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Host–parasite relationship of *Ortholinea lauquen* sp. nov. (Cnidaria: Myxozoa) and the fish *Galaxias maculatus* in northwestern Patagonia, Argentina

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ABSTRACT: *Galaxias maculatus* (Jenyns, 1842) is a widespread freshwater fish and an important component of the economically important whitebait fisheries across the Southern Hemisphere. We report a new myxosporean parasite (Cnidaria: Myxozoa) infecting the kidney of *G. maculatus* from northwestern Patagonia (Argentina). *Ortholinea lauquen* sp. nov. was characterized using myxospore morphology, morphometrics and small subunit rDNA (ssrDNA) sequence data. Our ssrDNA phylogenetic analyses showed that *O. lauquen* sp. nov. is a member of the oligochaete–freshwater urinary tract clade and basal to a clade containing 4 different spore morphotypes (*Chloromyxum*, *Myxidium*, *Zschokkella*, *Hoferellus*). We explored host–parasite relationships at the macro- and microscale by analyzing the distribution, tissue tropism and pathology of *O. lauquen* sp. nov. Prevalence was relatively low (7%) by microscopy, but PCR detection revealed hidden levels of infection (49%), with the highest detection in lakes Morenito and Moreno (63–90%, Río Negro Province). The only locality negative by both microscopy and PCR was the Caleufu River (Neuquén Province), suggesting differences in fish life history traits (landlocked vs. potamodromous) or preference of the putative obligate invertebrate host for lentic habitats. *O. lauquen* sp. nov. sporulates in the renal tubules and occasionally in the glomerular space. The plasmodia frequently occluded the tubule lumina, and cellular necrosis and disintegration of the epithelium were observed. *O. lauquen* sp. nov. could represent a potential threat to *G. maculatus* culture under intensive farming conditions.

KEY WORDS: Myxozoa · *Galaxias maculatus* · *Ortholinea* · Urinary system · South America · Patagonia · Histopathology

1. INTRODUCTION

Galaxias maculatus (Jenyns, 1842) (Osmeriformes: Galaxiidae; known as inanga by the Māori or puyen chico in Spanish) is one of the most naturally widely distributed freshwater fish (Berra et al. 1996). This amphidromous species is found throughout the Southern Hemisphere temperate zone, in coastal environments, rivers and lakes, with diadromous and landlocked populations, e.g. in the Andean lakes. *G. maculatus* is one of the species regarded as whitebait and is an important component of this commercial and recreational fishery as food fish in New Zealand and Chile (David et al. 2014, Froese & Pauly 2018, Gomon & Bray 2019). Studies have been undertaken on its biology and reproduction for commercial uses,

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as a laboratory animal, and for repopulation of habitats where the fish has been extirpated through overexploitation or depredation by introduced salmonids; research into culturing programs is known as galaxiculture (Mitchell 1989, Vega et al. 2013).

Myxozoans are a widespread group of parasitic cnidarians that have complex life cycles which alternate between an intermediate host (vertebrates, mainly fish) and a definitive host (annelid or bryozoan) (Eszterbauer et al. 2015). These spore-forming parasites are responsible for diseases that can cause significant impacts on fish health in both wild populations and aquaculture and affect commercial fisheries (Dyková & Lom 1982, Molnár et al. 1989, Hedrick et al. 1993, Whipps 2011). The genus *Ortholinea* Shulman, 1962 contains some 20 species, which develop myxospores mostly in the excretory system of marine fishes. The known species form a polyphyletic group scattered through the oligochaete–freshwater urinary tract clade of myxozoans (Fiala 2006, Rangel et al. 2014, 2017, Holzer et al. 2018). This heterogeneous clade contains at least 7 different genera with very different spore morphologies. Several representatives from this clade were reported to cause disease and/or histopathological changes in the urinary system of their hosts (e.g. kidney enlargement disease in goldfish, Molnár et al. 1989; inter-renal disease in bluegill, Whipps 2011). Few data exist on the host–parasite interaction of members of the genus *Ortholinea* in the urinary system of their hosts.

Using general metazoan and/or myxozoan primers, we screened kidney samples of *G. maculatus* collected from 5 localities in northwestern Patagonia. We identified and characterized a novel *Ortholinea* species using myxospore morphology, morphometrics and small subunit rDNA (ssrDNA) sequence data. At macro- and microscales, we explored host–parasite relationships by studying the distribution of infections in *G. maculatus* populations from the different localities and pathology of the parasite where it sporulated in the kidney. The DNA data were used to determine phylogenetic relationships among our novel *Ortholinea* and other members of the oligochaete–freshwater urinary tract myxozoan clade.

2. MATERIALS AND METHODS

2.1. Fish collection and dissection

In 2017 and 2018, we collected 114 puyen chico from 5 localities in Patagonia, Argentina, comprising the Andean glacial lakes Moreno, Morenito, Escondido and Gutiérrez in Río Negro Province and the Caleufu River in the Patagonian steppe of Neuquén Province (Table 1). Fish were captured alive using hand nets and baited traps, transported in an aerated cooler to the Laboratorio de Parasitología (INBIOMA: CONICET-UNCo) and killed by neural pithing before dissection. We examined wet mounts of brain, gills, gall bladder and kidney for myxozoan parasites using a light microscope at 400 to 1000× magnification. This study focuses on the parasites found in the kidney. Kidney samples were preserved in ethanol for later DNA analyses or fixed for histology or electron microscopy.

2.2. Morphological analysis of myxozoans

Digital images of fresh myxozoan stages were obtained at 1000× magnification under bright field with a Zeiss AxioCamERc5s camera mounted on a Zeiss Primo Star compound microscope. Spore measurements followed published recommendations (Lom & Arthur 1989; but using the more structurally accurate term polar tubule instead of polar filament, Ben-David et al. 2016). Measurements in micrometers were taken from the images using ImageJ (v. 1.47, National Institutes of Health, Bethesda, MD, USA) and are presented as the mean followed by SD and range in parentheses. Parasite myxospores were air dried directly onto glass slides, stained with Diff-Quik and mounted with DPX (Sigma-Aldrich). Archival smears and histological sections were deposited at the Invertebrate Collection of Museo de La Plata, FCNyM-UNLP, La Plata, Buenos Aires, Argentina.

For scanning electron microscopy (SEM), mature myxospores were washed out of one infected kidney with 0.1M cacodylate buffer and then fixed with glutaraldehyde to a final concentration of 2.5%. The spores were left to settle on a 0.4 µm Nucleopore filter (Whatman), dehydrated in a graded ethanol series for 15 min each step, and then critical point dried before being sputtercoated with gold:palladium (40:60 ratio). Myxospores were examined using an FEI Quanta 600 FEG scanning electron microscope at Oregon State University's Electron Microscopy Facility.

For histology, one infected kidney was fixed in 10% neutral buffered formalin, dehydrated in a graded alcohol series and then embedded in paraffin. Sections were cut at 6 µm and stained with haematoxylin and eosin or Giemsa.

Table 1. *Ortholinea lauquen* sp. nov. prevalence, as revealed by light microscopy and by PCR of *Galaxias maculatus* kidneys collected from northwestern Patagonia, and number of small subunit rDNA isolates sequenced, sequence lengths and GenBank accession numbers

Locality	Month and year	No. of fish	Prevalence by microscopy	Prevalence by PCR	No. of sequences	Sequence length and GenBank accession no.
Lake Morenito (41°3′17.17″S, 71°31′7.41″W)	July 2017	5	0% (0/5)	100% (5/5)[a]	5	685 bp (GM12_6; MN128726) 763 bp (GM12_8; MN128725) 763 bp (GM12_9; MN128724) 685 bp (GM12_10; MN128723)
	January 2018	13	8% (1/13)	NA	–	–
	November 2018	5	0% (0/5)	80% (4/5)	1	–
Lake Escondido (41°3′37.64″S, 71°33′58.86″W)	July 2017	10	0% (0/10)	100% (9/9)[a]	9	750 bp (GM11_3; MN128728) 786 bp (GM12_4; MN128727)
	March 2018	8	0% (0/8)	25% (2/8)	–	–
	November 2018	8	0% (0/8)	50% (4/8)	2	–
Lake Moreno (41°3′34.67″S, 71°33′50.82″W)	March 2018	20	15% (3/20)	45% (9/20)	4	2040 bp (GM3; MN128729) type
	November 2018	12	25% (3/12)	92% (11/12)	1	–
Caleufu River (40°23′52.94″S, 70°44′16.47″W)	March 2018	21	0% (0/21)	0% (0/21)	–	–
Lake Guitérrez (41°10′37.32″S, 71°24′54.73″W)	November 2018	12	8% (1/12)	42% (5/12)	–	–

[a]Samples analysed using nested PCR

2.3. Molecular data and phylogenetic analyses

Kidney tissues (n = 100) preserved in 96–100% ethanol were first air dried, and then total DNA was extracted using a phenol–chloroform protocol (Holzer et al. 2004) or the DNeasy blood and tissue kit (Qiagen). Extracted DNA was re-suspended in 100–200 µl RNAse- and DNAse-free water. Kidney samples from July 2017 were screened using a fully nested PCR with a first round of primers ERIB1 (5′-ACC TGG TTG ATC CTG CCA G-3′) and ERIB10 (5′-CTT CCG CAG GTT CAC CTA CGG-3′) (Barta et al. 1997) followed by MyxGP2F (5′-WTG GAT AAC CGT GGG AAA-3′; Kent et al. 1998) and Act1R (5′-AAT TTC ACC TCT CGC TGC CA-3′; Hallett & Diamant 2001). Samples from 2018 were screened in single round PCR using 18e (5′-CTG GTT GAT CCT GCC AGT-3′; Hillis & Dixon 1991) and novel primer ACTATK1r (5′-ATG GAA ACG GTC TTG ACA AAT GCC-3′). Additional primers 18e and MYX4R (5′-CTG ACA GAT CAC TCC ACG AAC-3′; Hallett & Diamant 2001) were used to obtain the longest ssrDNA type sequence. PCRs were conducted in 10 µl reactions with 2 U Titanium *Taq* DNA polymerase and 10× buffer which contained 1.5 mM $MgCl_2$ (BD Biosciences Clontech), 0.5× *Redi*Load dye (Invitrogen), 25 ng μl^{-1} BSA, 0.2 mM each dNTP, 0.25 µM each primer, PCR-grade water and 10 to 150 ng of template DNA or 1/6 diluted 1st-round PCR product. ERIB1–ERIB10 PCR cycling conditions consisted of 95°C for 2 min; followed by 35 cycles of 94°C for 50 s, 60°C for 1 min 20 s and 68°C for 2 min; and a final extension at 68°C for 10 min. All other PCR cycling conditions consisted of 95°C for 3 min; followed by 35 cycles of 94°C for 20 s, 62°C for 20 s and 68°C for 25 s; and a final extension at 68°C for 7 min. DNA amplicons were visualized with a 1% agarose gel in sodium acetate or TAE buffer and then purified for sequencing using a Gel/PCR DNA fragments extraction kit (Geneaid Biotech) or a QIAquick PCR purification kit (Qiagen) or by direct sequencing of a diluted 1:10 PCR product. Sequences were obtained with an ABI PRISM 3130x1 or ABI3730 genetic analyzer sequencer (Applied Biosystems). We trimmed overlapping partial sequences of ssrDNA and assembled them into consensus contigs using Geneious 7.0.6. (Biomatters).

We used our longest contig, 2040 bp, in a BLAST search of GenBank to identify the closest relatives to our novel myxozoan (>80% identity). We then downloaded available sequences of *Ortholinea* spp. and species of other related genera above this similarity

threshold, all of which belonged to the oligochaete–freshwater urinary tract clade (Fiala 2006, Holzer et al. 2018). Basal myxozoans *Myxidium lieberkuehni* Bütschli, 1882 and *Chloromyxum legeri* Tourraine, 1931 were used as outgroup taxa. Alignment and phylogenetic analyses were performed with programs as plugins in Geneious 7.0.6. We aligned the sequence files using MAFFT v. 7.017, with the L-INS-I algorithm and default parameters. We used the GTR + G substitution model, which was determined to be one of the best fitting models according to Modeltest (Posada & Crandall 1998) using the FindModel web implementation: http://hiv.lanl.gov/content/sequence/findmodel/findmodel.html. Phylogenetic analyses comprised maximum likelihood (ML) using RAxML v. 7.2.8 (Stamatakis et al. 2005), maximum parsimony (MP) using PAUP v. 4.0 (Swofford 2002) and Bayesian inference (BI) using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003). ML and MP analyses were conducted using heuristic searches with random taxa addition, TBR swapping algorithm, all characters treated as unordered and gaps treated as missing data. Clade support values were calculated from 1000 bootstrap replicates for both ML and MP analyses. For BI, posterior probabilities were calculated over 1 000 000 generations via 2 independent runs of 4 simultaneous Markov chain Monte Carlo algorithms with every 200th tree saved. Burn-in was set to 100 000 generations. We calculated interspecific ssrDNA identity (%) using PAUP v. 4.0 using 5′ and 3′ ends trimmed and removed inserts of *M. streisingeri* Whipps, Murray & Kent, 2015 (GenBank Accession No. KM001684) from the alignment.

3. RESULTS

Of the 114 puyen chico collected, 32 were females, 16 were males and 66 were of undetermined sex, with average (±SD) total length 5.3 ± 1.3 cm and weight 0.9 ± 0.9 g. Myxozoan plasmodia and spores were found in the kidney of 7% (8/114) of the fish, and this is described as a new species from *Galaxias maculatus*, with a differential diagnosis.

3.1. *Ortholinea lauquen* sp. nov. taxonomic summary

Type host: *G. maculatus* (Jenyns, 1842).

Type locality: Lago Moreno (41° 3′ 34.67″ S, 71° 33′ 50.82″ W), San Carlos de Bariloche, Río Negro, Argentina.

Site of infection: Renal tubules.

Prevalence: 7% (8/114) microscopic detection, 49% (49/100) molecular detection.

Etymology: From the word 'lauquen', meaning 'lake' in the language of the Mapuches, one of the indigenous peoples of Patagonia.

Material deposited: Invertebrate Collection of Museo de La Plata, FCNyM-UNLP, La Plata, Buenos Aires, Argentina; 2 Diff-Quik stained slides of air-dried spores (Catalog Nos. MLP-Oi 4195 and MLP-Oi 4196) and 1 histological section of infected kidney (Catalog No. MLP 4197).

Molecular data: Partial ssrDNA sequence, 2040 bp (GenBank Accession No. MN128729).

3.2. Description of myxospores

Based on 82 myxospores from the kidneys of 7 hosts and spores examined by SEM from 1 host. Myxospores subspherical (Figs. 1A–F & 2), width 7.3 ± 0.4 (6.5–8.3) µm, length 7.6 ± 0.4 (6.6–8.8) µm (both in valvular view, n = 47) and thickness 7.5 ± 0.6 (6.3–8.8) µm (sutural view, n = 35). Two valves, joined at straight suture, with surface ridges 15 to 20, semi-concentric, some bifurcated, occupying entire myxospore surface (Fig. 1F). Polar capsules, 2, pyriform 3.3 ± 0.3 (2.2–4.0) µm × 2.4 ± 0.3 (1.8–3.1) µm (n = 92) located towards one end of the spore, each in different valves, with openings in opposite directions (Figs. 1C & 2), each capsule contains a coiled tubule with 3 to 4 turns. Sporoplasm binucleate.

3.3. Description and tissue location of plasmodia

Based on 14 plasmodia from 3 hosts. Amoeboid, spherical and pyriform plasmodia (Fig. 1G–H), 24.7 ± 12.4 (14.4–55.4) µm × 16.7 ± 4.6 (9.3–28.1) µm, in lumina of renal tubules. Occasionally multiple plasmodia occluded the lumina of renal tubules. Myxospores develop in pairs, 2 to 6 spores per plasmodium. Plasmodia with posterior pseudopodium.

3.4. Remarks

Based on myxospore and polar capsule shape, number of polar capsules and their position with respect to the straight sutural plane, we place the new species in the genus *Ortholinea*. *Ortholinea lauquen* sp. nov. is unique with respect to all other

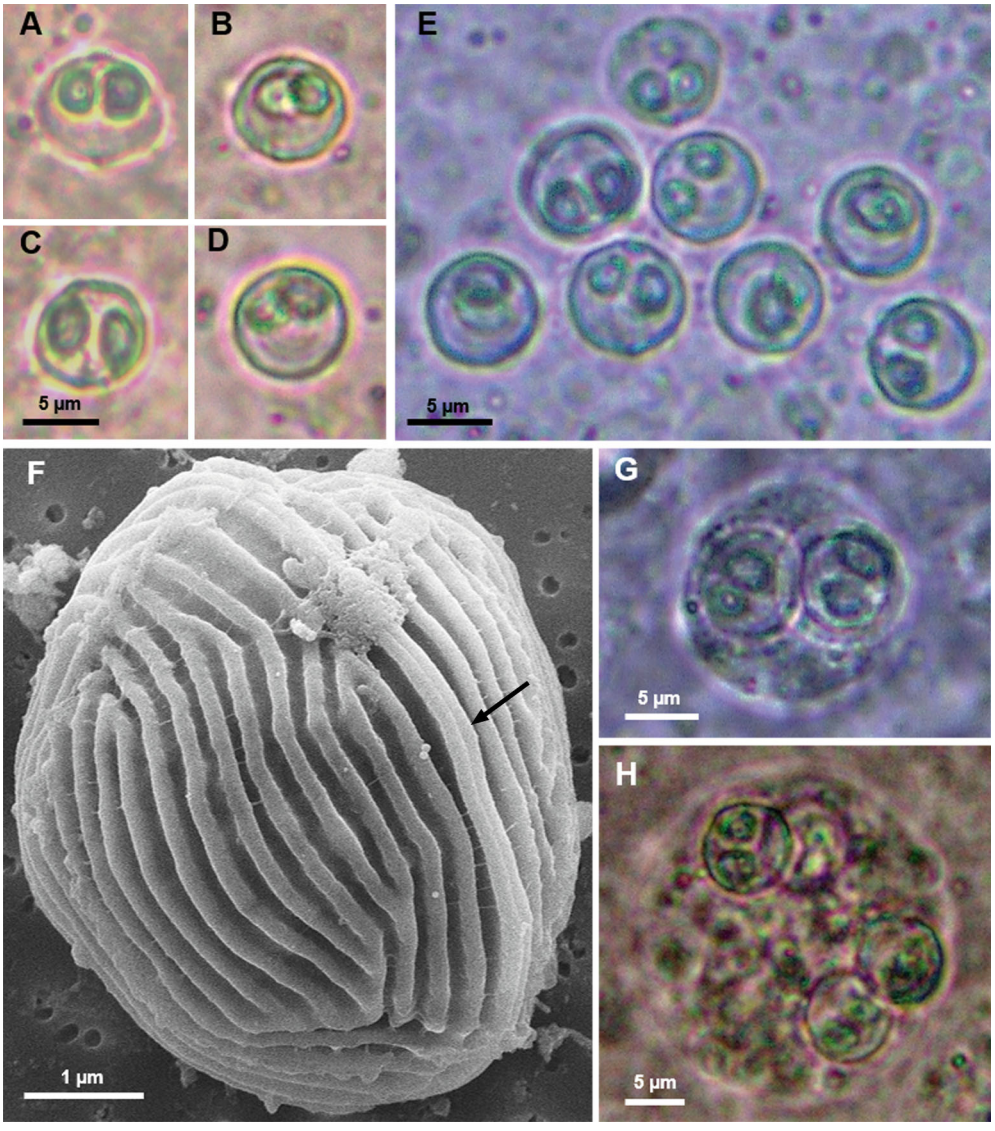

Fig. 1. *Ortholinea lauquen* sp. nov. myxospores and plasmodia (light microscopy and scanning electron microscopy) from the kidney of *Galaxias maculatus*. Myxospores in (A,B) side sutural view, (C) apical sutural view and (D) valvular view. (E) Group of mature myxospores in different orientations. (F) Scanning electron micrograph of myxospore showing ridges on surface of valve cell and prominent straight suture (arrow) between the 2 valve cells. Round plasmodia with (G) 2 or (H) 4 developing myxospores

Ortholinea species in fish host and geographic location (Table 2). It is morphologically and morphometrically similar to several members of the genus: *O. labracis* Rangel et al., 2017; *O. africanus* Abdel-Ghaffar et al., 2008; *O. antipae* Moshu & Trombitsky, 2006; and *O. gobiusi* Naidenova, 1968, with the only difference being spore thickness and shape of the polar capsule (pyriform vs. subspherical or round) (Table 2). *O. lauquen* sp. nov. differed in spore width from *O. clupeidae* Aseeva, 2000. The new species is differentiated from *O. fluviatilis* Lom & Dyková, 1995 and *O. undulans* Meglitsch, 1970 by polar capsule shape (pyriform vs. subspherical) and the maximum number of polar tubule turns. *O. lauquen* sp. nov. and *O. orientalis* (Shulman & Shulman-Albova 1953) from clupeid and gadid fishes can be distinguished by their disparate geographical distribution (Patagonia vs. Denmark and Bering Sea). Measurements of the myxospores of *O. orientalis* overlapped with *O. lauquen* sp. nov., but some differed in length (Shulman & Shulman-Albova 1953, Aseeva 2000, 2002, Karlsbakk & Køie 2011).

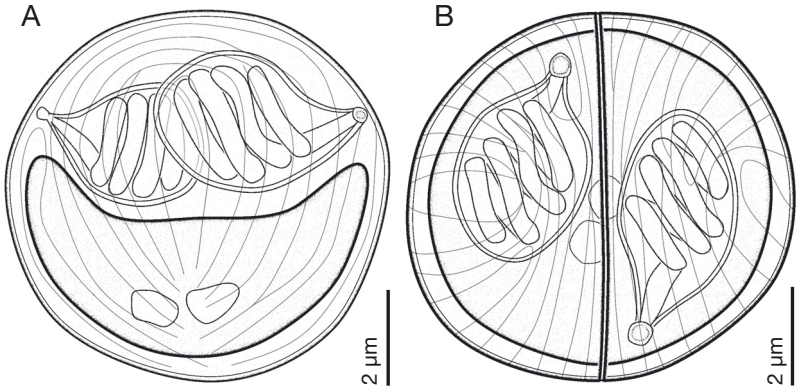

Fig. 2. Drawing of *Ortholinea lauquen* sp. nov. myxospore from *Galaxias maculatus*. (A) Valvular view; (B) apical sutural view. Surface ridges are represented schematically

Most *Ortholinea* spp. have been described with ornamentation of the outside of their valves, of different numbers and patterns of ridges. The number of ridges in *O. lauquen* sp. nov. is similar to *O. concentrica* Alama-Bermejo & Hernández-Orts, 2018; *O. auratae* Rangel et al., 2014; *O. striateculus* Su & White, 1994; and *O. undulans*. The species *O. africanus*, *O. antipae*, *O. basma* Ali, 2000 and *O. australis* Lom et al., 1992 all have fewer surface ridges than the new species (Table 2).

O. concentrica is the only other *Ortholinea* species described in Patagonia. The 2 Patagonian species differ in length, polar capsule shape (pyriform vs. subspherical) and host (freshwater *G. maculatus* vs. marine seabass *Acanthistius patachonicus*).

3.5. Histopathology

No gross clinical signs were observed. Plasmodia containing spores were located mostly in renal tubules (Fig. 3A), and occasionally spores were observed in the glomerular space (Fig. 3B). Plasmodia partially or completely adhered to the microvillar zone of the tubular epithelium and frequently occluded the lumina of the renal tubules (Fig. 3C–F). In some tubules, picnotic nuclei were observed at the base of the epithelium, suggesting cellular necrosis (Fig. 3C), and others showed epithelial disintegration associated with parasite stages (Fig. 3D). Swollen kidney tubules were observed frequently, with tubule epithelial cells enlarged with rounding of the plasma membrane (Fig. 3E) and apical blebbing (Fig. 3F), but swollen tubules were not associated with *O. lauquen* sp. nov. infection exclusively.

3.6. Parasite prevalence

By microscopy, we observed plasmodia and myxospores of *O. lauquen* sp. nov. in the lumina of *G. maculatus* kidney tubules at prevalences of 18.8% (6/32) in Lake Moreno, 8.3% (1/12) in Lake Guitérrez and 4.4% (1/23) in Lake Morenito; Lake Escondido (0/26) and the Caleufu River (0/21) were negative visually.

PCR detection (Table 1) revealed higher infection levels than visual detection in some localities. The highest PCR detection was 90.0% (9/10) in Lake Morenito, followed by 62.5% (20/32) in Lake Moreno, 60.0% (15/25) in Lake Escondido and 41.7% (5/12) in Lake Gutiérrez. Fish from Lake Escondido were positive by PCR only. The Caleufu River was the only locality negative by both microscopy and PCR.

3.7. Molecular and phylogenetic results

Partial ssrDNA sequences obtained in this study are listed in Table 1. In total, 23 isolates were obtained from *O. lauquen* sp. nov. from kidneys of different geographic locations. Intraspecific sequence identity was between 98.7 and 100% (up to 9 bp over a 656 bp alignment). One isolate from Morenito Lake (July 2017) showed the highest variability (1.1–1.3%, 7–9 bp different) (685 bp; GenBank Accession No. MN128723).

BLAST searches showed that the closest sequence matches of *O. lauquen* sp. nov. were *O. auratae* (GenBank Accession No. KR025869; query coverage 87%; maximum identity 89%) and *O. labracis* (GenBank Accession No. KU363831; query coverage 89%; maximum identity 88%).

Interspecific ssrDNA identities to members of the oligochaete–freshwater urinary tract clade (over an 835 bp alignment) varied widely. *O. lauquen* sp. nov. had the highest sequence similarity to *Chloromyxum shurovi* Shul'man & Ieshko, 2003 (82.8%), *Myxidium giardi* (Cépede, 1906) (82.7%) and *Zschokkella* sp. ex *Anguilla anguilla* (L.) (82.7%), followed by *Ortholinea* sp. ex *Alosa alosa* (L.) (82.6%), *O. orientalis* (82.4%) and *Hoferellus alosae* Wünnemann, Holzer, Pecková, Bartošová-Sojková, Eskens & Lierz, 2016 (82.4%). Sequence similarity to the only other species of *Ortholinea* described in Argentina, *O. concentrica*, was 78.7%.

Table 2. Comparison of *Ortholinea* spp. host, localities, myxospore and polar capsules. Measurements in micrometers. SPL: spore length; SPW: spore width; SPT: spore thickness; PCS: polar capsule shape; PCL: polar capsule length (or diameter); PCW: polar capsule width; PTC: polar tubule coil; ND: not determined

Species	Reference	Host	Locality	SPL	SPW	SPT	Ridges	PCS	PCL	PCW	PTC
O. orientalis	Shulman & Shulman-Albova (1953)	*Clupea harengus* Linnaeus, 1758/ *Eleginus navaga* Walbaum, 1792	Russia	7.5–8.5/ 8.5–11.5	7.5–7.6/ 6.8–9.8	5.1/ 6.6–8.0	No[a]	–	2.2–2.9/ 3.0–4.2	–	–
O. gobiusi	Naidenova (1968)	*Zosterisessor ophiocephalus* (Pallas, 1814)	Russia	7.7–9.8	7.0–7.2	4.8–5.0	Yes, ND	Round	1.8–2.1	–	–
O. undulans	Meglitsch (1970)	*Arnoglossus scapha* (Forster, 1801)/*Perthorhamphus novae-zeelandiae* Günther, 1862	New Zealand	7.0–10.0/ 7.0–9.0	6–9/7–8	5–8/6–7	Yes, 20	Pyriform	2.0–4.0/ 3.0	2.0–3.0/ 2.0–3.0	5
O. australis	Lom et al. (1992)	*Acanthopagrus australis* (Günther, 1859)	Australia	7.8–10.4	7.3–9.5	6.2–7.3	Yes, 1–3 + 5–9	Oval, anteriorly pointed	2.8–4.4	2.3–3.2	3–4
O. striateculus	Su & White (1994)	*Leptatherina presbyteroides* (Richardson, 1843)	Australia	9.1–10.5	8.9–10.4	–	Yes, 18–20	Pyriform	3.4–3.6	2.8–3.1	5–7
O. fluviatilis	Lom & Dyková (1995)	*Dichotomyctere fluviatilis* (Hamilton, 1822)	SE Asia	7.9–8.4	7.3–8.0	6.8	Yes, ND	Subspherical	2.8–3.3	–	4–6
O. clupeidae	Aseeva (2000)	*Clupea pallasii* Valenciennes, 1847; *Konosirus punctatus* (Temminck & Schlegel, 1846)	Sea of Japan	7.4–9.5	5.5–6.3	–	Yes, ND	Pyriform	2.5–3.0	–	–
O. basma	Ali (2000)	*Clinus agilis* Smith, 1931	S Africa	12.0–15.0	11.8–13.0	–	Yes, 12–13	Pyriform	4.0–4.8	3.0–4.3	4–5
O. antipae	Moshu & Trombitsky (2006)	*Alosa tanaica* (Grimm, 1901)	Ukraine and Republic of Moldova	6.8–7.5	6.2–7.2	5.0–5.4	Yes, 10–16	Spherical	1.8–2.5	–	3–4
O. africanus	Abdel-Ghaffar et al. (2008)	*Oreochromis niloticus* (Linnaeus, 1758)	Egypt	6.93–8.47	6.93–8.47	3.85–4.62	Yes, 10–14	Spherical	2.31–3.85	–	4–5
O. auratae	Rangel et al. (2014)	*Sparus aurata* Linnaeus, 1758	Portugal	8.2–10.1	7.5–9.1	6.3–8.4	Yes, 19	Subspherical	2.9–3.6	2.4–2.9	3–4
O. labracis	Rangel et al. (2017)	*Dicentrarchus labrax* (Linnaeus, 1758)	Portugal	6.8–8.7	6.7–7.7	5.8–7.7	Yes, –	Subspherical	2.6–3.4	2.0–2.9	4–5
O. concentrica	Alama-Bermejo & Hernández-Orts (2018)	*Acanthistius patachonicus* Jenyns, 1840	Argentina	8.2–11.0	7.9–11.0	7.7–9.0	Yes, 17–20	Subspherical	2.4–3.8	2.3–3.6	3–4
O. lauquen sp. nov.	Present study	*Galaxias maculatus* (Jenyns, 1842)	Argentina	6.5–8.3	6.6–8.8	6.3–8.8	Yes, 15–20	Pyriform	2.2–4.0	1.8–3.1	3–4

[a]Ridges observed later by Karlsbakk & Køie (2011)

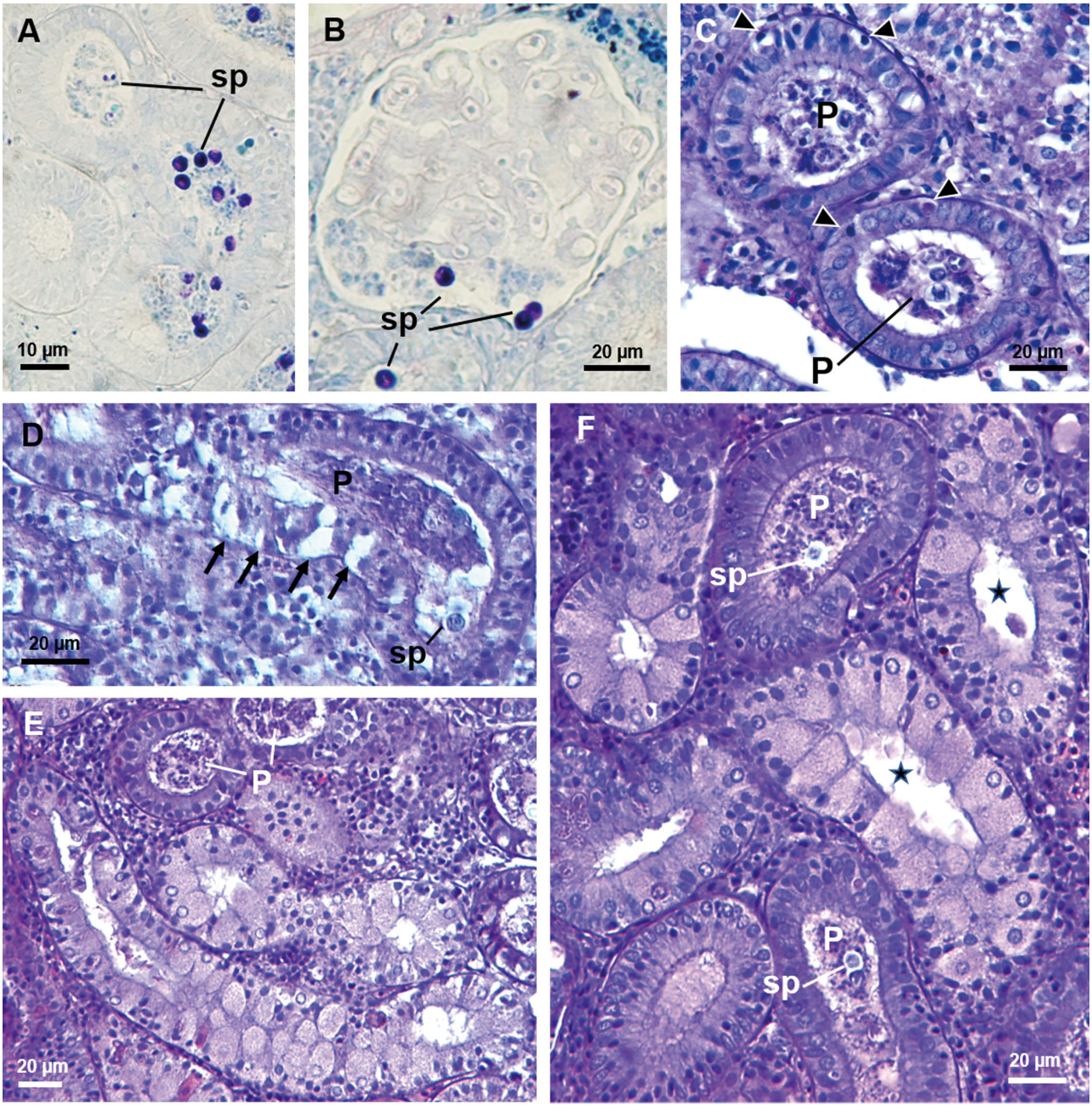

Fig. 3. Stained histological sections of *Ortholinea lauquen* sp. nov. from kidney of *Galaxias maculatus*. (A) Kidney tubules occluded by plasmodia and spores. (B) Spores in a glomerulus. (C) Plasmodia completely and partially attached to the microvillar zone of tubular epithelia, which have picnotic nuclei (arrowheads). (D) Kidney tubule with signs of epithelial disintegration (arrows) associated with the parasite. (E,F) Swollen kidney tubules, with enlarged epithelial cells showing rounded plasma membrane and apical blebbing (star). (A,B) Giemsa stain; (C–F) Haematoxylin-eosin stain. sp: spore; P: plasmodia (most with spores)

BI and MP phylogenetic analyses placed *O. lauquen* sp. nov. as basal taxa to a clade containing *C. shurovi*, *M. giardi*, *Zschokkella* sp. and *H. azevedoi* (Fig. 4). The position of the new species was unresolved using ML analyses.

4. DISCUSSION

Given the unique host, morphometrics, DNA sequence and phylogenetic position, we ascribed *Ortholinea lauquen* sp. nov. as a novel myxozoan

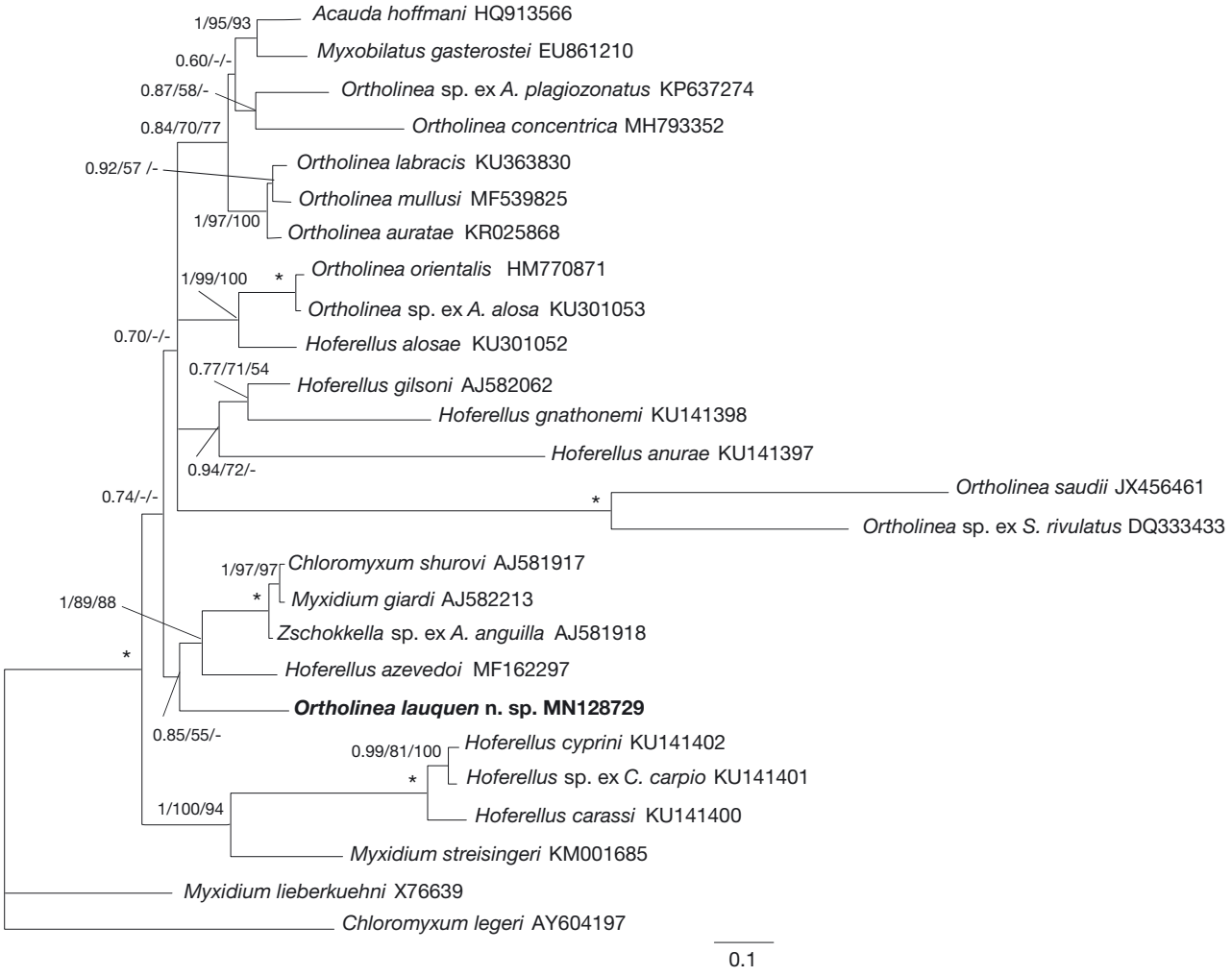

Fig. 4. Bayesian inference (BI) tree showing the phylogenetic position of *Ortholinea lauquen* sp. nov. within the oligochaete–freshwater urinary tract clade (as defined by Fiala [2006] and Holzer et al. [2018]). The new species is indicated in **bold**. *Myxidium lieberkuehni* and *Chloromyxum legeri* were used as outgroups. Numbers at nodes represent posterior probabilities and bootstrap values (BI/maximum likelihood [ML]/maximum parsimony [MP]). Asterisks indicate nodes with maximum supports (BI = 1, MP/ML = 100). Dashes at nodes represent nodal support BI < 0.5 and MP/ML < 50 or node not present in the MP or ML trees

parasite of *Galaxias maculatus* and showed this to be the second species of *Ortholinea* described from Argentina. Five myxozoan species were known from *G. maculatus*: 4 *Myxobolus* spp. and *Myxidium biliare* Viozzi & Flores, 2003, which sporulates in the gall bladder of *G. maculatus* in Patagonia, Argentina (Szidat 1953, Hine 1976, Kalavati et al. 2000, Flores & Viozzi 2001, 2007, Viozzi & Flores 2003). *O. lauquen* sp. nov. is the first myxozoan known to infect the urinary system of *G. maculatus*.

We detected a pattern of parasite prevalence in the fish populations, depending on sampled environment: all the infected fish were from oligotrophic lakes of glacial origin, and the only negative sample group was from a river. We consider several factors may contribute to this uneven prevalence. Different life histories of lake vs. river fish populations correlated with infection. Unlike the isolated lake populations, fish from the Calefu River have potamodromous migration (i.e. within freshwater), migrating from Piedra del Águila Reservoir to the river (Barriga et al. 2007); yet despite greater potential to encounter the parasite in different habitats, these river fish were infection free. Nothing is known about the putative invertebrate host of *O. lauquen* sp. nov., but we suspect it has a preference for lentic habitats. Further research is needed to understand the distribution of *O. lauquen* sp. nov. in water bodies of northwestern Patagonia and to identify if this correlates more with invertebrate rather than vertebrate host availability.

The genus *Ortholinea*, like most myxozoan genera, was established on the basis of myxospore morphology (Lom & Dyková 2006). The addition of molecular sequence data has shown the genus to be less cohesive, with phylogenetic analyses revealing *Ortholinea* to be polyphyletic, with species spread among multiple clades dominated by other genera or by species of mixed genera (Fiala 2006, Rangel et al. 2014, 2017, Alama-Bermejo et al. 2016). Our new species is further evidence of the polyphyletic nature of *Ortholinea*, with *O. lauquen* sp. nov. being less closely related to other *Ortholinea* and instead falling basal to a small clade of myxozoans with 4 different spore morphologies: *Chloromyxum*, *Myxidium*, *Zschokkella*, *Hoferellus*. Further morphological characterization of *Ortholinea* spores including ultrastructure is needed to determine if there are common morphological features among this myxozoan clade and to test the hypothesis that an *Ortholinea* morphotype is ancestral. Character evolution analyses by Fiala & Bartošová (2010) suggest that a *Chloromyxum* morphotype represents the ancestral form, but *Ortholinea* species were not included in that analysis. Considering the morphology of *Chloromyxum* and *Ortholinea* myxospores, we speculate that *Chloromyxum* could be considered a twinned *Ortholinea* having double the valve cells and capsules but with similar distinctive valve cell surface ridges and phylogenetically basal positions. Given that myxospores with 4 valves are atypical for the majority of genera, we suggest that *Ortholinea* could represent the most ancestral freshwater form. Again, to test this hypothesis, *Chloromyxum* and *Ortholinea* species need to be reassessed at the ultrastructural level and combined in molecular and character evolutionary analyses.

Our histopathology analyses revealed that *O. lauquen* sp. nov. caused both physical and pathological changes to the host kidneys, with plasmodia occluding the tubule lumina and cellular necrosis and disintegration of the tubular epithelium. Similar signs of necrosis of the renal tubules have been reported for other urinary system-infecting myxozoans, and acute disease is observed with a few species (Molnár et al. 1989, Whipps 2011, Kodádková et al. 2014). Whether kidney damage caused by *O. lauquen* sp. nov. can manifest as disease, and if this can cause population-level impacts, is unknown. We have shown that the parasite is present at high prevalences in wild populations of puyen chico in northwestern Patagonian lakes; therefore, we suggest that given this high prevalence, the parasite could become a problem if other stressors are introduced. For example, if galaxiculture is established in this region, and cultured fish have similar prevalence of the parasite, the farming conditions may exacerbate the effects of *O. lauquen* sp. nov. infections, and disease could emerge. Future surveillance of the fish populations, particularly under localized stressful conditions (low water flow, higher temperatures), could reveal signs of population-level effects and inform risk assessments for this fish.

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