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Source: Journal of Parasitology, 103(1) : 95-102

Published By: American Society of Parasitologists

URL: https://doi.org/10.1645/16-24

J. Parasitol., 103(1), 2017, pp. 95–102

TWO NOVEL MICROSPORIDIA IN SKELETAL MUSCLE OF PIKE-PERCH *SANDER LUCIOPERCA* AND BURBOT *LOTA LOTA* IN FINLAND

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ABSTRACT: Two new species of Microsporidia were recognized in skeletal muscle of freshwater fishes from Finland. *Myosporidium spraguei* n. sp. from pike-perch *Sander lucioperca* occurred as mature spores within sporophorous vesicles (SPVs) within a xenoma. The ovoid spores were 3.8 μm long and 2.4 μm wide, based on transmission electron micrographs (TEM). The exospore and endospore were equally thick, the nucleus was monokaryotic and the polar filament was isofilar with 12 coils in a single rank, entirely adjacent to the prominent posterior vacuole. Small subunit (SSU) rDNA sequence confirmed the presence of *M. spraguei* n. sp. in burbot *Lota lota*. The second species, *Microsporidium luciopercae* n. sp., also from pike-perch, occurred within SPVs that occupied only a fraction of the volume of the otherwise intact myocyte; no xenoma was produced. Myocyte degeneration and necrosis occurred as mature spores dispersed into direct contact with the sarcoplasm. The ovoid spores were 4.6 μm long and 2.8 μm wide (based on TEM); they were monokaryotic and the polar filament was isofilar with 25 coils in a single rank in the posterior of the spore. The exospore was relatively thin with an irregular profile. Neither infection elicited an inflammatory response, although degenerate spores were observed within host cells, suggesting phagocytosis. Phylogenetic analysis of SSU sequences placed both organisms on distinct clades within the Marinosporidia.

The phylum Microsporidia (kingdom Fungi) includes obligate intracellular, spore-forming parasites of vertebrate and invertebrate animals (Corradi and Keeling, 2009). The number of microsporidian species known to infect fishes is estimated to range from 100 to 156 belonging to 14 to 17 genera (Lom and Nilsen, 2003; Kent et al., 2014; Phelps et al., 2015). Taxonomic groupings of microsporidia are primarily based on light and electron microscopic assessments of parasite structure and patterns of development. In addition, phylogenetic relationships among taxa can be inferred from ribosomal DNA nucleotide sequences, however, these relationships only occasionally agree with the morphological taxonomic groupings (Stentiford et al., 2013). Microsporidia are transmitted among fish hosts via the microspore, which injects the sporoplasm into the host cell via the polar tube. The parasite then undergoes sequential cycles of merogony and sporogony. There is tremendous variation among parasite taxa in the elaboration of structures within which the parasite develops: development may occur freely in the cytoplasm or enclosed within parasite-derived sporophorous vesicles, which in turn may lie within a larger, parasite-derived sporophorocyst. The xenoma is a hypertrophic infected host cell, which no longer resembles the native state (Lom and Dyková, 2005). Space-occupying lesions caused by microsporidia are grossly visible as nodules or cysts, which may elicit capsule formation and ultimately a granulomatous response by the host. Thus microsporidian infections have economic consequences when they cause disease in commercially valuable fish (Michel et al., 1989; Pulsford and Matthews, 1991; Kent et al., 2014; Phelps et al., 2015).

In Finland, lakes and rivers support valuable sport, recreational, and commercial fisheries. The pike-perch (*Sander lucioperca*) inhabits fresh and brackish waters throughout much of northern Eurasia and in Finland; a total catch of 3.9×10^6 kg was landed in 2013. Similarly, burbot (*L. lota*) occur in the fresh and brackish waters of northern Eurasia and northern North America. In Finland, fisheries occurring mainly in the winter landed 0.7×10^6 kg of burbot in 2013 (https://www.luke.fi/en/). The purpose of the present study was to describe and identify the microsporidia infecting skeletal muscle of *S. lucioperca* and *L. lota* collected from freshwater lakes in Finland. The morphology and developmental stages of the parasites were described with the use of light and electron microscopy, and inferences of phylogenetic affinity were made from analyses of small subunit ribosomal DNA (SSU) sequences.

MATERIALS AND METHODS

Collections

Lakes Päijänne (1,083 km^2) and Leppävesi (64 km^2) belong to the River Kymijoki catchment, whereas Lakes Haukivesi (560 km^2) and Pielinen (894 km^2) belong to the River Neva catchment, both draining to the Gulf of Finland, Baltic Sea (Fig. 1). Between 2010 and 2014, fish were collected by seine or gill nets from the 4 lakes, transported to the laboratory either alive in aerated lake water or dead and on ice, and examined within 6–24 hr of capture. During necropsy, infections with microsporidia were observed as areas of pallor within the skeletal muscle. Samples of the affected muscle were dissected and preserved in neutral buffered 10% formalin (NBF, 1-cm^3 pieces) for light microscopy, 2.5% glutaraldehyde in 0.1 M Sörensen's phosphate buffer (SPB) (1 mm^3) for electron microscopy or 95% ethanol (1–5 mm^3) for DNA extraction.

Histology

Tissue fixed in NBF was trimmed and dehydrated in an alcohol gradient, cleared in xylene, embedded with paraffin, and sectioned at 3 μm. Each section was mounted onto a glass slide, stained with hematoxylin and eosin or Gram stains, cover-slipped, and

Received 20 March 2016; revised 2 August 2016; accepted 22 August 2016.

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DOI: 10.1645/16-24

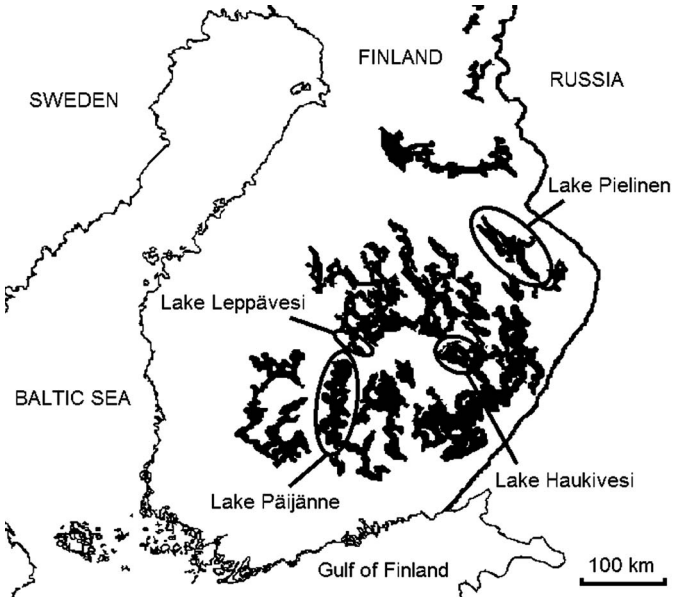

FIGURE 1. Locations of lakes in Finland from which samples were collected.

examined with the use of a compound microscope (Zeiss Axio Imager, Carl Zeiss Canada, Toronto, Ontario, Canada) equipped with a digital imaging system (Axiocam MRc5, Carl Zeiss Canada).

Electron microscopy

Glutaraldehyde-fixed tissue was washed in SPB, postfixed in 1% osmium tetroxide for 1 hr, washed, and dehydrated through a graded ethanol series into 100% acetone. Samples were incubated overnight in a 50:50 mixture of acetone and epoxy resin (JEMBED 812 Embedding Kit, Canemco Inc., Lakefield, Quebec, Canada) followed by 2 changes of epoxy before polymerization overnight at 60 C. Semithin (1–2 μm) sections (Reichert-Jung Ultracut E ultramicrotome, Leica Microsystems, Richmond Hill, Ontario, Canada) were stained with toluidine blue for light microscopic examination. Ultrathin (70–90 nm) sections were mounted on uncoated copper grids (Canemco Inc.), stained with uranyl acetate for 7 min, washed 3 times in ddH_2O, stained with lead citrate for 3 min, and washed. Sections were viewed with the use of a Hitachi H-7000 transmission electron microscope at 75 kV and digital images captured with the use of an AMT 2 k CCD camera (Advanced Microscopy Techniques, Woburn, Massachusetts). Assessments of microspore morphology and dimensions were made from ultrathin preparations viewed in electron micrographs.

DNA extraction, amplification, and sequencing

DNA was extracted (DNeasy, Qiagen Inc., Toronto, Ontario, Canada) from approximately 35 mg of ethanol-preserved muscle into AE buffer and quantified with the use of a Nanodrop-1000 spectrophotometer (Thermo Scientific, Wilmington, Delaware). Oligonucleotide primers used to amplify and to sequence partial small-subunit (SSU) rDNA sequences are listed in Table I, and methods followed Jones et al. (2012). PCR products were cleaned with the use of ExoSap-IT (USB Corporation, Cleveland, Ohio) and sequencing reactions were performed with the use of BDT V3.1 (Applied Biosystems, Foster City, California). Reaction products were purified with the use of Qiagen Dye-Ex 2.0 kits, and sequences were obtained from a 16-capillary 3130xl Genetic Analyzer (Applied Biosystems).

Sequencing, alignment, and phylogenetic analyses

Sequences were edited and assembled in Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, Michigan) and contigs were subjected to BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine identity with archived sequences. A pairwise identity matrix was constructed to compare sequences (Huang and Miller, 1991), and the locations of nucleotide differences were described relative to reference sequence AF387331 (*Heterosporis anguillarum*). The most closely related sequences obtained by BLAST search from GenBank (*Pleistophora mulleri* EF119339, *Pleistophora typicalis* AF044387, *Pleistophora hippoglossoides* AJ252953, *Heterosporis sutherlandae* KC137548, *Heterosporis anguillarum* AF387331, *Glugea plecoglossi* AB623035, *Glugea atherinae* GAU15987, *Glugea hertwigi* GQ203287, *Glugea anomala* AF044391, *Loma embiotocia* AF320310, *Loma salmonae* LSU78736, *Ichthyosporidium weissii* JQ062988, *Pseudoloma neurophila* AF322654, *Microsporidium cerebralis* JQ316511, *Microsporidium prosopium* AF151529, *Heterosporis* sp. KC137554, *Myosporidium merluccius* AY530532), and an outgroup sequence (*Basidiobolus ranarum* AY635841) were imported to MEGA6 for phylogenetic analysis. The sequences were aligned by Muscle 3.7 with default parameters and manually trimmed, resulting in a data set of 843 phylogenetically informative positions. Phylogenetic relationships among aligned sequences were inferred from a maximum-likelihood analysis with the use of the general-time-reversible (GTR) model with gamma distribution set to 3, without gaps or missing data. Confidence in tree topology was based on 1,000 bootstrap replications.

DESCRIPTION

Myosporidium spraguei n. sp.

(Figs. 2, 3)

Diagnosis: Spores monokaryotic, ovoid, 3.8 μm long (range 3.2–4.8 μm), 2.4 μm wide, based on TEM (range 2.2–2.6 μm) (n = 15). Exospore and endospore approximately equal in thickness, polar filament isofilar, 12 coils (range 11–13) in single rank entirely adjacent to the prominent posterior vacuole. Spore aggregates within sporophorous vesicles (SPV) within xenoma. Xenomas 250–275 μm long, 50–175 μm wide, based on histological preparations (n = 3), with laminated wall of variable thickness, without microvilli or other irregularities.

Taxonomic summary

Type host: Sander lucioperca (Linnaeus, 1758).
Site of infection: Skeletal muscle.
Type locality: Lake Päijänne (61°29′N, 25°26′E), Finland.
Other host: Lota lota (Linnaeus, 1758).
Other localities: Lake Haukivesi (62°06′N, 28°26′E), Finland.
Specimens deposited: Hapantotype (1) and parahapantotype (1) slides, containing stained sections of infected pike-perch muscle have been lodged in the Parasitology Collection of the

TABLE I. Oligonucleotide primers used to amplify the microsporidian ribosomal DNA.

Name	5′–3′ sequence	Reference
530F	GTGCCAGC(A/C)GCCGCGG	Vossbrinck et al. (1993)
580R	GGTCCGTGTTTCAAGACGG	Vossbrinck et al. (1993)
MicroLH-F	CGATAACGACGGGCGGTGTGT	Present study
MicroLH-R	GGGGAGTACACGCGCAAGC	Present study
PomportF	GGTTGATTCTGCCTGACGT	Baker et al. (1994)
PomportR	GACGGGCGGTGTGTACAAAG	Pomport-Castillon et al. (1997)
McerF	CTAGGCGCGAGCGAGGTTCG	Present study
McerR	CGTCGTCCCGCGAGCCAAAA	Present study
HeteF	GTCCGGGAAGTAGTGAGACC	Present study
HeteR	GCCGCTACTACAGGAATCCT	Present study

Canadian Museum of Nature with catalogue number CMNPA 2016-0001.

Etymology: The species is named after Professor Victor Sprague, for contributions to the study of microsporidia of aquatic organisms.

Molecular sequences: Small subunit ribosomal DNA sequences are deposited in GenBank with accession numbers KX351970, KX351971, and KU302781.

Remarks

The presence of a xenoma and a polar filament with a mean of 12 coils readily distinguished *M. spraguei* n. sp. from *M. luciopercae* n. sp. in the skeletal muscle of pike-perch. *Myosporidium spraguei* n. sp. was distinguished from xenoma-forming parasites of the genus *Glugea* spp., in which the xenoma wall is thickly laminated with sloughed-off host cell material and stratified into a cortex of early developmental stages and a medullary region of mature spores. Similarly, the parasite was distinguished from *Heterosporis* spp. by the absence of an obvious sporophorocyst, and from most *Pleistophora* spp. by the formation of a xenoma. The thick-walled cysts produced by *Pleistophora senegalensis* and *Pleistophora hippoglossoideos* (Morrison et al., 1984; Faye et al., 1990) may be the result of encapsulation by the host. The structure of the xenoma in *M.*

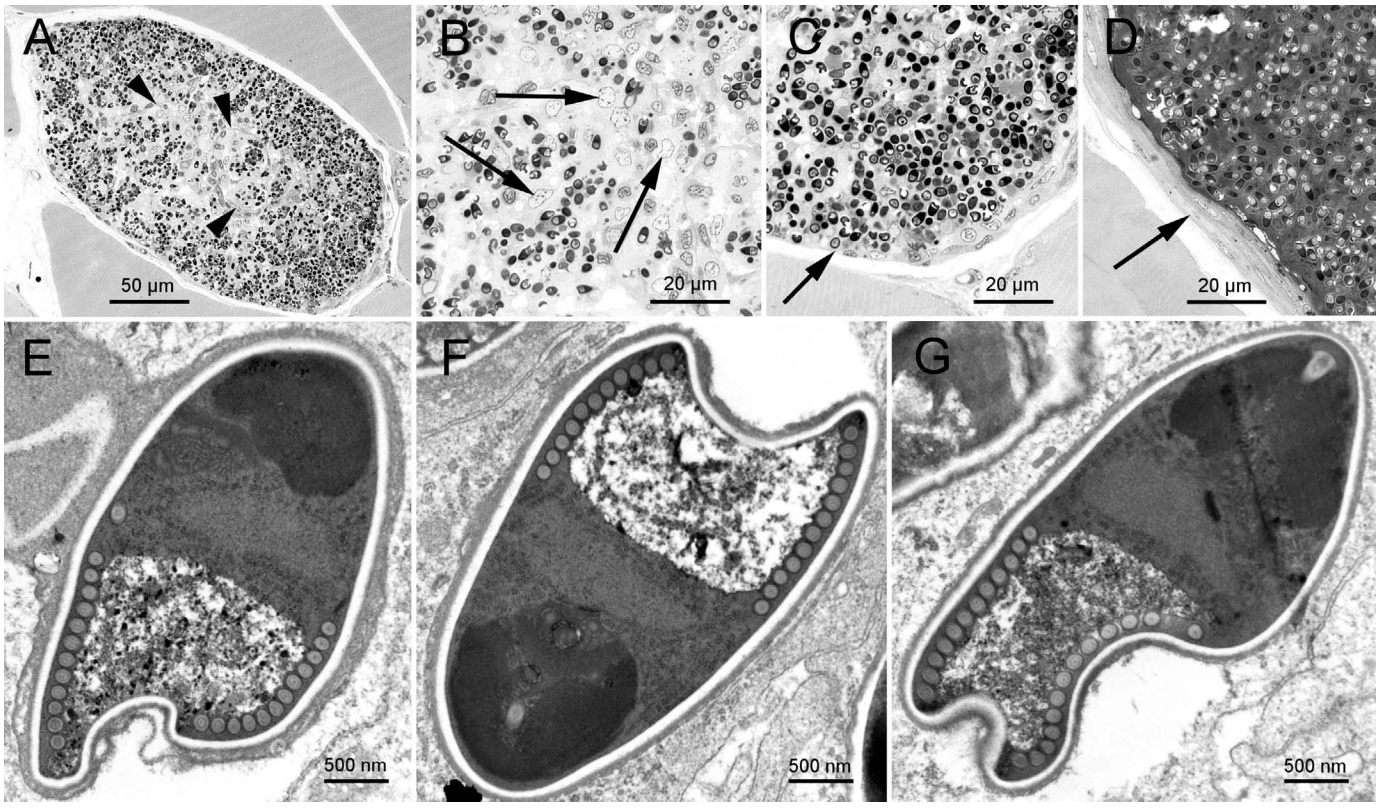

FIGURE 2. Pike-perch *Sander lucioperca* skeletal muscle showing infection with *Myosporidium spraguei* n. sp. (**A**) Xenoma showing clear demarcation from surrounding muscle cells and membrane-associated spore aggregates (arrowheads). (**B**) Xenoma showing detail of host-cell nuclei or nucleus fragments (arrows). (**C**) Thin-walled xenoma (arrow). (**D**) Thick-walled xenoma, showing laminated fibrocyte structure of wall (arrow). (A–D) are light micrographs of toluidine blue-stained semithin resin preparations. (**E–G**) Electron micrographs of microspores within xenoma showing structural detail.

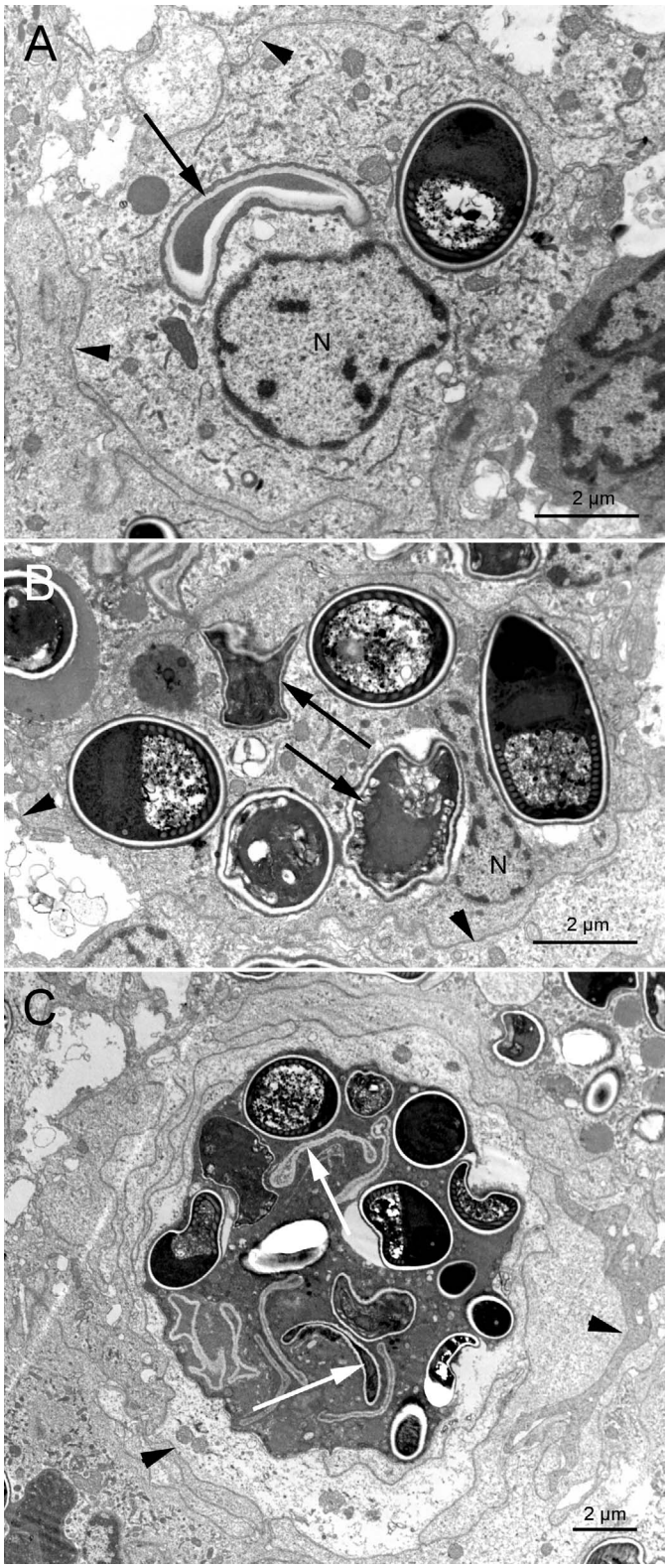

FIGURE 3. Electron micrographs of phagocytosed *Myosporidium spraguei* n. sp. within xenoma. (**A**, **B**) Intact and degenerate (arrows) microspores within nucleated (N) host cell, containing organelles and an electron-dense cytoplasmic matrix. Host cell membrane (arrowheads) is shown. (**C**) Membrane-bound cluster of intact and degenerate spores (arrows) within host cell (arrowhead shows cell membrane).

spraguei n. sp. was reminiscent of *Myosporidium merluccius*, a parasite of hake (*Merluccius capensis*/*paradoxus* complex) off the coast of Namibia, in which both thin- and thick-walled xenomas are also produced (Baquero et al., 2005).

Two small-subunit ribosomal RNA gene (SSU) sequence types were associated with *M. spraguei* n. sp. Type A was represented by 2 sequences: KX351970, assembled from *S. lucioperca* (n = 1) and *L. lota* (n = 2) from Lake Haukivesi; and KU302781, assembled from *L. lota* (n = 2) from Lake Haukivesi. Type B was represented by KX351971, assembled from *S. lucioperca* (n = 8) from Lake Päijänne. Histological analysis confirmed the presence of *M. spraguei* n. sp. in a fish with a type B sequence. Percent identity between these sequence types was 98.3% (Table II), corresponding to nucleotide (nt) differences at 25 sites and a 4-nt insert in KX351971 at position nt1342. Phylogenetic analysis provided strong support for placement of the *M. spraguei* n. sp. sequences, along with those from other myotropic microsporidia (*Microsporidium* sp. from mountain whitefish *Prosopium williamsoni*, *Heterosporis* sp. from cisco *Coregonus* sp. and *M. merluccius*), into a clade similar to that previously described by Diamant et al. (2010) (Fig. 4). Infections with *Microsporidium* sp. in fibrocytes of the skeletal endomesium of *P. williamsoni* are diffuse with poorly defined perimeters (Kent et al., 1999) and the parasite from cisco has not been described.

Microsporidium luciopercae n. sp.

(Figs. 5, 6)

Diagnosis: Spores monokaryotic, ovoid, 4.6 µm long (range 3.9–5.0 µm) and 2.8 µm wide, based on TEM (range 2.2–3.2 µm) (n = 20). Irregular exospore thinner than endospore, polar filament isofilar, 25 coils (range 24–28) in single rank in posterior of spore. Sporogony within SPVs without sporophorocyst or xenoma formation. Mature spores in direct contact with sarcoplasm.

Taxonomic summary

Type host: Sander lucioperca (Linnaeus, 1758).

Site of infection: Skeletal muscle.

Type locality: Lake Haukivesi (62°06′N, 28°26′E), Finland.

Comments: Mycocyte degeneration associated with spore maturation.

Other localities: Lakes Pielinen (63°15′N, 29°42′E) and Leppävesi (62°13′N, 25°56′E), Finland.

Specimens deposited: Hapantotype (1) and parahapantotype (1) slides, containing stained sections of infected pike-perch muscle have been lodged in the Parasitology Collection of the Canadian Museum of Nature with catalogue number CMNPA 2016-0002.

Etymology: The species is named for its host, *S. lucioperca*.

Molecular sequences: SSU ribosomal DNA sequences are deposited in GenBank with accession numbers KX351969 and KU302782.

Remarks

A single-SSU sequence type associated with *M. luciopercae* n. sp. was represented by 2 sequences: KX351969, assembled from *S. lucioperca* from Lake Haukivesi (n = 4), Lake Pielinen (n = 1) and Lake Leppävesi (n = 1); and KU302782, assembled from *S. lucioperca* from Lake Pielinen (n = 2) and Lake Haukivesi (n = 1).

TABLE II. Pairwise percent identity matrix among ribosomal DNA sequences from microsporidia.

Sequence*	1	2	3	4
1				
2	98.3			
3	78.7	78.4		
4	99.8	98.3	78.6	
5	78.5	78.3	99.7	79.3

* 1, KX351970: *Myosporidium spraguei* n. sp. type A; 2, KX351971: *M. spraguei* type B; 3, KX351969: *Microsporidium luciopercae* n. sp.; 4, KU302781: *M. spraguei* n. sp. type A; 5, KU302782: *M. luciopercae* n. sp.

Histological analysis confirmed the presence *M. luciopercae* n. sp. in 1 fish from Lake Haukivesi. KX351969 shared 78.7% and 78.4% identity with the *M. spraguei* n. sp. sequences KX351970 and KX351971. This, along with the absence of a xenoma and a polar filament with 25 rather than 12 coils, clearly distinguished *M. luciopercae* n. sp. from *M. spraguei*, n. sp. Unlike *T. brevifilum* (Matthews and Matthews, 1980), spores of *M. luciopercae* n. sp. did not possess an electron-dense inclusion within the sporoplasm, nor were the spores diplokaryotic as in *Ichthyosporidium* spp. (Sanders et al., 2012). Although the SPV aggregates in *M. luciopercae* n. sp. were superficially similar to some *Pleistophora* spp. in which the infections are often diffuse and associated with myodegeneration (Kent et al., 2014), BLAST and phylogenetic analyses did not support a relationship of *M. luciopercae* n. sp. with other non-xenoma-forming myotropic microsporidia of fish belong to the genera *Pleistophora*, *Heterosporis*, *Kabatana*, *Myosporidium*, and *Dasyatispora* (Baquero et al., 2005; Dyková, 2006; Diamant et al., 2010). Rather, the SSU sequences of *M. luciopercae* n. sp. clustered with the neurotropic species *M. cerebralis* and *P. neurophila* (Fig. 4), which develop as SPV aggregates in the spinal cord and/or hind brain of marine farmed Atlantic salmon *Salmo salar* in Western Canada and in the central nervous system and skeletal muscle of zebrafish *Danio rerio* in laboratory colonies, respectively (Brocklebank et al., 1995; Matthews et al., 2001; Cali et al., 2011; S. Jones, unpubl. data).

DISCUSSION

Microsporidia in skeletal muscle of *S. lucioperca* and *L. lota* collected from freshwater lakes in Finland were found to belong to 2 previously unrecognized species. Small subunit ribosomal DNA (SSU) sequences placed these morphologically distinct species into two well-supported phylogenetic clades. Two SSU sequence types from *M. spraguei* n. sp. shared approximately 79% identity with a sequence from *M. luciopercae* n. sp.; however, all three sequences clustered within the Marinosporidia, which are primarily parasites of marine hosts (Vossbrinck and Debrunner-Vossbrinck, 2005; Vossbrinck et al., 2014). The 2 sequence types from *M. spraguei* n. sp. shared 98.3% identity with each other, consistent with intraspecific variation in SSU rDNA sequences in species of *Kabatana* and *Dictyocoela* (Terry et al., 2004; Barber et al., 2009). The occurrence of sequence variants from Lakes Päijänne and Haukivesi indicates that distinct *M. spraguei* n. sp. genotypes occur in these lakes, suggesting that mixing of parasites between these catchment basins is limited or absent. A test of this hypothesis requires further research, particularly given the single-

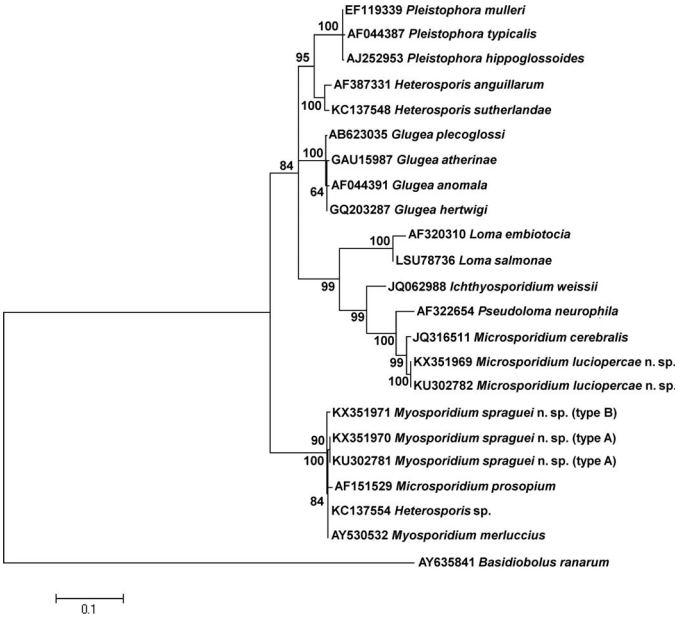

FIGURE 4. Maximum-likelihood analysis of partial small-subunit ribosomal RNA gene sequences from select microsporidians of fish, based on an alignment of 843 informative positions. Branch nodes show bootstrap support and scale shows number of substitutions per site.

SSU sequence type associated with *M. luciopercae* n. sp. in *S. lucioperca* from Lakes Haukivesi, Pielinen, and Leppävesi. The 2 parasites also demonstrated clear structural and developmental differences. Although the spores of these parasites were superficially similar in size, they differed in the number of coils of the polar filament, the prominence of the posterior vacuole and the relative thickness of the exospore. However, some meristic and morphological attributes of the spores may have been affected by processing for electron microscopy. *Microsporidium luciopercae* n. sp. also differed from *M. spraguei* n. sp. by developing within myocytes without the formation of a xenoma, and the infection ultimately resulted in degeneration of the affected cell following loss of parasite-associated membranes and release of mature spores. Internalization and degradation of spores or SPV-like complexes within host cells indicates phagocytosis and was evident during infections with *M. spraguei* n. sp. or *M. luciopercae* n. sp., consistent with the conclusion of Dyková and Lom (1980) that in fish, resolution of infections with microsporidians includes a phagocytic host response. The evidence from electron micrographs indicates that host-cell nuclei within the *M. spraguei* n. sp. xenoma belong to infiltrating phagocytes rather than being evidence of a syncytial xenoma, as reviewed by Lom and Dyková (2005).

Microsporidia previously reported from *S. lucioperca* and *L. lota* or from related fish species include *Glugea luciopercae*, from ovary, gill and intestinal submucosa of *S. lucioperca* in northern Eurasia (Lom, 2002). The parasite is an important pathogen of juvenile fish and xenomas rarely occur elsewhere in the body (Lom and Dyková, 1992). *Glugea fennica* occurs as xenomas in subcutaneous tissues and fins of *L. lota* from northern Eurasia (Lom and Dyková, 1992; Lom, 2002). *Heterosporis sutherlandae* was described from skeletal muscle of walleye, *Sander vitreus* in the United States (Phelps et al., 2015). The parasite develops as

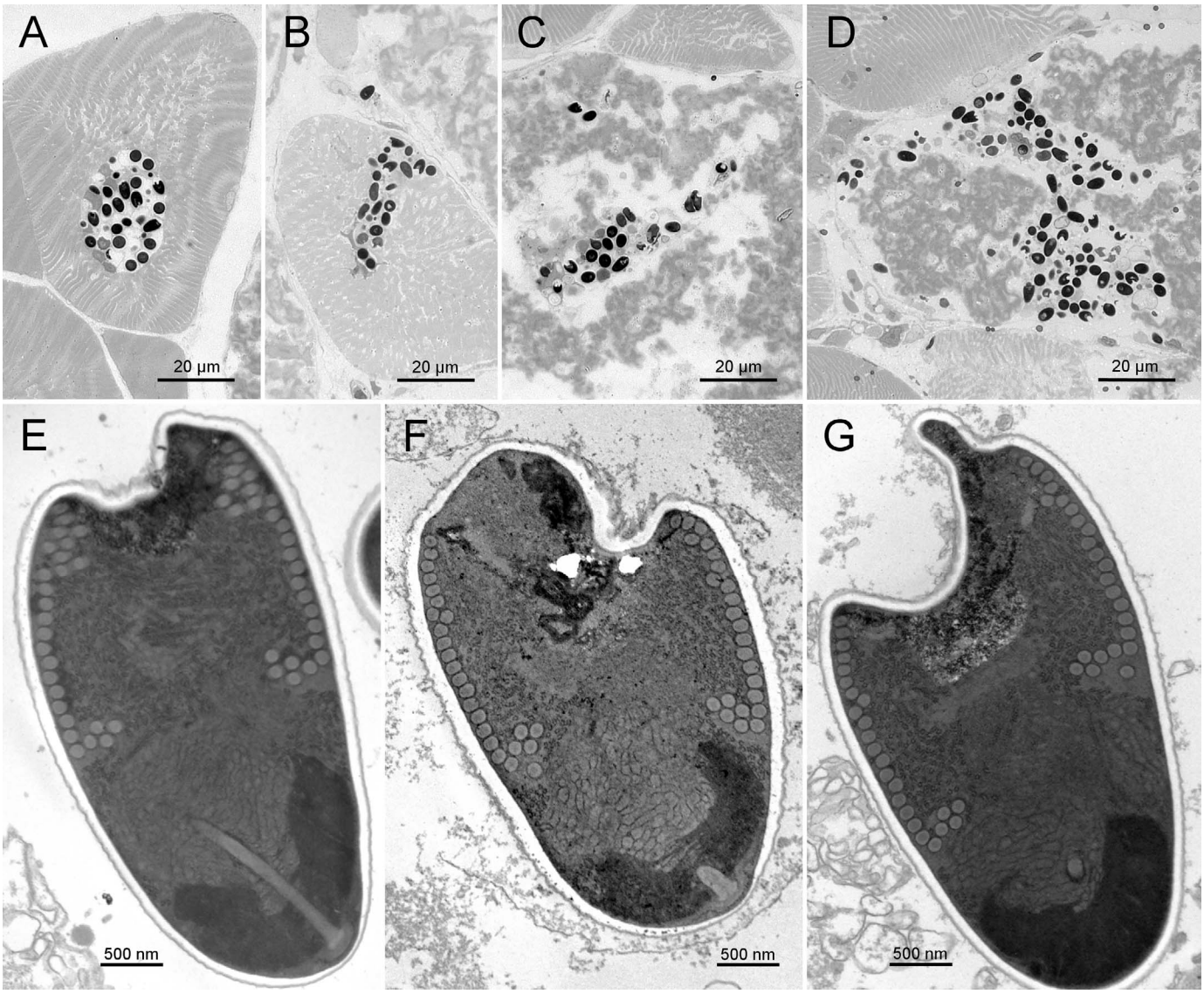

FIGURE 5. Pike-perch *Sander lucioperca* skeletal muscle showing infection with *Microsporidium luciopercae* n. sp. (**A**) Intact sporophorocyst within normal myocyte; spores within membrane-bound sporophorous vesicles. (**B**) Spores no longer constrained within membranous structures; some vacuolation of myocyte. (**C**, **D**) Free spores associated with degenerate myocyte. (A–D) are light micrographs of toluidine blue-stained semithin resin preparations. (**E**–**G**) Electron micrographs of microspores showing structural detail.

sporophorocysts containing numerous SPVs. *Pleistophora ladogensis* occurs as pansporoblasts in skeletal muscle of *L. lota* and the smelt *Osmerus eperlanus eperlanus* from freshwater lakes in western Russia (Voronin, 1978, 1981). An unnamed and undescribed microsporidian was reported from gill, musculature and/or skin of *L. lota* from western Canada (McDonald and Margolis, 1995). Our conclusion of *M. spraguei* n. sp. in skeletal muscle of *L. lota* was based on molecular data. Combined morphological and molecular data are required to describe and identify microsporidia in skeletal muscle of *L. lota* adequately, and to better understand the relationship between *M. spraguei* n. sp. and *P. ladogensis*.

Microsporidian infections of the skeletal muscle of finfish are associated with changes to the appearance and texture of the fillet and may cause a reduction in the commercial value. The extent of visible change is related to the severity of the infection and resulting tissue damage, or to the host response. In the present study *M. spraguei* n. sp. developed within xenomas at the limit of visual resolution, whereas mature spores of *M. luciopercae* n. sp. occurring freely within the sarcoplasm were associated with myocyte degeneration and necrosis. An acute inflammatory cellular infiltrate was not observed in either infection, although in both, spores were observed within macrophage-like cells. Pike-perch fishermen observe no changes in fillets infected with *M. spraguei* n. sp. In contrast, *M. luciopercae* n. sp.–infected fillets are rigid and opaque with conspicuous pale patches. In severe cases there is a grossly visible loss of structure and the fillet is said to resemble ground meat. In another study, infections with *H. sutherlandae* were shown to cause multifocal to locally extensive necrosis of skeletal muscle in walleye from Minnesota (Phelps et

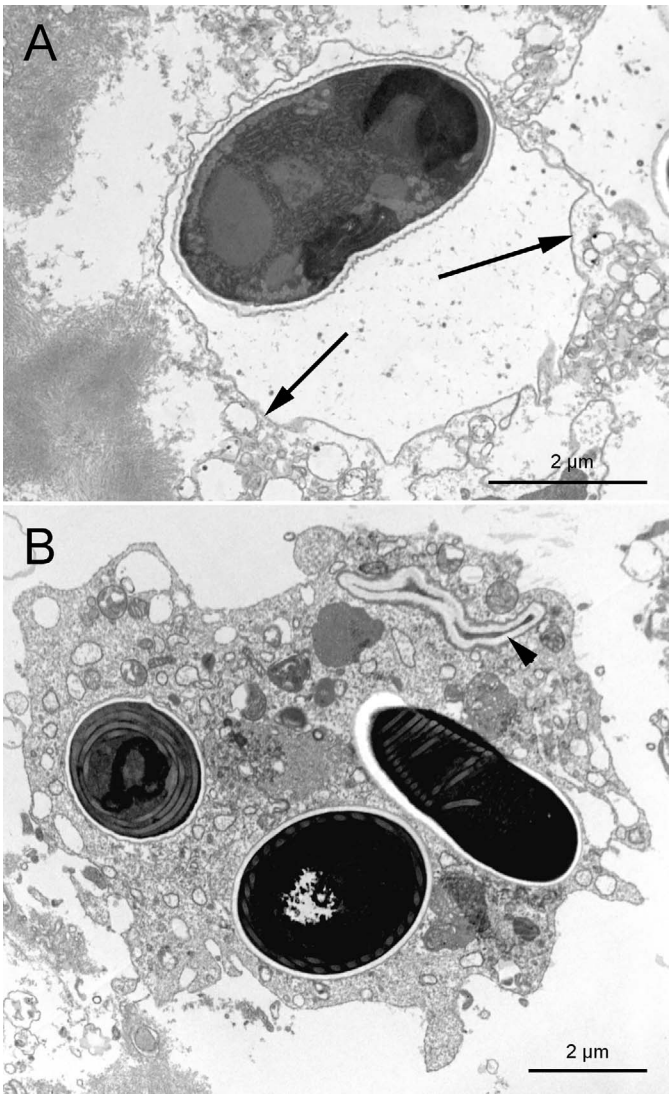

FIGURE 6. Electron micrographs of *Microsporidium luciopercae* n. sp. within membrane-bound structures. (**A**) Intact microspore within a sporophorous vesicle (arrows). (**B**) Intact and degenerate (arrowhead) microspores within host phagocyte.

al., 2015). Similarly, there are numerous examples of infections with *Pleistophora* spp. causing lesions in fillets that render them unfit for human consumption (Lom and Dyková 1992). Although infections with *M. spraguei* n. sp. and *M. luciopercae* n. sp. are likely to be directly transmitted among fish via ingestion of microspores, nothing is known about the demographic composition of affected fish. Knowledge of age-associated changes in prevalence will assist in managing the impacts of these infections.

ACKNOWLEDGMENTS

We thank Amelia Mahony and Gina Prosperi-Porta, Fisheries and Oceans Canada (DFO) for assistance with PCR, sequencing and phylogenetic analyses. Steve Cho, DFO, and Dorota Wadowska, University of Prince Edward Island, provided electron micrographs. We thank Henna Majuri, Markku Kettunen, Lauri Kakkonen, and Arto Tuomainen for collection of fish. We are grateful to the late E. T. Valtonen for comments on the manuscript and to Nina Honkanen, JYU, for help in the laboratory. We acknowledge the European Fisheries Fund (Central Finland ELY Centre and North Savo ELY Centre) and the Finnish Women's Science Foundation, Olvi Foundation, Finnish Cultural Foundation (Central Finland regional fund) and Finnish Food and Drink Industries' Federation for funding. The comments of 2 anonymous reviewers helped improve the manuscript.

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