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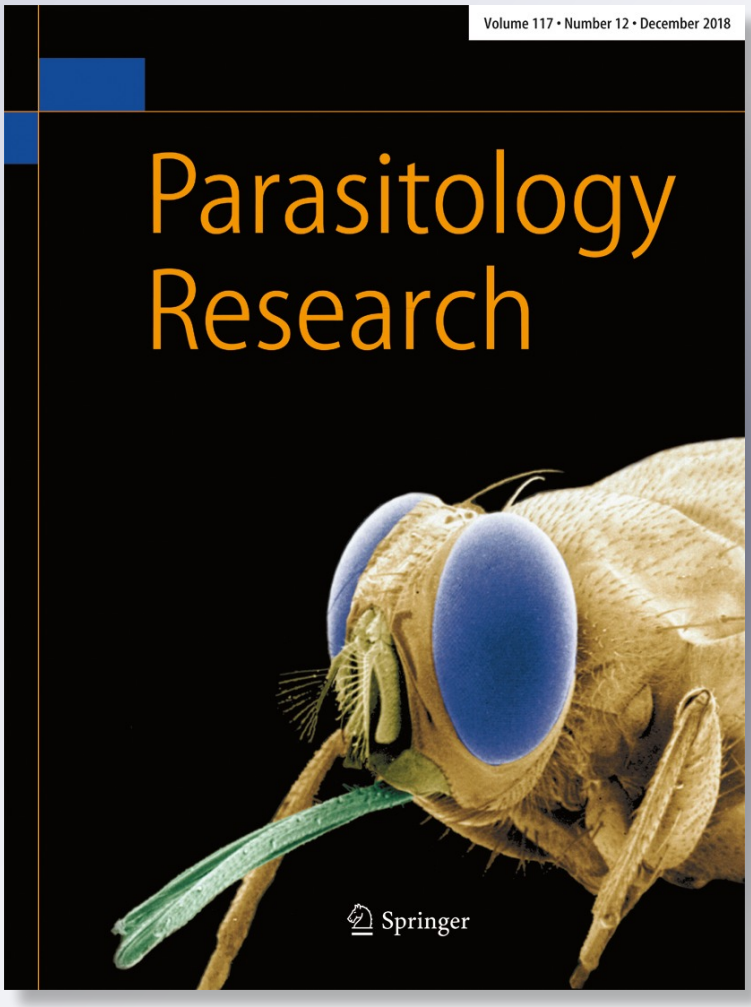

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ORIGINAL PAPER

Ortholinea concentrica n. sp. (Cnidaria: Myxozoa) from the Patagonian seabass *Acanthistius patachonicus* (Jenyns, 1840) (Perciformes: Serranidae) off Patagonia, Argentina

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Abstract

The Patagonian seabass *Acanthistius patachonicus* (Jenyns, 1840) (Serranidae) is a marine fish valued for commercial and sport fisheries from Argentina. We report a new myxosporean (Cnidaria: Myxozoa) infecting the urinary system of the Patagonian seabass from San Antonio Bay, San Matías Gulf, on the Atlantic Ocean. The mature myxospores were subspherical, 8.2–11.0 μm × 7.9–11.0 μm and 7.7–9.0 μm in thickness; two subspherical polar capsules, 2.4–3.8 μm × 2.3–3.6 μm, with 3 to 4 turns of the polar tubule; openings on different valves in almost opposite directions. Ornamented shell valves exhibited 17–20 concentrically organized surface ridges. SSU rDNA phylogenetics analyses placed the new species in the freshwater urinary tract clade, clustering in a clade formed by *Myxobilatus gasterostei* (Parisi, 1912), *Acauda hoffmani* Whipps, 2011, and other *Ortholinea* spp. Based on spore morphology, site of infection, and molecular data, we described this myxozoan as *Ortholinea concentrica* n. sp.

Keywords Myxozoa · Marine fish · Urinary system · South America · Taxonomy · SSU rDNA · Phylogeny

Introduction

Myxozoans are microscopic cnidarian endoparasites from aquatic environments. They have complex life cycles, that alternate between an intermediate host (mainly fish) and a definitive host (annelids or bryozoans). They are a diverse and widespread group of spore-forming parasites, with approx. 2400 species described, representing 18% of all known cnidarian species (Zhang 2011; Okamura et al. 2015). Taxonomy and systematics of this group of parasites are challenging, since the traditional classification, based on spore morphology, does not always correlate with the more recent molecular phylogeny-based systematics (Fiala et al. 2015a). Recent large-scale cophylogenetic studies determined that the invertebrate host is the strongest defining character for myxozoans evolution, with two large clades: polychaete infecting myxozoans (marine clade) and oligochaete infecting myxozoans (freshwater clade) (Holzer et al. 2018).

The oligochaete-freshwater urinary tract clade (also known as freshwater urinary bladder clade, see Fiala 2006 and Holzer et al. 2018) is a heterogeneous clade containing representatives from at least 7 different genera, each of them with very different spore morphotype (Whipps 2011; Karlsbakk and Køie 2011; Fiala et al. 2015a). *Ortholinea* Shulman, 1962 is a representative of this clade and a genus known to have ancestors that likely reinvaded marine habitats (Karlsbakk and Køie 2011; Fiala et al. 2015a, b). Due to its phylogenetical proximity, *Ortholinea* was recently transferred from Ortholineidae to Myxobilatidae (Karlsbakk et al. 2017). *Ortholinea* contains approx. 20 species, most of them inhabiting the excretory system of marine fishes. Only two species have their complete life cycle elucidated, using marine oligochaetes as definitive invertebrate hosts (Rangel et al. 2015, 2017).

The Patagonian seabass *Acanthistius patachonicus* (Jenyns, 1840) (Serranidae) is a common rocky reef fish in South-Western Atlantic valuable for both commercial and

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recreational fisheries (http://www.fishbase.org; Irigoyen et al. 2008; Galván et al. 2009). *A. patachonicus* has no reported myxozoan infections, and only one species of the genus *Ortholinea*, *O. basma* Ali, 2000 was reported in the South Atlantic (Mackenzie & Kalavati 2014). During a parasitological survey, myxozoan parasites were detected in the urinary bladder of the Patagonian seabass. Herein, we describe a new species of myxozoan from the urinary system of the Patagonian seabass using morphological and molecular data to establish its identity and determine its phylogenetic relationships within *Ortholinea* and other members of the freshwater urinary system clade.

Material and methods

Fish collection

Between May and June 2017, 10 Patagonian seabass *A. patachonicus* (15.0 ± 3.5 cm total length and 68.1 ± 53.5 g in weight, 1 female and 9 undetermined sex) were caught by rod from Punta Verde (40° 43′ 47″ S, 64° 54′ 45″ W) in San Antonio Bay, San Matías Gulf, Argentina. Fish were captured during low tide in a rocky area, kept refrigerated and examined within 24 h of capture. Wet mounts of the skin, gills, gall bladder, intestine, and urinary system (kidney, ureter, and urinary bladder) were examined for parasites using light microscopy at × 400–1000 magnification. This study focuses on the parasites detected in the urinary system.

Morphological analysis

Digital images of myxozoan stages were obtained at × 400–1000 magnification with a Nikon Cool P5100 camera mounted on a Nikon Eclipse E200 microscope. Morphological descriptions and measurements of spores followed the recommendations of Lom and Arthur (1989). Measurements were taken from digital images using the software ImageJ 1.47v (National Institutes of Health, Bethesda, USA) and calibrated against a digital image of a graticule. Drawings of spores were made using photomicrographs. Measurements are given in micrometers and are presented as the mean followed by the standard deviation and the range in parenthesis. Archival smears were stained with Diff Quik and mounted with DPX (Sigma-Aldrich, Missouri, USA).

Urine collected from the urinary bladder containing myxospores was fixed in 2.5% glutaraldehyde in 0.1 M PBS and processed for scanning electron microscopy (SEM), by adhering the spores to a poly-D-lysine coated coverslip, followed by fixation, postfixation and dehydratation according to Alama-Bermejo et al. (2012). Samples were critical point dried, gold sputtered-coated and examined using a JEOL JSM-7401F Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan) at the Laboratory of Electron Microscopy, Institute of Parasitology, Czech Academy of Sciences.

Molecular analysis

The kidney ($n = 10$), ureter ($n = 1$), and urinary bladder ($n = 8$) were preserved in 96% ethanol. Tissue was air-dried and re-suspended in TNES (10 mM Tris-HCl (pH 8), 125 mM NaCl, 10 mM EDTA, 0.5% SDS, 4 M urea), digested with 100 μg/ml of proteinase K, overnight at 55 °C, and extracted following a standard phenol-chloroform protocol. The extracted DNA was re-suspended in 50–100 μL RNAse/DNAse-free water. SSU rDNA amplicons were first amplified using primers 18e (5′-CTG GTT GAT CCT GCC AGT-3′; Hillis and Dixon 1991) and 18R (5′-CTA CGG AAA CCT TGT TAC G-3′; Whipps et al. 2003), followed by a nested PCR with MYX1F (5′-GTG AGA CTG CGG ACG GCT CAG-3′; Hallett and Diamant 2001) and MX3 (5′-CCA GGA CAT CTT AGG GCA TCA CAG A-3′; Andree et al. 1998). PCRs were conducted in 10–20 μl reactions with 0.025Uμl^{-1} Titanium Taq DNA polymerase and 10× buffer which contained 1.5 mM $MgCl_2$ (BD Biosciences Clontech, Shiga, Japan), with 0.2 mM of each dNTP, 0.5 mM of each primer, and 10–150 ng of template DNA. PCR cycling conditions consisted of 95 °C for 3 min, followed by 30 cycles of 94 °C for 50 s, 58–60 °C for 50 s and 68 °C for 1 min 30 s to 2 min, and final extension 68 °C for 10 min. DNA amplicons were visualized with a 1% agarose gel in sodium acetate buffer and purified for sequencing using a Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan). Nested PCR primers were used for sequencing. Sequences were obtained with an ABI PRISM 3130 × 1 automatic sequencer (Applied Biosystems, Foster City, USA). The overlapping partial sequences of SSU rDNA were trimmed and assembled into consensus contigs using Geneious 7.0.6. (Biomatters Ltd., Auckland, New Zealand).

Phylogenetic analyses

SSU rDNA sequences were submitted to the Basic Local Alignment Search Tool (BLAST) on GenBank to identify the closest relatives. Newly generated sequences were aligned together with sequences for species of *Ortholinea* and species of other related genera retrieved from GenBank (see Table 1 for details). Alignment and phylogenetic analyses were performed with programs as plugins in Geneious 7.0.6. An alignment was created using MAFFT v. 7.017, with L-INS-I algorithm and default parameters. All similar sequences were within the freshwater urinary bladder clade according to Fiala (2006). The basal freshwater myxosporean *Myxidium lieberkuehni* Bütschli, 1882 was used as outgroup. No regions

Table 1 Taxa included in the phylogenetic analyses with data on the host, locality, and GenBank accession number (SSU rDNA)

Species	Host	Locality	GenBank accession no.	Source
Genus *Acauda* Whipps, 2011				
A. hoffmani Whipps, 2011	*Lepomis macrochirus* Rafinesque, 1819	Cazenovia Lake, New York (USA)	HQ913566	Whipps (2011)
Genus *Chloromyxum* Mingazzini, 1890				
Chloromyxum sp.	*Salmo salar* Linnaeus, 1758	Amhainnan Stratha Bhig River, Scotland (UK)	AJ581917	Holzer et al. (2004)
Genus Hoferellus Berg, 1898				
H. azevedoi Matos, da Silva, Hamoy & Matos, 2018	*Chaetobranchus flavescens* Heckel, 1840	Marajó Island, State of Pará (Brazil)	MF162297	Matos et al. (2018)
H. alosae Wünnemann, Holzer, Pecková, Bartošová-Sojková, Eskens & Lierz (2016)	*Alosa alosa* (Linnaeus, 1758)	Dordogne/Garonne River (France)	KU301052	Wünnemann et al. (2016)
H. anurae Mutschmann, 2004	*Hyperolius kivuensis* Ahl, 1931	Kakamega (Kenya)	KU141397	Alama-Bermejo et al. (2016)
H. carassii Achmerov, 1960,	*Carassius gibelio* (Bloch 1782)	Jihlava (Czech Republic)	KU141400	Alama-Bermejo et al. (2016)
H. cyprini (Doflein, 1898)	*Cyprinus carpio* Linnaeus, 1758	Jihlava (Czech Republic)	KU141402	Alama-Bermejo et al. (2016)
H. gnathonemi Alama-Bermejo, Jirků, Kodádková, Pecková, Fiala & Holzer, 2016	*Gnathonemus petersii* (Günther, 1862)	Nigeria, Africa (not exact location, fish obtained from pet shop).	KU141398	Alama-Bermejo et al. (2016)
H. gilsoni (Debaisieux, 1925)	*Anguilla anguilla* (Linnaeus, 1758)	Amhainnan Stratha Bhig River, Scotland (UK)	AJ582062	Holzer et al. (2004)
Hoferellus sp.	*C. carpio*	Chřeštóvice (Czech Republic)	KU141401	Alama-Bermejo et al. (2016)
Genus *Myxidium* Buetschli, 1882				
M. giardi (Cépede, 1906)	*A. anguilla*	Amhainnan Stratha Bhig River, Scotland (UK)	AJ582213	Holzer et al. (2004)
M. lieberkuhni Bütschli, 1882	*Esox lucius* Linnaeus, 1758	Europe	X76639	Schlegel et al. (1996)
M. streisingeri Whipps, Murray & Kent, 2015	*Danio rerio* (Hamilton, 1822)	Baltimore, Maryland (USA)	KM001685	Whipps et al. (2015)
Genus *Myxobilatus* Davis, 1944				
M. gasterostei (Parisi, 1912)	*Gasterosteus aculeatus* Linnaeus, 1758	Willamette River, Oregon (USA)	EU861210	Atkinson and Bartholomew (2009)
Genus *Ortholinea* Shulman, 1962				
O. auratae Rangel, Rocha, Borkhanuddin, Cech, Castro, Casal, Azevedo, Severino, Székely & Santos, 2014	*Limnodriloides agnes* Hrabě, 1967	Portimão, Algarve (Portugal)	KR025868	Rangel et al. (2015)
O. labracis Rangel, Rocha, Casal, Castro, Severino, Azevedo, Cavaleiro & Santos, 2017	*Dicentrarchus labrax* (Linnaeus, 1758)	Portimão, Algarve (Portugal)	KU363830	Rangel et al. (2017)
O. mullusi Gürkanlı, Okkay, Çiftçi, Yurakhno & Özer, 2018	*Mullus barbatus* Linnaeus, 1758	off Sinop, Black Sea (Turkey)	MF539825	Gürkanlı et al. (2018)
O. orientalis (Shulman & Shulman-Albova, 1953)	*Clupea harengus* Linnaeus, 1758	Øresund (Denmark)	HM770871	Karlsbakk and Køie (2011)
O. saudii Abdel-Baki, Soliman, Saleh, Al-Quraishy & El-Matbouli, 2015	*Siganus rivulatus* Forsskål & Niebuhr, 1775	off Jeddah, Red Sea, (Saudi Arabia)	JX456461	Abdel-Baki et al. (2015)
Ortholinea sp.	*Aequidens plagiozonatus* Kullander, 1984	Brazil	KP637274	Unpublished
Ortholinea sp	*A. alosa*	Dordogne/Garonne River (France)	KU301053	Wünnemann et al. (2016)

Table 1 (continued)

Species	Host	Locality	GenBank accession no.	Source
Ortholinea sp.	*S. rivulatus*	Israel	DQ333433	Unpublished
Genus *Zschokkella* Auerbach, 1909				
Zschokkella sp.	*A. anguilla*	Amhainnan Stratha Bhig River, Scotland (UK)	AJ581918	Holzer et al. (2004)

were excluded from the analysis. Phylogenetic analyses used included maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI). ML was implemented in RAxML v. 7.2.8 (Stamatakis et al. 2005), MP in PAUP v4.0 (Swofford 2002) and BI in MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003). GTR + I + G was the best-fit model according to jModeltest 2.1.10 (Darriba et al. 2012). ML and MP analyses were conducted using heuristic searches with random taxa addition and the TBR swapping algorithm. All characters were treated as unordered and gaps as missing data. Bootstrap support was calculated from 1000 replicates. For BI, posterior probabilities were calculated over 1000,000 generations via two independent runs of four simultaneous Markov chain Monte Carlo with every 200th tree saved. Burn-in was set to 100,000 generations. Identity of bases which are identical (%) were calculated with PAUP v4.0. The alignment was 5′ and 3′ ends trimmed and inserts of *Myxidium streisingeri* Whipps, Murray & Kent, 2015 (GenBank accession no. KM001684) were excluded.

Results

Ortholinea concentrica n. sp.

Taxonomic summary

Type host: *Acanthistius patachonicus* (Jenyns, 1840) (Perciformes: Serranidae), Patagonian seabass.

Type locality: Punta Verde, San Antonio Bay, San Matías Gulf (40°43′47”S, 64°54′45”W) Rio Negro, Argentina.

Site in host: urinary system (kidney, ureters and urinary bladder).

Prevalence: 30% (3/10) microscopic detection, 60% (6/10) molecular detection.

Etymology: named for the concentric pattern of ridges on the shell valve of the spores.

Material deposited: Invertebrate Collection of Museo de La Plata, FCNyM-UNLP, La Plata, Buenos Aires, Argentina (two Diff Quik stained slides of air-dried spores, Cat. No. MLP-Oi 4184 and 4185).

Molecular data: partial SSU rDNA sequence 1627 bp (GenBank Acc. Number MH793352).

Description

Myxospores typical of the genus *Ortholinea*, and abundant pre-sporogonic and early sporogonic stages were observed in the urinary bladder. No parasite stages were visually detected in the kidney smears or ureter, but only SSU DNA was detected (Table 2). No clinical signs were observed.

Description of the myxospores Based on 41 myxospores from the urinary bladders of two hosts and spores examined under SEM. Subspherical myxospores (Fig. 1a–f and 2) measured 8.9 ± 0.6 (8.2–11.0) × 8.7 ± 0.6 (7.9–11.0) (valvular view), and 8.3 ± 0.4 (7.7–9.0) in thickness (sutural view). Two valves, joined by transverse, straight suture, with 17 to 20 surface ridges partially organized concentrically, some bifurcated, occupying entire valve surface (Fig. 3). Different surface ridges organization observed: i) spiraling center near shell valve margin (Fig. 3a); ii) spiraling center at mid-level of shell valve (Fig. 3b); or iii) with two spiraling centers (Fig. 3c). Two subspherical polar capsules, 3.1 ± 0.3 (2.4–3.8) × 2.7 ± 0.2 (2.3–3.6). Polar capsule openings at anterior end (Fig. 3), each of them opening in a different valve in almost opposite directions. Polar tubule (filament) had 3 to 4 coils. Sporoplasm binucleate.

Description and localization of the plasmodia Based on 31 plasmodia from one host. Motile amoeboid round and pyriform plasmodia, 46.6 ± 23.4 (13.7–111.1) × 32.6 ± 13.6 (13.0–65.4), in lumina of urinary bladder (Fig. 1g–i). Polysporic plasmodia, spores developing in pairs. Profuse budding and/or long pseudopods in some plasmodia. Sometimes plasmodia clustered together (Fig. 1i).

Remarks

Ortholinea concentrica n. sp. is morphologically similar to *O. auratae* Rangel, Rocha, Borkhanuddin, Cech, Castro,

Table 2 *Ortholinea concentrica* n. sp. prevalence, as revealed by light microscopy and by PCR of urinary system of *Acanthistius patachonicus*, number of sequences, sequence length and GenBank accession number

Tissue	Prevalence-microscopy	Prevalence-PCR	Number of sequences	Sequences length and GenBank Acc. Number
Kidney	0/10	4/10 (40%)	3	1623 bp (MH793344), 1606 bp (MH793349), 1608 bp (MH793351)
Ureter	0/1	1/1	1	1094 bp (MH793345)
Urinary bladder	3/10 (30%)	6/8 (75%)	7	853 bp (MH793343), 1616 bp (MH793346), 1622 bp (MH793348), 938 bp (MH793347), 1623 bp (MH793350), 1099 bp (MH793353), 1627 bp (type - MH793352)

Casal, Azevedo, Severino, Székely & Santos, 2014, *O. mullusi* Gürkanlı, Okkay, Çiftçi, Yurakhno & Özer, 2018 and *O. labracis* Rangel, Rocha, Casal, Castro, Severino, Azevedo, Cavaleiro & Santos, 2017 (see Table 3). The new species could not be separated by morphology to *O. auratae* and *O. mullusi*, with only differences in spore thickness (7.7–9.0 vs 6.3–8.4 and 7.5–7.9). In contrast, *O. labracis* clearly differs from the new species by being smaller (6.8–8.7 × 6.7–7.7 vs 8.2–11.0 × 7.9–11.0) and thinner (5.8–7.7 vs 7.7–9.0). Additionally, *O. labracis* differs *O. concentrica* n. sp. in the number of coils of polar tubules (4 to 5 vs 3 to 4) (see Ben-David et al. 2016 for polar tubule term). While morphologically similar, the molecular data (see below), as well as the different geographic location and host, support the distinct species status of *O. concentrica* n. sp.

The new species can be differentiated from *O. saudii* Abdel-Baki, Soliman, Saleh, Al-Quraishy & El-Matbouli, 2015 in having narrower spores (7.9–11.0 vs 11.0–13.0), in the shape of the polar capsule (subspherical vs spherical), the maximum number of coils of polar tubule (4 vs 3) and the presence of ornamented shell valves in *O. concentrica* n. sp.

Spores of *O. orientalis* (Shulman & Shulman-Albova, 1953) have been described from several clupeids and gadids fishes (Shulman and Shulman-Albova 1953; Aseeva 2000, 2002; Karlsbakk and Køie 2011). The new species differ from *O. orientalis* ex *Clupea harengus* Linnaeus, 1758 (type host)

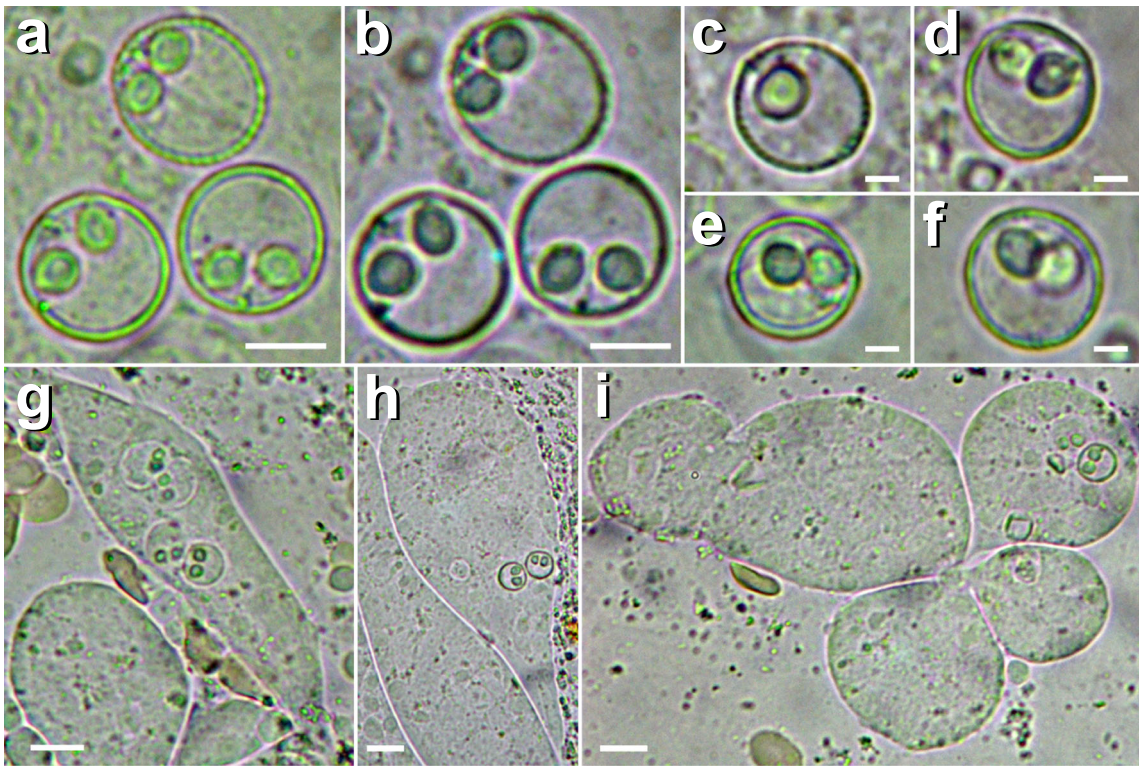

Fig. 1 *Ortholinea concentrica* n. sp. spores and plasmodia (LM) from the urinary bladder of *Acanthistius patachonicus*. a–b Three myxospores in valvular view at different depths. c Myxospore sutural view. d–f Myxospores showing overlapped polar capsules. e Apical view of a myxospore. g Plasmodia developing four spores. h Pyriform plasmodia with two spores. i Large plasmodia, with one main lobe and three smaller buds, one of them developing two spores. *Scale bar* a–b, 5 μm, c–f, 2 μm, g–i, 10 μm

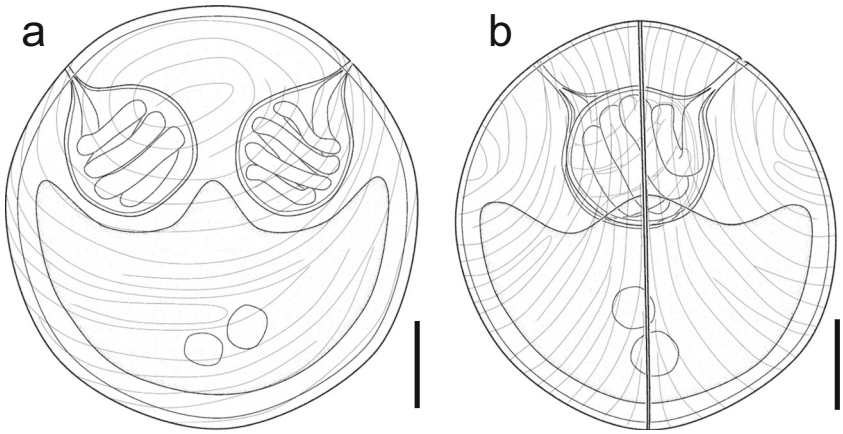

Fig. 2 Drawing of *Ortholinea concentrica* n. sp. spore from *Acanthistius patachonicus*. a Valvular view. b Sutural view. Surface ridges are schematically represented. *Scale bar* 2 μm

in the size (8.2–11.0 × 7.9–11.0 vs 7.5–8.5 × 7.5–7.6) and thickness (7.7–9.0 vs 5.1) of the myxospores. *O. concentrica* n. sp. can be differentiated from *O. orientalis* ex *Eleginus navaga* (Walbaum, 1792) by having thicker myxospore (7.7–9.0 vs 6.6–8.0). Measurements of the myxospores of *O. orientalis* from other fish hosts and descriptions (see Table 2 in Karlsbakk and Køie 2011) appear overlapped with those of *O. concentrica* n. sp. However, both species can be distinguished by their disparate geographical distribution (Denmark and Bering Sea vs Patagonia). No ornamentation of the shell valve was reported for *O. orientalis* by Shulman and Shulman-Albova (1953) and Aseeva (2000, 2002), although ridges were later observed by Karlsbakk and Køie (2011).

Previous to our description, *O. basma* was the only species of *Ortholinea* reported in South Atlantic. This species was described from the urinary bladder of *Clinus agilis* Smith, 1931 from tide pools in the Atlantic coast of South Africa (Ali 2000). *O. basma* can be distinguished from *O. concentrica* n. sp. in its distinctly larger spores (12.0–

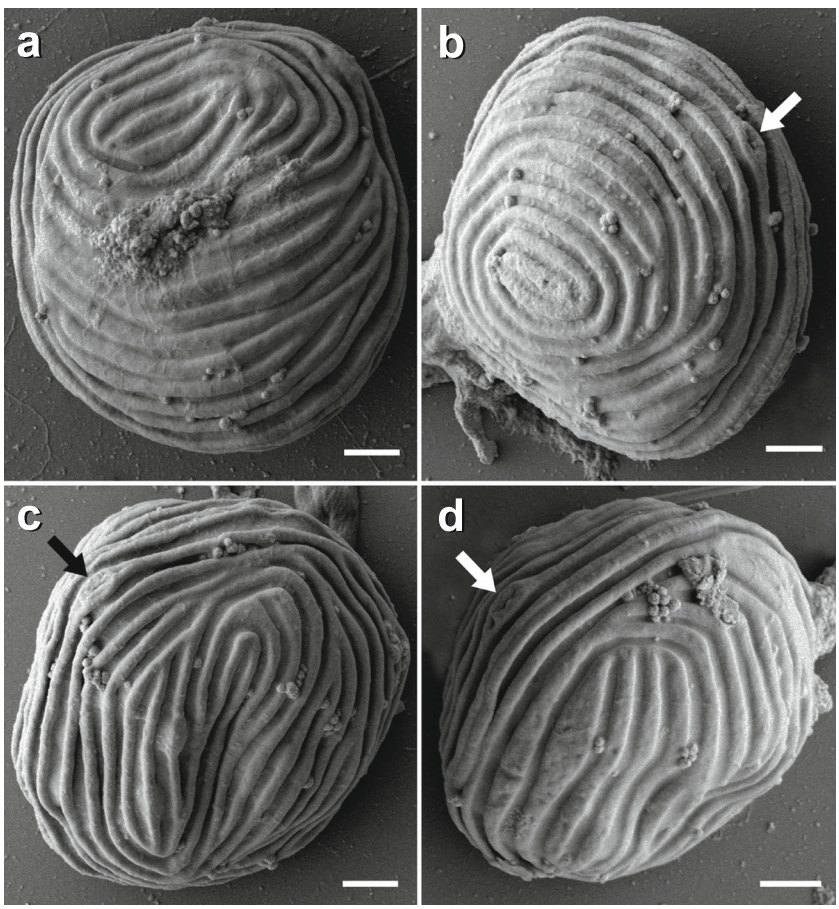

Fig. 3 Scanning electron micrographs of *Ortholinea concentrica* n. sp. spores from the urinary bladder of *Acanthistius patachonicus*. a–d External ornamentation showing different ridge arrangements on the shell valves. a Spiraling center near shell valve margin. b Spiraling center at mid-level of shell valve. c Spore with two spiraling centers. Arrow points the polar capsule opening. *Scale bar* 1 μm

Table 3 Comparison of *Ortholinea* spp. host, localities, spore, 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 coils

Species	Reference	Host	Locality	SPL	SPW	SPT	Ridges	PCS	PCL	PCW	PTC
O. orientalis	Shulman and 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*	–	2.2-2.9/3.0–4.2	–	–
O. undulans	Meglitsch 1970	*Arnoglossus scapha* (Forster, 1801) / *Perthorhamphus novaezeelandiae* 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 and Dyková 1995	*Dichotomyctere fluviatilis* (Hamilton, 1822)	Southeast Asia	7.9–8.4	7.3–8.0	6.8	Yes, –	Subspherical	2.8–3.3	–	4–6
O. basma	Ali 2000	*Clinus agilis* Smith, 1931	South Africa	12.0–15.0	11.8–13.0	–	Yes, 12–13	Pyriform	4.0–4.8	3.0–4.3	4–5
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. saudii	Abdel-Baki et al. 2015	*Siganus rivulatus* Forsskål & Niebuhr, 1775	Saudi Arabia	9–11	11–13	–	No	Spherical	4.0–5.0	–	3
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. mullusi	Gürkanlı et al. 2018	*Mullus barbatus* Linnaeus, 1758	Turkey	9.0–9.7	8.2–9.3	7.5–7.9	Yes, –	Pyriform	3.0–3.2	2.4–2.6	3–4
O. concentrica n. sp.	Present study	*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

*Ridges were observed later by Karlsbakk and Køie (2011)

15.0 × 11.8–13.0 vs 8.2–11.0 × 7.9–11.0), the shape of the polar capsules (pyriform vs subspherical), and in the number of ridges on the surface of the valves (12–13 vs 17–20).

Currently, most *Ortholinea* spp. have been described with ornamented shell valves, including both marine and freshwater species (Thélohan 1895; Davis 1917; Shulman and Shulman-Albova 1953; Naidenova 1968; Meglitsch 1970; Wierzbicka 1986; Lom et al. 1992; Kovalyova et al. 1993; Padma and Kalavati 1993; Su and White 1994; Lom and Dyková 1995; Ali 2000; Moshu and Trombitsky 2006; Abdel-Ghaffar et al. 2008; Rangel et al. 2014, 2017; Gürkanlı et al. 2018). However, only in *O. auratae*, *O. australis* Lom, Rohde & Dyková, 1992, *O. basma* and *O. concentrica* n. sp. the valve ornamentation was described in detail using SEM photomicrographs. Lom and Dyková (1995) provided diagrammatic schemes for the ridges arrangement of *O. fluviatilis* Lom & Dyková, 1995, which are similar to the patterns observed in *O. concentrica* n. sp. The new species has a similar number of surface ridges (i.e., between 17 to 20) to *O. auratae*, *O. undulans* (Meglitsch, 1970) and *O. striateculus* Su & White, 1994. Other species of *Ortholinea*, such as *O. africanus* Abdel-Ghaffar, El-Toukhy, Al-Quraishy, Al-Rasheid, Abdel-Baki, Hegazy & Bashtar, 2008, *O. australis* and *O. basma* differs from the new species in having fewer surface ridges (between 10 to 16). In *O. australis*, surface ridges were described as 1 to 3 circular ridges + either smooth rest of the shell valve or 5 to 9 longitudinal ridges, evidencing different ornamentations patterns (see Lom et al. 1992).

Molecular results

Partial SSU rDNA sequences obtained in this study are listed in Table 2. Eleven isolates were obtained from *O. concentrica* n. sp. from different parts of the urinary system from six hosts. All sequences were identical except for two urinary bladder isolates (ME3UB GenBank Acc. Numb. MH793343 - 853 bp and ME7UB GenBank Acc. Numb. MH793350 - 1623 bp) that showed an intraspecific variability of 0.3–0.5% (2 bp of a 837 bp alignment).

Interspecific SSU rDNA identity to other members of the urinary freshwater clade (715 bp alignment) revealed high variability. *O. concentrica* n. sp. showed the lowest sequence divergence to *O. labracis* (17.2%), *Acauda hoffmani* Whipps, 2011 (17.5%) and *Myxobilatus gasterostei* (Parisi, 1912) (17.9%), followed by *O. mullusi* (18.1%), *O. auratae* (18.2%) and *Ortholinea* sp. ex *Aequidens plagiozonatus* Kullander, 1984 (18.3%).

All the phylogenetic analyses placed the new species in a clade containing other marine species of *Ortholinea* (i.e., *O. labracis*, *O. auratae* and *O. mullusi*), and *A. hoffmani*, *M. gasterostei* and the freshwater *Ortholinea* sp. ex *A. plagiozonatus* (see Fig. 4). The relationship between *O. concentrica* n. sp. and *Ortholinea* sp. ex *A. plagiozonatus* was not stable and was not observed in all tree topologies or had < 50% support.

The analysis of interspecific SSU sequence distances (Fig. 5) revealed large differences in the minimum sequence dissimilarity between *Ortholinea* spp. and other members of the freshwater urinary bladder clade. Three different groups were observed: (i) high minimum interspecific distance (> 30%), *O. saudii* and *Ortholinea* sp. ex *Siganus rivulatus* Forsskål & Niebuhr, 1775; (ii) medium minimum interspecific distance (10–20%), *O. concentrica* n. sp. and *Ortholinea* sp. ex *A. plagiozonatus*; (iii) low minimum interspecific distance (< 10%), *O. auratae*, *O. labracis*, *O. mullusi*, *O. orientalis* and *Ortholinea* sp. ex *Alosa alosa* (Linnaeus, 1758).

Discussion

To date, all *Ortholinea* spp. with molecular data available are marine taxa, except for two undescribed species: *Ortholinea* sp. ex *A. alosa*, from an anadromous fish, and *Ortholinea* sp. ex *A. plagiozonatus*, from a freshwater fish. Interestingly, all sequenced taxa morphologically identified as belonging to *Ortholinea* clustered in the oligochaete-freshwater urinary tract clade. This situation is not uncommon: almost one quarter of species in the freshwater lineage were obtained from marine hosts and habitats (Holzer et al. 2018).

Different genera cluster in the oligochaete-freshwater urinary tract clade and *Ortholinea* is not the only polyphyletic genera in the clade (Fig. 4). One of its representatives, the genus *Hoferellus* Berg, 1898 was divided in two: *Hoferellus,* sensu stricto, containing the type species *H. cyprini* (Doflein, 1898), and *Hoferellus* sensu *lato*, which contains all other *Hoferellus* species that show phylogenetic affinities to other genera in the clade (see Alama-Bermejo et al. 2016; Wünnemann et al. 2016; Matos et al. 2018). Unfortunately, *O. divergens* (Thélohan, 1895), the type species of *Ortholinea*, has not been sequenced yet, so no further divisions can be defined at the present time.

The SSU rDNA distances revealed a slightly different grouping than the one observed in the phylogenetic analysis, probably influenced by the high divergence (long branch attraction) of the clade formed by *Ortholinea* spp. infecting *S. rivulatus*. The analysis of the distances seemed to support some relationship between *O. concentrica* n. sp. and *Ortholinea* sp. ex *A. plagiozonatus*. A possible affinity could be due to some relative geographical proximity, since both taxa were collected in South America (Videira 2015).

The traditional spore-based taxonomy and SSU rDNA phylogeny have limitations on defining the relationships within members in this heterogenous clade (Fiala et al.

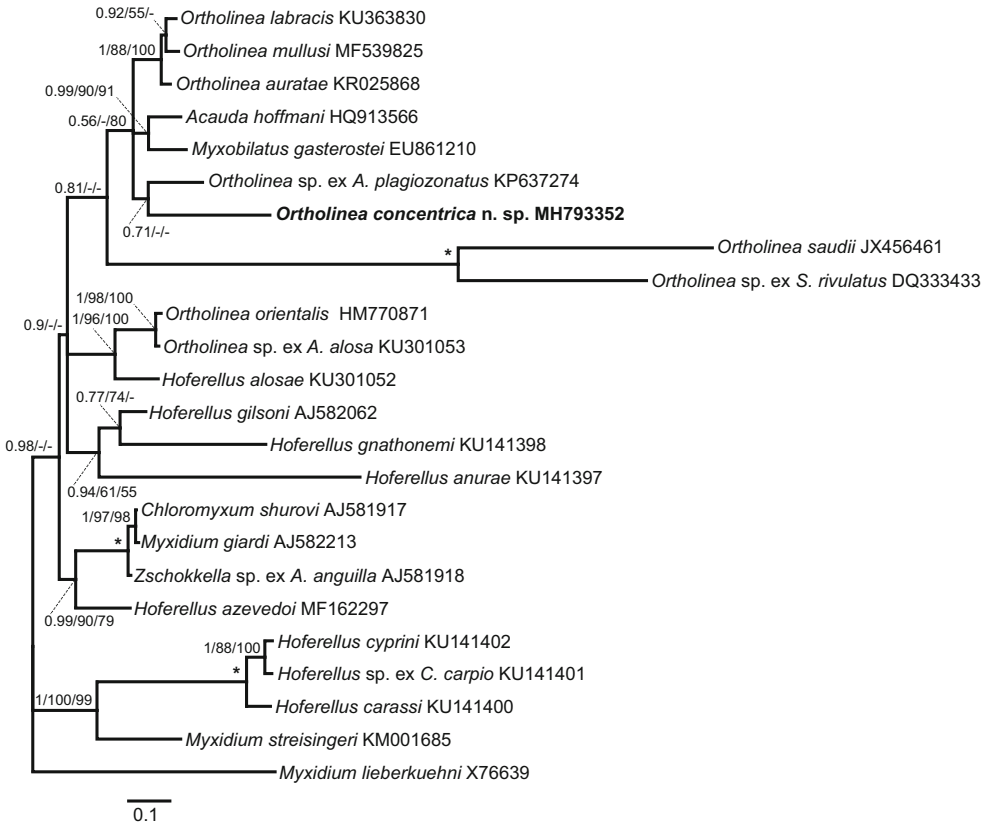

Fig. 4 Bayesian inference (BI) tree showing the phylogenetic position of *Ortholinea concentrica* n. sp. within the freshwater urinary bladder clade as defined by Fiala (2006). *Myxidium lieberkuehni* was used as outgroup. Numbers at nodes represent Bayesian posterior probability and bootstrap values (BI/ML/MP). Dashes at nodes represent nodal support BI < 0.5 and MP/ML < 50 or node not present in the maximum parsimony or maximum likelihood trees. The newly generated sequence is indicated in bold. Asterisk indicates a node with maximum nodal supports (BI = 1, MP/ML = 100)

2015a), that contains both marine and freshwater species, in fish and amphibians vertebrate hosts (i.e., *H. anurae* Mutschmann, 2004) and up to seven different myxospore morphologies/genera (Fiala 2006; Karlsbakk and Køie 2011; Whipps 2011). The only unifying criteria to date for this clade are oligochaete as invertebrate host (Groβheider & Körting 1992; El-Matbouli et al. 1992; Benajiba & Marques 1993; Yokoyama et al. 1993; Trouillier et al. 1996; Holzer et al. 2006; Atkinson & Bartholomew 2009; Rangel et al. 2015, 2017) and tissue tropism, urinary tract. Future studies on *O. concentrica* n. sp. should focus on identifying the invertebrate host, probably a marine oligochaete in the intertidal zone.

Using a combination of morphological and molecular data, we reported a new species *Ortholinea concentrica* n. sp. from the urinary system of Patagonian seabass. To the best of our knowledge, there are no previous records of a myxozoan parasite infecting the Patagonian seabass. *O. concentrica* n. sp. is the first species of *Ortholinea* described in Argentina and the second *Ortholinea* species in the South Atlantic.

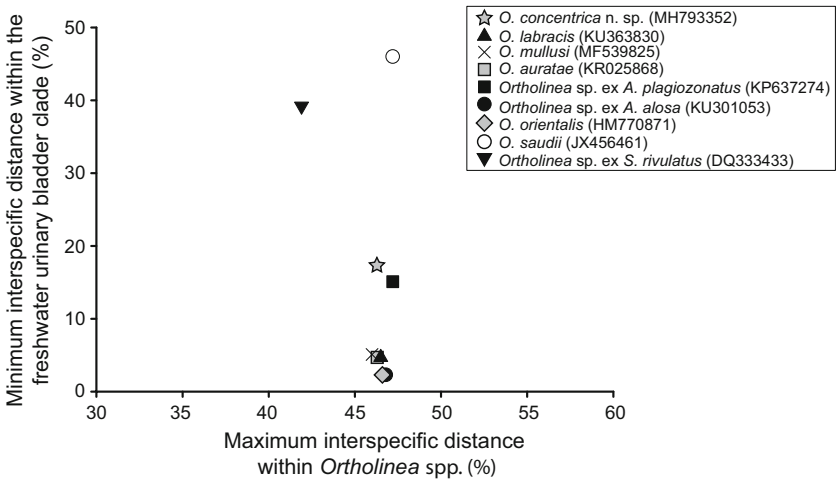

Fig. 5 Graphic interpretation of the SSU rDNA minimum interspecific distances (dissimilarities) between *Ortholinea* spp. and other members of the freshwater urinary bladder clade plotted against maximum interspecific distances within *Ortholinea* spp.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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