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# Morphology and molecular phylogeny of *Ortholinea mullusi* sp. nov. (Myxozoa) in *Mullus barbatus* from the Black Sea

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ABSTRACT: Myxosporeans of the genus *Ortholinea* have a worldwide distribution and infect organs and tissues of exclusively marine fishes. Here we describe the morphological and molecular characteristics of *Ortholinea mullusi* sp. nov. parasitizing the urinary bladder and kidney tubules of red mullet *Mullus barbatus* collected from the coastal zone of Sinop in the Black Sea, Turkey. Polysporic plasmodia with immature spores were either elongate, 37.0 ± 4.5 SD (30–50) µm long and 45.0 ± 3.8 (40–55) µm wide, or were round, up to 100.0 µm in diameter. Mature, free spores were spherical in the frontal view and measured 9.3 ± 0.2 (9.0–9.7) µm in length, 8.7 ± 0.3 (8.2–9.3) µm in width and 7.7 ± 0.1 (7.5–7.9) µm in thickness. We observed 2 polar capsules of equal size, which measured 3.1 ± 0.1 (3.0–3.2) µm long by 2.5 ± 0.1 (2.4–2.6) µm wide, and the tips of the polar capsules were open towards the sutural line. The prevalence of infection by *O. mullusi* sp. nov. was 24.5%. Phylogenetic analysis based on nuclear small subunit ribosomal DNA (SSU rDNA) clearly suggested *O. mullusi* to be a new species, clustered within a lineage comprising *O. labracis* and *O auratae*. Pairwise nucleotide similarities and DNA distance values between *O. mullusi* sp. nov. and sister *Ortholinea* species also supported this suggestion.

KEY WORDS: *Ortholinea* · Red mullet · Black Sea · Myxozoa · Phylogeny



## INTRODUCTION

Myxozoan parasites of fishes have a cosmopolitan distribution. Among these, the genus *Ortholinea* Shul'man, 1962 includes 22 nominal species which are mostly coelozoic in the excretory system of mainly marine fishes (Lom & Dyková 1992, Rangel et al. 2014, 2015, 2017). Members of this genus are described as spherical to subspherical with a prominent sutural ridge and containing 2 subspherical to pyriform polar capsules and a binucleate sporoplasm (Lom & Dyková 2006). Most members of this genus have external striations or ridges on their shell valves (Ali 2000).

The red mullet *Mullus barbatus* L., 1758 (Perciformes: Mullidae) is a teleost fish of great economic value in the North Atlantic, Mediterranean Sea and Black Sea (Debenedetti et al. 2013). This benthic species inhabits sandy and muddy bottoms of the continental shelf (Hureau 1986). In Turkey, the red mullet is one of the main target species in small- and large-scale fishery due to its commercial value (Özbilgin et al. 2004). Despite several studies conducted on the parasites of this species, mainly along the Mediterranean coasts (Carreras-Aubets et al. 2011, 2012, Debenedetti et al. 2013), studies on myxosporean parasites in *M. barbatus* yielded only 2 species, namely *O. orientalis* and *Fabespora nana*,

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in the Black Sea (Yurakhno 1993, 1994, Özer et al. 2015a,b).

Here we describe the morphological and molecular identification of a new myxosporean parasite species found in *M. barbatus* collected from the coast of the Black Sea in the Sinop Province of Turkey.

## MATERIALS AND METHODS

### Sampling and microscopy

Samples of red mullet (n = 200) collected from the Sinop coast of the Black Sea (42° 05′ 68″ N, 35° 10′ 55″ E) during September 2015 and March 2016 were investigated for myxosporean parasites. Gills, fins, skin, urinary bladder, kidney, gall bladder, intestine and gonads were examined using a light microscope at 400× and 1000× magnifications. Parasite species were studied in detail using an Olympus microscope (BX53) equipped with a digital camera (DP50) and a differential interference contrast attachment. Measurements were based on 30 fresh spores, and morphological terminology used in the descriptions follows the definitions of Lom & Dyková (1992). Some of the fresh smears were treated with 5% KOH solution for the extrusion of polar filaments. All measurements include the mean SD value along with the range of variation in parentheses. Prevalence of infection was determined according to Bush et al. (1997), and the intensity of infection of myxozoans was semiquantitatively evaluated following a scale from + to ++++++, based on the number of myxosporean parasites per microscopic field at 300× magnification, as described by Alvarez-Pellitero et al. (1995). This protocol was modified for 200× magnification: (+) 1–5; (++) 6–10; (+++) 11–25; (++++) 26–50; (+++++) 51–100; (++++++) >100. All applicable international, national and institutional guidelines for the care and use of animals were followed.

### Molecular analyses

Total genomic DNA was isolated from infected urinary bladder tissue of *Mullus barbatus* using an Invitrogen PureLink® Genomic DNA Mini Kit (USA). Extracted genomic DNA was stored at −20°C prior to use. As a genetic marker, we used the small subunit of nuclear ribosomal DNA (nuclear SSU rDNA) because nuclear SSU rDNA sequences of different *Ortholinea* species are already available in GenBank for comparison. To eliminate the host SSU rDNA, we used the parasite-specific primers OrthoF1 (Karlsbakk & Køie 2011) and Ortho_Int_Rew (this study, 5′-CCA ACC ACG AGC ATT TCW A-3′), in addition to the universal primers SR-1 (Nakayama et al. 1996) and NS-8 (White et al. 1990) for PCR amplifications. A Techne (TC-Plus) thermal cycler was used for PCR amplifications as per the conditions given in Table 1. A 50 µl PCR reaction was prepared using genomic DNA (50 ng), 1.5 mM $MgCl_2$, 1.25 U *Taq* polymerase (New England BioLabs), 2.5 mM dNTP mix (Thermo Scientific), 5 µl of 10× PCR buffer, 0.5 pmol (final concentration) of each primer and $ddH_2O$. The PCR products were stained with ethidium bromide and visualized using a Vilber Lourmat Imaging System. Nucleotide sequencing was performed commercially with the same primers used for PCR amplifications.

To assemble the sequences from both strands, BioEdit (Hall 1999) was employed. For analyses we created a nuclear SSU rDNA data set using the *Ortholinea* species showing the highest Basic Local Alignment Search Tool (BLAST) similarity with the new *Ortholinea* species described here, and multiple nucleotide sequence alignments were performed using ClustalX (Thompson et al. 1997). To determine the best fitting evolutionary model(s), Akaike's information criterion (AIC) (Akaike 1974) and Bayesian information criterion (BIC) tests were performed using the jModelTest v. 0.1 package (Guindon & Gascuel 2003, Posada 2008). To construct the phyloge-

Table 1. PCR conditions used for amplification of nuclear small subunit ribosomal DNA (SSU rDNA) of *Ortholinea mullusi* sp. nov. ID: initial denaturation; C: number of PCR cycles; D: denaturation; A: annealing; E: extension; FE: final extension

| Gene | Primer | ID | | C | D | | A | | E | | FE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | °C | Time (min) | | °C | Time (min) | °C | Time (min) | °C | Time (min) | °C | Time (min) |
| 18S rDNA | SR1[a], Ortho_Int_Rew[b] | 95 | 3 | 35 | 94 | 1 | 50 | 1 | 72 | 1.5 | 72 | 10 |
| 18S rDNA | OrthoF1[c], NS8[d] | 95 | 3 | 35 | 94 | 1 | 59 | 1 | 72 | 1.5 | 72 | 10 |

[a]Nakayama et al. (1996). [b]This study. [c]Karlsbakk & Køie (2011). [d]White et al. (1990)

nies, neighbor-joining (NJ, Saitou & Nei 1987), maximum parsimony (MP, Eck & Dayhoff 1966, Fitch 1977) and maximum likelihood (ML) algorithms were employed. Both NJ and MP analyses were performed using the software program PAUP* v. 4.0b10 (Swofford 1998). A heuristic search approach using the TBR swapping algorithm with 10 random repetitions was performed for MP analyses. ML analysis was conducted using PhyML 3.0 software (Guindon & Gascuel 2003). To test the reliability of the trees, bootstrap tests (Efron 1982, Felsenstein 1985) were run with 10 000 pseudoreplicates for NJ and 1000 pseudoreplicates for MP and ML analyses. BioEdit was used to calculate the nucleotide sequence similarities. The nuclear SSU rDNA sequence of the new *Ortholinea* isolate was submitted to GenBank under accession number MF539825.

## RESULTS

### Taxonomic summary

Name: *Ortholinea mullusi* sp. nov. (Fig. 1).

Type host: Red mullet *Mullus barbatus* L, 1758 (Perciformes: Mullidae).

Type locality: Coast of Sinop, Black Sea, Turkey (42° 02′ 51″ N, 35° 02′ 56″ E).

Site of infection: Urinary bladder and kidney tubules.

Type material: One holotype (MyxoOM 2017.1) and one paratype (MyxoOM 2017.2) were deposited in the Sinop University, Faculty of Fisheries and Aquatic Sciences Parasitological Collection, Sinop, Turkey.

Etymology: The specific epithet 'mullusi' recalls the name '*Mullus*', the genus of the host fish species in which this parasite was found.

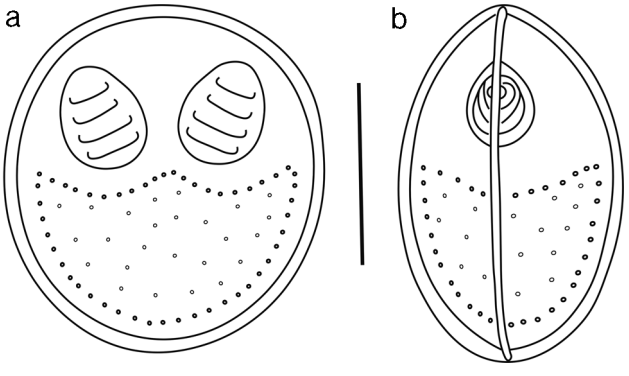



Fig. 1. *Ortholinea mullusi* sp. nov. mature spore. (a) frontal and (b) sutural view. Scale bar = 5 µm

### Description

#### Vegetative stages

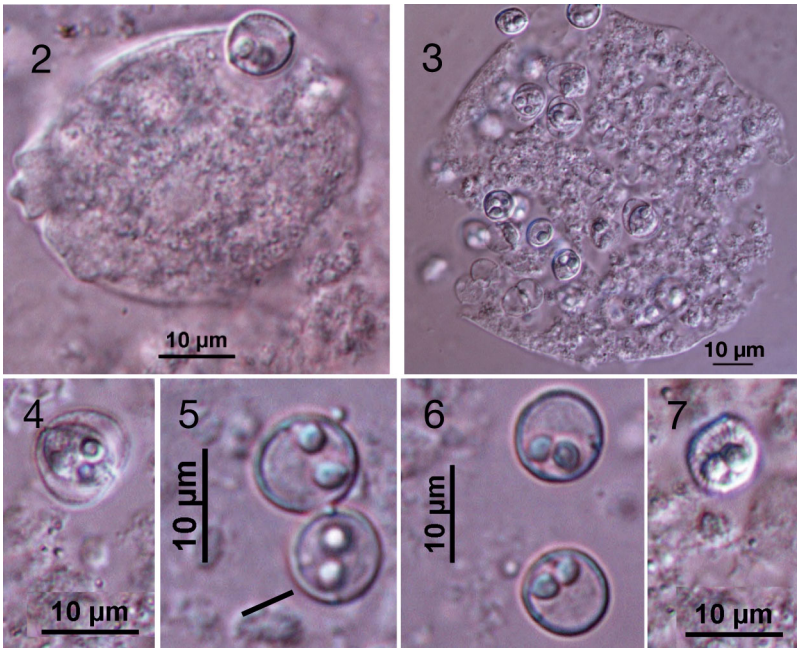
Numerous polysporic plasmodia with many either immature or developing spores were observed. Spores are either elongate, measuring 37.0 ± 4.5 (30–50) µm long by 45.0 ± 3.8 (40–55) µm wide, or they are round, up to 100.0 µm in diameter (Figs. 2 & 3).

#### Spores

Immature and developing spores are irregular in valvular view, but contain polar capsules (Fig. 4). Mature, free spores are spherical in frontal view, with rounded anterior and posterior poles (Fig. 5), and ellipsoidal in sutural view (Fig. 6). Spore surfaces have external ridges (Fig. 7). Polar capsules are pyriform and equal in size (Figs. 5 & 6). Tips of the polar capsules open towards the sutural line. Polar filaments are coiled with 3–4 turns. Extended length of polar filaments is about 10–12 µm. Spore body measures 9.3 ± 0.2 (9.0–9.7) µm in length by 8.7 ± 0.3 (8.2–9.3) µm in width and 7.7 ± 0.1 (7.5–7.9) µm in thickness. Polar capsules are 3.1 ± 0.1 (3.0–3.2) µm long by 2.5 ± 0.1 (2.4–2.6) µm wide.

### Remarks on differential diagnosis

A comparison of spore characters of *O. mullusi* sp. nov. with that of *O. labracis* and *O. auratae* (Rangel et al. 2014, 2017) shows that *O. mullusi* sp. nov. has ellipsoidal spores in sutural view, while spores of *O. labracis* and *O. auratae* are more rounded and smaller in all dimensions. Moreover, polar capsules of *O. mullusi* sp. nov. are larger than those of *O. labracis* but smaller than those of *O. auratae*. Polar filaments of *O. mullusi* sp. nov. have 3–4 turns while those of *O. labracis* have 5 turns. *O. divergens* from rusty blenny *Parablennius sanguinolentus* (Özer et al. 2015b) have smaller polar capsules and shorter, but wider, spores compared to *O. mullusi* sp. nov. When compared with *O. basma* from agile klipfish *Clinus agilis* (Ali 2000), *O. mullusi* sp. nov. differs in the organization of striations on the spore body and in having fewer polar filament turns. Further, the present species differs from *O. basma* in having smaller spores and polar capsules. *O. saudii* from marbled spinefoot *Siganus rivulatus* (Abdel-Baki et al. 2015) possess large polar capsules, more polar filament coils and large, subspherical, somewhat triangular spores when com-




Figs. 2–7. *Ortholinea mullusi* sp. nov. parasitizing the urinary bladder and kidney of *Mullus barbatus*. Figs. 2 & 3. Plasmodia of different sizes in the urinary bladder and kidney of the host fish. Fig. 4. Immature spore. Fig. 5. Mature spores in the urinary bladder (arrow indicates apical view). Fig. 6. Mature spores in the kidney (frontal view). Fig. 7. External ridges on the spore body

pared to *O. mullusi* sp. nov. Spore and polar capsule dimensions of *O. mullusi* sp. nov. are larger than those of *O. orientalis*, the only other *Ortholinea* species reported from *M. barbatus* (Özer et al. 2015a). Spores of *O. gobiusi* from round goby *Neogobius melanostomus* (Özer et al. 2015b) and *O. antipae* from Caspian shad *Alosa caspia* (Moshu & Trombitsky 2006) differ from *O. mullusi* sp. nov. in the dimensions of the spores and polar capsules and in having pointed posterior regions. In addition, polar capsules of *O. antipae* are rounded and smaller, whereas those of *O. mullusi* sp. nov. are pyriform and larger. *O. mullusi* sp. nov. can be differentiated from *O. irregularis* (Kabata 1962), *O. alata* (Kent & Moser 1990) and *O. striateculus* (Su & White 1994) based on the dimensions of the spores and polar capsules. Spores of *O. mullusi* sp. nov. are shorter than those of *O. gadusiae* (Sarkar 1999), whereas polar capsule width and size are larger than those of *O. gadusiae*.

### Molecular analyses

We sequenced approximately 1850 bp of the nuclear SSU rDNA locus of our isolate AO-2 (*O. mullusi* sp. nov.). In the BLAST search, in addition to other members of the genus *Ortholinea*, *O. mullusi* sp. nov. showed significant similarities with myxosporeans belonging to the genera *Myxobilatus*, *Hoferellus* and *Zschokkella*. We established a data set for phylogenetic analyses using these sequences. Phylogenetic analyses of our data set were performed using 1573 aligned nucleotides with 482 segregating sites. As a result of the model test, AIC and BIC values suggested TIM2+G (G: 0.224) and TPM2uf+G (G: 0.222) as substitution models, respectively. The NJ (Fig. 8) and ML trees created with the TPM2uf+G model were considered in this study because they gave higher bootstrap values. MP analysis produced a single most parsimonious tree with 1096 steps (consistency index [CI]: 0.746; retention index [RI]: 0.774, homoplasy index [HI]: 0.254). In all phylogenetic trees created using NJ (Fig. 8), MP and ML algorithms, the topology was almost the same except for the position of *H. gilsoni* and *Zschokkella* sp., but this difference did not affect the relationship of *O. mullusi* sp. nov. with its sister *Ortholinea* species. In all phylogenetic trees, *O. mullusi* sp. nov. appeared as a sister to *O. labracis* with 95.8% nucleotide sequence similarity. This relationship was supported with 88, 73 and 87% bootstrap values in the NJ, MP and ML trees, respectively. *O. auratae* appeared as a sister to the lineage comprising *O. labracis* and *O. mullusi* sp. nov. with 100% bootstrap values in all trees. Nucleotide sequence similarity between *O. mullusi* sp. nov. and *O. auratae* was 94.8%. *M. gasterostei* was the closest relative to the lineage above, with 98, 99 and 100% bootstrap values in the NJ, MP and ML trees, respectively. The 2 taxa that exhibited topological differences between phylogenetic trees, i.e. *H. gilsoni* and *Zschokkella* sp., grouped within the same lineage along with *O. mullusi* sp. nov. *O. labracis*, *O. auratae* and *M. gasterostei* in the NJ (Fig. 8) and ML trees but grouped with *O. orientalis* in the MP tree.

### Prevalence and intensity of *O. mullusi* sp. nov

Of the 200 red mullet specimens screened, 49 individuals (24.5%) were infected by *O. mullusi* sp. nov. with an intensity of +++ per infected host. Plasmodia

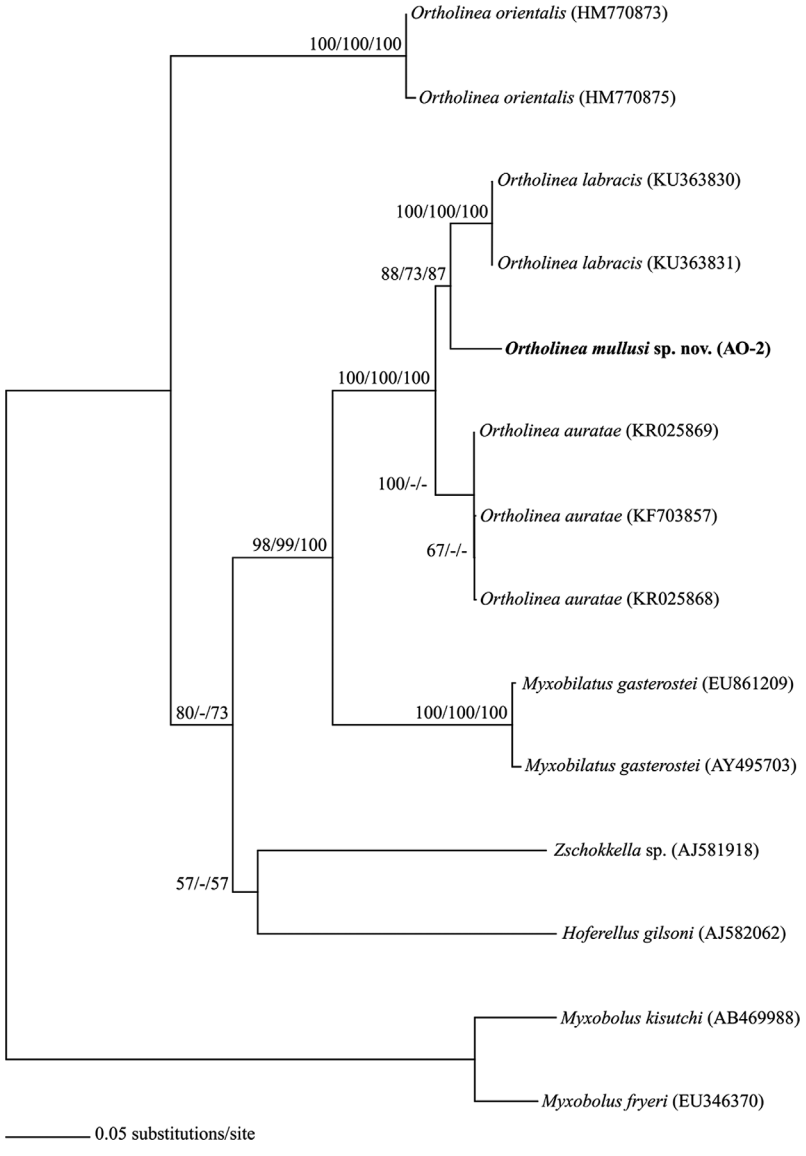



Fig. 8. Neighbor-joining tree showing the phylogenetic relations between *Ortholinea mullusi* sp. nov. (isolate AO-2) and related species of Myxozoa downloaded from GenBank (given with GenBank accession numbers, see Table A1 in the Appendix). The tree was created with the TPM2uf+G substitution model and is rooted with *Myxobolus kisutchi* and *M. fryeri*

and spores of myxosporeans were found floating free in the urine. No clinical signs were observed in parasitized hosts.

## DISCUSSION

Members of the genus *Ortholinea* have a worldwide distribution and infect exclusively marine fish species. The total number of *Ortholinea* species described to date is only 22 out of the 2280 species of myxosporeans reported from fishes (Lom & Dyková 1992, Rangel et al. 2014, 2015, Whipps et al. 2015). However, there has been an increase in the number of newly identified *Ortholinea* species in recent years (Rangel et al. 2014, 2017, Abdel-Baki et al. 2015). Studies on myxosporean parasites of mullid fish are very limited (Yurakhno 1993, 1994, Özer et al. 2015a), and only 2 species of myxosporean parasites have so far been reported from *Mullus barbatus* in the Black Sea. One of these is *O. orientalis* (Özer et al. 2015a); thus, *O. mullusi* sp. nov. represents the second report of an *Ortholinea* species from *M. barbatus*.

Members of the genus *Ortholinea* infect mainly the ureter, urinary bladder, kidney and gall bladder of host fishes. Most of the *Ortholinea* species reported, including the new species described herein, infect the urinary bladder (Table 2). The overall prevalence of 24.5% observed in the present study, however, falls between the ranges reported by several authors for other *Ortholinea* species from various fish species elsewhere (Table 2).

Myxosporean taxonomy is primarily based on morphology and spore structure, and we have therefore classified the present myxosporean under the genus *Ortholinea* based on specific morphological criteria. On the other hand, some authors contend that traditional classification appears artificial, may not be consistent and does not reflect phylogenetic relationships, and have suggested that other biological features, such as the life cycle, morphology of actinosporean stages, host specificity and infection site tropism should also be taken into account (Fiala 2006, Shin et al. 2014). We therefore used nuclear SSU rDNA as a molecular marker for the construction of the phylogenetic tree. Phylogenetic analyses based on nuclear SSU rDNA placed *O. mullusi* sp. nov. alongside other members of the genus *Ortholinea*, within the clade of freshwater Myxozoa that infect the urinary tract (Rangel et al. 2017). On the other hand, members of other genera, including *Myxobilatus*, *Hoferellus* and *Zschokkella*, also appeared in the lineage, indicating

Table 2. Site of infection, hosts, geographical localities and dimensions (µm, ±SD) of species of the genus *Ortholinea* found in marine fish. PFC: number of polar filament coils. –: no data

| Species | Spore body | | | Polar capsule | | | PFC | Site of infection | Prevalence (%) | Host species | Locality | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Length | Width | Thickness | Length | Width | Diameter | | | | | | |
| *Ortholinea mullusi* sp. nov. | 9.3 ± 0.2 (9.0–9.7) | 8.7 ± 0.3 (8.2–9.3) | 7.7 ± 0.1 (7.5–7.9) | 3.1 ± 0.1 (3.0–3.2) | 2.5 ± 0.1 (2.4-2.6) | – | 3–4 | Urinary bladder, kidney | 24.5 | *Mullus barbatus* | Black Sea coast, Sinop, Turkey | This study |
| *O. labracis* | 7.6 ± 0.3 (6.8–8.7) | 7.2 ± 0.2 (6.7–7.7) | 6.5 ± 0.4 (5.8–7.7) | 3.0 ± 0.2 (2.6–3.4) | 2.4 ± 0.1 (2.0–2.9) | – | 4–5 | Urinary bladder, kidney | 11.0 | *Dicentrarchus labrax* | Alvor estuary, near the Atlantic coast, Portugal | Rangel et al. (2017) |
| *O. auratae* | 9.0 ± 0.3 (8.2–10.1) | 8.3 ± 0.4 (7.5–9.1) | 7.2 ± 0.5 (6.3–8.4) | 3.2 ± 0.1 (2.9–3.6) | 2.7 ± 0.1 (2.4–2.9) | – | 3–4 | Urinary bladder, kidney | 51.6 | *Sparus aurata* | Alvor estuary, near the Atlantic coast, Portugal | Rangel et al. (2014) |
| *O. saudii* | 10 ± 0.4 (9–11) | 12 ± 0.5 (11–13) | – | – | – | 4.5 ± 0.3 (4.0–5.0) | 3 | Kidney | 5.0 | *Siganus rivulatus* | Red Sea coast, Jeddah, Saudi Arabia | Abdel-Baki et al. (2015) |
| *O. basma* | 13.5 ± 1.0 (12.0–15.0) | 12.3 ± 0.5 (11.8–13.0) | – | 4.3 ± 0.3 (4.0–4.8) | 3.5 ± 0.5 (3.0–4.3) | – | 4–5 | Urinary bladder | 16.6 | *Clinus agilis* | Port Nolloth, South Africa | Ali (2000) |
| *O. orientalis* | 7.3 (7.1–7.5) | 7.0 (6.9–7.2) | 6.2 (6.0–6.4) | 2.7 (2.6–2.9) | 2.2 (2.1–2.3) | – | – | Urinary bladder | 33.3 | *Mullus barbatus* | Black Sea coast, Sinop, Turkey | Özer et al. (2015a) |
| *O. orientalis* | 7.4 (7.2–7.6) | 7.2 (7.0–7.4) | 6.2 (6.1–6.4) | 2.8 (2.7–3.0) | 1.9 (1.8–2.0) | – | – | Urinary bladder | 2.5 | *Alosa tanaica* | Black Sea coast, Sinop, Turkey | Özer et al. (2015a) |
| *O. orientalis* | 7.8 | 6.0 | – | 2.5 | 2.1 | – | – | Kidney, urinary and gall bladder | – | *Clupea* spp. | Northern Pacific | Shul'man & Shul'man-Albova (1953) |
| *O. gobiusi* | 8.3 (7.5–8.6) | 7.2 (6.8–7.5) | – | 4.9 (4.6–5.1) | 2.0 (1.9–2.2) | – | – | Urinary bladder | 4.1 | *Neogobius melanostomus* | Black Sea coast, Sinop, Turkey | Özer et al. (2015b) |
| *O. gobiusi* | 8.8 | 8.4 | – | 1.9 | 1.9 | – | – | Urinary bladder | – | *Gobius ophiocephalus* | Black Sea | Lom & Dyková (1992) |
| *O. divergens* | 9.0 (8.1–9.4) | 9.2 (8.4–9.7) | – | 2.0 (1.9–2.2) | 2.2 (1.9–2.4) | – | – | Urinary bladder | 2.7 | *Parablennius sanguinolentus* | Black Sea coast, Sinop, Turkey | Özer et al. (2015b) |
| *O. divergens* | 9.2 | 9.4 | – | 2.0 | 2.4 | – | – | Urinary bladder | 2.7 | *Hippoglossoides platessoides* | North Atlantic | Shul'man (1966) |
| *O. irregularis* | 10.6 (8.0–11.0) | 7.1 (6.0–9.0) | – | 2.2 | 2.2 | – | – | Urinary bladder | – | *Drepanopsetta platessoides* | North Sea | Kabata (1962) |
| *O. alata* | 12.6 | 9.6 | – | 4.6 | 4.6 | – | – | Kidney tubules | – | *Chaetodon rainfordi* | Australia | Kent & Moser (1990) |
| *O. striateculus* | 10.1 | 10.0 | – | 3.5 | 2.9 | – | – | Ureters | – | *Leptatherina presbyteroides* | Australia | Su & White (1994) |
| *O. gadusiae* | 10.8 (9.0–11.7) | 8.0 (7.2–9.0) | – | 3.0 (2.3–3.2) | No data | – | – | Urinary bladder | – | *Gadusia chapra* | Bay of Bengal, India | Sarkar (1999) |
| *O. antipae* | 6.8–7.5 | 5.0–5.4 | – | 1.8–2.5 | No data | – | 3–4 | Urinary bladder | 34.8 | *Alosa caspia* | Black Sea | Moshu & Trombitsky (2006) |

a paraphyly in the genus *Ortholinea* (Fig. 8). *O. mullusi* sp. nov. appeared as a sister to *O. labracis* and showed 95.8% nucleotide sequence similarity, which is much lower than intraspecific sequence similarities of related *Ortholinea* species (*O. orientalis*: 99.6%; *O. labracis*: 100%; *O. auratae*: 99.8%). This result indicates that *O. mullusi* sp. nov. is diverged enough from the closest species (*O. labracis*) to be considered as a separate new species.

To date, many morphological studies of myxosporean parasites have been performed in Black Sea fishes. The present study provides detailed morphological and molecular descriptions of a new species of the genus *Ortholinea*, namely *O. mullusi*, occurring in the urinary bladder and kidney tubules of red mullet *M. barbatus.*

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**Appendix.** Additional data for phylogenetic analyses

Table A1. Myxozoan species used in phylogenetic analyses

| Species | Host | Locality | GenBank acc. no. | Source |
|---|---|---|---|---|
| *Ortholinea mullusi* (AO-2) | *Mullus barbatus* | Turkey | MF539825 | This study |
| *Ortholinea orientalis* | *Clupea harengus* | Denmark | HM770873 | Karlsbakk & Køie (2011) |
| *Ortholinea orientalis* | *Sprattus sprattus* | Denmark | HM770875 | Karlsbakk & Køie (2011) |
| *Ortholinea labracis* | *Dicentrarchus labrax* | Portugal | KU363830 | Rangel et al. (2017) |
| *Ortholinea labracis* | *Tectidrilus* sp. | Portugal | KU363831 | Rangel et al. (2017) |
| *Ortholinea auratae* | *Limnodriloides agnes* | Portugal | KR025869 | Rangel et al. (2015) |
| *Ortholinea auratae* | *Sparus aurata* | Portugal | KF703857 | Rangel et al. (2017) |
| *Ortholinea auratae* | *Sparus aurata* | Portugal | KR025868 | Rangel et al. (2015) |
| *Myxobilatus gasterostei* | *Nais cummunis* | USA | EU861209 | Atkinson & Bartholomew (2009) |
| *Myxobilatus gasterostei* | *Gasterosteus aculeatus* | Germany | AY495703 | Hallett et al. (unpubl.) |
| *Zschokkella* sp. | *Anguilla anguilla* | UK | AJ581918 | Holzer et al. (2004) |
| *Hoferellus gilsoni* | *Anguilla anguilla* | UK | AJ582062 | Holzer et al. (2004) |
| *Myxobolus kisutchi* | *Oncorhynchus kisutch* | USA | AB469988 | Urawa et al. (2009) |
| *Myxobolus fryeri* | *Oncorhynchus kisutch* | USA | EU346370 | Ferguson et al. (2008) |

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