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A NEW SPECIES OF *LOMA* (MICROSPOREA) IN SHINER PERCH (*CYMATOGASTER AGGREGATA*)

R. W. Shaw*, M. L. Kent, M. F. Docker†, A. M. V. Brown*, R. H. Devlin†, and M. L. Adamson*
Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia V9R 5K6, Canada

ABSTRACT: *Loma embiotocia* n. sp. is described from the gills of shiner perch (*Cymatogaster aggregata*) from waters off Vancouver Island, British Columbia, Canada. Highest prevalence at a site was 15% and greatest intensity was 583 xenomas per fish. Xenomas averaged 0.13 mm in diameter (0.06–0.16 mm) and contained ovoid spores 4.8 × 2.6 (4.0–5.0 × 2.0–3.0) μm. Sporogonic stages were dispersed throughout the xenomas. The xenoma wall was smooth lacking invaginations into the cytoplasm; sporoblasts were not highly vacuolated, and the sporophorous vesicle formed before sporogony. In addition to differences in host and geographic location the new species is distinguished from *Loma salmonae,* the only other species in the genus known from British Columbia, by its internal transcribed spacer (ITS) ribosomal DNA sequence.

Species of the microsporidian genus *Loma* have been described from freshwater and saltwater fishes (Morrison and Sprague, 1981a, 1983). Species of *Loma* most often infect endothelial cells of vascular tissue and appear as white cysts termed xenomas in fresh gill tissue (Canning and Lom, 1986; Kent, 1992). One species, *Loma salmonae,* formerly *Pleistophora salmonae* (Putz, Hoffman, and Dunbar, 1965), is an important pathogen of salmonid fishes reared in fresh and salt water (Wales and Wolf, 1955; Kent et al., 1989). Nonsalmonid fishes have been suggested as potential reservoirs for infections of *L. salmonae* acquired by seawater-reared salmon (Kent et al., 1995). Shiner perch *Cymatogaster aggregata* Gibbons, 1854 are numerous in and around salmon netpen farms in British Columbia, some of which have experienced severe outbreaks of *L. salmonae.* A species of *Loma* was found in the gills of shiner perch that were being examined as part of a study of potential reservoir hosts for *L. salmonae.* This species of *Loma* can be distinguished from *L. salmonae* by host and by geographic, morphological, and molecular (rDNA) characters. We, therefore, describe this microsporean as a new species.

MATERIALS AND METHODS

Specimen collection

Cymatogaster aggregata were collected from 3 localities off Vancouver Island, British Columbia, a commercial dock at Tofino and a netpen salmon farm at Eagle Bay (both in Clayoquot Sound 13 km apart) and a beach at Departure Bay, Nanaimo. Fish were collected by seine in Nanaimo, by angling at the Clayoquot Sound localities and by dipnet at the fish farm.

Fish were killed with tricaine methanesulfonate (MS-222), then stored on ice or frozen. Fish were weighed and measured (total length), and the first 3 gills arches on the left side of each fish were examined for the presence of the parasite.

Gill material for spore and xenoma measurements and abundance was collected at all sites. Material for histology was collected in July for the Eagle Bay sample, whereas material for electron microscopy was collected from the Tofino dock sample. Material for molecular biology was collected at all sites and frozen at −70 C.

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* Department of Zoology, 6270 University Boulevard, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4.
† Department of Fisheries and Oceans, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, British Columbia, Canada V7V 1N6.

Measurements and abundance

Xenomas and spores were measured from wet mounts of fresh material. For size comparison, 30 spores of *L. salmonae* from seawater-reared chinook salmon *Oncorhynchus tshawytscha* Walbaum, 1792 (British Columbia) were measured under identical conditions. Prevalence was determined by examination of gills in wet mount preparations, except for the July sample from Eagle Bay, in which prevalence was determined from histological sections. Intensity was determined by counting the total number of xenomas in all gill arches of the infected fish collected from Eagle Bay in September. Wet mount preparations for prevalence and intensity were examined at ×100. Fish were separated into underyearlings (≤1 yr old) and adults (>1 yr old) based on frequency plots and reproductive biology (Hart, 1973).

Histology

Gills were removed from fish and placed in Davidson's solution for histological examination (Humason, 1979). Histological sections were prepared from these gills and stained with hematoxylin and eosin.

Electron microscopy

Infected lamellae were fixed for 24 hr in 4% glutaraldehyde, then transferred to Millonig's solution for an additional 24 hr. Lamellae were postfixed in 1% osmium tetraoxide, embedded in epoxy resin, sectioned, stained with lead citrate and uranyl acetate, and examined with a transmission electron microscope.

Polymerase chain reaction (PCR) and DNA sequencing

DNA was extracted from infected shiner perch gill tissue from all 3 sites by proteinase K digestion at 37 C for 6 hr (10 mM Tris, pH 8.0; 10 mM EDTA; 1% SDS; 150 mM NaCl; 200 μg/mL proteinase K). The suspension was extracted twice with a 50:50:1 ratio, respectively, of phenol, chloroform, and isoamyl alcohol (pci), and the DNA precipitated on ice with ethanol. The DNA was resuspended in TE, and stored at 4 C.

Fragments of the above DNA were amplified by PCR with 2 sets of primers developed from the rDNA sequence of *L. salmonae* in chinook salmon (Docker, Devlin et al., 1996). The first set, the forward primer Loma f (5′-ATTAGTGAGACCTCAGCC-3′) and the reverse primer LS-2 (5′-ATGACATCTCACATAATTGTG-3′), is located in the small subunit (SSU) and large subunit (LSU) rDNA genes, respectively; they amplify a 627-base pair (bp) fragment in *L. salmonae* that spans the internal transcribed spacer (ITS) region. The second PCR assay used the reverse primer LS-2 and a second forward primer, LS-1 (5′-CTGGATCAGACCGATTTATAT-3′), which was developed from the hypervariable ITS region in *L. salmonae* and amplifies a 272-bp fragment in this species. Docker, Devlin et al. (1996) found the second PCR assay (LS-1/LS-2) to be highly sensitive, capable of reliably detecting as few as 0.01 *L. salmonae* spores per 50-μl PCR reaction (or 40 spores per g of chinook salmon gill tissue). The first set of primers, Loma f and LS-2, was 3 orders of magnitude less sensitive for *L. salmonae* under these conditions.

Gene amplification was accomplished using standard PCR buffer (Gibco BRL, Gaithersburg, Maryland), 1.5 nM $MgCl_2$, and 0.2 mM

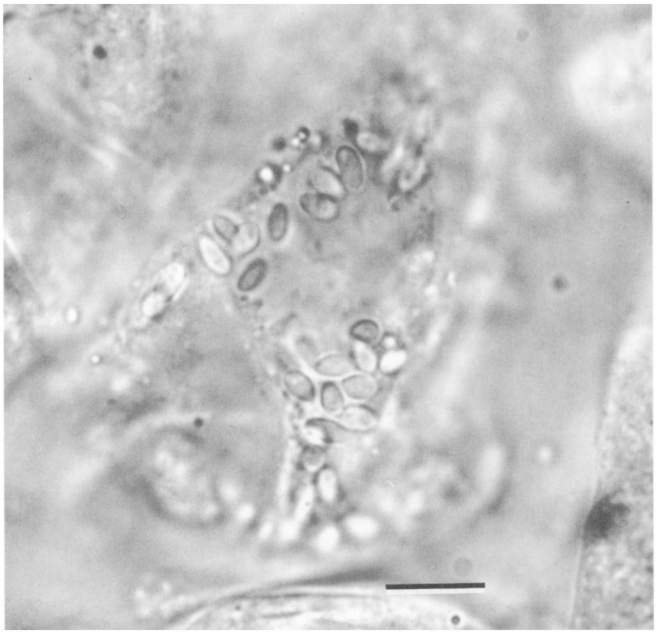


FIGURE 1. Fresh spores of *Loma embiotocia* n. sp. observed under oil immersion. Bar = 10 μm.

dNTP (Saiki, 1990). Each 50-μl reaction contained 25 pmol of each primer, 1.25 units of Taq DNA polymerase, and 0.6 μg of genomic DNA. The reactions were run in a PTC-200 thermocycler (MJ Research, Watertown, Massachusetts) for 35 cycles of 94 C for 45 sec, 53 C for 45 sec, and 72 C for 90 sec; these were preceded by a 3-min denaturation at 95 C and followed by a 10-min 72 C extension.

The PCR product generated by primers Loma f and LS-2 in the Tofino shiner perch sample was sequenced using the Sequenase PCR Product Sequencing Kit (United States Biochemical, Cleveland, Ohio), according to the manufacturer's instructions, and in the other 2 samples by Taq terminator sequencing using fluorescent dye-labeled terminators on the 373 DNA automated sequencer. Internal primers also generated from *L. salmonae* sequence were used to sequence from 47 to 97% of the product in both directions.

DESCRIPTION

Loma embiotocia n. sp.

(Figs. 1–6)

Spores (n = 30) 4.8 μm long (4.0–5.0 μm) × 2.6 μm wide (2.0–3.0 μm). Xenomas (n = 10) 0.13 mm (0.06–0.16 mm). Xenomas in endothelial cells of secondary gill lamellae. Spores (n = 20) with 14–18 coils of the polar filament. Earlier developmental stages (meronts, sporoblasts) present. Small tubules present. Sporoblasts not highly vacuolated. Sporophorous vesicle formed before sporogony. Smooth xenoma wall lacking complex invaginations into xenoma cytoplasm.

Taxonomic summary

Type host: *Cymatogaster aggregata.*

Type locality: Eagle Bay, Clayoquot Sound, Vancouver Island, British Columbia.

Site of infection: Secondary lamellae of gills.

Distinguishing characters: Round xenomas in gill, spore size, polar filament count, and rDNA sequence.

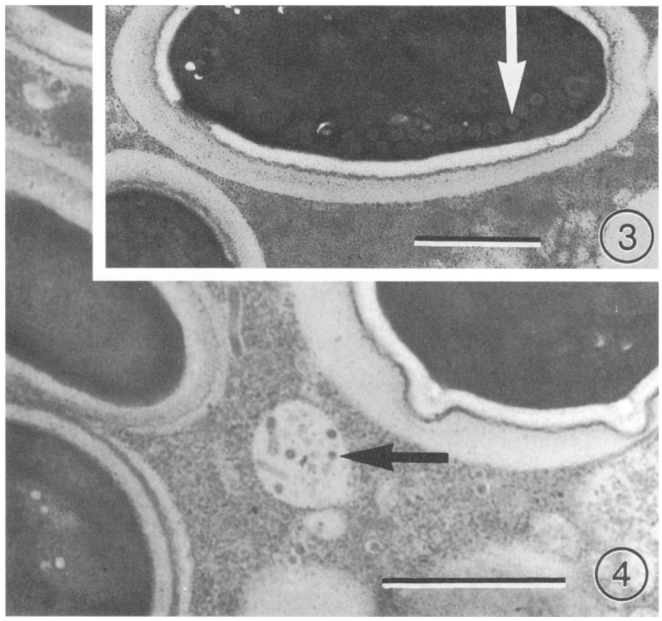
Material deposited: Hapantotypes in histological sections, Canadian Museum of Nature, Invertebrate Collection (Parasites), Ottawa, Canada; sequence information as GenBank accession number U78815.

Etymology: Named after family name for shiner perch, Embiotocidae.

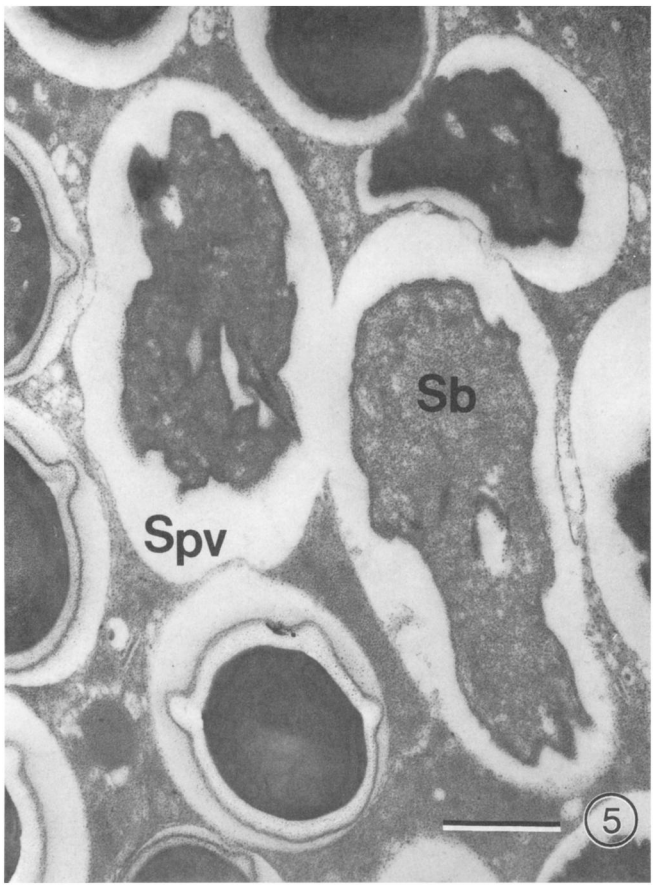
Ecology

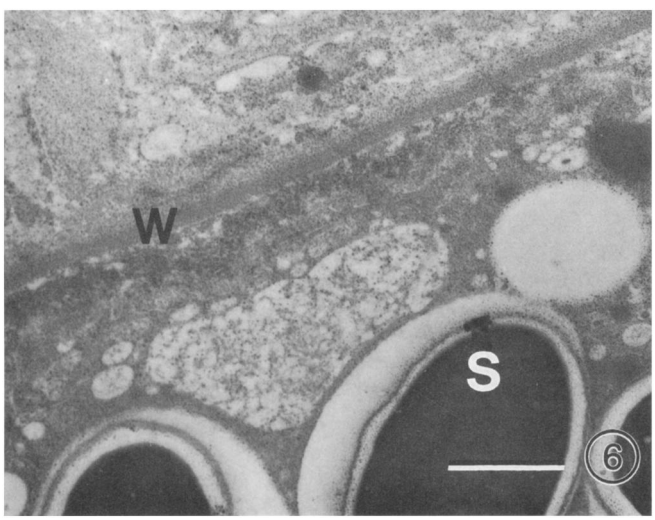
Loma embiotocia n. sp. was detected in the gills of shiner perch from all 3 locations (Table I). Shiner perch examined ranged from 6.1 to 15.5

FIGURE 2. Xenoma of the hypertrophic cell type formed by *Loma embiotocia* n. sp. associated with gill secondary lamellae. N = host cell nucleus remnants. Bar = 10 μm.



FIGURES 3–4. Transmission electron micrographs of *Loma embiotocia* n. sp. spores and xenoma structure. Bars = 1 μm. **3.** Mature spore with 17 coils of the polar filament (arrow). **4.** Small tubules (arrow) within xenoma cytoplasm.





FIGURES 5–6. **5.** Sporoblasts (Sb) with minimal vacuolation within sporophorous vesicles (Spv). **6.** Smooth xenoma wall (W) containing spores (S). Transmission electron micrographs of *Loma embiotocia* n. sp. spores and xenoma structure. Bars = 1 μm.

cm in length and 2.8 to 58.1 g in weight, which represented 2 yr classes (underyearlings, 6.1–9.4 cm; adults, 10.5–15.5 cm). Both year classes were infected with *L. embiotocia.* Prevalence was as high as 15.2% in underyearlings and 13.5% in adults (Table I). Intensity of *L. embiotocia* in underyearling fish (n = 12) from Eagle Bay (September), ranged from 4 to 583 xenomas per fish.

TABLE I. Infection statistics for *Loma embiotocia* n. sp. found in *Cymatogaster aggregata* Gibbons, 1854 collected at 3 sites around Vancouver Island, British Columbia in 1995.

Site	Month	Number of fish	Prevalence (%)		
			Underyearlings	Adults*	Total
Eagle Bay	Jul	23			2/23 (8.7)
	Aug	54	1/40 (2.5)	1/14 (7.1)	2/54 (3.7)
	Sep	80	12/79 (15.2)	0/1 (0.0)	12/80 (15.0)
Tofino Dock	Sep	38	0/1 (0.0)	5/37 (13.5)	5/38 (13.2)
Nanaimo	Sep	60	0/60 (0.0)	0/0 (0.0)	0/60 (0.0)
	Oct	60	1/60 (1.7)	0/0 (0.0)	1/60 (1.7)

* Adults are greater than 1 yr of age.

Remarks

Spore size, the formation of xenomas of the cell-hypertrophy type, location in the gill, and mixture of developmental stages randomly throughout xenomas place this parasite within the genus *Loma* (Canning and Lom, 1986). Species of *Loma* show extreme overlap in spore size, number of polar filament turns, and other characters (Table II). *Loma embiotocia* resembles *Loma diplodae* Bekhti and Bouix, 1985 and *L. salmonae* in having a smooth xenoma wall lacking invaginations of the wall (interdigitated) into the cytoplasm, a low level of sporoblast vacuolation, and in the developmental timing of the sporophorous vesicle. Note that *Loma branchialis* (Nemeczek, 1911) is insufficiently known for comparison (Table II). *Loma embiotocia* is distinguished from *L. diplodae* by host and geographic location. Host and rDNA sequence separate *L. embiotocia* from *L. salmonae.*

The PCR primers Loma f and LS-2 both developed from the relatively conserved regions of rDNA sequence of *L. salmonae,* yielded positive assays for the shiner perch infected with *L. embiotocia* from all 3 sites; the size of the fragment was approximately the same as that from *L. salmonae* (Fig. 7). Repeated assays with primers LS-1, designed from the hypervariable ITS region of *L. salmonae,* and LS-2 consistently failed to amplify DNA from any of the infected shiner perch (Fig. 7).

Of the 635-bp amplified by primers Loma f and LS-2 in the 3 localities of shiner perch, 564 bp was sequenced and consisted of 334 bp of SSU rDNA sequence, 45 bp of ITS, and 185 bp of LSU rDNA, although the exact boundaries of the ITS are not known. As the PCR results suggested, the SSU rDNA and LSU rDNA regions in *L. embiotocia* were similar to those of *L. salmonae,* differing by only 4 and 2 base substitutions, respectively. All 6 substitutions were transitions, and all were found in the 3 different *L. embiotocia* isolates. There were no differences between these isolates in the SSU and LSU gene fragments, although multiple nucleotide signals were observed at 1 SSU rDNA site in the Nanaimo (position 101, Fig. 8) and Eagle Bay (position 317, Fig. 8) samples. These sites may indicate that several slightly different copies were present, either due to the presence of different *L. embiotocia* isolates in the pooled gill samples or to differences among dispersed members of the ribosomal gene array. To determine this, cloning and sequencing of individual repeats is required.

In contrast to the SSU and LSU gene fragments, the short ITS region (45 and 37bp, respectively) differed significantly between *L. embiotocia* and *L. salmonae* by having 3 indels, 4 transitions, and 1 transversion (Fig. 8). The lack of amplification with primer LS-1 in infected shiner perch from all 3 sites reflects these sequence differences, many of which are at the 3′ end of the LS-1 primer site. The ITS region was identical among *L. embiotocia* from different localities, except for 2 sites each with 2 nucleotide signals in the Eagle Bay sample (Fig. 8).

DISCUSSION

In species of *Loma* that infect gills, most descriptions were based on spore measurements taken from formalin fixed spores

TABLE II. Comparison of *Loma embiotocia* n. sp. host, morphological, and developmental characteristics with *L. branchialis* (Nemeczek, 1911), *L. diplodae* Bekhti and Bouix, 1985, *L. fontinalis* Morrison and Sprague, 1983, *L. morhua* Morrison and Sprague, 1981, and *L. salmonae* (Putz, Hoffman, and Dunbar, 1965). Characteristics of turns in filament, xenoma wall, sporoblast vacuolation, and sporophorous vesicle (SPV) are ultrastructural. SPV formation is summarized as occurring before or after spore formation is complete.

Species (morphological reference)	Host	Distribution*	Spore		Xenoma wall	Sporoblast vacuolation	SPV
			Size (μm)†	Turns in filament			
L. embiotocia (This study)	*Cymatogaster aggregata* Gibbons, 1854	Vancouver Island, British Columbia, M	4.8 × 2.6 a	14–18	Smooth	Low	Before
L. branchialis (Nemeczek, 1911)	*Melanogrammus aeglefinus* L.	Nova Scotia, M	6.3 × 3.5 b				
L. diplodae (Bekhti and Bouix, 1985)	*Diplodus sargus* L.	France, M	4.2 × 2.2 b	17–18	Smooth	Low	Before
L. fontinalis (Morrison and Sprague, 1983)	*Salvelinus fontinalis* Mitchill, 1815	Nova Scotia, FW	3.7 × 2.2 b	14–15	Smooth	High	After
L. morhua (Morrison and Sprague, 1981a)	*Gadus morhua* L.	Nova Scotia, M	4.2 × 2.0 b	16–17	Interdigitated	Low	Before
(Morrison and Sprague, 1981b)	*Melanogrammus aeglefinus*	Nova Scotia, M	4.4 × 2.1 b	16–18			
L. salmonae (Morrison and Sprague, 1983)	*Oncorhynchus mykiss* Walbaum, 1792	California, FW	4.5 × 2.2 c	14–17	Smooth	Low	Before
(Kent et al., 1989)	*Oncorhynchus kisutch* Walbaum, 1792	Washington, M	4.4 × 2.3 c	14–17			
(This study)	*Oncorhynchus tshawytscha* Walbaum, 1792	British Columbia, FW	5.5 × 2.7 a				

* FW, freshwater; M, marine.
† Measurement given as a mean of length × thickness; spores measured were fresh (a), from resin sections (b), or preserved in formalin (c).

or from sections of resin-embedded material (Table II). This makes comparison of the size of our spores with those of other species difficult. The number of polar filament coils overlaps, and developmentally there are only minor differences between most species of *Loma* (Table II). Morrison and Sprague (1983) realized that the structure of spores of *L. salmonae* and *Loma morhua* Morrison and Sprague, 1981 contained no differences of taxonomic value, and these species were separated based on differences in hosts and habitats. These species may be valid, as transmission studies by Kent et al. (1995) suggest that *L. salmonae* is host specific, i.e., marine fishes, including shiner perch, were not susceptible to experimental infection by *L. salmonae*.

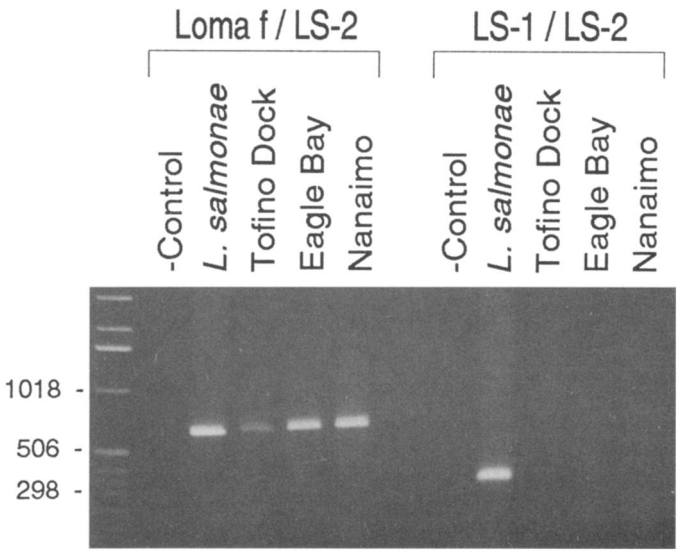


FIGURE 7. PCR of DNA from chinook salmon gill infected with *Loma salmonae* and from gills of shiner perch infected with *L. embiotocia* n. sp. Shiner perch were collected at Eagle Bay, Tofino Dock, and Nanaimo sites, and parasite DNA amplified with primers Loma f and LS-2 or primers LS-1 and LS-2 (1% agarose gel, ethidium-bromide stained). Negative control used distilled water instead of template DNA. Molecular weight markers (bp) are shown in the left lane.

The ribosomal DNA sequence comparisons presented here (Fig. 8) further demonstrate that *L. embiotocia* n. sp. is distinct from *L. salmonae,* although the 2 species appear to be very closely related. Intraspecific differences in SSU rDNA sequence have been studied in a number of microsporeans and have been shown to range from 0.2 to 11.4% within the genera *Encephalitozoon, Varimorpha,* and *Nosema* (Baker et al. 1995). The 1.2% difference (4 base pairs in 334) found between *L. embiotocia* and *L. salmonae* SSU rDNA fragments approaches the lower end of the observed range and thus reflects a close relationship between these species. The relatively low level of variation nevertheless is greater than expected due to intraspecific variation (Didier et al. 1995). Although comparison between *Enterocytozoon bieneusi* SSU rDNA sequence generated by 2 different studies (Zhu et al., 1994; Hartskeerl et al., 1995) yielded differences as high as 1.73%, levels of intraspecific variation in the SSU gene of microsporidians is generally believed to be negligible (Didier et al., 1995; Docker, Kent et al., 1996). Similarly, the more substantial differences in the ITS region of *L. embiotocia* and *L. salmonae* also surpass that expected due to intraspecific variation. ITS sequence differences

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L. salmonae     CAGGTGCGCA AAGCACAGGA AGGATGGGTC AAGGACAGGT CAGTGATGCC
L. embio Tofino CAGGTGCGCA AAGCACAGGA AGGATGGGTC AAGGACAGGT CAGTGATGCC
L. embio EagleB CAGGTGCGCA AAGCACAGGA AGGATGGGTC AAGGACAGGT CAGTGATGCC
L. embio Nanaim CAGGTGCGCA AAGCACAGGA AGGATGGGTC AAGGACAGGT CAGTGATGCC

                                                        *    *
L. salmonae     CTTAGATGGT CCGGGCTGCA CGCGCACTAC AGTGGTCGCC GAAATTACCT
L. embio Tofino CTTAGATGGT CCGGGCTGCA CGCGCACTAC AGTGGTCGCC GGAATTRCCT
L. embio EagleB CTTAGATGGT CCGGGCTGCA CGCGCACTAC AGTGGTCGCC GGAATTRCCT
L. embio Nanaim CTTAGATGGT CCGGGCTGCA CGCGCACTAC AGTGGTCGCC GGAATTRCCT

                +
L. salmonae     GATAATTATA AAGGCGATCG AGAGGGAATG AGCTTTGTAA GAGGCTCAGG
L. embio Tofino GATAATTATA AAGGCGATCG AGAGGGAATG AGCTTTGTAA GAGGCTCAGG
L. embio EagleB GATAATTATA AAGGCGATCG AGAGGGAATG AGCTTTGTAA GAGGCTCAGG
L. embio Nanaim RATAATTATA AAGGCGATCG AGAGGGAATG AGCTTTGTAA GAGGCTCAGG


L. salmonae     AACGAGGAAT TGCTAGTAAT CGCGGACTCA TTAAGACGCG ATGAATACGT
L. embio Tofino AACGAGGAAT TGCTAGTAAT CGCGGACTCA TTAAGACGCG ATGAATACGT
L. embio EagleB AACGAGGAAT TGCTAGTAAT CGCGGACTCA TTAAGACGCG ATGAATACGT
L. embio Nanaim AACGAGGAAT TGCTAGTAAT CGCGGACTCA TTAAGACGCG ATGAATACGT

                                                           *
L. salmonae     CCCTGTTCTT TGTACACACC GCCCGTCGTT ATCGAAGATG AAGATAGGCG
L. embio Tofino CCCTGTTCTT TGTACACACC GCCCGTCGTT ATCGAAGATG AAGACAGGCG
L. embio EagleB CCCTGTTCTT TGTACACACC GCCCGTCGTT ATCGAAGATG AAGACAGGCG
L. embio Nanaim CCCTGTTCTT TGTACACACC GCCCGTCGTT ATCGAAGATG AAGACAGGCG

                                        *
L. salmonae     CGAACGATCT ACCAGAAAGT GAGCGCAGGT TTTTAGATCT GATACAAGTC
L. embio Tofino CGAACGATCT ACCAGAAAGT GAGCGCAGGT CTTTAGATCT GATACAAGTC
L. embio EagleB CGAACGATCT ACCAGAAAGT GAGCGCAGGT CTTTAGATCT GATACAAGTC
L. embio Nanaim CGAACGATCT ACCAGAAAGT GAGCGCAGGT CTTTAGATCT GATACAAGTC

                              +                            +
L. salmonae     GTAACAAGGT AGCTGTAGGA GAACCTGTAG CTGGatcaga ccga------
L. embio Tofino GTAACAAGGT AGCTGTAGGA GAACCTGTAG CTGGatcaga ccgacaaaaa
L. embio EagleB GTAACAAGGT AGCTGTWGGA GAACCTGTAG CTGGatcaga ccgacmaaaa
L. embio Nanaim GTAACAAGGT AGCTGTAGGA GAACCTGTAG CTGGatcaga ccgacaaaaa

                    * *  *   + *   *
L. salmonae     ----tttata taatctttgt atgaatgtaa tCTCTGCGCA AGGGATCTTT
L. embio Tofino gaaactcatg t-atatt-at atgaatgtaa tCTCTGCGCA AGGGATCTTT
L. embio EagleB gaaactcatg t-wtatt-at atgaatgtaa tCTCTGCGCA AGGGATCTTT
L. embio Nanaim gaaactcatg t-atatt-at atgaatgtaa tCTCTGCGCA AGGGATCTTT


L. salmonae     TGGTTCGCTA GACGAAGAAG GGCGCAGCGG AATGCGAAAT GTGCAGGAGT
L. embio Tofino TGGTTCGCTA GACGAAGAAG GGCGCAGCGG AATGCGAAAT GTGCAGGAGT
L. embio EagleB TGGTTCGCTA GACGAAGAAG GGCGCAGCGG AATGCGAAAT GTGCAGGAGT
L. embio Nanaim TGGTTCGCTA GACGAAGAAG GGCGCAGCGG AATGCGAAAT GTGCAGGAGT

                          *
L. salmonae     CGCAGCGAAG ATAGCACATG CCTGAAATCA CGAGAGTGAG ACTACCCCTT
L. embio Tofino CGCAGCGAAG ACAGCACATG CCTGAAATCA CGAGAGTGAG ACTACCCCTT
L. embio EagleB CGCAGCGAAG ACAGCACATG CCTGAAATCA CGAGAGTGAG ACTACCCCTT
L. embio Nanaim CGCAGCGAAG ACAGCACATG CCTGAAATCA CGAGAGTGAG ACTACCCCTT

                                                    *
L. salmonae     TGAATTAAGC ATATGAGTAA AGGGAGGAAA AGAAACTAAC AAGGATTCCT
L. embio Tofino TGAATTAAGC ATATGAGTAA AGGGAGGAAA AAAAACTAAC AAGGATTCCT
L. embio EagleB TGAATTAAGC ATATGAGTAA AGGGAGGAAA AAAAACTAAC AAGGATTCCT
L. embio Nanaim TGAATTAAGC ATATGAGTAA AGGGAGGAAA AAAAACTAAC AAGGATTCCT

L. salmonae     GTAGTAGCGG CGAGCG
L. embio Tofino GTAGTAGCGG CGAGCG
L. embio EagleB GTAGTAGCGG CGAGCG
L. embio Nanaim GTAGTAGCGG CGAGCG
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FIGURE 8. Sequence alignment of the gene fragments sequenced here, including a portion (334 bp) of the small subunit ribosomal DNA (SSU rDNA) and part (185 bp) of the large subunit DNA (LSU rDNA), and the internal transcribed spacer (ITS), for *Loma salmonae* and *L. embiotocia* n. sp. Lowercase letters denote the presumptive ITS. Differences between *L. salmonae* and *L. embiotocia* are marked with "*." Differences between *L. embiotocia* from different localities are marked with +. R indicates both G and A, W indicates both A and T, and M indicates both C and A.

between the 3 isolates of *L. embiotocia* were minimal, and intraspecific variation in the second ITS region of mosquitos, flukes, and lake trout has also been shown to be negligible (Porter and Collins, 1991; Adlard et al., 1993, Zhuo et al., 1994). Even where intraspecific variation has been shown to be more substantial (e.g., Fritz et al., 1994; Vogler and DeSalle, 1994; Didier et al., 1995; Docker, Kent et al., 1996), it was not of the magnitude noted in this study between *L. embiotocia* and *L. salmonae*.

PCR assays used in the present study were useful both in detecting species of *Loma* infections that were not readily observed in wet mount preparations (primers Loma f and LS-2) and in distinguishing between *L. salmonae* and *L. embiotocia* (LS-1 and LS-2). In chinook salmon infected with *L. salmonae,* however, the assay using primers Loma f and LS-2 was found to be several orders of magnitude less sensitive than that using LS-1 and LS-2. A more sensitive PCR assay for detection of *L. embiotocia* in shiner perch could be developed by replacing Loma f with an *L. embiotocia* specific primer in the region of the LS-1 site in *L. salmonae*. Such an assay could speed transmission and cross-infection studies of potential reservoir hosts.

In conclusion, based on host, geographic location, spore morphology, and rDNA sequence, the microsporidium found in shiner perch and described here is a new species within the genus *Loma*. To clarify the relationships and validity of other described species of *Loma,* further comparisons should be done of inter- and intraspecific differences in rDNA combined with transmission studies for members of the genus *Loma*.

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LITERATURE CITED

ADLARD, R. D., S. C. BARKER, D. BLAIR, AND T. H. CRIBBS. 1993. Comparison of the second internal transcribed spacer (ribosomal DNA) from populations and species of Fasciolidae (Digenea). International Journal for Parasitology **23:** 423–425.

BAKER, M. D., C. R. VOSSBRINCK, E. S. DIDIER, J. V. MADDOX, AND J. A. SHADDUCK. 1995. Small subunit ribosomal DNA phylogeny of various microsporidia with emphasis on AIDS related forms. Journal of Eukaryotic Microbiology **42:** 564–570.

BEKHTI, M., AND G. BOUIX. 1985. *Loma salmonae* (Putz, Hoffman et Dunbar, 1965) et *Loma diplodae* n. sp., microsporidies parasites de branchies de poissons téléostéens: Implantation et données ultrastructurales. Protistologica **21:** 47–59.

CANNING, E. U., AND J. LOM. 1986. The microsporidia of vertebrates. Academic Press, New York, New York, 289 p.

DIDIER, E. S., C. R. VOSSBRINCK, M. D. BAKER, L. B. ROGERS, D. C. BERTUCCI, AND J. A. SHADDUCK. 1995. Identification and characterization of three Encephalitozoon cuniculi strains. Parasitology **111:** 411–421.

DOCKER, M. F., R. H. DEVLIN, J. RICHARD, AND M. L. KENT. 1996. Sensitive and specific polymerase chain reaction assay for detection of *Loma salmonae* in chinook salmon *Oncorhynchus tsawytscha.* Diseases of Aquatic Organisms (in press).

———, M. L. KENT, D. M. L. HERVIO, J. S. KHATTRA, L. M. WEISS, A. CALI, AND R. H. DEVLIN. 1996. Ribosomal DNA sequence of *Nucleospora salmonis* (Microsporea: Enterocytozooidae): Implications for phylogeny and nomenclature. Journal of Eukaryotic Microbiology (in press).

FRITZ, G. N., J. CONN, A. COCKBURN, AND J. SEAWRIGHT. 1994. Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from populations of *Anopheles nuneztovari* (Diptera: Culicidae). Molecular Biology and Evolution **11:** 406–416.

HART, J. L. 1973. Pacific fishes of Canada. Bulletin 180, Fisheries Research Board of Canada, 740 p.

HARTSKEERL, R. A., A. R. SCHUITEMA, T. VAN GOOL, AND W. J. TERPSTRA. 1993. Genetic evidence for the occurrence of extra-intestinal *Enterocytozoon bieneusi* infections. Nucleic Acids Research **21:** 4150.

HUMASON, G. L. 1979. Animal tissue techniques, 4th ed. W. H. Freeman and Company, San Francisco, California, 470 p.

KENT, M. L. 1992. Diseases of seawater netpen-reared salmonid fishes in the Pacific Northwest. Canadian Special Publications of Fisheries and Aquatic Sciences No. 116.

———, S. C. DAWE, AND D. J. SPEARE. 1995. Transmission of *Loma salmonae* (Microsporea) to chinook salmon in sea water. Canadian Veterinary Journal **36:** 98–101.

———, D. G. ELLIOTT, J. M. GROFF, AND R. P. HEDRICK. 1989. *Loma salmonae* (Protozoa: Microspora) infections in seawater reared coho salmon *Oncorhynchus kisutch.* Aquaculture **80:** 211–222.

MORRISON, C. M., AND V. SPRAGUE. 1981a. Electron microscopical study of a new genus and new species of microsporida in the gills of Atlantic cod *Gadus morhua* L. Journal of Fish Diseases **4:** 15–32.

———, AND ———. 1981b. Light and electron microscope study of microsporida in the gill of haddock, *Melanogrammus aeglefinus* (L.). Journal of Fish Diseases **4:** 179–184.

———, AND ———. 1983. *Loma salmonae* (Putz, Hoffman and Dunbar, 1965) in the rainbow trout, *Salmo gairdneri* Richardson, and *L. fontinalis* sp. nov. (Microsporida) in the brook trout, *Salvelinus fontinalis* (Mitchill). Journal of Fish Diseases **6:** 345–353.

NEMECZEK, A. 1911. Beiträge zur kenntnis der myxo und microsporidien der fische. Archiv für Protistenkunde **22:** 143–169.

PORTER, C. H., AND F. H. COLLINS. 1991. Species-diagnostic differences in a ribosomal DNA internal transcribed spacer from the sibling species *Anopheles freeborni* and *Anopheles hermsi* (Diptera: Culicidae). American Journal of Tropical Medicine and Hygiene **45:** 271–279.

PUTZ, R. E., L. HOFFMAN, AND C. E. DUNBAR. 1965. Two new species of *Plistophora* (Microsporidea) from North American fish with a synopsis of Microsporidea of freshwater and euryhaline fishes. Journal of Protozoology **12:** 228–236.

SAIKI, R. S. 1990. Amplification of genomic DNA. *In* PCR protocols, M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (eds.). Academic Press, San Diego, California, p. 13–20.

VOGLER, A. P., AND R. DESALLE. 1994. Evolution and phylogenetic information content in the ITS-1 region in the tiger beetle *Cicindela dorsalis.* Molecular Biology and Evolution **11:** 393–405.

WALES, J. H., AND H. WOLF. 1955. Three protozoan diseases of trout in California. California Fish and Game **41:** 183–187.

ZHU, X., M. WITTNER, H. B. TANOWITZ, A. CALI, AND L. M. WEISS. 1994. Ribosomal RNA sequences of *Enterocytozoon bieneusis, Septata intestinalis* and *Ameson michaelis*: Phylogenetic construction and structural correspondence. Journal of Eukaryotic Microbiology **41:** 204–209.

ZHUO, L., S. L. SAJDAK, AND R. B. PHILLIPS. 1994. Minimal intraspecific variation in the sequence of the transcribed spacer regions of the ribosomal DNA of lake trout (*Salvelinus namaycush*). Genome **37:** 664–671.