# RIBOSOMAL DNA SEQUENCE DATA REVEAL THE MYXOSPOREAN GENUS *LEPTOTHECA* TO BE POLYPHYLETIC

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## Introduction

The type species of the genus *Leptotheca agilis* (Ceratomyxidae), a gall bladder parasite of the southern stingray (*Dasyatis* sp.), was first described by Thélohan in 1895, after he initially incorrectly classified it into the genus *Ceratomyxa* in 1892. Difficulties in interpreting certain spore morphologies have continued to blur the distinction between the genera *Ceratomyxa*, *Leptotheca* and *Sphaerospora*, which has led to the necessary reclassification of some species. Currently, more than 55 species are assigned to the genus *Leptotheca*, most of them infecting marine fishes but also found infecting reptiles and amphibians.

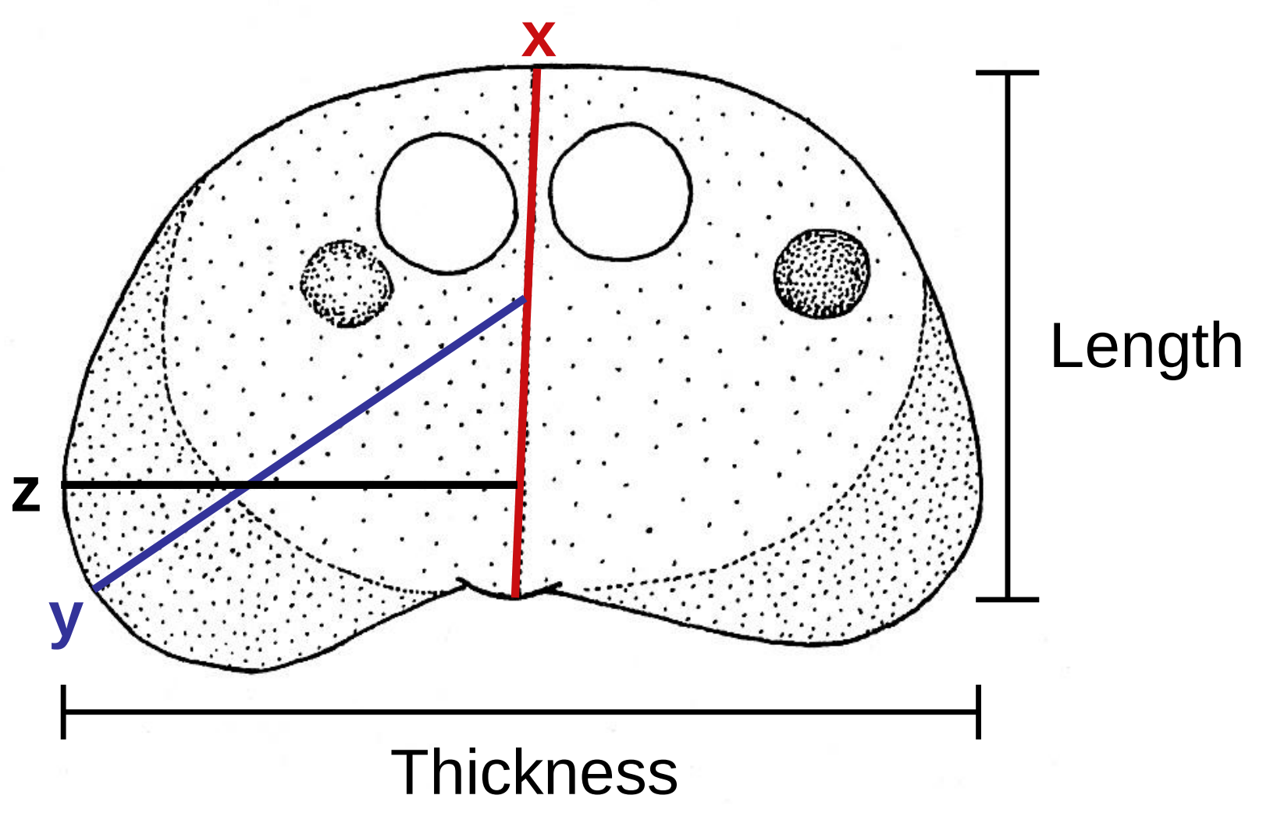

Fig. 1 Official dimensions defining *Leptotheca* spp. using the original line drawing of *Leptotheca fugu* in valvular view

Line drawing of *L. fugu* spore modified from Tun et al. (2000) Fish Pathol. **35** (3) 145-156

The red axis **x** represents the spore length and also the sutural diameter in the valvular view. The black axis **z** represents half of the spore thickness and the blue axis y represents the individual valve length. The axis x must be greater than y to be *Leptotheca;* if y is longer it becomes *Ceratomyxa*. The axis y must also be greater than half of axis x otherwise it becomes *Sphaerospora*. The width of the spore is measured only in the apical view (see Fig. 2 below). However, the majority of *Leptotheca* spp. are more bean-shaped than arcuate as in *L. fugu* (Fig. 1) making both y and **z** almost equal and hence less borderline to *Ceratomyxa*.

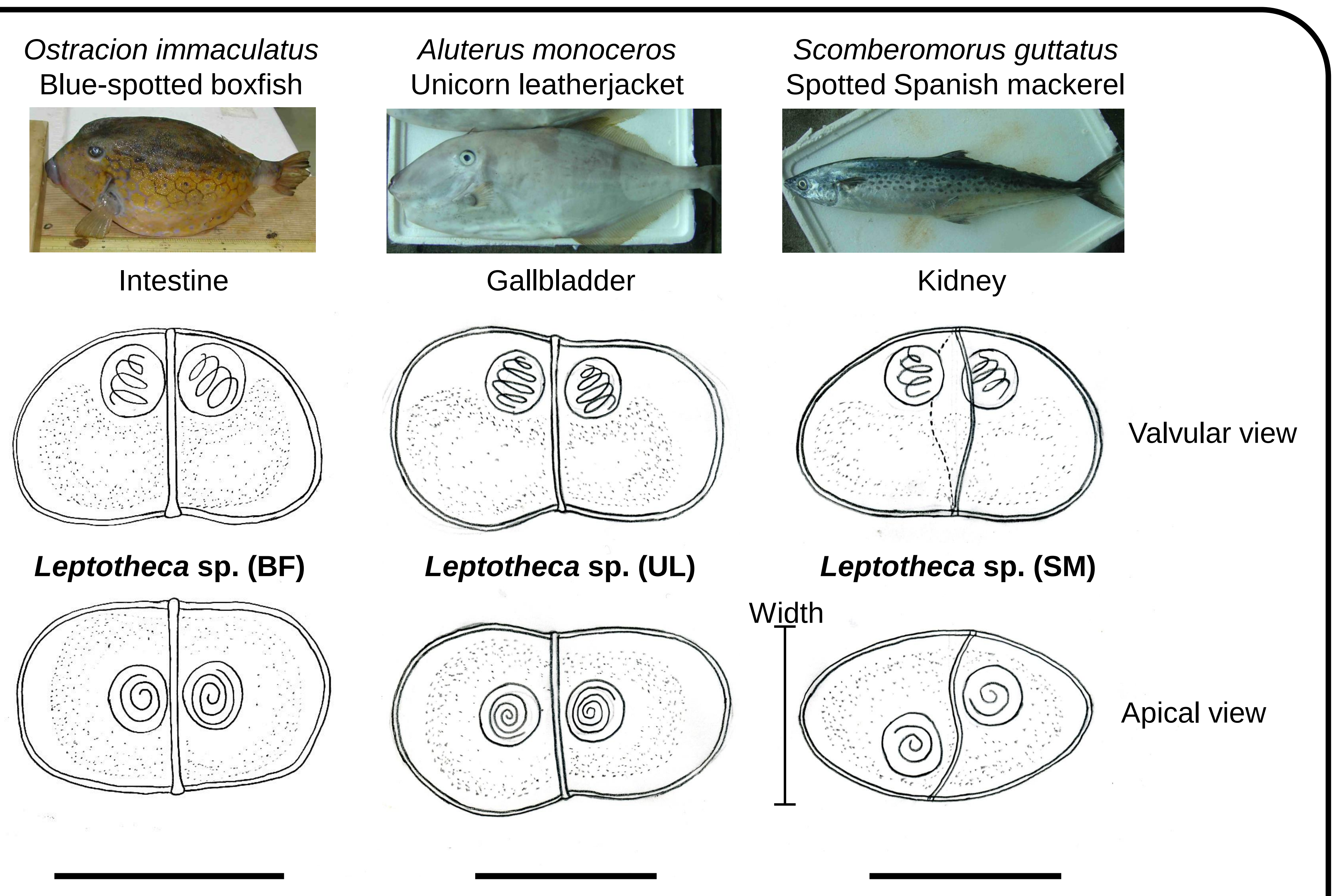

Fig. 2 Line drawings of three novel *Leptotheca* spp. spores, detailing fish host and site of infection, scar bars represent 10 µm

## Discussion and conclusions

Although the three novel myxosporeans from the present study all conform to the current morphological requirements for the genus *Leptotheca*, two of them and *Leptotheca fugu* have been shown, using phylogenetic analyses, to belong to three separate groups within the Myxozoa. Two are marine: the Ceratomyxidae and the urinary / kidney-infecting group; and one freshwater: the basal sphaerosporid clade. The *Ceratomyxa* and *Sphaerospora* have polyphyletic distributions in the Myxozoa. However, most *Ceratomyxa* (except the unusual *C. shasta*) do form a monophyletic clade in the marine group and the majority are found infecting the gallbladder of marine fish. The grouping of *Leptotheca fugu* at the base of the *Sphaerospora* clade is interesting and it is unfortunate that is was not possible to obtain a SSU rDNA sequence for *Leptotheca* sp. (BF), in order to confirm that marine histozoic species infecting the intestinal epithelium with trapezium-shaped / arcuate spores can be robustly placed at the base of the sphaerosporid clade. The urinary / kidney-infecting group contains two genera, *Parvicapsula* and *Zschokkella* and will possibly include other myxosporean genera that infect the renal system of marine fish, as has been demonstrated here for *Leptotheca* sp. (SM); reinforcing the importance of the host tissue / organ that is infected in phylogenetic groupings.

Some subtle morphological differences are apparent between the three species of *Leptotheca* in this study, which may as yet become useful taxonomic features. There is a marked difference in the prominence and nature of the sutural line between species. In *Leptotheca* sp. (SM) the sutural line is fairly conspicuous but clearly sinuous between obliquely positioned polar capsules when viewed from an apical aspect. But the sutural line is straight and extremely prominent, running between directly opposing polar capsules in *Leptotheca* sp. (UL), which is a typical feature of the Ceratomyxidae.

Although it is hard to place taxa using DNA sequence data when no data is available for the type species of the genus involved, we conclude that *Leptotheca* sp. (UL) is most likely a true member of the genus *Leptotheca,* due to its spore morphology, location within the Ceratomyxidae in the phylogenetic analyses and its infection site in the gall bladder. *Leptotheca* sp. (BF), *Leptotheca fugu* and *Leptotheca* sp. (SM) are only included in the genus *Leptotheca* due to their relative spore morphologies. Using spore morphology as a sole criteria for placing novel myxosporeans in a group such as *Leptotheca* is artificial and not sufficiently far-sighted to be of use for myxozoan systematics. Therefore, using other data such as tissue tropism and DNA sequence data will be necessary in order to successfully reclassify certain myxosporean groups.

## Materials and methods

In the present study, spore morphologies, tissue tropism and small subunit rDNA (SSU rDNA) sequences of three novel myxosporeans, all conforming to the current morphological description of *Leptotheca,* are compared. *Leptotheca* sp. (BF) is histozoic in the intestine of the blue-spotted boxfish (*Ostracion immaculatus*) in southern Japan, *Leptotheca* sp. (SM) is coelozoic in the kidney tubules of the Spanish mackerel (*Scomberomorus* spp.) from Malaysia and *Leptotheca* sp. (UL) is coelozoic in the gall bladder of the unicorn leatherjacket (*Aluterus monoceros*) also from Malaysian waters. In addition, *Leptotheca fugu* infecting the intestine and causing emaciation disease in the tiger puffer (*Takifugu rubripes*) from southern Japan has been included in the study due to its similarities with *Leptotheca* sp. (BF), and a SSU rDNA sequence for this species was obtained during an earlier study.

## Results

Table 1. Comparison of *Leptotheca* spp. from the present study with some related species in the genus found in marine fishes. Mean and range (in parentheses) are expressed in micrometres.

| Species | Spore |  |  |  | Polar capsule L x W or diameter | Site of infection | Host |
|---|---|---|---|---|---|---|---|
|  | length | thickness | width | General shape |  |  |  |
| *Leptotheca* sp. (UL) | 10.6<br>(8.8-12.2) | 17.8<br>(15.6-20.6) | 9.5<br>(8.2-11.4) | Peanut or gourd-shaped, with central constriction | 4.2 x 4.3 | Gallbladder (coelozoic) | *Aluterus monoceros* |
| *Leptotheca* sp. (SM) | 9.2<br>(8.1-10.4) | 16.1<br>(14.8-17.0) | 9.6<br>(7.9-12.9) | Bean-shaped | 3.0 | Kidney (coelozoic) | *Scomberomorus* spp. |
| *Leptotheca* sp. (BF) | 8.0<br>(7.3-8.4) | 14.2<br>(13.1-15.2) | 8.3<br>(7.7-9.2) | Trapezium-like | 3.2<br>(2.5-3.7) | Intestine (histozoic) | *Ostracion immaculatus* |
| *L. fugu* | 9.0<br>(8.3-9.5) | 14.0<br>(13.0-15.0) | 7.9<br>(7.0-8.0) | Trapezium-like | 2.8 | Intestine (histozoic) | *Takifugu rubripes* |
| *L. elongata* | 9.58<br>(9.9-10.35) | 14.04<br>(13.5-14.4) | nd | Ellipsoid | 3.06 | Gallbladder | *Merluccius senegalensis* |
| *L. informis* | 10 | 18-20 | 9 | Bean-shaped | 3-4 | Gallbladder | *Molva vulgaris* |
| *L. koreana* | 8.59 | 13.42 | 8.13 | Bean-shaped | 3.86 | Renal tubule | *Sebastes schlegeli* |
| *L. lutjani* | 6.06<br>(4.5-7.6) | 8.95<br>(6.75-10.1) | nd | Oval | 2.12 | Kidney | *Lutjanus fulgens* |
| *L. sparidarum* | 6.02<br>(5-7.1) | 10.65<br>(8.8-12.3) | 5.98<br>(5.88-6.18) | Bean-shaped | 2.91 | Kidney | *Dentex dentex* |

nd: not determined

Line drawings and relative spore dimensions of the three novel *Leptotheca* spp. from this study can be found in Fig. 2 and Table 1. *Leptotheca* sp. (BF) has slightly trapezium-shaped spores with spherical polar capsules, with an indistinct sutural line, it shows some similarities to *L. fugu* (Fig. 1). *Leptotheca* sp. (SM) has more bean-shaped spores, valves with rounded ends of slightly differing sizes, round polar capsules that are obliquely positioned in the apical view with an indistinct sinuous sutural line. *Leptotheca* sp. (UL) has peanut or gourd-shaped spores with a constriction at the centre. The two valves have rounded ends of differing sizes; a prominent sutural line runs between the two spherical polar capsules. SSU rDNA sequences reveal that *Leptotheca* have a polyphyletic distribution among the myxosporeans (Fig. 3). No sequence was generated for *Leptotheca* sp. (BF), however, a similar intestinal species, *Leptotheca fugu,* groups with the basal *Sphaerospora* clade which includes *S. ranae* (formerly *Leptotheca ranae*). *Leptotheca* sp. (SM) groups within the marine clade that contains kidney and urinary bladder-infecting species *Zschokkella* and *Parvicapsula*. *Leptotheca* sp. (UL) groups within the Ceratomyxidae and all sequenced members of the genus *Ceratomyxa* that infect gall bladders.

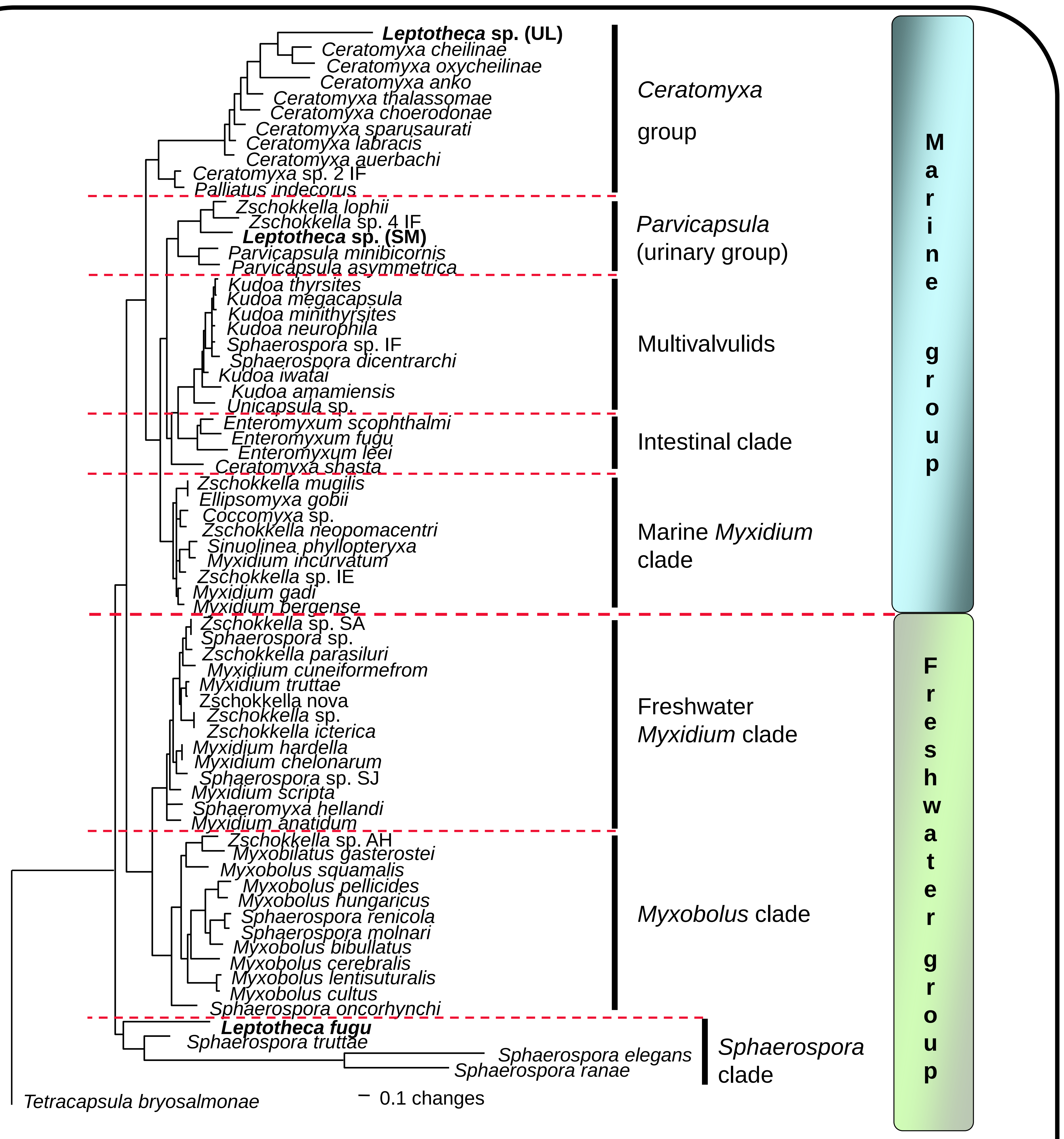

Fig. 3 Bayesian inference analysis generated phylogenetic tree of the Myxozoa. The taxa form the generally accepted distribution of clades, with *Leptotheca* spp. showing a clear polyphyletic distribution pattern throughout the tree.