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Parvicapsula curvatura n. sp. in cultured olive flounder *Paralichthys olivaceus* and phylogenetic characteristics of the genus *Parvicapsula*

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ABSTRACT: *Parvicapsula curvatura* n. sp. (Myxozoa; Bivalvulida) was found in the urinary bladder of olive flounder *Paralichthys olivaceus* cultured in a fish farm on Jeju Island, ROK. When laterally viewed, the parasite has asymmetrical curved spores that measure 9.6–11.6 µm in length. Furthermore, it has 2 subspherical polar capsules at the apex. Based on the phenotypical traits, it is most similar to *P. limandae* but differs in the shape of polar capsule, locality, and host specificity (family level). BLAST analysis indicated that *P. curvatura* was closest to *P. unicornis* and *P. petuniae* via 18S and 28S rDNA sequences, respectively. The 18S rDNA from *P. curvatura* was used in molecular phylogenetic analyses of *Parvicapsula* spp. to examine the congruence of phylogeny with spore morphology, locality, and host specificity. The results demonstrated that the spore morphotype was correlated with the phylogeny of the genus *Parvicapsula*, and the parasites have speciated into an oblong and semicircular spore type.

KEY WORDS: *Parvicapsula curvatura* · Spore morphology · Locality · Host specificity

INTRODUCTION

Myxosporeans are parasites that infect vertebrates (mainly fish) and invertebrates (annelids). These parasites evolved from a cnidarian ancestor (Chang et al. 2015) and diverged into 3 main distinctive lineages: marine, freshwater, and *Sphaerospora* sensu stricto (Fiala 2006, Bartosová et al. 2013). The marine and freshwater lineages constitute clades of species according to infection site such as the gall bladder, urinary system, and various tissues (Fiala et al. 2015). The genus *Parvicapsula* mainly infects the urinary system of marine and anadromous fishes (Lom & Dyková 2006), but some species have been reported in the gall bladder, intestine, and pseudobranch (Kovaleva & Gaevskaya 1981, Dorothy & Kalavati 1993, Karlsbakk et al. 2002). *Parvicapsula* species occasionally recognized as potential pathogens include *P. minibicornis* and *P. pseudobranchicola*, both of which cause mortality in salmonid fish (Sterud et al. 2003, Bradford et al. 2010). Furthermore, *P. anisocaudata* infections can occasionally cause opaque urine with white spots in olive flounder *Paralichthys olivaceus* (Zhao et al. 2000).

Olive flounder is an important fish species in Korean aquaculture. However, the olive flounder aquaculture industry has suffered from parasitic diseases and infections of myxosporeans involving *Enteromyxum leei*, *Kudoa septempunctata*, and *P. anisocaudata*, which cause food poisoning and economic losses (Yasuda et al. 2005, Jung et al. 2012, Kawai et al. 2012, Song et al. 2013, Kim et al. 2015, Sekiya et al. 2016). To prevent and control parasitic diseases, the parasites must be exactly identified and classified based on morphological or molecular data. The Fish Vaccine Research Center of Jeju National

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University, ROK, has been monitoring parasitic infections of olive flounder cultured on Jeju island and has isolated a novel *Parvicapsula* species from the fish samples. The aim of the present study was to identify this new *Parvicapsula* species and to determine the relationship between its phylogenetic position and biological characteristics such as host, spore morphotype, and locality.

MATERIALS AND METHODS

Parasite samples and partial purification

Olive flounder samples (n = 15; total length = 32.8 ± 9.5 cm) were obtained from 5 olive flounder farms on Jeju island. The fish showed a distension of the urinary bladder with urine that was aseptically extracted using a syringe. The urine suspensions were filtered using a 40 µm cell strainer and then centrifuged at 10 000 × *g* (3 min at 20°C). The pellets were resuspended in lysis buffer (RIPA, Merck) for 5 min and the suspensions centrifuged at 10 000 × *g* (1 min at 20°C). The supernatant was removed and the pellets resuspended in phosphate-buffered saline. The samples were collected and preserved at 4°C.

Morphological identification

The urine suspensions and partially purified parasites were wet mounted and observed under a light microscope and photographed at 1000× magnification. Myxospores were measured from 20 individual spores using the ImageJ image-processing program (http://rsb.info.nih.gov/ij/) according to Karlsbakk et al. (2002).

Molecular identification and phylogenetic analysis

DNA was extracted from partially purified parasites using the AccuPrep Genomic DNA Extraction Kit (Bioneer) following the manufacturer's instructions. Portions of the 18S and 28S rDNA were amplified by PCR using a combination of primers designed by the authors (ParviK500F, ParviK950R, ParviK1800F, and ParviK2700F) and other sources (18e, ERIB 10, Myxo28S1F[mo], NLF1050, NLR1694[mo], 28S3R, and NLR3113[mo]) (Table 1). PCR was performed with the following cycling parameters: initial denaturation at 95°C for 5 min; followed by 35 cycles at 95°C for 30 s, 58°C for 30 s, 72°C for 60 or 90 s; and a final extension at 72°C for 7 min. PCR products were treated with an AccuPrep Genomic PCR Purification Kit (Bioneer) to remove excess primers and dNTPs and were then directly sequenced with BigDye™ Terminator v3.1 in an ABI 3730xl Sequencer. Multiple alignments of the 18S rDNA sequence were carried out by Clustal X 2.0 (Larkin et al. 2007) with homologous sequences of other *Parvicapsula* species based on similar spore morphotypes. Pairwise sequence distances and similarities of the *Parvicapsula* spp. based on 18S rDNA were calculated in MEGA 7.0 (Kumar et al. 2016) and Clustal Omega (Sievers et al. 2011). Bayesian inference (BI) was used to reconstruct the phylogenetic tree from datasets containing 13 sequences of the 18S rDNA from *Parvicapsula* spp. with *Tetracapsuloides bryosalmonae* (KF731712), which was used as an outgroup. Ambiguously aligned regions in 18S rDNA datasets were removed using Gblocks v0.91b (Castresana 2000) under default parameters, allowing up to half of the taxa to have gaps. For BI analysis, nucleotide substitution models were selected using Akaike's information criterion (AIC), and the Bayesian information criterion (BIC)

Table 1. Oligonucleotide primers used for molecular analysis in this study

Target	Name	Sequence (5′–3′)	Position	Reference
18S rRNA	18e	CTG GTT GAT CCT GCC AGT	3	Hillis & Dixon (1991)
	ERIB10	CTT CCG CAG GTT CAC CTA	1733	Barta et al. (1997)
	ParviK500F	GGT GAG CCA CTG GTT CAC TAT	550	This study
	ParviK950R	CGG ACA CTG ACC GTT TGA	950	This study
28S rRNA	Myxo28S1F(mo)[a]	AGT AAC TGC GAG TGA AGC G	100	Bartosová et al. (2009)
	NLF 1050	AAT CGA ACC ATC TAG TAG CTG G	1050	Bartosová et al. (2009)
	NLR1694(mo)[a]	GTT AGG CAA TGG CTT AGG ACC	1674	Bartosová et al. (2009)
	ParviK1800F	CGT TGT GTC GGA TTG CAG TG	1880	This study
	ParviK2700F	CCA AAG CCA TGC CGT AAA C	2900	This study
	28S3R	GAG CAC TGG GCA GAA ATC	2490	Whipps et al. (2004b)
	NLR 3113(mo)[a]	GTC TAA ACC CAG CTC ACG TTC	3310	Bartosová et al. (2009)

[a]Primer sequence was slightly modified in the present study

implemented in jModeltest 2.1.7 (Guindon & Gascuel 2003, Darriba et al. 2012), GTR + I + G and TrN + G were chosen as the best-fit nucleotide substitution models for the 18S rDNA data sets. The metropolis-coupled Markov chain Monte Carlo (MCMC) algorithm implemented in MrBayes 3.2.4 (Ronquist et al. 2012) was performed for a sufficient number of generations until the average standard deviation of the split frequencies was <0.05. The sampling frequency was set at every 100 generations for 1 000 000 samples. The first 100 000 samples from each run were discarded as burn-in, and the remaining were analyzed using the 'sumt' command in MrBayes. Gaps were treated as missing data. A consensus tree was created using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The association between the phylogeny and biological traits was tested using a Bayesian MCMC approach implemented in Bayesian tip-association significance testing (BaTS) v0.1.1 (Parker et al. 2008). This program analyzes the posterior set of 9000 trees generated by MrBayes 3.2.4, accounting for phylogenetic error (Ronquist et al. 2012). Correlations for the presence of the phylogenetic traits (morphological differences, cyst/pseudocyst formation, and infection site tropism) were assessed using the parsimony score, association index, and maximum monophyletic clade size, and the complete list of sequences is shown in Table S1 in the Supplement at www.int-res.com/articles/suppl/d130p199_supp.pdf. The null hypothesis of random phylogenetic trait associations was rejected at a significance level of 0.05 ($p < 0.05$).

RESULTS

Description of disporic plasmodia and mature spores

Disporic plasmodia and mature spores were found in the urine suspension; spherical disporogonic plasmodia had 2 spores, 9–12 µm in length (Fig. 1A). The spores were asymmetrical (curved) in the lateral view (Fig. 1B,C) and measured (mean ± SD) 10.5 ± 0.6 µm (range: 9.6–11.6 µm) in length and 4.9 ± 0.6 µm (3.9–5.8 µm) in thickness, whereas the spores had an elliptical shape in the medial plane (Fig. 1D). A suture line passed between polar capsules and along lateral sides to the convex side, which was a distance from the posterior pole, and then went across the bottom to the apex (Figs. 1E,F & 2). Furthermore, the sporoplasm was binucleate. Two sub-

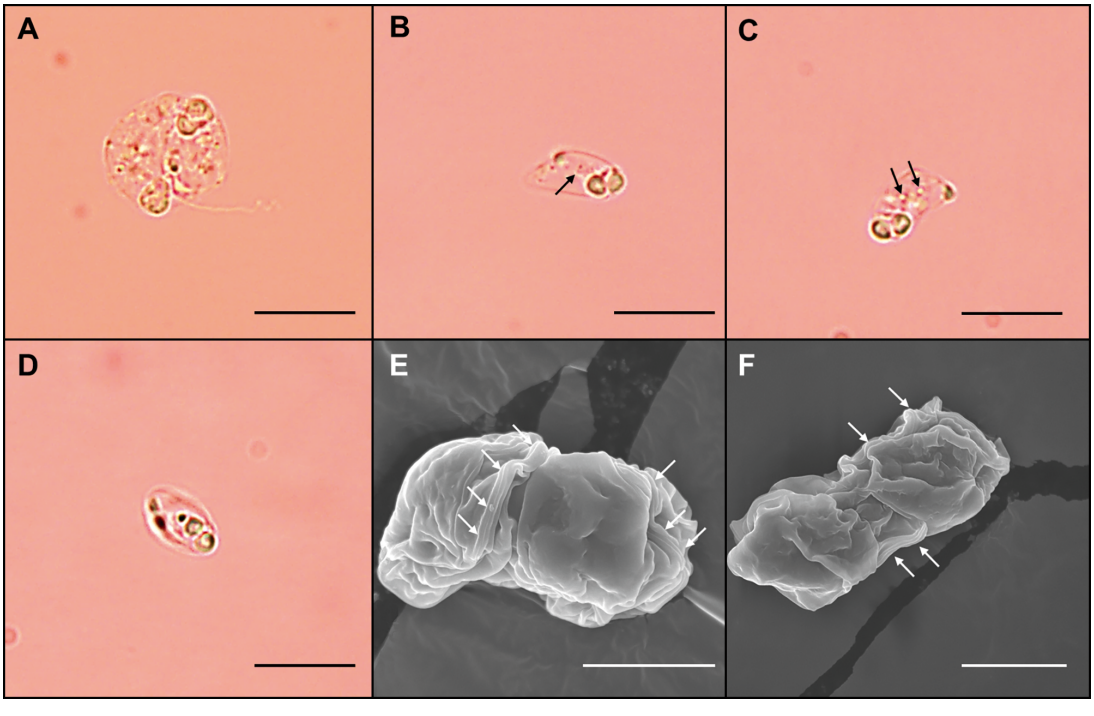

Fig. 1. *Parvicapsula curvatura* n. sp. in the urinary bladder of *Paralichthys olivaceus*. (A) Disporic plasmodium. (B,C) Mature spores with sutural line (B, black arrow) and 2 circular nuclei (C, arrows). (D) Mature spore in frontal view, seen from the convex side. (E,F) Scanning electron microscopy images of mature spores with sutural line (white arrows) in the lateral and frontal views, as observed from the concave side. Scale bars = (A–D) 10 µm, (E,F) 5 µm

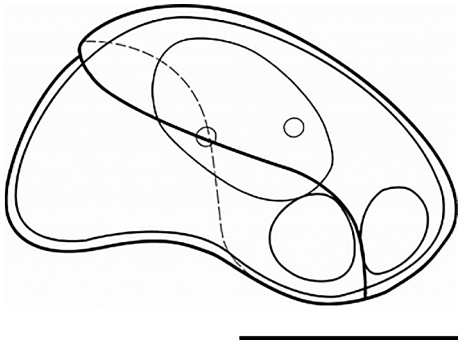

Fig. 2. Line drawing of a *Parvicapsula curvatura* spore. Scale bar = 5 µm

spherical capsules, with a diameter of 4.3 ± 0.4 µm (3.8–5.0 µm), were arranged anteriorly side by side and were open at the concave side.

According to the shape and size of the spores, 4 species, *Parvicapsula pseudobranchicola* (Karlsbakk et al. 2002), *P. spinachiae*, (Køie 2003), *P. kabatai* (Jones et al. 2006), and *P. limandae* (Køie et al. 2007), exhibited similarities to the present species (Table 2). *P. limandae* was most similar in spore shape, size, and host specificity (Order: Pleuronectiformes). However, the isolate in this study had subspherical (ovoidal) polar capsules, whereas *P. limandae* has spherical polar capsules. In addition, there were differences in locality (Asia vs. Europe) and at the family level of host specificity (Paralichthyidae vs. Pleuronectidae). *P. spinachiae* was similar in spore shape and size; however, there were differences in the shape of the polar capsule (subspherical vs. spherical), locality (Asia vs. Europe), and at the order level of host specificity (Pleuronectiformes vs. Gasterosteiformes). *P. pseudobranchicola* and *P. kabatai* have different host specificities (order: Salmoniformes) and spore size and locality. In addition, *P. pseudobranchicola* infects mainly pseudobranchs.

Molecular identification and phylogenetic analysis

Partial sequences of the 18S rDNA (1763 bp) and 28S rDNA (3310 bp) were obtained from the isolate and had 100% similarities with GenBank accession numbers MF161398 and MF161399, respectively. A BLAST search indicated that this sequence differed from all available sequences in GenBank. The analysis of Max Score by BLAST indicated that the 18S rDNA gene of *P. unicornis* (AY584190) and the 28S rDNA gene of *P. petuniae* (KF874223) were the most similar sequences. The sequence of the isolate that

Table 2. Comparison of *Parvicapsula curvatura* with other *Parvicapsula* spp. that have a similar spore morphotype. Length and thickness of spores are given as mean (±SD) (range) in µm. Pyriform polar capsule size is given as mean length × width, and spherical polar capsule size is given as mean diameter (range), in µm

Species	Host	Spore total length	Spore thickness	Spore shape	Polar capsule	Infection site	Locality	Reference
P. curvatura	*Paralichthys olivaceus*	10.5 ± 0.6 (9.6–11.6)	4.9 ± 0.6 (3.9–5.8)	Semicircular	2.2 × 1.6	Urinary bladder	Jeju island, ROK	Present study
P. pseudobranchicola	*Salmo salar*	12.4 (11.1–13.8)	6.2 (5.2–7.3)	Semicircular	2.4 (2.1–2.9)	Pseudobranch	Lyngen, Norway	Karlsbakk et al. (2002)
P. spinachiae	*Spinachia spinachia*	10 (9–12)	5 (4–7)	Semicircular	1.5	Kidney, urinary bladder	Øresund, Denmark	Køie (2003)
P. kabatai	*Oncorhynchus gorbuscha*	12.3 (10.7–14.0)	6.0 (4.9–8.0)	Semicircular	1.8 (1.2–2.2)	Kidney	British Columbia, Canada	Jones et al. (2006)
P. limandae	*Limanda limanda*	9.5 (8.3–10.8)	4.7 (4.3–5.4)	Semicircular	1.5–1.6	Kidney, urinary bladder	Øresund, Denmark	Køie et al. (2007)

differed from the aligned sequences of *Parvicapsula* species had a similar spore shape available in GenBank at the 1620 nucleotide alignments and had a maximum genetic similarity of 90.4%, being closest to *P. limandae* (Table 3). Based on the morphological and molecular identification, the myxospores provided compelling evidence that this is a new species of *Parvicapsula.*

Parvicapsula curvatura n. sp. taxonomic summary

Host: Olive flounder *Paralichthys olivaceus* (Pleuronectiformes; Paralichthyidae)
Locality: Olive flounder culture farm, Jeju self-governing province, Republic of Korea (33° 16′ N, 126° 40′ E)
Site of infection: Urinary bladder
Date of sampling: January and February 2018
Host size: 22–51 cm
Prevalence: 8 of 15 fish examined were infected (53.3%)
Type material: Diff-Quik stained smears were deposited in the parasitological collection of the Fish Vaccine Research Center, Jeju National University, under accession number PCFVRC20180301A.
Etymology: The specific epithet refers to the shape of the spore, which is curved.
Diagnosis: The morphology of *P. curvatura* n. sp. is similar to that of *P. limandae*, *P. spinachiae*, *P. pseudobranchicola*, and *P. kabatai*. However, it differs from *P. limandae* and *P. spinachiae* by having a different polar capsule shape (subspherical vs. spherical) and from *P. pseudobranchicola* and *P. kabatai* by having a smaller spore size (9–11 vs. 11–14 µm). In addition, *P. curvatura* differs from *P. anisocaudata*, found occasionally together in the urinary bladder of olive flounder, by having a different spore shape (semicircular type vs. oblong type) and a smaller spore size (9–11 vs. 12–15 µm).

The phylogenetic tree divided 2 groups, and *P. curvatura* was clustered with *P. kabatai*, *P. spinachiae*, *P. limandae*, *P. asymmetrica*, *P. pseudobranchicola*, and *P. unicornis* (Fig. 3). Based on the BaTS result, the spore morphotype of the *Parvicapsula* spp. showed a significant relationship in the phylogenetic tree, whereas no significant differences were observed for locality and host specificity (Table 4; Tables S2 & S3).

DISCUSSION

We previously isolated a myxosporean similar to *Sinuolinea capsularis* from urine samples of cultured olive flounder and obtained partial sequences of 18S and 28S rDNA from the samples. The sequences were deposited in GenBank under accession numbers MF161398 and MF161399. However, the sequences showed high similarity with *Parvicapsula* spp. but not *Sinuolinea* spp. In addition, we identified sequences that were also amplified in the urine of olive flounder samples that did not contain parasites such as *S. capsularis* (data not shown). Interestingly, Dyková et al. (2013) also reported an 18S rDNA sequence of *Parvicapsula* sp. (accession number JX460907) that apparently belongs to an undetermined *Parvicapsula* species that was not observed in the material examined with light microscopy. In the present study, we isolated a new *Parvicapsula* sp. that was morphologically different from *P. anisocaudata*, well known from cultured olive flounder on Jeju Island, and obtained 18S and 28S rDNA sequences from the parasite. The sequences have 100% similarity with MF161398 and MF161399.

The traditional approach to classifying myxosporeans uses phenotypic differences, such as spore morphology and infection site tropism (Whipps et al. 2004b, Lom & Dykova 2006, Kodádková et al. 2015). The genus *Parvicapsula* was defined by previous

Table 3. Genetic distance (p-distance; below diagonal) and percent sequence similarity (%; above diagonal) obtained from the distance matrix based on a 1620 bp 18S rDNA sequence of *Parvicapsula curvatura* (present isolate) with *Parvicapsula* species with similar spore morphotype. GenBank accession numbers are shown in parentheses

	P. curvatura (MF161398)	*P. limandae* (EF429096)	*P. pseudobranchicola* (AY308481)	*P. unicornis* (AY584190)	*P. spinachiae* (AY431928)	*P. kabatai* (DQ515821)
P. curvatura (MF161398)		90.4	89.2	88.5	88.0	88.3
P. limandae (EF429096)	0.103		95.2	92.0	86.9	85.2
P. pseudobranchicola (AY308481)	0.120	0.054		90.9	87.4	86.9
P. unicornis (AY584190)	0.121	0.084	0.088		65.8	87.4
P. spinachiae (AY431928)	0.124	0.137	0.134	0.133		89.4
P. kabatai (DQ515821)	0.127	0.156	0.138	0.130	0.101	

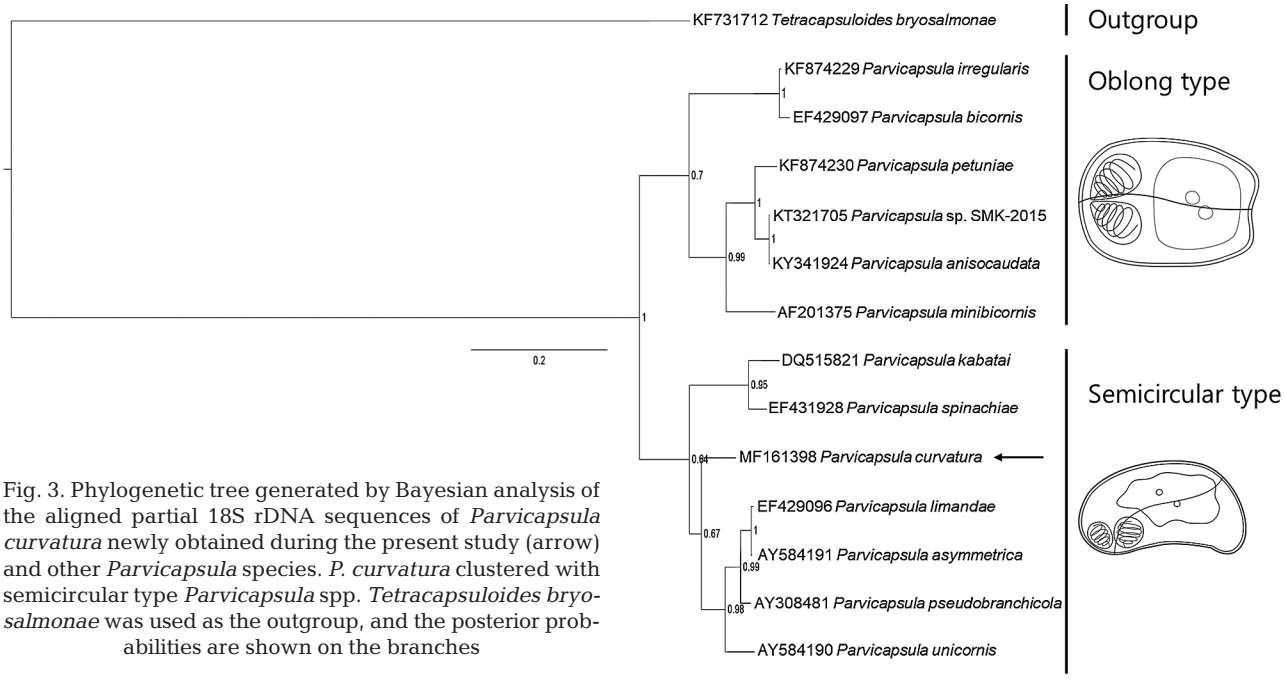

Fig. 3. Phylogenetic tree generated by Bayesian analysis of the aligned partial 18S rDNA sequences of *Parvicapsula curvatura* newly obtained during the present study (arrow) and other *Parvicapsula* species. *P. curvatura* clustered with semicircular type *Parvicapsula* spp. *Tetracapsuloides bryosalmonae* was used as the outgroup, and the posterior probabilities are shown on the branches

Table 4. Significant associations between 18S rDNA sequence phylogeny and morphotype of *Parvicapsula* spp. identified by Bayesian tip-association significance testing. AI: association index; PS: parsimony score; MC: monophyletic clade

Statistic	Observed mean	Lower 95% CI	Upper 95% CI	Null mean	Lower 95% CI	Upper 95% CI	Significance
AI	0.001	0.000	0.003	0.908	0.443	1.347	0.000
PS	2.174	2.000	3.000	7.697	5.665	9.615	0.000
MC (semicircular type)	6.144	4.000	7.000	1.959	1.000	3.971	0.001
MC (oblong type)	5.335	4.000	6.000	1.679	1.000	2.995	0.001
MC (outgroup)	1.000	1.000	1.000	1.000	1.000	1.000	1.000

studies (Shulman 1953, Køie 2003, Lom & Dyková 2006) as follows: myxosporeans with spores elongated, asymmetrical, and somewhat curved. Additional characteristics include 2 small spherical to pyriform polar capsules at the anterior end of the spores, 1 relatively large binucleate sporoplasm, and disporic trophozoites coelozoic in the urinary system or histozoic in kidneys. However, some *Parvicapsula* spp. were not in accordance with this definition (Køie 2003). Interestingly, the BaTS result suggested that spore morphotype is an important factor to identify and classify *Parvicapsula* spp. (Table 4). A previous study suggested the possibility that spore morphology of the genus *Parvicapsula* correlates with genetic relatedness (Nylund et al. 2005), and we support the correlation by dividing the spore morphotype into semicircular and oblong types from a lateral perspective. The semicircular type has curved spores, whereas the oblong type does not. *P. curvatura* was included with the semicircular type. A relationship between the biological traits and phylogenetic clusters was also observed in another myxosporean group, genus *Kudoa* (Shin et al. 2016). In addition, myxobolid-infected cyprinids also have particular phylogenetic characteristics (Shin et al. 2014), although not as strict as genus *Parvicapsula* and *Kudoa.* Therefore, spore morphotype is an important factor to identify and classify myxosporeans as well as to investigate the speciation and evolution of these parasites.

Previous phylogeographical studies of myxozoans reported that intraspecific differences depend on the geographical distribution of *Tetracapsuloides bryosalmonae*, *Myxobolus cerebralis*, *K. thyrsites*, and *P. minibicornis* (Henderson & Okamura 2004, Whipps et al. 2004a, Whipps & Kent 2006, Atkinson et al. 2011). We investigated the relationship between locality and species of *Parvicapsula* based on the 18S

rDNA phylogeny. The BaTS result showed a significant association among *Parvicapsula* spp. found in Asia and Europe, whereas there were no significant associations for the parasites found in North America (Table S2). It is therefore difficult to claim that there is a significant relation between locality and phylogeny of *Parvicapsula* spp. because the p-value of the parasite found in Europe is only marginally significant ($p = 0.048$), and only 2 and 3 species found in North America and Asia, respectively, were investigated. In addition, *P. pseudobranchicola* was detected in North America (Miller et al. 2014). Other various factors should also be considered, because the phylogeography of the parasite is related to the distribution and speciation of the host as well as to the host specificity of the parasite.

Host specificity is an important factor used to characterize myxozoans. The host specificity of most myxozoan species is narrow, with the parasites able to develop only in a single or closely related host species. However, some myxozoans (typically marine species), such as *K. thyrsites* and *Enteromyxum leei*, have broad host ranges (Burger & Adlard 2011, Yanagida 2017). In the present study, the BaTS result revealed no significant association between the host specificity (order level) and phylogeny of *Parvicapsula* spp. (Table S3). In addition, we found a co-infection of *P. curvatura* with other myxosporeans such as *P. anisocaudata* (Parvicapsulidae), *Sinuolinea* sp. (Sinoulineidae), *Myxodavisia* sp. (Sinoulineidae), and *Ortholinea* sp. (Ortholineidae) in the olive flounder samples (data not shown). Interestingly, the freshwater myxosporeans *Myxobolus* spp. and *Henneguya* spp. have a strict host specificity (order/family level), whereas the marine myxosporean Kudoidae has low host specificity (Burger & Adlard 2011, Carriero et al. 2013, Shin et al. 2014). We could not identify a reason for why there was a difference in host specificity between marine and freshwater myxosporeans. However, we speculate that when the parasites come into contact with a new fish species, they adapt to infect the new species; the parasites then cospeciate with the new host (Shin et al. 2014). Since the speciation and extinction rate of freshwater fish is higher than in marine fish (Bloom et al. 2013), this environmental (biological) pressure results in a strict relationship between parasite and host. Interestingly, a previous study suggested that high host specificity increases genetic structure of the parasite (Huyse et al. 2005). Further, freshwater fish and myxosporeans have larger genomes than marine fish and myxosporeans (Smith & Gregory 2009, Yang et al. 2014, Chang et al. 2015).

More myxozoan species, including the genus *Parvicapsula*, need to be sequenced to reveal the relationship between biological traits and phylogeny as well as the evolution of the parasite. The phylogenetic analysis methods used in this study can be applied to reveal the relationship between phenotypic traits and the phylogeny of other myxosporean species.

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LITERATURE CITED

Atkinson SD, Jones SR, Adlard RD, Bartholomew JL (2011) Geographical and host distribution patterns of *Parvicapsula minibicornis* (Myxozoa) small subunit ribosomal RNA genetic types. Parasitology 138:969–977

Barta JR, Martin DS, Liberator PA, Dashkevicz M and others (1997) Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J Parasitol 83: 262–271

Bartosová P, Fiala I, Hypsa V (2009) Concatenated SSU and LSU rDNA data confirm the main evolutionary trends within myxosporeans (Myxozoa: Myxosporea) and provide an effective tool for their molecular phylogenetics. Mol Phylogenet Evol 53:81–93

Bartosová P, Fiala I, Jirku M, Cinkova M and others (2013) *Sphaerospora* sensu stricto: taxonomy, diversity and evolution of a unique lineage of myxosporeans (Myxozoa). Mol Phylogenet Evol 68:93–105

Bloom DD, Weir JT, Piller KR, Lovejoy NR (2013) Do freshwater fishes diversify faster than marine fishes? A test using state-dependent diversification analyses and molecular phylogenetics of New World silversides (Atherinopsidae). Evolution 67:2040–2057

Bradford MJ, Lovy J, Patterson DA, Speare DJ, Bennett WR, Stobbart AR, Tovey CP (2010) *Parvicapsula minibicornis* infections in gill and kidney and the premature mortality of adult sockeye salmon (*Oncorhynchus nerka*) from Cultus Lake, British Columbia. Can J Fish Aquat Sci 67: 673–683

Burger MA, Adlard RD (2011) Low host specificity in the Kudoidae (Myxosporea: Multivalvulida) including seventeen new host records for *Kudoa thalassomi*. Folia Parasitol 58:1–16

Carriero MM, Adriano EA, Silva MRM, Ceccarelli PS, Maia AAM (2013) Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American species. PLOS ONE 8:e73713

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552

Chang ES, Neuhof M, Rubinstein ND, Diamant A, Philippe H, Huchon D, Cartwright P (2015) Genomic insights into the evolutionary origin of Myxozoa within Cnidaria. Proc Natl Acad Sci USA 112:14912–14917

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772

Dorothy KP, Kalavati C (1993) *Parvicapsula hoffmani* sp.n. (Myxozoa: Parvicapsulidae) from the mullet, *Liza macrolepis* (Smith). Acta Protozool 32:123–125

Dyková I, Kodádková A, de Buron I, Fiala I, Roumillat WA (2013) *Sinuolinea* infections in the urinary system of *Cynoscion* species (Sciaenidae) and phylogenetic position of the type species of *Sinuolinea* Davis, 1917 (Myxozoa: Myxosporea). Int J Parasitol Parasites Wildl 2:10–17

Fiala I (2006) The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. Int J Parasitol 36:1521–1534

Fiala I, Hlavnicková M, Kodádková A, Freeman MA, Bartosová-Sojková P, Atkinson SD (2015) Evolutionary origin of *Ceratonova shasta* and phylogeny of the marine myxosporean lineage. Mol Phylogenet Evol 86:75–89

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Henderson M, Okamura B (2004) The phylogeography of salmonid proliferative kidney disease in Europe and North America. Proc R Soc B 271:1729–1736

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66:411–453

Huyse T, Poulin R, Theron A (2005) Speciation in parasites: a population genetics approach. Trends Parasitol 21: 469–475

Jones S, Prosperi-Porta G, Dawe S (2006) A new parvicapsulid (Myxosporea) species in adult pink salmon, *Oncorhynchus gorbuscha*, from the Quinsam River, British Columbia, Canada. J Parasitol 92:1313–1318

Jung SH, Choi HS, Do JW, Kim MS and others (2012) Monitoring of bacteria and parasites in cultured olive flounder, black rockfish, red sea bream and shrimp during summer period in Korea from 2007 to 2011. J Fish Pathol 25:231–241

Karlsbakk E, Sæther PA, Høstlund C, Fjellsøy KR, Nylund A (2002) *Parvicapsula pseudobranchicola* n. sp. (Myxozoa), a myxosporidian infecting the pseudobranch of cultured Atlantic salmon (*Salmo salar*) in Norway. Bull Eur Assoc Fish Pathol 22:381–387

Kawai T, Sekizuka T, Yahata Y, Kuroda M and others (2012) Identification of *Kudoa septempunctata* as the causative agent of novel food poisoning outbreaks in Japan by consumption of *Paralichthys olivaceus* in raw fish. Clin Infect Dis 54:1046–1052

Kim SM, Jun LJ, Park MA, Jeong HD, Jeong JB (2015) Characterization of the myxosporean parasite isolated from emaciated olive flounders *Paralichthys olivaceus* on Jeju Island. Korean J Fish Aquat Sci 48:337–345 (in Korean with English summary)

Kodádková A, Bartosová-Sojková P, Holzer AS, Fiala I (2015) *Bipteria vetusta* n. sp. an old parasite in an old host: tracing the origin of myxosporean parasitism in vertebrates. Int J Parasitol 45:269–276

Køie M (2003) *Parvicapsula spinachiae* n.sp. (Myxozoa, Parvicapsulidae) in *Spinachia spinachia* (L.) (Teleostei, Gasterosteidae) from Denmark. Parasitol Res 90:445–448

Køie M, Karlsbakk E, Nylund A (2007) *Parvicapsula bicornis* n. sp. and *P. limandae* n. sp. (Myxozoa, Parvicapsulidae) in Pleuronectidae (Teleostei, Heterosomata) from Denmark. Dis Aquat Org 76:123–129

Kovaleva AA, Gaevskaya AV (1981) On new findings of myxosporidia of the genus *Parvicapsula* in the Atlantic fishes. Zool Zh 60:771–773 (in Russian)

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Larkin MA, Blackshields G, Brown NP, Chenna R and others (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Lom J, Dyková I (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol 53:1–36

Miller KM, Teffer A, Tucker S, Li S and others (2014) Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evol Appl 7:812–855

Nylund A, Karlsbakk E, Sæther PA, Koren C and others (2005) *Parvicapsula pseudobranchicola* (Myxosporea) in farmed Atlantic salmon *Salmo salar*: tissue distribution, diagnosis and phylogeny. Dis Aquat Org 63:197–204

Parker J, Rambaut A, Pybus OG (2008) Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. Infect Genet Evol 8:239–246

Ronquist F, Teslenko M, van der Mark P, Ayres DL and others (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542

Sekiya M, Setsuda A, Sato H, Song K, Han JK, Kim GJ, Yeo IK (2016) *Enteromyxum leei* (Myxosporea: Bivalvulida) as the cause of myxosporean emaciation disease of farmed olive flounders (*Paralichthys olivaceus*) and a turbot (*Scophthalmus maximus*) on Jeju Island, Korea. Parasitol Res 115:4229–4237

Shin SP, Nguyen VG, Jeong JM, Jun JW and others (2014) The phylogenetic study on *Thelohanellus* species (Myxosporea) in relation to host specificity and infection site tropism. Mol Phylogenet Evol 72:31–34

Shin SP, Shirakashi S, Hamano S, Kato K, Lasso LT, Yokoyama H (2016) Phylogenetic study of the genus *Kudoa* (Myxozoa: Multivalvulida) with a description of *Kudoa rayformis* sp. nov. from the trunk muscle of Pacific sierra *Scomberomorus sierra*. Mol Phylogenet Evol 98:337–345

Shulman SS (1953) New and poorly studied myxosporidians of the White Sea. Zool Zh 32:384–394 (in Russian)

Sievers F, Wilm A, Dineen D, Gibson TJ and others (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539

Smith EM, Gregory TR (2009) Patterns of genome size diversity in the ray-finned fishes. Hydrobiologia 625:1–25

Song JY, Choi JH, Choi HS, Jung SH, Park MA (2013) Monitoring of *Kudoa septempunctata* in cultured olive flounder and wild fish in Jeju Island during 2012. J Fish Pathol 26:129–137

Sterud E, Simolin P, Kvellestad A (2003) Infection by *Parvicapsula* sp. (Myxozoa) is associated with mortality in sea-caged Atlantic salmon *Salmo salar* in northern Norway. Dis Aquat Org 54:259–263

Whipps CM, Kent ML (2006) Phylogeography of the cosmopolitan marine parasite *Kudoa thyrsites* (Myxozoa: Myxosporea). J Eukaryot Microbiol 53:364–373

Whipps CM, El-Matbouli M, Hedrick RP, Blazer V, Kent ML (2004a) *Myxobolus cerebralis* internal transcribed spacer 1 (ITS-1) sequences support recent spread of the parasite to North America and within Europe. Dis Aquat Org 60: 105–108

Whipps CM, Grossel G, Adlard RD, Yokoyama H, Bryant MS, Munday BL, Kent ML (2004b) Phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based on comparative ribosomal DNA sequence analysis. J Parasitol 90: 618–622

Yanagida T (2017) Myxosporean emaciation disease. Fish Pathol 52:63–67

Yang Y, Xiong J, Zhou Z, Huo F and others (2014) The genome of the myxosporean *Thelohanellus kitauei* shows adaptations to nutrient acquisition within its fish host. Genome Biol Evol 6:3182–3198

Yasuda H, Ooyama T, Nakamura A, Iwata K, Palenzuela O, Yokoyama H (2005) Occurrence of the myxosporean emaciation disease caused by *Enteromyxum leei* in cultured Japanese flounder *Paralichthys olivaceus.* Fish Pathol 40:175–180

Zhao Y, Ma C, Song W (2000) Description of two new species of *Parvicapsula* Shulman, 1953 (Myxosporea: Parvicapsulidae) parasitic in the urinary bladder of marine fishes, *Paralichthys olivaceus* and *Kareius bicoloratus*, from the coast of the Yellow Sea, China. Acta Protozool 39:157–162

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