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TWO MYXOZOANS FROM THE URINARY TRACT OF TOPSMELT, *ATHERINOPS AFFINIS*

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ABSTRACT: Two myxozoan species were observed in the kidney of topsmelt, *Atherinops affinis*, during a survey of parasites of estuarine fishes in the Carpinteria Salt Marsh Reserve, California. Fish collected on 3 dates in 2012 and 2013 were sectioned and examined histologically. Large extrasporogonic stages occurred in the renal interstitium of several fish from the first 2 collections (5/8, 11/20, respectively) and, in some fish, these replaced over 80% of the kidney. In addition, presporogonic and polysporogonic stages occurred in the lumen of the renal tubules, collecting ducts, and mesonephric ducts. The latter contained subspherical spores with up to 4 polar capsules, consistent with the genus *Chloromyxum*. For the third collection (15 May 2013, n = 30), we portioned kidneys for examination by histology, wet mount, and DNA extraction for small subunit ribosomal (SSU rDNA) gene sequencing. Histology showed the large extrasporogonic forms in the kidney interstitium of 3 fish and showed 2 other fish with subspherical myxospores in the lumen of the renal tubules with smooth valves and 2 spherical polar capsules consistent with the genus *Sphaerospora*. *Chloromyxum*-type myxospores were observed in the renal tubules of 1 fish by wet mount. Sequencing of the kidney tissue from this fish yielded a partial SSU rDNA sequence of 1,769 base pairs (bp). Phylogenetic reconstruction suggested this organism to be a novel species of *Chloromyxum*, most similar to *Chloromyxum careni* (84% similarity). In addition, subspherical myxospores with smooth valves and 2 spherical polar capsules consistent with the genus *Sphaerospora* were observed in wet mounts of 2 fish. Sequencing of the kidney tissue from 1 fish yielded a partial SSU rDNA sequence of 1,937 bp. Phylogenetic reconstruction suggests this organism to be a novel species of *Sphaerospora* most closely related to *Sphaerospora epinepheli* (93%). We conclude that these organisms represent novel species of the genera *Chloromyxum* and *Sphaerospora* based on host, location, and SSU rDNA sequence. We further conclude that the formation of large, histozoic extrasporogonic stages in the renal interstitium represents developmental stages of *Chloromyxum* species for the following reasons: (1) Large extrasporogonic stages were only observed in fish with *Chloromyxum*-type spores developing within the renal tubules, (2) a DNA sequence consistent with the *Chloromyxum* sp. was only detected in fish with the large extrasporogonic stages, and (3) several *Sphaerospora* species have extrasporogonic forms, but they are considerably smaller and are composed of far fewer cells.

During a survey of parasites of estuarine fishes in the Carpinteria Salt Marsh Reserve, a Pacific Ocean estuary located on the southern California coast, 2 novel species of myxosporeans were found infecting the topsmelt, *Atherinops affinis*. This site has been used in past studies of parasite ecology, including how parasites impact food webs (Lafferty et al., 2006a, 2006b) and affect behavior of their hosts (Lafferty and Morris, 1996), and the current research adds to the knowledge of parasite diversity in that system. The California topsmelt, *A. affinis* (Ayres, 1860) Atherinidae, lives along the Pacific coast of North America. A schooling fish, they generally inhabit bays, estuaries, and kelp forests, feeding on zooplankton and macroalgae. In recent years we have used histopathology, in addition to wet mount preparations, to survey fishes for novel parasites (Rodnick et al., 2008; Ferguson et al., 2011; Kent et al., 2013). Here, we applied a similar approach with topsmelt and discovered a heavy infection of the kidney by a myxozoan. Examination of additional fish using histology, wet mount preparations, and small subunit ribosomal (SSU rDNA) sequence revealed novel *Chloromyxum* and *Sphaerospora* species, both of which are described here. These are the first myxozoans described from *A. affinis*.

MATERIALS AND METHODS

Sample collection

We seined California topsmelt, *A. affinis*, in Carpinteria Salt Marsh, California (34.40°N, 119.53°W) on 6 February 2012 (n = 8), 15 March 2012 (n = 20), and 15 May 2013 (n = 30). Fish were acclimated in the laboratory at the University of California Santa Barbara in flow-through tanks for 2 days. Fish were euthanized by submersion into a lethal dose (250 mg/L) of tricaine methanesulfonate (MS-222, Argent Finquel®, Redmond, Washington), placed in individual bags, and shipped on ice overnight to Oregon State University where fish were dissected and kidney tissues were removed. A small portion was examined by wet mount, a portion was fixed in Dietrich's fixative for histology, and a portion was removed for DNA extraction.

Wet mount

Pieces of kidney were placed on a glass slide with a small amount of saline, a glass coverslip was overlaid, and the specimen was compressed. Slides were examined by bright field, phase contrast, and Nomarski phase interference. Representative myxozoan spores were photographed and measured using a SPOT camera and software (Diagnostic Instruments, Sterling Heights, Michigan).

Histology

Kidney tissues were preserved in Dietrich's fixative and processed for histology and embedded in paraffin. Two 5-μm sections were cut for each individual tissue. One section was stained with standard hematoxylin and eosin and the other with Giemsa stain. Stained sections were examined by light microscopy.

DNA isolation and sequencing

DNA was extracted using the Qiagen Blood and Tissue Extraction kit (Qiagen, Valencia, California), according to the manufacturer's protocol, from part of the kidney from fishes in which either spores or presporogonic myxozoan stages were observed by wet mount or in histological sections.

PCR was performed using the general myxozoan primers MyxoSpecF 5′-TTCTGCCCTATCAACTWGTTG-3′ (Fiala, 2006) and 18R 5′-CTACGGAAACCTTGTTACG-3′ (Whipps et al., 2003) to amplify a region of the SSU rDNA gene. All reactions were performed in 50-μl volumes using Platinum PCR Supermix (Invitrogen, Carlsbad, California) which contains 22 U/ml recombinant Tag DNA polymerase, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM MgCl2, 220 μM dGTP, 220 μM dATP, 220 μM dTTP, 220 μM dCTP, 0.9 mmol of each primer, and 5 μl of each DNA extraction. Amplifications were performed on a Peltier 200 thermocycler (MJ Research, Watertown, Massachusetts) with an initial denaturation at 94 C for 2 min, 40 cycles of 94 C for 30 sec, 55 C for 30 sec, and 72 C for 2 min with a final extension at 72 C for 10 min. The

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resulting PCR products were purified using the QIAquick PCR Purification kit (Qiagen), sequenced directly, and products from 2 specimens in which representative myxozoan spores were observed by wet mount were cloned into TOPO TA cloning vectors (Invitrogen). Two clones for each species were sequenced in both directions using primers flanking the inserted sequence, M13F 5′-TGT AAA ACG ACG GCC AGT-3′ and M13R 5′-CAG GAA ACA GCT ATG ACC-3′. The resulting, overlapping sequences were manually assembled using BioEdit (Hall, 1999). All DNA analyzed in the study was sequenced at the Core Laboratory at Oregon State University on an ABI Prism® 3730 Genetic Analyzer with the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California).

Phylogenetic analyses

The rDNA sequences obtained from samples were compared with those in the National Center for Biotechnology Information's GenBank database using the BLASTN version 2.2.29 program (Altschul et al., 1997). Sequences from the most-closely related species returned by the BLASTN program, and representative species from closely related myxozoan genera based on previous phylogenetic analyses of the genera *Chloromyxum* (Jirků et al., 2011) and *Sphaerospora* (Bartošová et al., 2013) and 2 outgroup taxa, *Buddenbrockia plumatellae* and *Tetracapsuloides bryosalmonae*, were selected and aligned using the ClustalW (Thompson et al., 1994) algorithm with default parameters (penalty for gap opening 10; penalty for gap extension 0.2) in the Mega version 6.06 software (Tamura et al., 2013). Forty-five sequences were included in the alignment. The multiple sequence alignment was visually examined and poorly aligned or ambiguous regions were removed using the Gblocks program version 0.91b on the webserver available at http://molevol.cmima.csic.es/castresana/Gblocks_server.html (Castresana, 2000; Talavera and Castresana, 2007). The resulting multiple sequence alignment was analyzed using the jModelTest program version 2.1.4 (Guindon and Gascuel, 2003; Darriba et al., 2012) to determine the most likely model of nucleotide substitution. Based on the results of the jModelTest analysis, phylogenetic reconstruction using the maximum likelihood method was performed using PhyML (Guindon et al., 2005) on the webserver available at http://www.atgc-montpellier.fr/phyml/ using the generalized time-reversible model with gamma-distributed rate variation among sites. The analysis was run using 100 bootstrap replicates.

DESCRIPTION

Chloromyxum kurisi n. sp.

(Figs. 1–10)

Diagnosis: Histozoic extrasporogonic stages in the renal interstitium and rarely in spleen, up to 600 μm in diameter, containing numerous vegetative and generative cells, often with daughter cells but no myxospores. Presporogonic plasmodia and sporogonic polysporic stages in renal tubules, collecting ducts, and mesonephric ducts. Mature spores rarely seen, subspherical, with approximately 15 linear vertical ridges (Fig. 1). Four pyriform polar capsules, containing filaments with 3–4 coils. Mean spore dimensions in micrometers (μm) with standard deviation followed by range in parentheses as follows: spore length (n = 5) 9.0 (0.23, 8.7 = 9.2); spore width (n = 8) 8.1 (0.17, 7.9–8.3); spore thickness (n = 9) 7.9 (0.19, 7.6–8.2); polar capsule length (n = 16) 2.3 (0.16, 2.1–2.5); polar capsule width (n = 16), 1.8 (0.09, 1.6–2.0).

Taxonomic summary

Type host: A. affinis (Ayres, 1860).

Site of infection: Kidney and mesonephric ducts (occasionally spleen).

Prevalence: 59% (16/27) based on February and March 2012 collections.

Type locality: Carpinteria Salt Marsh, California (34.40°N, 119.53°W).

Specimens deposited: Slides of histological sections from whole fish were deposited in the collections of the Queensland Museum, Brisbane, Australia, (syntypes G465697 and G465698). GenBank accession number for partial SSU rDNA sequence KJ526212.

Etymology: Named after parasitologist Armand Kuris, University of California, Santa Barbara, in recognition of his contributions to the field of aquatic parasite ecology.

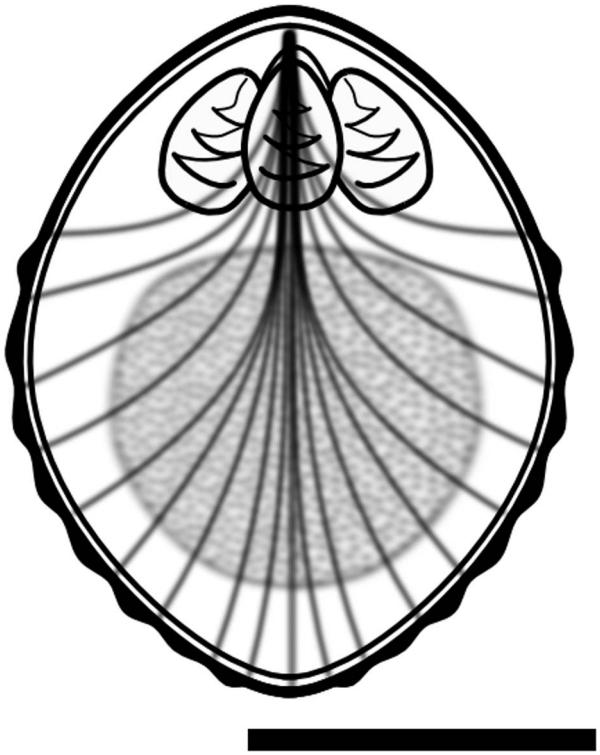


FIGURE 1. Line drawing of *Chloromyxum kurisi* n. sp. Bar = 5 μm.

Remarks

Large extrasporogonic forms of *C. kurisi* n. sp. were observed in the kidney interstitium by histology (Figs. 2–5) at all 3 sample collection times as follows: 5 of 7, 6 February 2012; 11 of 20, 6 March 2012; and 3 of 30, 15 May 2013. We conclude that these stages are extrasporogonic due to the lack of evidence of polar capsule development seen after careful examination. Several fish exhibited massive infections in which the extrasporogonic forms replaced approximately 80–90% of the kidney (Figs. 2, 3). One fish had these stages within the spleen.

Three of the 11 fish with the extrasporogonic forms also exhibited presporogonic forms in the renal interstium and presporogonic and sporogonic stages in the renal tubules, collecting, and mesonephric ducts (Figs. 4–6). Giemsa stains revealed polysporogonic forms containing subspherical spores with up to 4 polar capsules (Figs. 7, 8). *Chloromyxum*-type spores were observed in a wet mount preparation of 1 of 30 kidneys examined in the 2013 collection (Fig. 9).

Molecular analysis revealed SSU rDNA sequences similar to those of other *Chloromyxum* spp. in the 4 samples exhibiting extrasporogonic forms and in the 1 sample with myxospores seen in wet mounts collected in 2013 (Fig. 10). Sequence was completed in 1 sample (1,769 bp length, GenBank KJ526212), and the other 3 partial sequences (866, 811, and 907 bp) were identical over homologous regions. The sequence was most similar to *Chloromyxum careni*, with 84% similarity.

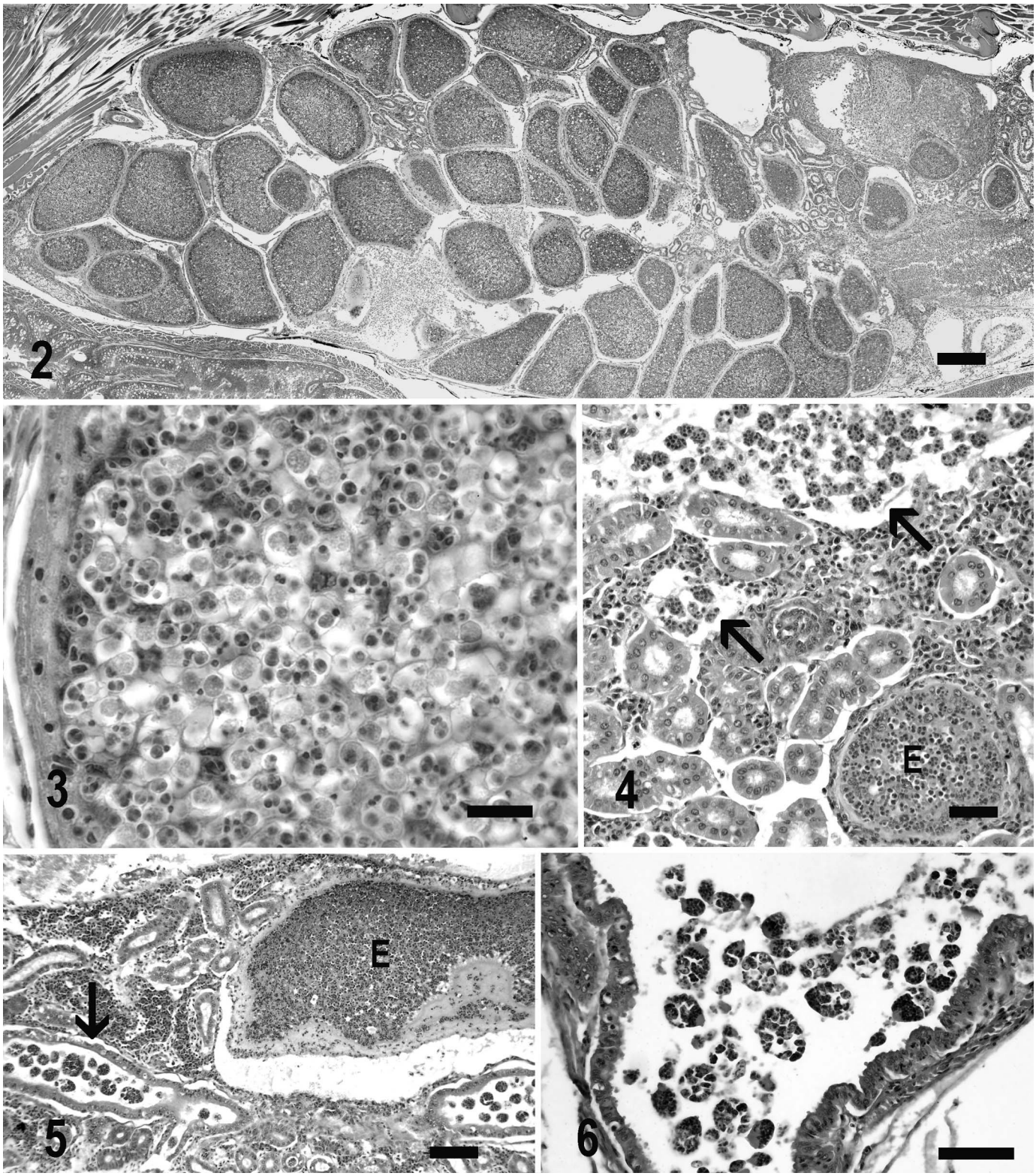
Sphaerospora olsoni n. sp.

(Figs. 11–14)

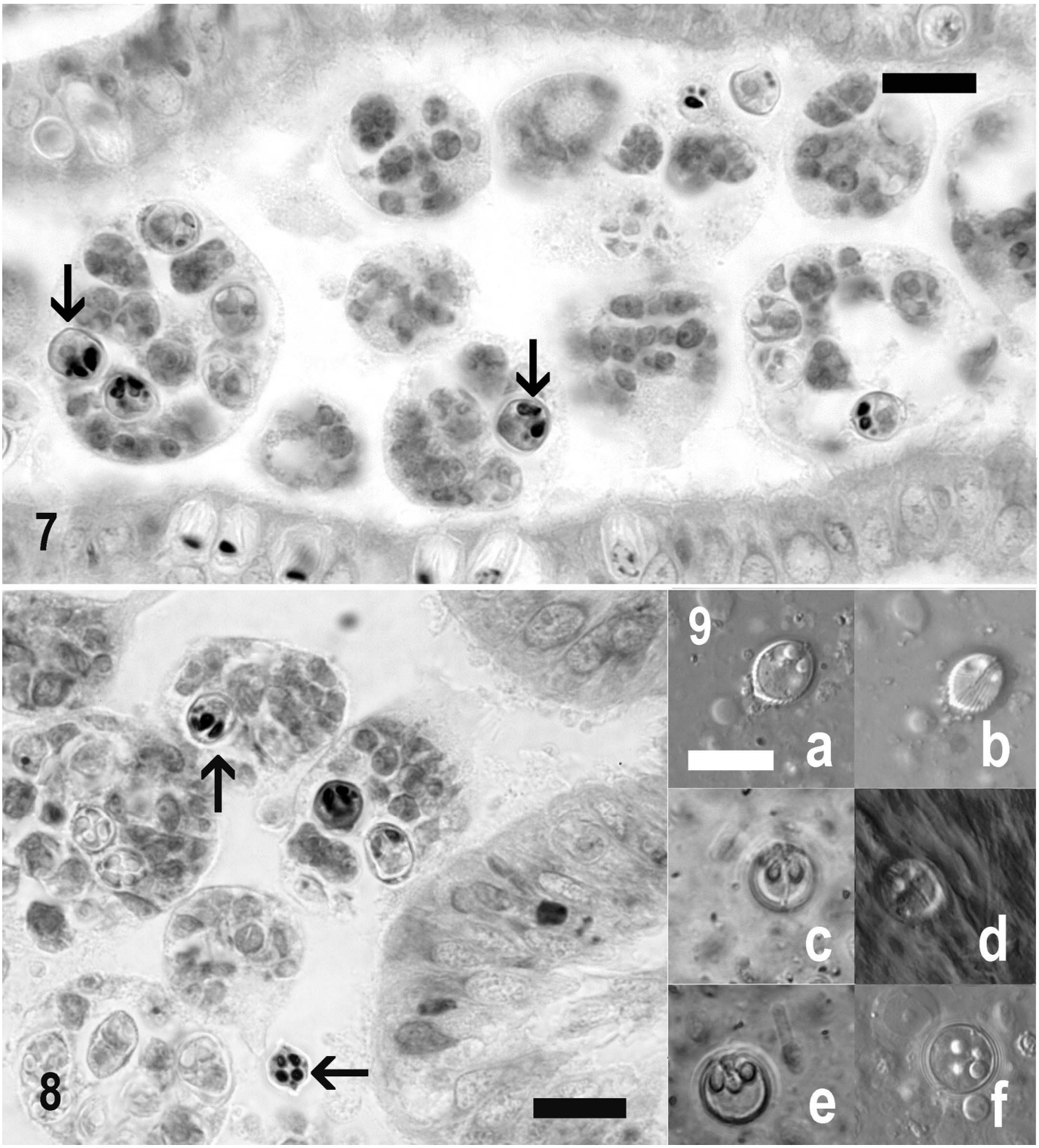
Diagnosis: Coelozoic sporogonic stages and disporic spores in renal tubules. Mature spores subspherical, with smooth valves (Fig. 11), 2 uninucleate sporoplasms. Two spherical polar capsules, containing filaments with 3–5 coils. Mean spore dimensions in micrometers (μm) with standard deviation followed by range in parentheses: Spore length (n = 23) 6.0 (0.50, 5.2–7.1); spore width (n = 5) 5.8 (0.10, 5.7–6.0); spore thickness (n = 19) 7.3 (8, 5.8–8.4). Polar capsule diameter (n = 32) 2.0 (0.14, 1.8–2.4).

Taxonomic summary

Type host: A. affinis (Ayres, 1860).



FIGURES 2–6. Histological sections of topsmelt, *Atherinops affinis*, infected with *Chloromyxum kurisi* n. sp. Hematoxylin and eosin stain. (**2**) Sagittal section through anterior half of the kidney in which extrasporogonic forms have replaced most of the kidney tissue. Bar = 200 μm. (**3**) Higher magnification of extrasporogonic form in the kidney showing vegetative and daughter cells but no spores nor sporoblasts. Bar = 10 μm. (**4**) Extrasporogonic form (E) and small plasmodia (arrows) in renal interstitium. Bar = 50 μm. (**5**) Extrasporogonic (E) and sporogonic forms in renal tubule (arrow). Bar = 50 μm. (**6**) Ureter containing sporogonic stages. Bar = 50 μm.



FIGURES 7–9. Histological sections and wet mount preparations of topsmelt, *Atherinops affinis*, urinary tract infected with *Chloromyxum kurisi* n. sp. Histological sections of renal tubule (**7**) and ureter (**8**) with sporogonic stages and polysporous spores (arrows). Giemsa. Bar = 10 μm. (**9a–f**) Wet mounts of individual spores. Nomarski phase interference. Bar = 10 μm. (9a, b) Shows arrangement of spore valve ridges; (c, d) spores with prominent sutures; and (e, f) demonstrates the orientation of the 4 polar capsules.

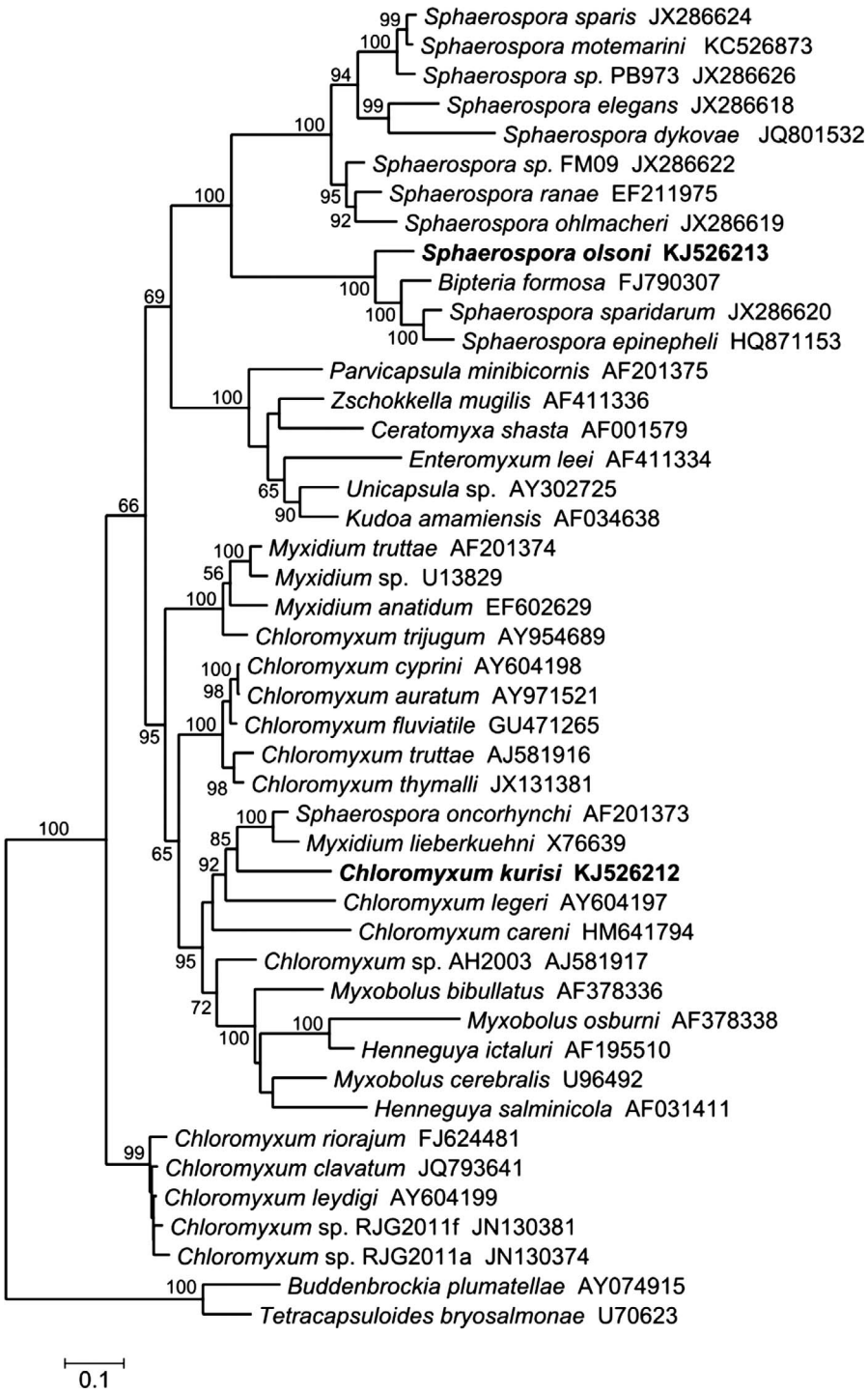


FIGURE 10. Phylogenetic tree generated from maximum likelihood analysis of small subunit ribosomal rDNA gene sequences for *Chloromyxum kurisi* n. sp. and *Sphaerospora olsoni* n. sp. GenBank accession numbers are listed adjacent to species names. Support values in percent units at branching points, values under 50% not shown.

Site of infection: Coelozoic in the kidney tubules.

Prevalence: 10% (2/20) based on May 2013 sample.

Type locality: Carpinteria Salt Marsh, California (34.40°N, 119.53°W).

Specimens deposited: Slides of histological sections from whole fish were deposited in the collections of the Queensland Museum, Brisbane, Australia (syntypes G465699 and G465700). GenBank accession number for partial SSU rDNA sequence KJ526213.

Etymology: Specific name in honor of Dr. Andrew C. Olson Jr., Professor Emeritus of Zoology, San Diego State University, in recognition of his contributions to the field of aquatic parasitology.

Remarks

Examination of histological (Fig. 12) and wet mount preparations (Figs. 13, 14) revealed sporogonic forms containing 2 spores within renal

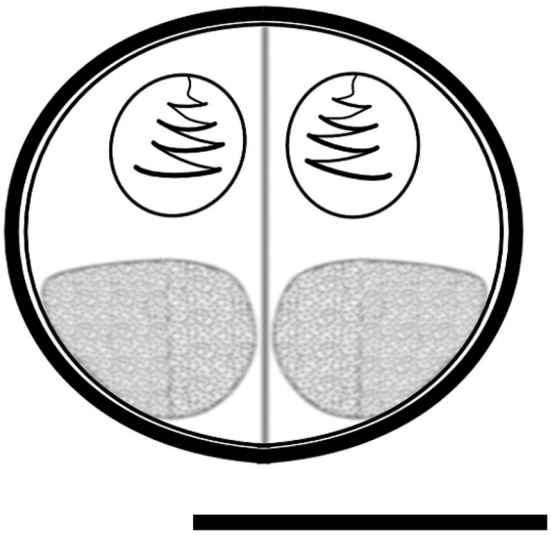


FIGURE 11. Line drawing of *Sphaerospora olsoni* n. sp. Bar = 5 μm.

tubules in 2 of 20 fish. Approximately 10% of the kidney tubule lumens were replete with sporogonic stages containing these disporous spores. Neither of these fish exhibited the large extrasporogonic forms as observed with the *Chloromyxum kurisi* n. sp. infections. We sequenced myxosporean SSU rDNA from the 2 fish with these infections; one at 1,934 bp (GenBank KJ526212) and the other at 579 bp, and they were identical over homologous regions. Molecular analysis revealed SSU rDNA sequences similar to those of other *Sphaerospora* species with the *Sphaerospora* sensu stricto clade as described by Bartošová et al. (2013) (Fig. 10). It was most-closely related to *Sphaerospora sparidarum* (89%), *S. epinepheli* (93%) and *Bipteria formosa* (92%).

DISCUSSION

The massive replacement of the kidney by a myxozoan is remarkable. Extensive examination of the extrasporogonic stages in the renal interstitium seen in several fish, including application of Giemsa stains, revealed no developing or mature spores. Hence, these forms correspond to an extrasporogonic stage distinguished from presporogonic and sporogonic forms that occur in a different location (i.e., the renal tubules and mesonephric ducts). This type of development is found in several other phylogenetically diverse myxozoans that are linked to each other in that these extrasporogonic stages often cause significant disease in their fish hosts. This is most notably seen in *T. bryosalmonae*. Indeed, this parasite was named "PKX" for decades because the disease it causes, proliferative kidney disease, is associated only with extrasporogonic stages which occur in the kidney interstitium and other tissues. In the fish host, immature spores of *T. bryosalmonae* are observed in the renal tubules as the infection progresses (Kent and Hedrick, 1986). Mature spores are rare and have only been found in the urine (Hedrick et al., 2004).

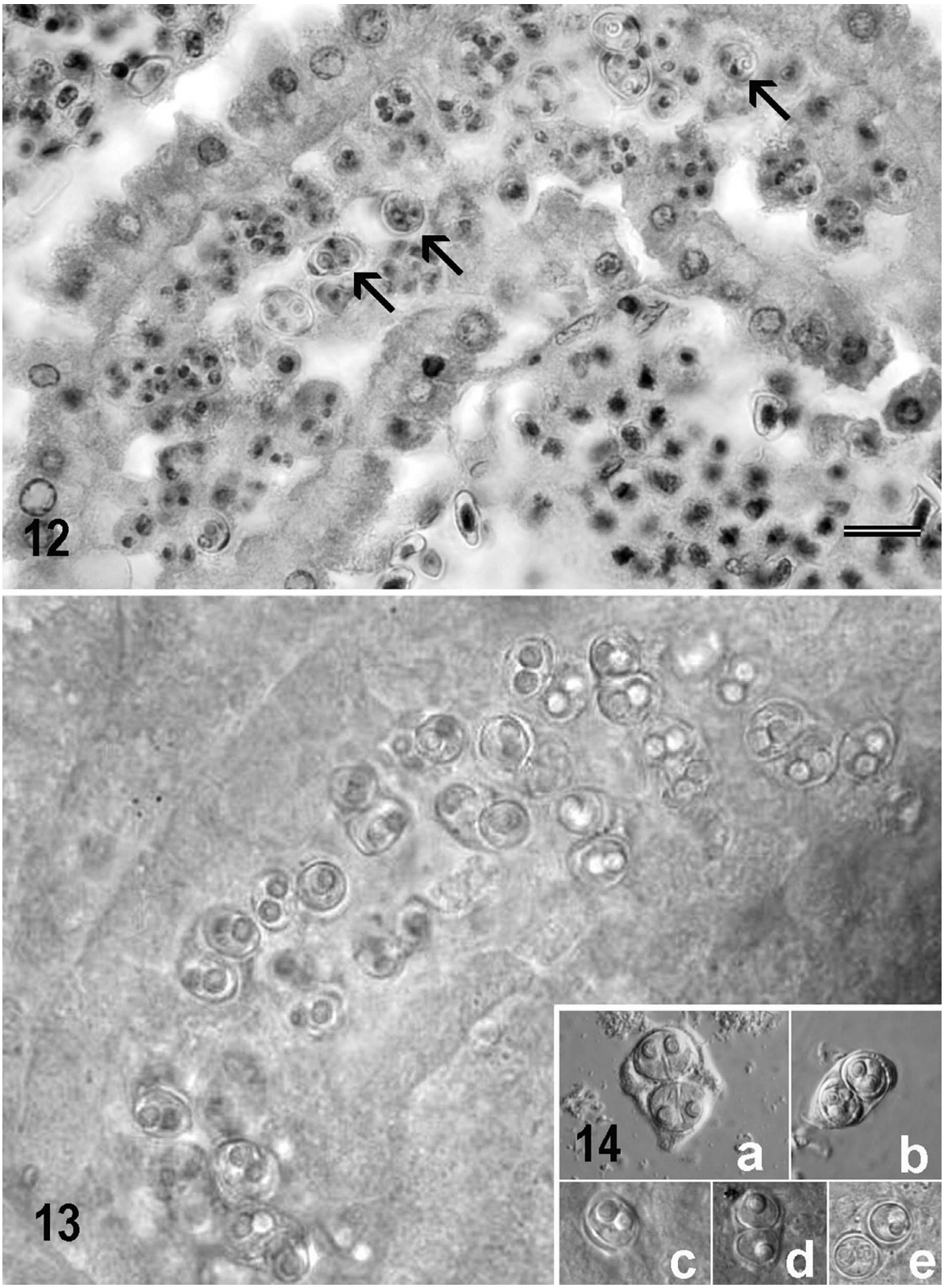
Other myxozoan genera that exhibit similar development include *Sphaerospora, Hoferellus*, and *Myxidium* species (Feist and Longshaw, 2006). In the genus *Sphaerospora*, *Sphaerospora dykovae* has been shown to form extrasporogonic stages that occur in the epithelial cells of renal tubules (Dyková and Lom, 1982) and in the swimbladder and blood (Molnár, 1980; Kovács-Gayer et al., 1982; Lom et al., 1983). Extrasporogonic blood stages have also been described in *Sphaerospora truttae*, with histocytic stages observed early in the infection in kidney, spleen, and liver. However, these stages appear to break down rapidly within these tissues (Holzer et al., 2003). *Hoferellus carassii* causes kidney enlargement disease in goldfish (*Carassius auratus*) due to massive proliferation of presporogonic forms in the renal tubule epithelium, with spores occurring more distally in the urinary tract (Molnár et al., 1989). These stages are replete with extrasporogonic proliferating cells, but no spores. Similar stages are present within collecting duct epithelial cells. Sporogonic development occurs within the lumen of kidney tubules, collecting ducts, and urinary bladder (Lom et al., 1989). Members of the genus *Acauda* (Mitraspora) sporulate in renal tubules and also produce large interstitial stages. However, these represent end stage development and contain spores within an apparent inflammatory, granulomatous matrix (Whipps, 2011).

Myxidium lieberkuehni, which infects the kidney and urinary tract of the pike (*Esox lucius*), exhibits development quite similar to *C. kurisi* n. sp.; it forms large cystic structures in the renal glomeruli, resulting in hypertrophic host cells equivalent to xenomas within the cytoplasm. One of the 2 closest relatives to *C. kurisi* n. sp. based on our SSU rDNA comparisons was *M. lieberkuehni*. Spores of members of the genus *Myxidium* are dramatically different than those of *Chloromyxum*, as the former are characterized by polar capsules at the opposing ends of the spores. This is yet another example supporting recent phylogenetic studies that indicate the tissue location and type of development before sporulation may have more significance than spore morphology (Fiala and Bartošová, 2010).

Various lines of evidence led us to conclude that the massive extrasporogonic forms in the topsmelt kidneys correspond to the *Chloromyxum* spores found in the lumen of the urinary tract—but discovery of a second myxozoan, *Sphaerospora olsoni* n. sp., in the 2013 samples presented a problem—i.e., could these massive developmental forms belong to this second species? Histology, however, provided a clear progression of development between the large extrasporogonic and sporogonic forms containing *Chloromyxum*-like spores in the lumens of the tubules and mesonephric ducts.

Ribosomal DNA sequences have been used to link developmental and life cycle stages in many parasites, including myxozoans, but mixed infections can result in errors. For example, Kent et al. (1993) ascribed spores of *Sphaerospora oncorhynchi* to the PKX myxosporean, but it was later shown that they represent distinct species (Kent et al., 1998). Based on rDNA analyses, *S. oncorhynchi* is distinct from all other *Sphaerospora* species. *Myxidium salvelini* is very common in this sockeye salmon (*Oncorhynchus nerka*) population (Higgins et al., 1993; Kent et al., 1993), and given that the *S. oncorhynchi* sequence is quite close to *M. lieberkuehni* and distinct from other *Sphaerospora* species, it is possible that the sequence is from *M. salvelini* and not *S. oncorhynchi*. Similarly, Eszterbauer (2011) showed that the original rDNA sequence assigned to *Sphaerospora renicola*, which infects kidneys of cyprinid fishes, was actually derived from a co-infection by another myxozoan. Holzer et al. (2013) examined numerous myxozoans from cyprinids and showed that often the extrasporogonic stages assigned to *Sphaerospora* spp. actually represent developmental stages of other myxozoan species.

Fortunately, sequences obtained from the 2 myxozoans in our study were quite distinct and corresponded to the appropriate clades for these genera. We are confident that the sequence that we are assigning to *S. olsoni* n. sp. is correct, as it falls with the



FIGURES 12–14. Histological sections and wet mounts of topsmelt, *Atherinops affinis*, kidney infected with *Sphaerospora olsoni* n. sp. Bar = 10 μm. (**12**) Renal tubule replete with disporous sporogonic stages containing spores (arrow). Hematoxylin and eosin. (**13**) Kidney squash preparation showing numerous spores within renal tubule. Bright-field microscopy. (**14a–e**) Spores in kidney wet mounts. Nomarski phase interference (a–d) and bright field (e). (a) Two maturing spores with prominent sutures bisecting the polar capsules within a sporoblast. (b) Two spores within a sporoblast. (c) Free spore. (d) Two spores in sporoblast, side view. (e) Bright field image of 2 spores within sporoblast remnant.

Sphaerospora sensu stricto clade lineage A (Bartošová et al., 2013), and this group is distinguished from all other myxozoans by the presence of extensive insertions within the variable regions of the SSU rDNA (Holzer et al., 2007; Jirků et al., 2007). Comparison of a portion of the *S. olsoni* SSU rDNA gene sequenced in this study (1,764 bp) with the same regions of the SSU rDNA genes of neighboring taxa available from GenBank: *S. sparidarum*, *S. epinepheli*, and *Bipteria formosa*, showed them to be of similar lengths (1,685 bp, 1,773 bp, and 1,729 bp, respectively). Moreover, using myxozoan universal primers, this sequence was only obtained from the sample in which *Sphaerospora* was detected by microscopy. Conversely, *Chloromyxum*-like sequence was only obtained from fish infected with the large extrasporogonic forms or from fish with the *Chloromyxum*-type spores seen in wet mounts.

With confidence that we are assigning the correct SSU rDNA sequences to their corresponding spore type, we conclude that *Chloromyxum kurisi* n. sp. is a previously undescribed species for the following reasons. Eiras et al. (2012) provided a synopsis of the genus *Chloromyxum*. Of the approximately 140 nominal species of *Chloromyxum*, 10 infect kidneys or the urinary tract of fishes. Five of these species infect marine or anadromous hosts: *Chloromyxum granulosum* from *Tylosurus marinus* (family Belonidae), Atlantic Ocean off the United States; *Chloromyxum inexpectatum* from *Acipenser ruthenus* (family Acipenseridae) from Hungary; *Chloromyxum kotorense* of *Liza aurata* (family Mugilidae) off Montenegro; *Chloromyxum menticirrhi* of *Menticirrhus americanus* (family Sciaenidae) off Brazil, *C. renalae* of *Fundulus majalis* (family Fundulidae) Atlantic Ocean off the United States; and *Chloromyxum schurovi* of *Salmo salar* (Salmonidae) in Russia, Norway, and Finland. Hence, *C. kurisi* n. sp. is the only species that infects atherinid fishes and is the first species described from the urinary tract of a marine fish in the eastern Pacific. Regarding rDNA sequence comparisons, *C. kurisi* n. sp. fell into a clade containing 3 *Chloromyxum* spp. and was most-closely related to *Chloromyxum legeri* and *C. careni* (84% sequence similarity). *Chloromyxum legeri* infects the gall bladder of common carp (*Cyprinus carpio*) in France and *C. careni* infects the kidney tubules of long-nosed horned frog (*Megophrys nasuta*) in Indonesia (Mutschmann, 1999; Jirků et al., 2011).

It has been previously observed that most myxosporean genera represented in SSU rDNA phylogenetic trees are polyphyletic (Kent et al., 2001). This is especially true for the genus *Chloromyxum*, which has been noted as forming 3 separate lineages (Fiala and Dyková, 2004). We are assigning *C. kurisi* n. sp. to the genus *Chloromyxum* based on the combination of morphological characteristics and SSU rDNA that places it in within the *Chloromyxum* sensu lato based on the conservative approach recommended by others (Jirků et al., 2011; Gleeson and Adlard, 2012) who have noted that the freshwater-associated *Chloromyxum* spp. will likely need to be reassigned to new genera, but that this should occur only after extensive sampling and phylogenetic and morphological analyses have been completed.

We also conclude that *S. olsoni* n. sp. is a novel species. The genus *Sphaerospora* contains about 100 nominal species (Lom and Dyková, 2006; Gunter and Adlard, 2010), and rDNA sequence analyses have shown that it is polyphyletic (Bartošová et al., 2013). Two *Sphaerospora* species have been described from atherinid fishes; *Sphaerospora mayi* from the gallbladder of *Atherinomorus capricornensis* off eastern Australia (Moser et al., 1989) and *Sphaerospora undulans* was reported from the urinary tract of *Arnoglossus* (*Caulopsetta*) *scapha* off New Zealand (Meglitsch, 1970; Hewitt and Hine, 1972). *Sphaerospora olsoni* n. sp. is distinguished from *S. mayi* by site of infection, and *S. undulans* has distinct striations on its valves and the polar capsules are divergent.

No *Sphaerospora* spp. has been described from atherinid fishes off the west coast of the United States (Love and Moser, 1983) or Canada (McDonald and Margolis, 1995). Renal *Sphaerospora* species from eastern Pacific marine or anadromous fish are as follows: *Sphaerospora armatura* from *Albatrossia pectoralis* (family Macrouridae); *Sphaerospora compressa* from *Rimicola eigenmanni* (family Gobiesocidae); *Sphaerospora aculeatus* in sticklebacks *Gasterosteus aculeatus* from British Columbia; and *S. oncorhynchi* in sockeye salmon, *O. nerka* (family Salmonidae) from British Columbia. *Sphaerospora olsoni* n. sp. has spores that are considerably shorter in length from the former 3 species. It is also somewhat shorter in length than that reported for *S. oncorhynchi*, and the latter is monosporous. Small subunit ribosomal DNA sequence is available for 37 species of *Sphaerospora* in the GenBank database and phylogenetic analysis showed that *S. olsoni* n. sp. occurs within the *Sphaerospora* "sensu stricto" clade as described by Bartošová et al. (2013). The three most-closely related taxa, *B. formosa*, *S. sparidarum*, and *S. epinepheli* all infect the kidney tubules of their hosts, *Merlangius merlangus* (Gadiformes), *Sparus aurata* (Perciformes), and *Epinephelus malabaricus* (Perciformes), respectively.

Our study highlights the importance of combining non-specific screening methods such as wet mounts with histological analysis when performing comprehensive parasitological surveys. The 2 novel myxozoan species we describe herein would likely not have been detected by traditional parasite screening methods, such as the direct examination of tissues by wet mount alone, especially in light of the relative scarcity of mature myxospores. As has been noted with other myxozoan species such as *Ceratomyxa shasta*, the difficulty of visualization of presporogonic stages in wet mounts actually makes this a less-sensitive method of detection (Kent et al., 2013).

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