i) (Katoh et al. 2019). Columns with gaps in more than 20% of the sequences were removed using trimAl v1.4 (Capella-Gutiérrez et al. 2009), resulting in an alignment with 552 distinct site patterns and 390 parsimony informative sites. The best evolutionary model was inferred to be a GTR+FO substitution model using ModelFinder (Kalyaanamoorthy et al. 2017). A maximum likelihood tree was then generated in IQ-Tree (Nguyen et al. 2015), and bootstrap values were generated using 1000 iterations in UFBoot2 (Hoang et al. 2018).

3. RESULTS

3.1. Wet mount cytology

The microsporidium was first detected as a large aggregate of spores in a wet mount of the brain during a broader parasite health surveillance study (Fig. 1a). Higher magnification revealed typical microsporidial pyriform spores with a prominent posterior vacuole (Fig. 1b). Microsporidia were observed in a total of 11/25 (44%) of swamp guppies that were examined under light microscopy. The use of squash preparations made it difficult to discern the exact locations of the parasites, but some appeared to be in the neural parenchyma. Spore measurements (mean ± SD; n = 30) were as follows: length 3.6 ± 0.2 µm (range 3.0–4.1 µm); width 2.1 ± 0.2 µm (range 1.7–2.6 µm).

We combined measurements of 113 *Pseudoloma neurophilia* spores from 3 zebrafish using 2 camera systems, which showed a mean of 4.86 µm, with a range of 3.9–5.9 µm (Table 1). The redescription of *P. neurophilia* by Cali et al. (2012) used the original spore lengths reported by Matthews et al. (2001) (average 5.4 µm). However, we calculated spore lengths from 10 spores from the wet mount images from Cali et al. (2012) and found measurements more similar to our new measurements: length 4.53 µm (range 4.17–4.75 µm).

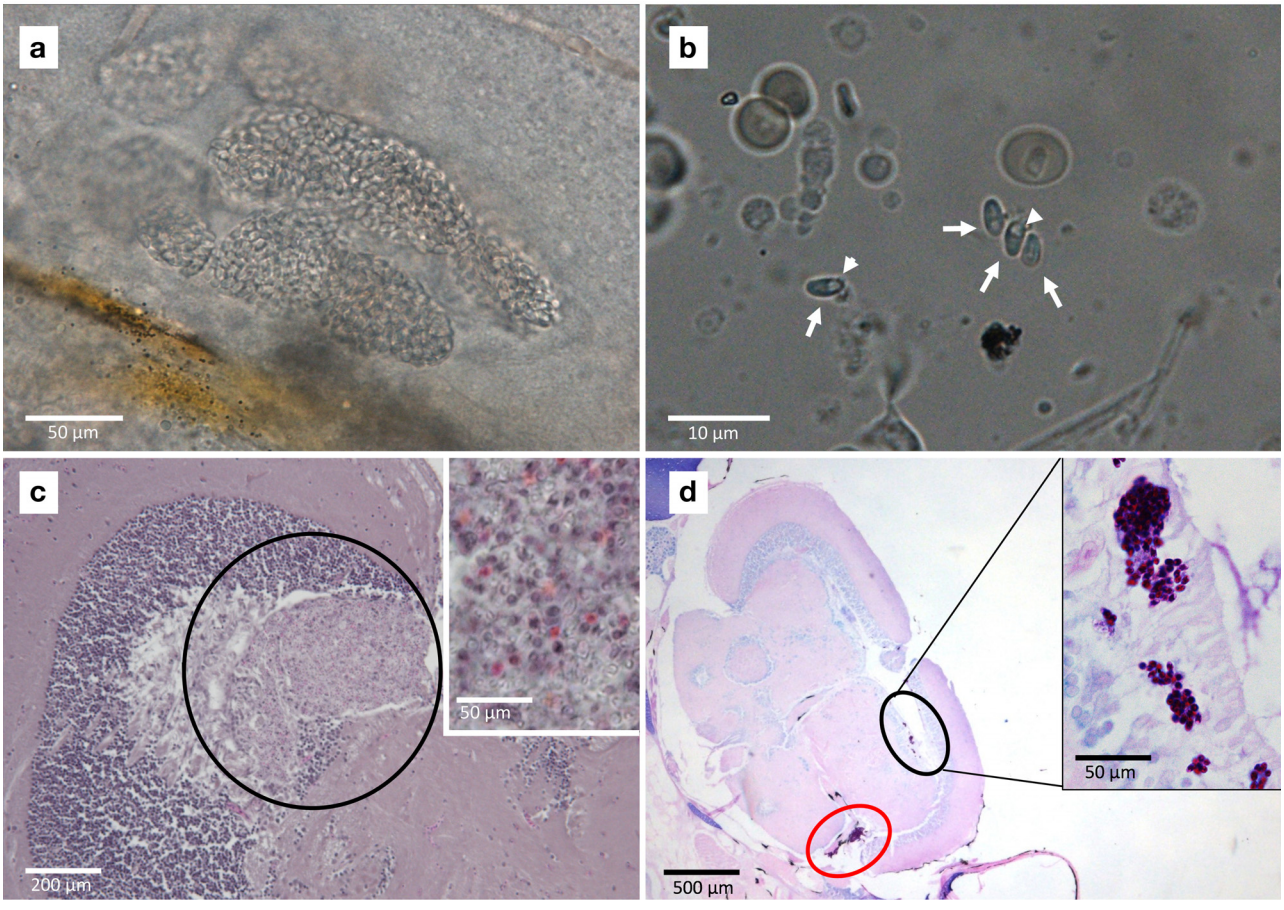

Fig. 1. Microsporidium in the brain of a swamp guppy *Micropoecilia picta*. (a) Wet mount cytology with multiple large aggregates of microsporidia spores. (b) Individual spores (arrows). Arrowheads: polar vacuole. (c) Histologic section (H&E) showing large mass of spores in the granular layer of the optic tectum (circle). (d) Histologic section (acid-fast) with acid-fast positive spores (ovals) within the superficial neural tissue

Table 1. Characteristics of microsporidium related to swamp guppy microsporidium (SGM). The 16S rDNA signature sequence (SS) for the genus *Pseudoloma* is in the small subunit rDNA at position 168–174, TTT TGT T and at position 1271–1276, TTA TTT. NA: not available. CNS: central nervous system; *M.*: *Microsporidium*

	GenBank	*P. neurophila* SS 5′	*P. neurophilia* SS 3′	CNS infection	Spore length (µm)	Reference(s)
SGM	OR398664	No	Yes	Yes	3.6 (3.0–4.1)	Present study
P. neurophilia original description	JQ316511	Yes	Yes	Yes	5.4 (4.8–5.9)	Matthews et al. (2001)
P. neurophilia emended description	NA	NA	NA	Yes	4.53 (4.17–4.75)[a]	Cali et al. (2012)
P. neurophilia new data	NA	Yes	Yes	Yes	4.86 (3.9–5.9)	Present study
M. cerebralis	JQ316511	No	No	Yes	5.7	Brocklebank et al. (1995), Jones et al. (2017)
M. luciopercae	KX351969	No	No	?	4.6 (3.9–5.0)	Jones et al. (2017)
M. luciopercae	KU302782	No	No	?	NA	Jones et al. (2017)

[a]Spore measurements from Cali et al. (2012) were calculated based on their original photographs

3.2. Histology

Transverse sections of swamp guppies demonstrated aggregates of oval to piriform microsporidia with an evident posterior vacuole in 3/10 (30%) examined fish. A large aggregate was observed in the granular layer of the optic tectum of the mesencephalon admixed with indistinct neural tissue and eosinophilic proteinaceous material (Fig. 1c). Multifocal packets of 8–16 microsporidia and scattered single spores were present at the periphery of the inferior lobe of the diencephalon and the tegmentum of the optic lobe near the lateral ventricle and within the submeninx space (Fig. 1d). There was no indication of xenoma formation. Spores stained with acid-fast (Fig. 1d), and no observable staining was evident with Gram's stain.

3.3. PCR testing

All 11 swamp guppy samples were evaluated for the presence of *P. neurophilia* using the qPCR designed to be specific for *P. neurophilia* (Sanders & Kent 2011) and all were negative. In contrast, 6 of these 11 samples (54.5%) were positive when tested at ZIRC, using the conventional PCR test described by Murray et al. (2011).

3.4. Molecular phylogeny

We obtained 1244 bp of SSU rDNA (GenBank OR398664) from one of the swamp guppy CNS samples. The SSU rDNA sequence placed the SGM as an immediate outlier of a clade comprised of *P. neurophilia*, *Microsporidium cerebralis, and M. luciopercae* (Fig. 2). However, the low bootstrap value for the clade containing SGM, *P. neurophilia* and *Ichthyosporidium weissii* indicates that this clade is poorly resolved. The SGM was dissimilar to the other related microsporidia, with the maximum homology being less 93% (Table 2). In contrast, *P. neurophilia* was close to 95% homologous to *M. cerebralis* and *M. luciopercae* (Table 2). However, the SGM contained 1 of the 2 signature sequences for the genus *Pseudoloma*. We also obtained 894 bp SSU rDNA of *P. neurophilia* recently obtained from a zebrafish in our laboratory, and the sequence was identical to the original sequence provided in Matthews et al. (2001), GenBank AF322654.

4. DISCUSSION

4.1. A distinct species of neurotropic microsporidia

Our initial observation of a microsporidium in the CNS of swamp guppies suggested to us that we discovered a wild fish host for *Pseudoloma neurophilia*, particularly because the zebrafish parasite also infects members of the family Poeciliidae (Sanders et al. 2016). However, various lines of evidence clearly demonstrate that it is a novel species that is phylogenetically related to *P. neurophilia* and another neurotropic species, *Microsporidium cerebralis,* of salmon. The SGM SSU rDNA is about 92% similar to these 2 parasites, which is consistent with not assign-

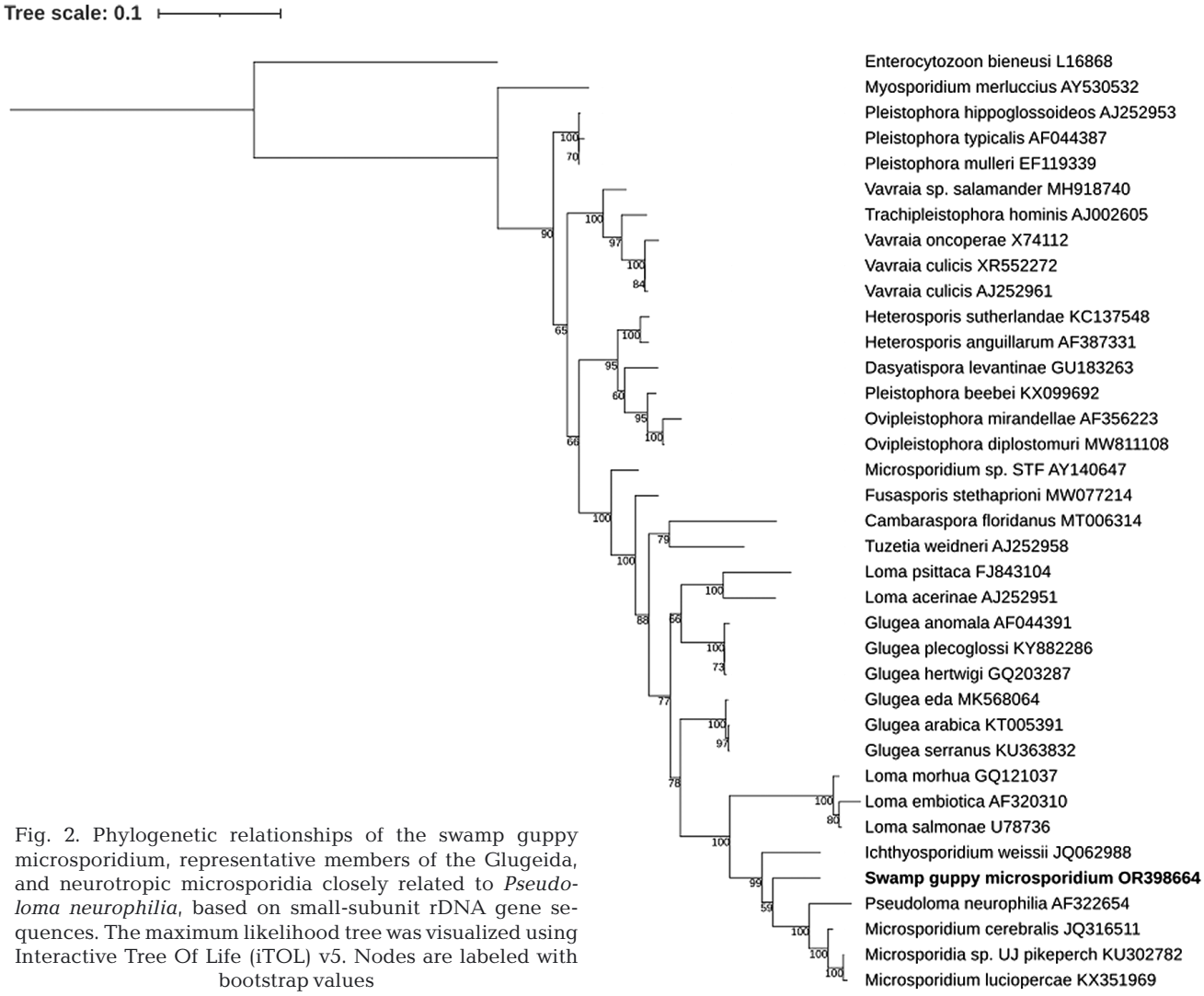

Fig. 2. Phylogenetic relationships of the swamp guppy microsporidium, representative members of the Glugeida, and neurotropic microsporidia closely related to *Pseudoloma neurophilia*, based on small-subunit rDNA gene sequences. The maximum likelihood tree was visualized using Interactive Tree Of Life (iTOL) v5. Nodes are labeled with bootstrap values

Table 2. Percent homology amongst microsporidia most closely related to the swamp guppy microsporidium (SGM) according to GenBank (cf. Fig. 2): *Pseudoloma neurophilia*, *Microsporidium cerebralis*, *M. luceopercae* (2 isolates), and *Ichthyosporidium weissii*

	SGM	*P. neurophilia*	*M. cerebralis*	*M. luciopercae* KX351969	*M. luciopercae* KU302782	*I. weissii*
SGM	100	91.9	92.6	92.4	92.7	92.3
P. neurophlia		100	94.9	94.7	94.9	90.6
M. cerebralis			100	97.4	97.7	90.6
M. luciopercae KX351969				100	99.7	89.6
M. luciopercae KU302782					100	90.4
I. weissii						100

ing the SMG to one of these species. Moreover, the spores of SGM are considerably shorter in length than the other species in this clade, including *M. luciopercae,* a muscle-infecting microsporidium of pike-perch (Jones et al. 2017). The SGM forms very large aggregates of spores, and based on histology, it appears that most of them occur at the periphery of neural tissues often along the ventricles with contact with the meninx, whereas *P. neurophilia* and *M. cerebralis* infect deep into the neural pile and cause associated lesions (Brocklebank et al. 1995, Spagnoli et al. 2015a). Indeed, given that *P. neurophilia* is so

common in zebrafish, we have observed this infection in over 1000 zebrafish (Spagnoli et al. 2015a), and this presentation of intact spore aggregates concentrated at the periphery of the brain has not been observed in zebrafish. This difference in location is unlikely to be a host effect as the various hosts infected with *P. neurophilia* described by Sanders et al. (2016) showed infections deep within neural tissues. Only muscle was examined in the description of *M. luciopercae* (Jones et al. 2017), so the possibility that this microsporidium also infects the CNS cannot be excluded. Indeed, whereas the CNS is the primary site of infection for *P. neurophilia*, it also frequently infects skeletal muscle (Spagnoli et al. 2015a). Our serendipitous discovery of a novel microsporidum from swamp guppies somewhat related to *P. neurophilia* and *M. cerebralis* suggests that that there are likely several species in this clade of mostly neurotropic microsporidia.

We reexamined *P. neurophilia*, given that the defining rDNA sequence and spore measurements were generated over 20 yr ago (Matthews et al. 2001). Comparison of spore sizes with the original description indicated the spores are actually smaller (4.5–4.8 µm long) compared to the length in the species description by Matthews et al. (2001). Discrepancies in spore measurements are not surprising, as microscopic measurements of such small objects inherently would include some minor variability between camera systems, microscopes, and readers.

4.2. Taxonomic implications

Given the clear differences between SGM and related species, we conclude that it is an undescribed species. Current phylogenetic evidence clearly places the SGM in a clade with *P. neurophilia*, *M. luceopercae* and *M. cerebralis.* However, the support for this clade is relatively weak in regards to the precise placement of *I. weissii,* which is also morphologically very different from the other members of this clade. We did not assign SGM to the genus *Pseudoloma*, as it did not meet the rDNA signature sequence criteria defined for the genus description by Matthews et al. (2001). If we had assigned SGM to the genus *Pseudoloma,* this would have to be applied to the other species in the clade (i.e. *M. cerebralis* and *M. luciopercae*), but these species also do not have the signature sequence for the genus that were erected by Matthews et al. (2001). Sprague (1977) established the name *Microsporidium* as a collective group generic name used for identifiable species of which generic positions for the time being are uncertain. We did not assign SGM to *Microsporidium*, and future studies will seek to describe the ultrastructure of the SGM and determine if it fits the description of the genus *Pseudoloma* (Cali et al. 2012). It is possible that all 4 species in the clade containing *P. neurophilia* and SGM might be placed into one genus, as 3 of the 4 infect the CNS and none of them produce true xenomas. In contrast, *I. weissii* is morphologically quite dissimilar than the other members of this clade as *Ichthysporidium* species form massive xenomas with very pleomorphic spores (Lom 2002)

4.3. Zebrafish laboratories

P. neurophilia is a very common and important parasite in laboratory zebrafish (Kent et al. 2020). The finding of a similar parasite in a freshwater tropical fish suggests that perhaps there are related microsporidia in zebrafish that could be confused with *P. neurophilia*. Indeed, *P. neurophilia* infects members of the family Poeciliidae, and hence the reverse should also be considered in surveillance efforts in zebrafish facilities. The presence of the parasite within fish or their facilities is now often determined using PCR assays. The SGM was detected by a conventional PCR assay for *P. neurophilia* (Murray et al. 2016) but was not detected by a commonly used qPCR assay (Sanders & Kent 2011). Therefore, it is conceivable that there are other related microsporidia capable of infecting zebrafish and other laboratory fishes that could confound research results or cross react with diagnostic assays for *P. neurophilia* in zebrafish facilities.

The impact of SGM on wild swamp guppy health and fitness is currently unknown. *P. neurophilia* infection in zebrafish has been associated with altered startle response and shoaling behavior (Spagnoli et al. 2015b, 2017). Evidence of neural infections within the optic tectum of swamp guppies suggests lesions could affect pathways associated with vision and pattern recognition. Additionally, Grenada supports native common guppies *Poecilia reticulata* in separate waterways. We examined 30 of these fish as they are in the same genus as the swamp guppy and saw no evidence of infection based on wet mount microscopy. This absence of infection may be secondary to lack of exposure or host resistance. Future studies will focus on conducting electron microscopy of the SGM to resolve its development and to conduct cross transmission studies with zebrafish and other poecilids to resolve its host specificity and potential pathogenicity in other fishes.

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LITERATURE CITED

Bojko J, Reinke AW, Stentiford GD, Williams B, Rogers MSJ, Bass D (2022) Microsporidia: a new taxonomic, evolutionary, and ecological synthesis. Trends Parasitol 38:642–659

Brocklebank JR, Speare DJ, Kent ML (1995) Microsporidian encephalitis of farmed Atlantic salmon (*Salmo salar*) in British Columbia. Can Vet J 36:631–633

Cali A, Kent M, Sanders J, Pau C, Takvorian PM (2012) Development, ultrastructural pathology, and taxonomic revision of the microsporidial genus, *Pseudoloma* and its type species *Pseudoloma neurophilia*, in skeletal muscle and nervous tissue of experimentally infected zebrafish *Danio rerio*. J Eukaryot Microbiol 59:40–48

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25:1972–1973

de Kinkelin PD (1980) Occurrence of a microsporidian infection in zebra danio *Brachydanio rerio* (Hamilton-Buchanan). J Fish Dis 3:71–73

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol 35:518–522

Jones SRM, Ahonen HS, Granlund L, Arsiola T, Taskinen J (2017) Two novel microsporidia in skeletal muscle of pike-perch *Sander lucioperca* and burbot *Lota lota* in Finland. J Parasitol 103:95–102

Kalyaanamoorthy S, Minh B, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589

Katoh K, Rozewick J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20:1160–1166

Kent ML, Shaw RW, Sanders JL (2014) Microsporidia in fish. In: Weiss LM, Becnel JJ (eds) Microsporidia: pathogens of opportunity. Wiley Blackwell, Oxford, p 493–520

Kent ML, Sanders JL, Spagnoli S, Al-Samarrie CE, Murray KN (2020) Review of diseases and health management in zebrafish *Danio rerio* (Hamilton 1822) in research facilities. J Fish Dis 43:637–650

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

Lom J (2002) A catalogue of described genera and species of microsporidians parasitic in fish. Syst Parasitol 53:81–99

Lovy J, Yanong RPE, Stilwell JM, Waltzek TB and others (2021) Tetra disseminated microsporidiosis: a novel disease in ornamental fish caused by *Fusasporis stethaprioni* n. gen. n. sp. Parasitol Res 120:497–514

Lucinda PHF (2003) *Poeciliidae* (livebearers). In: Reis RE, Kullander, SO, Ferraris CJ Jr (eds) Checklist of the freshwater fishes of South and Central America. ediPUCRS, Porto Alegre, p 555–581

Matthews JL, Brown AMV, Larison K, Bishop-Stewart JK, Rogers P, Kent ML (2001) *Pseudoloma neurophilia* n. g., n. sp., a new microsporidium from the central nervous system of the zebrafish (*Danio rerio*). J Eukaryot Microbiol 48: 227–233

Midttun HLE, Vindas MA, Whatmore PJ, Øverli Ø, Johansen IB (2020) Effects of *Pseudoloma neurophilia* infection on the brain transcriptome in zebrafish (*Danio rerio*). J Fish Dis 43:863–875

Murray KN, Dreska M, Nasiadka A, Rinne M and others (2011) Transmission, diagnosis, and recommendations for control of *Pseudoloma neurophilia* infections in laboratory zebrafish (*Danio rerio*) facilities. Comp Med 61:322–329

Murray KN, Varga ZM, Kent ML (2016) Biosecurity and health monitoring at the Zebrafish International Resource Center. Zebrafish 13:S30–S38

Nguyen LT, Schmidt HA, von Haeseler A, Minh B (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274

Ramsay JM, Watral V, Schreck CB, Kent ML (2009) *Pseudoloma neurophilia* infections in zebrafish *Danio rerio*: effects of stress on survival, growth, and reproduction. Dis Aquat Org 88:69–84

Sanders JL, Kent ML (2011) Development of a sensitive assay for the detection of *Pseudoloma neurophilia* in laboratory-populations of the zebrafish *Danio rerio.* Dis Aquat Org 96:145–156

Sanders JL, Watral V, Clarkson K, Kent ML (2013) Verification of intraovum transmission of a microsporidium of vertebrates: *Pseudoloma neurophilia* infecting the zebrafish, *Danio rerio*. PLOS ONE 8:e76064

Sanders JL, Peterson TS, Kent ML (2014) Early development and tissue distribution of *Pseudoloma neurophilia* in the zebrafish, *Danio rerio.* J Eukaryot Microbiol 61:238–246

Sanders JL, Watral V, Stidworthy MF, Kent ML (2016) Expansion of the known host range of the microsporidium, *Pseudoloma neurophilia.* Zebrafish 13(Suppl 1):S102–S106

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671–675

Schuster CJ, Sanders JL, Couch C, Kent ML (2022a) Recent advances with fish microsporidia. In: Weiss LM, Reinke AW (eds) Microsporidia: current advances in biology. Springer International Publishing, Cham, p 285–317

Schuster CJ, Kreul T, Al-Samarrie C, Peterson JT, Sanders JL, Kent ML (2022b) Progression of infection and detection of *Pseudoloma neurophilia* in zebrafish *Danio rerio* Hamilton by PCR and histology. J Fish Dis 45:1463–1475

Spagnoli ST, Xue L, Murray KN, Chow F, Kent ML (2015a) *Pseudoloma neurophilia*: a retrospective and descriptive study of nervous system and muscle infections, with new implications for pathogenesis and behavioral phenotypes. Zebrafish 12:189–201

Spagnoli S, Xue L, Kent ML (2015b) The common neural parasite *Pseudoloma neurophilia* is associated with altered

startle response habituation in adult zebrafish (*Danio rerio*): implications for the zebrafish as a model organism. Behav Brain Res 291:351–360

Spagnoli S, Sanders J, Kent ML (2017) The common neural parasite *Pseudoloma neurophilia* causes altered shoaling behaviour in adult laboratory zebrafish (*Danio rerio*) and its implications for neurobehavioural research. J Fish Dis 40:443–446

Sprague V (1977) Annotated list of species of microsporidia. In: Bulla LA, Cheng TC (eds) Comparative pathology, Vol 2: Systematics of microsporidia. Plenum Press, New York, NY, p 31–334

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