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Proliferative renal myxosporidiosis in spawning coho salmon (*Oncorhynchus kisutch*) in British Columbia and Washington

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Abstract. An unidentified myxosporean parasite (CKX) is described from the kidney of approximately 80% of spawning coho salmon *Oncorhynchus kisutch* (Walbaum) in British Columbia, Canada and Washington, United States of America. Morphological features were described using light and electron microscopy. Sequencing of polymerase chain reaction (PCR) amplified 18S ribosomal RNA gene and *in situ* hybridisation were used to further characterise CKX. The parasite occurred with a focal distribution within tubule epithelial cells, the tubule lumen and the interstitium as primary cells containing from one to at least 16 secondary cells. Luminal stages were degenerate and sporogony was not observed. *In situ* hybridisation using a digoxygenin-labelled DNA probe confirmed CKX to be the source of DNA used in PCR analyses. CKX 18S rDNA sequences were most similar (97%) to those of *Sphaerospora oncorhynchi*. Phylogenetic analysis revealed similarities among the 18S rDNA sequences of CKX, *S. oncorhynchi* and *Myxidium lieberkuehni*. CKX is hypothesised to be the abortive extrasporogonic development of a *Sphaerospora* sp. or *Myxidium* sp. occurring in immune-incompetent spawning and senescent salmon.

Elevated pre-spawn mortality was observed among returning stocks of adult coho salmon *Oncorhynchus kisutch* (Walbaum) migrating in streams along northern Vancouver Island. Analysis of specimens from the initial diagnostic case indicated that the furunculosis bacterium *Aeromonas salmonicida* contributed to the mortality. Routine histological examination of kidney from moribund and fresh-dead salmon also revealed foci of infection with intracellular parasites that resembled extrasporogonic myxosporean stages. This pattern of infection had not previously been noted in coho salmon. The present study describes the parasite in coho salmon, herein referred to as the unknown coho kidney parasite (CKX), and reports on its occurrence in Canada and the United States.

MATERIALS AND METHODS

Coho salmon locations and samples. Samples of posterior kidney, liver, spleen and gill were collected from fresh spawned salmon belonging to Marble, Cluxewe, Washlawis, Waukwaas, Quatse and Stephens Rivers stocks in northern Vancouver Island, British Columbia, Canada. All dissecting tools were carefully cleaned between fish by rinsing first with water, then bleach, then water, then 95% ethanol followed by flaming. From each organ sample, small (1–2 mm^3) pieces were preserved in 2% glutaraldehyde in 0.1 M Sörensen's phosphate buffer (pH 7.2) and two larger (10–20 mm^3) pieces were fixed in 95% ethanol and Davidson's solution, respectively. Similarly, duplicated 10–20 mm^3 kidney samples collected from the Quinault, Sooes, Methow and Wenatchee Rivers, Washington, United States of America were fixed in Davidson's solution and 95% ethanol, respectively.

Histological analysis. Tissues preserved in Davidson's solution were dehydrated through isopropanol, cleared in xylene and embedded in paraffin wax. Sections (5 μm) were mounted on glass slides and stained with haematoxylin and eosin, modified Pappenheim (Humason 1972), Gram or periodic acid Schiff (PAS) stains. Tissues fixed in ethanol were processed as described above and sections (5 μm) were mounted onto aminoalkylsilane (AAS) treated slides for *in situ* hybridisation (ISH). Control tissues for ISH (Atlantic salmon skeletal muscle infected with *Kudoa thyrsites*, sockeye salmon kidney infected with *Parvicapsula minibicornis*) were fixed in 95% ethanol and processed as described above.

Transmission electron microscopy (TEM). Glutaraldehyde-preserved kidney was rinsed twice in Sörensen's buffer and post-fixed in cold (4°C) 1% osmium tetroxide for 1.5 h. The tissue was rinsed, dehydrated through ethanol into acetone, embedded in epoxy resin (PELCO® Eponate 12 Kit, Ted Pella Inc.) and sectioned using an ultramicrotome (Ultracut, Reichert-Jung). Ultrathin sections mounted on copper grids were stained with uranyl acetate and lead citrate and examined with a transmission electron (Zeiss EM10) microscope.

Polymerase chain reaction (PCR). Template DNA was extracted from approximately 35 mg of ethanol-fixed samples (Qiagen DNeasy kits) into sterile deionized, distilled water (ddH_20). The primers used for PCR are listed in Table 1. *Parvicapsula minibicornis* SSU rDNA was amplified as

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Table 1. Oligonucleotide primers used to amplify segments of the small-subunit ribosomal RNA gene by polymerase chain reaction.

Primers[1]	5'-3' sequence	Location[2]	Reference
18e	ctg gtt gat cct gcc agt	–	Hillis and Dixon 1991
18g	ggt agt agc gac ggg cgg tgt g	–	Hillis and Dixon 1991
Parvi1f	gag gta aca caa gga gcc	167	Kent et al. 2000
Parvi2r	cct caa tct taa ttc ttc aga	1186	Kent et al. 2000
So1f	gca gtc aca cca gca tcg	148	Kent et al. 1998
So2r	caa ttg aaa cgt cgt cgg	297	Kent et al. 1998
Myxgp2f	wtg gat aac cgt ggg aaa	113	Kent et al. 1998
Sm2r	ctt ggt tag cat cta cct	325	Kent et al. 1998
So9r	caa aac gga aac caa cat cc	996	Present study
So10f	ttg gga gtg acg tac gga tt	910	Present study

[1]Parvi: *Parvicapsula minibicornis*, So: *Sphaerospora oncorhynchi*, Myxgp: myxozoa general primer, Sm: *Sphaerospora molnari*.
[2]Locations are relative to respective 18S rDNA sequences available in GenBank: *P. minibicornis*, accession number AF201375 and *Sphaerospora oncorhynchi*, accession number AF201373.

described earlier (Kent et al. 2000, St.-Hilaire et al. 2002). For amplification of *Sphaerospora oncorhynchi* SSU rDNA, 1.25 U Platinum Taq polymerase (Invitrogen), 1.5 mM $MgCl_2$ (Invitrogen), 1 × PCR buffer (Invitrogen), 0.5 µM of each primer, 0.2 mM of each dNTP, and 1.5 µl DNA template (≤20 µg/ml) in ddH_2O were used in reaction volumes of 25 µl. Reactions were conducted in a PTC-200 thermocycler (MJ Research). The amplification protocol was 95°C for 3 min, 40 cycles of 94°C for 45 s, 50–55°C for 45 s and 72°C for 45 s followed by 72°C for 10 min. PCR amplification products were electrophoretically resolved and visualised with ethidium bromide in 1.5% agarose gels by UV-transillumination.

Cloning and sequencing of PCR products. PCR products from the amplification of DNA from infected tissue (primers So1f and So2r, Myxgp2f and Sm2r) were directly sequenced. In addition, the product amplified using primers 18e and 18g was purified (QIAquick PCR Purification Kit, Qiagen) and cloned following manufacturer's protocols (TOPO TA Clone kit, Invitrogen Canada Inc.). Clones were screened using PCR (primers So1f and So2r) and positive inserts amplified using two overlapping primer pairs (M13f and So9r, So10f and 18g). Primer M13f recognised a plasmid sequence adjacent to the cloning site and was provided by the manufacturer. Purified PCR products were sequenced in a BigDye Terminator Cycle Sequencing Ready reaction (Applied Biosystems). Each 20 µl reaction contained 10–40 ng of PCR product, 3.2 pmols of each primer, 4 µl BigDye Terminator RR Mix, and 4 µl 2.5 × BDT buffer (200 mM Tris, pH 8.6, 5 mM $MgCl_2$) in ddH_2O. Reactions consisted of 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Extension products were purified (Centri-Sep chromatography, Princeton Separations, Inc.), rehydrated with ddH_2O and bidirectionally sequenced (ABI Prism 377, Applied Biosystems).

Individual sequences and contigs assembled using Sequencher 4.1.4 (Gene Codes Corporation) were BLAST analysed (http://www.ncbi.nlm.nih.gov/BLAST/) and then aligned (MultAlin, Corpet 1988) with those from other *Sphaerospora* spp. and phylogenetically related myxozoans as illustrated by Kent et al. (2001). Phylogenetic relationships between our sequences and related published sequences were estimated using neighbour-joining (NJ) and maximum parsimony algorithms in MEGA 2.1 (Kumar et al. 2001). Kimura 2-parameter analysis was used for the NJ analysis and bootstrap values, calculated as percentages over 1000 replicates, provided an estimate of confidence in branch nodes. Sequences from the following organisms (GenBank accession number) were used in the comparisons: *Sphaerospora oncorhynchi* (AF201373), *Sphaerospora molnari* (AF378345), *Myxidium truttae* (AF201374), *Myxidium lieberkuehni* (X76639), *Myxidium* sp. (U13829), *Kudoa thyrsites* (AF031412) and *Parvicapsula minibicornis* (AF201375).

Digoxygenin (DIG)-labelled probe and *in situ* hybridisation (ISH). Products from So1f and So1r primed PCRs were purified and 100 ng used as template DNA to prepare a DIG-labelled probe following the manufacturer's protocol (Roche PCR DIG Probe Synthesis Kit). The PCR protocol described above was followed but with an annealing temperature of 53°C. Labelled products from several reactions were pooled, purified, quantified (Genequant) and stored at –20°C. Tissue sections mounted on AAS slides were deparaffinised in xylene and baked overnight at 60°C. The probe was used at a concentration of 0.3 ng/µl and incubated at 40 ng per section following the method of Jones et al. (2003). Stained sections were examined with a compound microscope (400×). Micrographs were obtained with a Nikon Coolpix 995 digital imaging system mounted on a Leitz Dialux 22 compound microscope.

RESULTS

The myxosporean-like parasite (CKX) was present in all three of the initial diagnostic samples from the Quatse River, in one sample from the Waukwaas River but not in any from each of the Marble and Washlawis Rivers in British Columbia. Subsequent sampling in British Columbia and Washington revealed CKX in stained histological preparations from 78.9% of spawning coho from four of five streams in Northern Vancouver Island, British Columbia (BC) and the Olympic Peninsula, Washington (Table 2). In addition, the parasite occurred in all nine kidney samples collected from Cluxewe River (BC) coho but not in any of the seven from the Wenatchee River (Washington) or in any of the 11 from the Methow River (Washington).

Table 2. Histological and polymerase chain reaction (PCR) analyses of kidney from adult coho salmon collected in streams in British Columbia (BC) and Washington (WA).

Location	Histology[1]			PCR Primers[2]	
	CKX	*Myxidium*	*Sphaerospora*	So1f/So2r	Parvi1f/Parvi2r
Quatse River, BC	6/8[3]	1/8	0/8	8/8	0/8
Marble River, BC	0/3	n.d.	n.d.	0/3	0/3
Waukwaas River, BC	7/7	1/8	0/8	7/7	0/7
Quinault River, WA	10/10	3/10	0/10	10/10	2/10
Sooes River, WA	7/10	3/10	1/10	9/10	0/10

[1]CKX, unknown coho kidney parasite; *Myxidium*, mature spores in kidney tubule; *Sphaerospora*, mature spore in kidney tubule. Gram or modified Pappenheim-stained sections. [2]See Table 1 for primer sequence and specificity. So1f/So1r: *Sphaerospora oncorhynchi* – positive indicates production of a 150bp band; Parvi1f/Parvi2r: *Parvicapsula minibicornis* – positive indicates production of a 1019bp band. [3]Number positive / number examined.

In stained histological sections, CKX occurred as round to elongate cells within renal tubule epithelial cells, tubule lumina and occasionally, the renal interstitium (Figs. 1, 2). The distribution of the histological changes was focal within the posterior kidney. Infected epithelial cells were hypertrophic and the adjacent lumen was often stenotic. The organism was not evident in liver, spleen, heart and gill from five Quatse River coho. From one to as many as 16 secondary cells were observed within primary intraepithelial cells (Fig. 3). Secondary cells were round to ovate and their nuclei stained prominently with Gram (Fig. 3). As the number of secondary cells increased they became peripherally distributed within the primary cell and stained more intensely (Figs. 1, 2). Primary cells containing numerous intensely-stained secondary cells were frequently observed within the lumen of tubules (Fig. 1) and occasionally, protruding from the epithelium into the lumen (Fig. 2) and among interstitial cells adjacent to affected tubules. These primary cells were weakly PAS-positive. Inflammatory cells were rarely associated with affected tubules or interstitial stages.

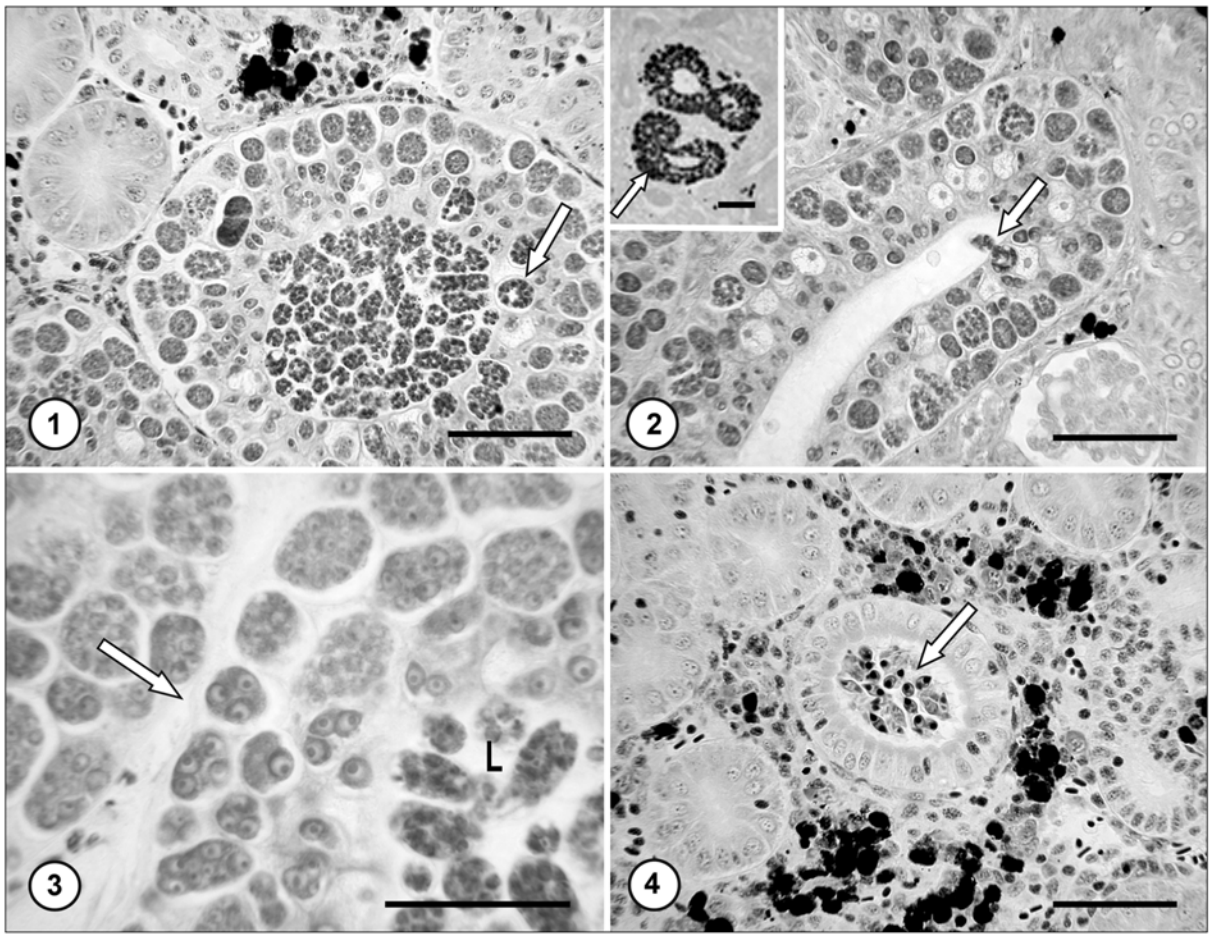
In addition, spores of a *Myxidium* sp. (Fig. 4) and a *Sphaerospora* sp. were observed in the renal tubule lumina of 22.2% and 2.7% respectively, of coho salmon infected with CKX (Table 2).

Transmission electron microscopy (TEM) revealed that infected tubule epithelial cells contained from one to several primary cells (Figs. 5, 6). The mitochondria of infected epithelial cells were distorted or absent, the cytoplasm was vacuolated, endoplasmic reticula were compressed adjacent to the primary parasitic cells and the host-cell nucleus was displaced apically with an enlarged perinuclear space (Fig. 5). The intraepithelial primary cell enveloped from one to several secondary cells (Figs. 5, 6). Membrane-bounded secondary cells occurred individually or as pairs in which one cell partially enveloped the other (Fig. 6). Secondary cells possessed a nucleus with a prominent nucleolus and several perinuclear mitochondria that were circular in profile with well-developed cristae. Structures resembling microtubules were occasionally observed adjacent to the nucleus of secondary cells (Fig. 7). Dividing stages of secondary cells were infrequently observed. The cytoplasm of secondary cells tended to be more electron-dense in intraluminal compared with intraepithelial primary cells (Fig. 8). Intraluminal primary cells also contained numerous amorphous, electron-dense structures (Figs. 5, 7) and occasionally appeared vacuolated (Fig. 8). Neither tertiary cells nor sporogony was observed by TEM.

Polymerase chain reaction (PCR) using primers So1f and So2r amplified a 150 base-pair (bp) DNA product from all 15 coho examined from two of three rivers on Vancouver Island (Table 2). This PCR also amplified DNA from all nine Cluxewe River coho, from all 10 Quinault River coho and from nine of 10 Sooes River coho. The negative sample was one of three from the latter site that were also negative by histology. Similarly, DNA was not amplified from three coho from Marble River in which CKX was not detected by histology. *Parvicapsula minibicornis* DNA was amplified from two of 10 coho from the Quinault River (Table 2) and not from any other sample.

Formazan staining was associated with CKX stages (intraepithelial, luminal and interstitial primary and secondary cells) following hybridisation with the 150bp digoxygenin-labelled probe (Fig. 2). Similar reactions were not produced following hybridisation of *Kudoa thyrsites* or *Parvicapsula minibicornis*-infected tissues from Atlantic salmon or sockeye salmon, respectively with this probe.

A consensus sequence of 141 bp of 18S rDNA amplified from three coho (2 Quatse River, 1 Cluxewe River) using primers So1f and So1r shared 91% identity with the homologous sequence of *Sphaerospora oncorhynchi*. Similarly, the 166 bp of sequence amplified from another Quatse River coho using primers Myxgp2f and Sm2r was most like that of *S. oncorhynchi* with 91% identity. A fragment of 18S rDNA amplified from a cloned segment (primers M13f and So9r), originally obtained from a fifth coho (Waukwaas River, B.C.), shared 95% identity with the homologous *S. oncorhynchi* sequence over 944 bp. The cloned CKX sequence has been deposited in GenBank (accession number AY 525343). Phylogenetic analyses using neighbour-joining

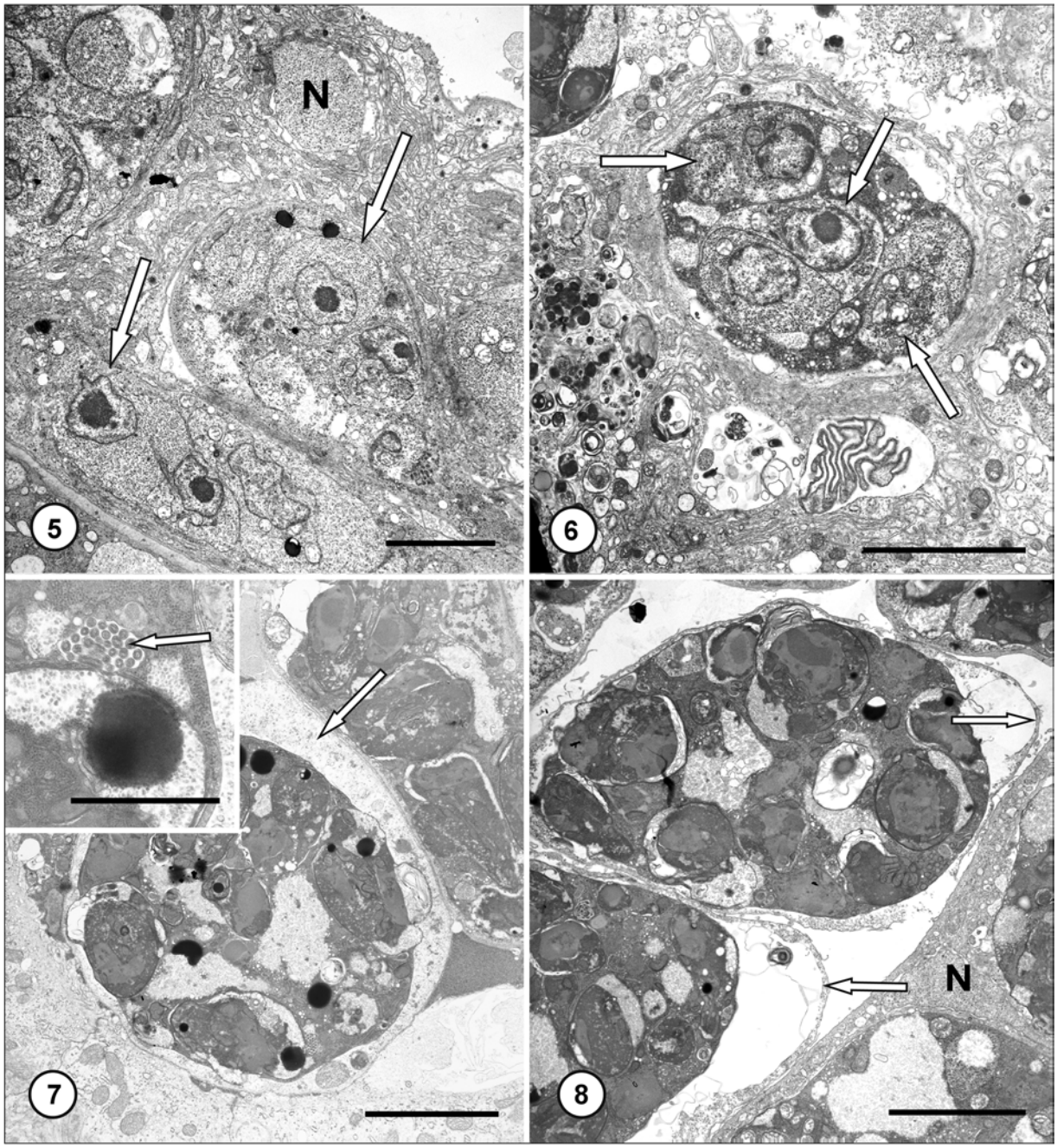


Figs. 1–4. Light micrographs of coho salmon (*Oncorhynchus kisutch*) kidney infected with the unknown coho kidney parasite (CKX) or with *Myxidium* sp. **Fig. 1.** Renal tubule with CKX in epithelial cells and lumen. Peripheral arrangement of intensely-stained secondary cells within primary cell (arrow). Primary cells in lumen with numerous, intensely-stained secondary cells. Gram stain. **Fig. 2.** Primary cell containing numerous, intensely-stained secondary cells protruding from epithelium into lumen (arrow). Modified Pappenheim stain. **Inset.** *In situ* hybridisation reaction of CKX-infected renal tubules (arrow). **Fig. 3.** CKX within tubule epithelial cells of adjacent tubules separated by connective tissue (arrow) and in the tubule lumen (L). Primary cells contain from one to several secondary cells. Gram stain. **Fig. 4.** *Myxidium* sp. spores (arrow) in tubule lumen of CKX-infected coho. Gram stain. Scale bars: Figs. 1, 2, 4 = 50 µm; Fig. 2, inset = 20 µm; Fig. 3 = 30 µm.

(NJ) (Fig. 9) and maximum parsimony (MP) (not shown) methods were conducted on homologous nucleotide sequences obtained from GenBank and on the cloned sequence obtained from the present study. The topologies of unrooted phylograms obtained using both methods were similar and indicated that the CKX and *S. oncorhynchi* grouping was most closely related to *M. lieberkuehni*.

DISCUSSION

Light and electron microscopic examination confirmed that CKX possessed some features typical of Myxosporea: cell-within-cell organisation and glycogen within secondary cells (Lom et al. 1982). Structures similar to the microtubule bundles observed in *M. lieberkuehni* by Lom and Puytorac (1965) were occasionally seen in CKX, however, their pleomorphic morphology was not completely consistent with microtubules. Generic and specific identifications within the class Myxosporea however are principally based on spore morphology. Extrasporogonic stages tend to be morphologically similar, limiting their taxonomic value. Morphological features suggested that the CKX organisms were extrasporogonic myxosporean stages, and thus provided little information on their specific identity. Similarly, the focal pattern of CKX development without evident inflammation was not similar to that described previously for myxosporeans in the kidney of North American salmonids. Histological observations suggested that CKX developed within renal tubule epithelial cells from primary cells containing one secondary cell to those containing 16 or more secondary cells. The latter stages



Figs. 5–8. Transmission electron micrographs of coho salmon (*Oncorhynchus kisutch*) kidney infected with the unknown coho kidney parasite. **Fig. 5.** Tubule epithelial cell containing two primary cells (arrows), each containing secondary cells. Host cell cytoplasm is vacuolated and the nucleus (N) is displaced apically. **Fig. 6.** Electron-dense primary cell containing single or paired secondary cells (arrows) in which one cell partially envelops the other. Degeneration of host cytoplasm and organelles is evident. **Fig. 7.** Intact intraluminal primary cell (arrow) containing electron-dense secondary cells. **Inset.** Higher magnification of Fig. 7 showing transverse section through a bundle of microtubule-like structures adjacent to a secondary cell (arrow). **Fig. 8.** Vacuolated intraluminal primary cells (arrows) containing electron-dense secondary cells. Nucleus of infected epithelial cell (N) is displaced apically. Scale bars: Figs. 5–8 = 5 µm; Fig. 7, inset = 1 µm.

stained more intensely in histological preparations, were more electron-dense and appeared to pass into the tubule lumen. The initial infective stage was not identified although subsequent primary cells may each have derived from a secondary cell pair in which one cell partially enveloped the other. Similarly, sporogonic stages were not observed and some intraluminal primary cells appeared degenerate, suggesting that development was abortive. Infection with CKX was unlike that of *Tetracapsula bryosalmonae* which causes proliferative kid-

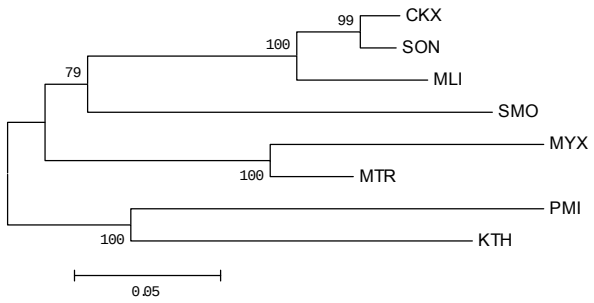


Fig. 9. Unrooted neighbour-joining phylogram showing relationships among 18S rDNA sequences of the unidentified coho salmon kidney parasite (CKX) and other myxosporeans. Comparisons were based on approximately 944 bp of 18S rDNA sequences. Numbers at nodes are bootstrap confidence levels based on 1000 resamplings. The divergence scale represents the proportion of sites that differ among sequences. Sequences compared included: SON – *Sphaerospora oncorhynchi*, MLI – *Myxidium lieberkuehni*, SMO – *Sphaerospora molnari*, MYX – *Myxidium* sp., MTR – *Myxidium truttae*, PMI – *Parvicapsula minibicornis*, KTH – *Kudoa thyrsites*.

ney disease (PKD), a severe, disseminated inflammation of the salmonid kidney (Hedrick et al. 1993). The development of other myxosporean species infecting the kidney of western North American *Oncorhynchus* spp. (*Myxidium salvelini*, *M. minteri*, *Chloromyxum majori*, *Sphaerospora oncorhynchi*, *Parvicapsula minibicornis*) occurs entirely within glomeruli, renal tubules, collecting ducts or the urinary bladder. In contrast, several histozoic, proliferative renal myxosporidioses have been reported from non-salmonid species, including those caused by *Sphaerospora renicola* in common carp (*Cyprinus carpio*) (Lom et al. 1982), *Myxidium lieberkuehni* in northern pike (*Esox lucius*) (Lom et al. 1989) and *Hoferellus cyprini* in *C. carpio* (Molnár and Kovács-Gayer 1986). CKX is strikingly similar to the extrasporogonic developmental stages of *S. renicola* (Lom and Dyková 1985).

Polymerase chain reaction (PCR) using *S. oncorhynchi*-specific primers amplified DNA from all specimens with histological criteria of CKX infection and from some that did not. The latter were undoubtedly light infections undetectable by histology. Sequencing the 18S ribosomal gene confirmed the myxosporean affinities of CKX and demonstrated its similarity to *S. oncorhynchi*. *Sphaerospora oncorhynchi* was described originally from sockeye salmon (*O. nerka*) in British Columbia (Kent et al. 1993), suggesting that it is a sister taxon of CKX. The 18S rDNA sequence of CKX was found also to be similar to *Myxidium lieberkuehni*, consistent with the phylogenetic relationships among some *Myxidium* and *Sphaerospora* species hypothesised by Kent et al. (2001). However, given that available SSU sequence data indicate these genera to be paraphyletic (Kent et al. 2001), the present analysis serves mainly to confirm the close relationship of CKX to both *S. oncorhynchi* and *M. lieberkuehni*. The similarities of CKX 18S rDNA sequences to those of *Sphaerospora* and *Myxidium* suggest that CKX might be a previously unrecognised proliferative stage of *M. salvelini*, *M. minteri* or an undescribed species of *Sphaerospora* or *Myxidium*.

In situ hybridisation using a DIG-labelled probe was used on tissue sections to identify the source and location of DNA used for PCR and sequencing studies. The pattern of CKX staining obtained by ISH was identical to that of traditional histological staining. Under conditions of relatively low stringency, hybridisation may occur between the probe and DNA from *S. oncorhynchi* or related organisms including *M. lieberkuehni*. Thus, while more work is required to derive a probe unique for the CKX organism, the probe failed to hybridize with uninfected kidney and with some other Myxosporea of salmon in western North America. Similarly, the low sequence identity between the CKX probe and the homologous *T. bryosalmonae* sequence indicates the low probability of cross-hybridisation with the latter organism.

Our inability to specifically identify CKX notwithstanding, a particularly noteworthy aspect of this study was the coincident first detection of CKX infections in coho salmon from British Columbia and Washington. It is possible that CKX is a species of myxosporean previously unrecognised despite extensive parasitological surveys of salmonids from Oregon to British Columbia. An alternative hypothesis is that CKX is a known species displaying an unusual pattern of development because of the influence of unknown factors. The recent finding of CKX in spawning pink salmon *Oncorhynchus gorbuscha* (Walbaum) in British Columbia (Jones, unpublished observations) suggested that infection of an inappropriate host does not explain the histozoic development. Most coho in this study were not examined for bacterial infections and a relationship between the original diagnosis of *Aeromonas salmonicida* and CKX infections in those coho is unknown. Hamilton (1980) suggested that mixed infections with *Sphaerospora* sp. and *A. salmonicida* increased the susceptibility of goldfish to subsequent infection. Spawning and post-spawn senescence among *Oncorhynchus* spp. is associated with systemic degeneration, including immune incompetence (Schreck 1996). We suggest that the histozoic development of CKX is a consequence of this physiological degeneration. Specifically, we hypothesise that immune incompetence among spawning and post-spawn salmon provided a permissive environment for histozoic development of a species that, in the competent host, undergoes a strict coelozoic cycle of development. The unidentified *Myxidium* or *Sphaerospora* spores observed in CKX-infected coho may be remnants of this earlier cycle. The degenerate appearance of intraluminal CKX stages with no evidence of sporogony indicated that the developmental cycle was not presporogonic and was probably abortive. Whether

extrasporogonic development among other myxosporean species necessarily precedes or is required for sporulation to occur is not well understood (Lom and Dyková 1995). *Sphaerospora renicola* undergoes an increased frequency of abortive extrasporogonic development coincident with colder months (Grupcheva et al. 1985), suggesting a role for temperature in this process. The findings of CKX among two species of spawning Pacific salmon indicated that the parasite is not host specific and that conditions promoting the extrasporogonic pattern of development are widespread in spawning fish, consistent with the senescence hypothesis. Understanding the factors that regulate potentially pathogenic extrasporogonic development among otherwise non-histozoic Myxosporea will be a valuable field of research. Furthermore, the fishery management implications of understanding senescence-associated disease in Pacific salmon have not gone unnoticed.

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