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Evidence of striking morphological similarity and tissue tropism of phylogenetically distant myxozoan genera: *Myxidium* and *Paramyxidium* in the kidney of the European eel

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Abstract: European eel, *Anguilla anguilla* (Linnaeus) (Elopomorpha: Anguilliformes), is a critically endangered fish of ecological and economic importance, hosting numerous parasites, including myxozoans (Cnidaria). Since its initial discovery in the kidney of European eel, *Myxidium giardi* Cépède, 1906 has been reported with numerous spore sizes and shapes from various tissues of multiple anguillid species. Morphological variability, wide host and tissue spectrum, and lack of sequence data raised doubts about the conspecificity of reported isolates. Subsequent studies provided 18S rDNA sequences of several isolates from anguillids and other elopiform fish, and demonstrated a split of parasite data into two distinct phylogenetic lineages, one comprising the *M. giardi* sequence, and the other all species infecting elopiform fishes classified under the recently established genus *Paramyxidium* Freeman et Kristmundsson, 2018. *Myxidium giardi* was, however, transferred to this genus as *Paramyxidium giardi* n. comb. and designated as the type species of the genus. In line with this change, the sequence originally identified as *M. giardi* was considered to have been incorrectly associated with this species. To shed light on the status of *M. giardi* originally described by Cépède (1906), we conducted microscopic and molecular examinations of various organs of 24 individuals of European eel, originating from diverse Czech habitats. Through morphometric and molecular analyses, we demonstrated that spore and polar capsule morphology, morphometry and tissue tropism of our European eel kidney parasite isolates matched the features of the original *M. giardi* description. Our isolates clustered in the lineage encompassing the first published *M. giardi* sequence. Thus, the originally described *M. giardi* indeed represents an existing species within the genus *Myxidium* Bütschli, 1882, which we formally resurrect and redescribe. Due to the morphological and molecular differences between *M. giardi* and *P. giardi* of Freeman et Kristmundsson (2018), we additionally rename the latter species as *Paramyxidium freemani* nom. nov.

Keywords: *Anguilla,* Myxozoa, phylogeny, taxonomy, PCR screening.

This article contains supporting tables (Supplementary Table 1) online at http://folia.paru.cas.cz/suppl/2024-71-013.pdf

Myxozoan parasites (Cnidaria: Myxozoa) are a widely distributed group of microscopic metazoans that primarily infect fish as intermediate hosts, and annelids or bryozoans as final hosts of their complex life cycle (Eszterbauer et al. 2015). To date, more than 2,600 myxozoan species have been described (Okamura et al. 2018), and it appears that this group remains strongly underestimated (Bartošová-Sojková et al. 2014, Okamura et al. 2015, Hartikainen et al. 2016, Lisnerová et al. 2023).

The European eel *Anguilla anguilla* (Linnaeus) (Elopomorpha: Anguilliformes) is a catadromous fish widely distributed in European brackish and freshwater environments that undergoes sexual reproduction in the marine environment, namely in the Sargasso Sea (van Ginneken and Maes 2005, Miller et al. 2015). Despite its wide distribution and increasing economic and ecologic importance as a farmed fish (Parzanini et al. 2021, Yuan et al. 2022), the European eel is a critically endangered species (IUCN 2023). The European Union has implemented specific legislation (The EC Eel Regulation [1100/2007]), requiring member states to develop eel management plans to facilitate the recovery of European eel.

Parasites are one of the reasons for the significant collapse of the eel populations (Feunteun 2002). Parasite diversity in this fish species encompasses 'monogeneans', cestodes, nematodes, acanthocephalans, arthropods, ciliates, cryptosporidians, coccidians and myxozoans (Cépède 1906, Køie 1988, Borgsteede 1999, Sures et al. 1999, Kirk 2003, Jakob et al. 2016). As for the last group, representatives of eight myxosporean genera, i.e.,

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Table 1. Data on *Anguilla anguilla* (Linnaeus) examined for *Myxidium giardi* Cépède, 1906 infections in the present study. Postitive localities in bold.

Locality	Coordinates	PCR screening (number of positive tissues / overall number of screened tissues)	Total prevalence
Švihov Reservoir, CZ	49.6743N, 15.1635E	bile = 0/3; gills = 0/7; kidney = 0/7	13% (3/24)
Jordánka River, CZ	50.5166N, 15.2012E	gills = 0/3; kidney = 2/3 (67%)	
Klíčava Reservoir, CZ	50.0699N, 13.9300E	bile = 0/1; gills = 0/3; kidney = 0/3	
Římov Reservoir, CZ	48.8329N, 14.4836E	bile = 0/2; gills = 0/4; kidney = 1/4 (25%)	
Soběkury, CZ	49.5761N, 13.2390E	bile = 0/6; gills = 0/6; kidney = 0/6	
Obecník pond, CZ	49.8129N, 15.4747E	bile = 0/1; gills = 0/1; kidney = 0/1	

Ceratomyxa Thélohan, 1892, *Hoferellus* Berg, 1898, *Myxidium* Bütschli, 1882, *Myxobolus* Bütschli, 1882, *Ortholinea* Shulman, 1962, *Paramyxidium* Freeman et Kristmundsson, 2018, *Sphaerospora* Thélohan, 1892, and *Zschokkella* Auerbach, 1910, have formally been described from the European eel (Cépède 1906, Jacob and Bremen 1953, Copland 1982, Lom et al. 1986, Wierzbicka 1987, Tuzet and Ormières 1957, Freeman and Kristmundsson 2018, Dzido et al. 2020).

Myxidium giardi Cépède, 1906 is a myxosporean parasite originally described from the European eel kidney. The parasite spores have typical *Myxidium*-like morphology, being spindle-shaped with two subspherical polar capsules located at the pointed poles of the spore (Cépède 1906). Since its discovery, *M. giardi* has been reported in numerous spore sizes and shapes under this name or as other *Myxidium* species, later synonymised with *M. giardi* (see Hine 1980), from additional tissues (dermis, digestive tube, fins, gall bladder, gas gland, gills, liver, muscle) of multiple anguillid fish species, i.e., American eel *Anguilla rostrata* (Lesueur), short-finned eel *Anguilla australis* Richardson, African longfin eel *Anguilla mossambica* (Peters), and New Zealand longfin eel *Anguilla dieffenbachii* Gray (Ishii 1915, Ghittino et al. 1974, Hine 1980, Copland 1981, Ventura and Paperna 1984, Treasurer and Cox 1997, Aguilar et al. 2005).

However, no sequence data have been made available to evaluate the conspecificity of these findings. A single sequence of *M. giardi* (GenBank: AJ582213) has been published by Holzer (2004) from kidney infections of European eel collected from a riverine system in Scottish highlands. Phylogenetic analyses cluster this sequence among myxosporeans that infect the urinary system of teleosts, including European eel (Holzer et al. 2004). Freeman and Kristmundsson (2018) reported *M. giardi* from a new Icelandic isolate occurring in the kidney of European eel, and sharing overall morphology with the original species description.

A novel 18S rDNA sequence was provided in that study that, however, clustered distantly from the original sequence obtained by Holzer et al. (2004), namely among elopiform fish-infecting isolates of the newly established genus *Paramyxidium*. Consequently, Freeman and Kristmundsson (2018) transferred *M. giardi* to this genus as a new combination. Additionally, the authors considered the sequence provided by Holzer et al. (2004) to have been misidentified.

In the present study, we examined European eel from several Czech reservoirs and rivers for the presence of myxozoan infections. We provide both the morphological and molecular data for species analysis and demonstrate that the originally described *M. giardi* should be placed in *Myxidium*. In addition, *Paramyxidium freemani* nom. nov. is proposed for the phylogenetically distinct isolate described by Freeman and Kristmundsson (2018).

MATERIALS AND METHODS

Sample origin and morphological analyses of myxozoan stages

Overall, twenty-four European eel individuals were collected from reservoirs, a river and pond-originated hatchery in the Czech Republic during the years 2016–2019. The fish were captured and treated in compliance with the laws of the Czech Republic. European eels were captured in open waters based on the following permits: i) Nature Conservation Agency of the Czech Republic (No. 08782/SOPK/17) for the Jordánka River; ii) Ministry of the Environment of the Czech Republic (No. MZP/2019/630/16) for Klíčava Reservoir, Švihov Reservoir and Římov Reservoir, and iii) under rules of the Czech Anglers Union (Decree No. 197/2004 on the Implementation of Act No. 99/2004 Coll with membership ID number 00147677) for pond Obecník. Other European eel specimens were bought in the Soběkury hatchery from Sádky Soběkury, Ltd. grown to legal size in local area.

In total, 60 samples of gills, kidney and gall bladder were freshly dissected and examined (details in Table 1). Bile was collected from the gall bladders at all localities, except the Jordánka River, by gently squeezing the bladder contents into the microtube. The presence of myxozoan infections was assessed by light microscopy and molecular screening. Digital images of myxozoan spores from fresh tissues were captured using an Olympus BX51 microscope equipped with an Olympus DP70 camera (Olympus, Shindzhuku, Japan). Morphometry was determined from digital images of 15 spores per isolate, following guidelines outlined by Lom and Arthur (1989), and using ImageJ 1.53e software (Schneider et al. 2012). Measurements are presented as the mean ± standard deviation (S.D.) with the minimum and maximum range included within parentheses.

Molecular analyses

Small pieces (approx. 5 mm^3) of all collected tissue samples and the bile (approx. 30 µl) were preserved in 400 µl of TNES urea buffer (10 mM Tris-HCl with pH 8; 125 mM NaCl; 10 mM EDTA; 0.5% SDS and 4 M urea). Genomic DNA was extracted using the phenol-chloroform method (Holzer et al. 2004). This extraction process involved an overnight digestion step with proteinase K (50 µg/ml; Serva, Heidelberg, Germany) at a temperature of 55 °C.

Table 2. Species of *Myxidium* Bütschli, 1882 and *Paramyxidium* Freeman et Kristmundsson, 2018 described from anguillid hosts including tissue preference and spore/polar capsule measurements (in µm).

Myxozoan species	Host species	Host tissue	Spore length	Spore width	Polar capsule length	Polar capsule width	Reference
M. acinum Hine, 1975	*A. australis, A. dieffenbachii*	Gills	5.1–5.8	2.5–3.9	1.4–2.8	1.2–2.0	Hine (1975)
M. anguillae Ishii, 1915 [quoted from Jayasri and Hoffman 1982]	*A. japonica*	Intestine	9.1	2.9	3.5	3.5	Eiras et al. (2011)
M. illinoisense Meglitsch, 1937	*A. bostoniensis*	Kidney	12.7–15.3	7.6–9.3	3.4–6.1	3.4–6.1	Eiras et al. (2011)
M. durum Hine, 1980	*A. australis*	Gills	6.5–8.0 (7.6)	3.5–4.5 (4.0)	3.0–4.0 (3.2)	1.5–3.0 (2.3)	Hine (1980)
M. enchelypterygii Hoshina, 1952	*A. japonica*	Fins	7.1–9.0 (7.8)	4.2–5.1 (4.7)	1.7–2.4 (2.1)	1.5–2.1 (1.7)	Eiras et al. (2011)
M. giardi Cépède, 1906	*A. anguilla*	Kidney	9.0–10.0	5.5–6.0	3.0–5.0	2.0	Cépède (1906)
***M. giardi* Cépède, 1906**	***A. anguilla***	**Kidney**	**9.0–10.9 (9.5)**	**4.8–6.6 (5.3)**	**3.4–4.5 (3.8)**	**2.7–3.9 (3.6)**	**Present study**
M. giardi Cépède, 1906	*A. anguilla*	Kidney	9.0	5.4	3.5	2.2	Holzer et al. (2004)
M. lentiforme (Fujita 1929) (syn. *M. fusiforme* Fujita, 1923)	*A. japonica*	Kidney	19	5	4	4	Hine (1980)
M. matsuii Fujita, 1929	*A. japonica*	Skin	12–13	7	5	5	Hine (1980)
M. orientale Schulman, 1962	*A. japonica*	Gall bladder, gills, kidney, intestine	10.1–11.0	6.0–7.0	3.5–4.0	3.0–3.5	Eiras et al. (2011)
M. pseudogobii Akhmerov, 1960	*A. japonica*	Gills, intestine	13–14	4.5–5.0	4–4.2	2.8–3	Chen and Ma (1998)
M. serum Hine, 1975	*A. dieffenbachii*	Gall bladder	16–18	9	5–5.5	5–5.5	Hine (1975)
M. truttae Léger, 1930	*A. anguilla*	Gall bladder, gills	11–12	7–7.3	3.7	3.7	Hine (1980)
M. uchiyamae Fujita, 1929	*A. japonica*	Kidney	13.5	8		6.5	Eiras et al. (2011)
M. zealandicum Hine, 1975	*A. australis*, *A. dieffenbachii*	Gills	6.4–10.5	3.3–4.5	2.8–4.6	1.5–2.9	Hine (1975)
P. branchialis Freeman et Kristmundsson, 2018	*A. anguilla*	Gills	10.7–12.3 (11.6)	6.6–7.8 (7.3)	3.9–4.5 (4.2)	3.2–4.2 (3.8)	Freeman and Kristmundsson (2018)
P. giardi (Cépède, 1906)	*A. anguilla*	Kidney	9.5–11.4 (10.6)	6.5–7.5 (7.0)	3.5–4.2 (4.0)	2.7–3.9 (3.6)	Freeman and Kristmundsson (2018)
P. magi Freeman et Kristmundsson, 2018	*A. anguilla*	Stomach wall	10.8–12.9 (11.6)	7.0–8.4 (7.6)	3.6–4.6 (4.0)	3.1–4.0 (3.6)	Freeman and Kristmundsson (2018)

All collected samples were PCR-screened for myxozoan infections. PCR reactions contained 10 µl of the AmpOne HS-Taq premix (GeneAll Biotechnology Ltd., Seoul, South Korea), 0.5 µl of each primer (25 pmol), 8 µl of DNAse-free water, and 1 µl of extracted DNA with a concentration of 50–200 ng/µl. Nested PCRs were employed for the 18S rDNA amplification. In the first run, the general eukaryotic primer pair 18e–18g (Hillis and Dixon 1991) was used. In the second run, general myxozoan primers Myxgp2f–ACT1r (Kent et al. 1998, Hallett and Diamant 2001) were used for screening all samples, and species-specific primer MgiarF was designed and used in combination with 18g (Supplementary Table 1) aiming to obtain the longest possible part of 18S rDNA.

The cycling parameters in the thermal cycler (Biometra, Göttingen, Germany) were set as follows: First run: denaturation at 95 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 64 °C for 1 min s, 72 °C for 2 min, and a terminal extension at 72 °C for 10 min, second run: denaturation at 95 °C for 3 min, followed by 33 cycles of 94 °C for 40 s, annealing temperature (Supplementary Table 1) for 50 s, 72 °C for 1 min 40 s, and a terminal extension at 72 °C for 10 min. PCR products were extracted from gel by Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan) and Sanger sequenced (SEQme, Dobříš, Czech Republic).

Obtained PCR products with low band intensity or unclear sequencing results were cloned into the pDrive Vector with a Qiagen PCR Cloning Kit (Qiagen, Hilden, Germany) and transformed into *Escherichia coli* competent cells. Plasmids were purified using a High Pure Plasmid Isolation Kit (Roche Applied Science, Penzberg, Germany) and five colonies per sample were Sanger sequenced (SEQme, Dobříš, Czech Republic).

Phylogenetic analyses

The 18S rDNA alignment comprised a total of 88 sequences, which included the newly obtained sequences of *Myxidium giardi* (two sequences, representative of the two different localities in which infection by *M. giardi* was detected) along with the previously published sequences of *M. giardi,* all *Paramyxidium* sequences, and sequences of other myxosporeans.

The alignment was prepared using the E-INS-i multiple alignment method in MAFFT v7.017 (Katoh et al. 2005) implemented in Geneious Prime 2019.0.4 (Kearse et al. 2012). The resulting alignment had a final length of 3,016 base pairs. Phylogenetic analyses were conducted using both Bayesian inference (BI) and maximum likelihood (ML) methods. ML analysis was performed with RAxML v7.2.8 (Stamatakis 2006) with a GTR + Γ model which was selected as the best-fitting model of evolution in jModelTest (Posada 2008).

Bootstrap support values were calculated based on 1,000 replicates. The BI analysis was carried out using MrBayes v3.0 (Ronquist and Huelsenbeck 2003) with the GTR + Γ model of evolution. MrBayes ran for one million generations, using two independent runs of four simultaneous Markov Chain Monte Carlo (MCMC) algorithms, with every 100th tree saved to estimate

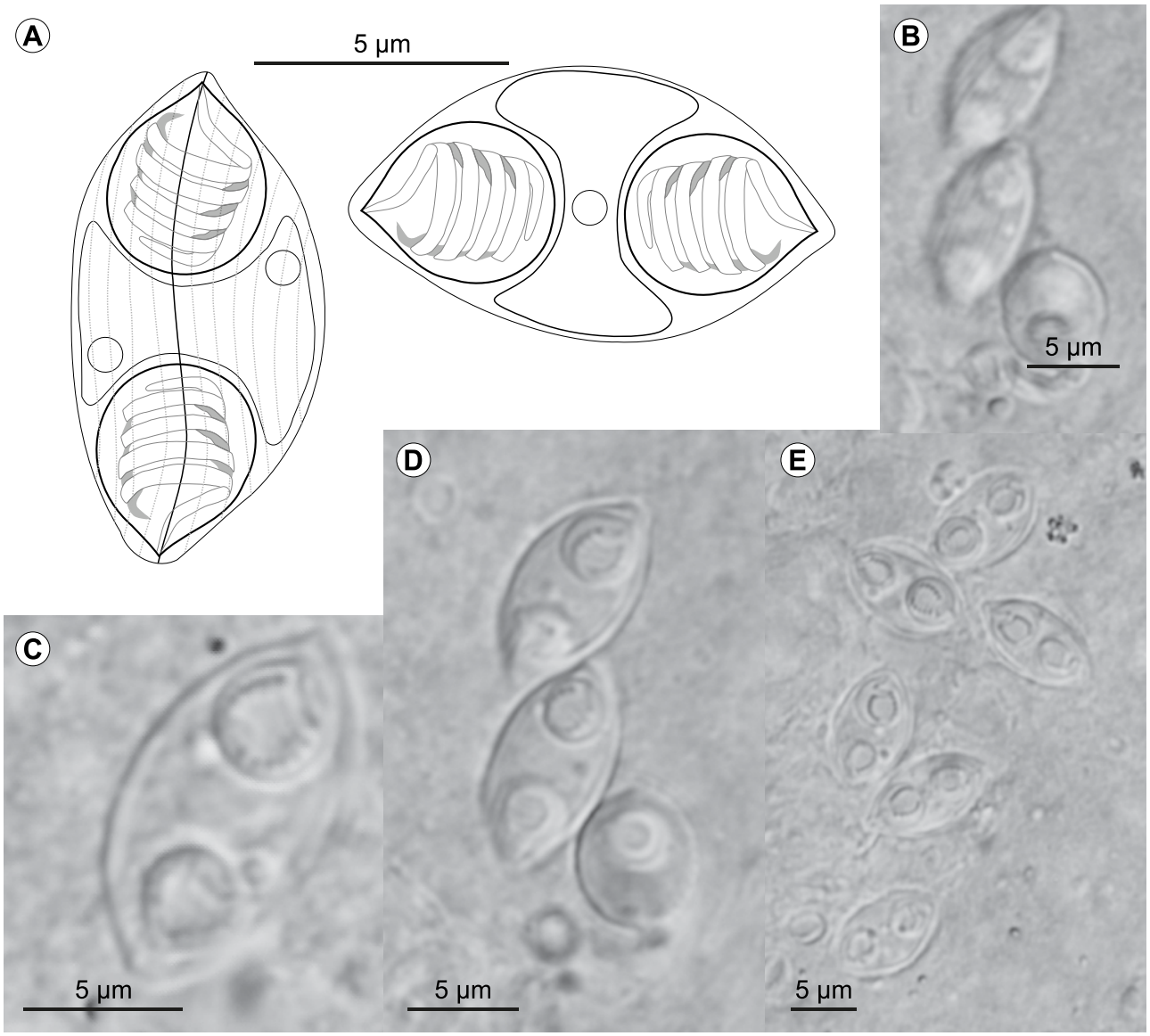

Fig. 1. Line drawings of mature spores of *Myxidium giardi* Cépède, 1906 in sutural (A left) and valvular view (A right) and light microscopy pictures of mature spores (B–E).

posterior probabilities. Species-specific divergences were identified based on proportional distances (in %). These distances were computed in Geneious Prime 2019.0.4 (Kearse et al. 2012), using the dataset previously used for ML analysis.

RESULTS

Light microscopy and PCR screening of myxozoans in European eel

Microscopic examination of fish samples revealed that kidneys of two European eel individuals (prevalence of 8%) were infected by spores of a myxozoan parasite matching the morphological diagnosis of *Myxidium giardi* as provided in the original species description (Figs. 1, 2, Table 2). Subsequent PCR screening of all our samples revealed infections by the myxozoan parasite molecularly identified by Holzer et al. (2004) as *M. giardi* in 13% of the European eel specimens, all present in fish kidney (three individuals from two localities), while no infections were detected in the bile and gills (details in Table 1).

Besides *M. giardi*, *Hoferellus gilsoni* (Debaisieux, 1925), *Myxobolus portucalensis* Saraiva et Molnár, 1990, and *Paramyxidium* spp. were both morphologically and molecularly identified in the examined European eel and these findings will be discussed in follow-up publications.

Phylogenetic analyses

The Czech isolates formed a closely related lineage to the AJ582213 sequence, previously identified as *M. giardi* by Holzer et al. (2004), and to other myxosporeans infecting the urinary system of freshwater fish. The 18S rDNA-based phylogenetic analyses uncovered that the eel-infecting *Paramyxidium giardi* (GenBank: MK635346, MH414925), *P. brachialis* Freeman et Kristmundsson, 2018 (GenBank: MH414926), and *P. magi* Freeman et Kristmundsson, 2018 (GenBank: MH414927) clustered apart from our isolates from European eel (Fig. 3). The intraspecific variation of

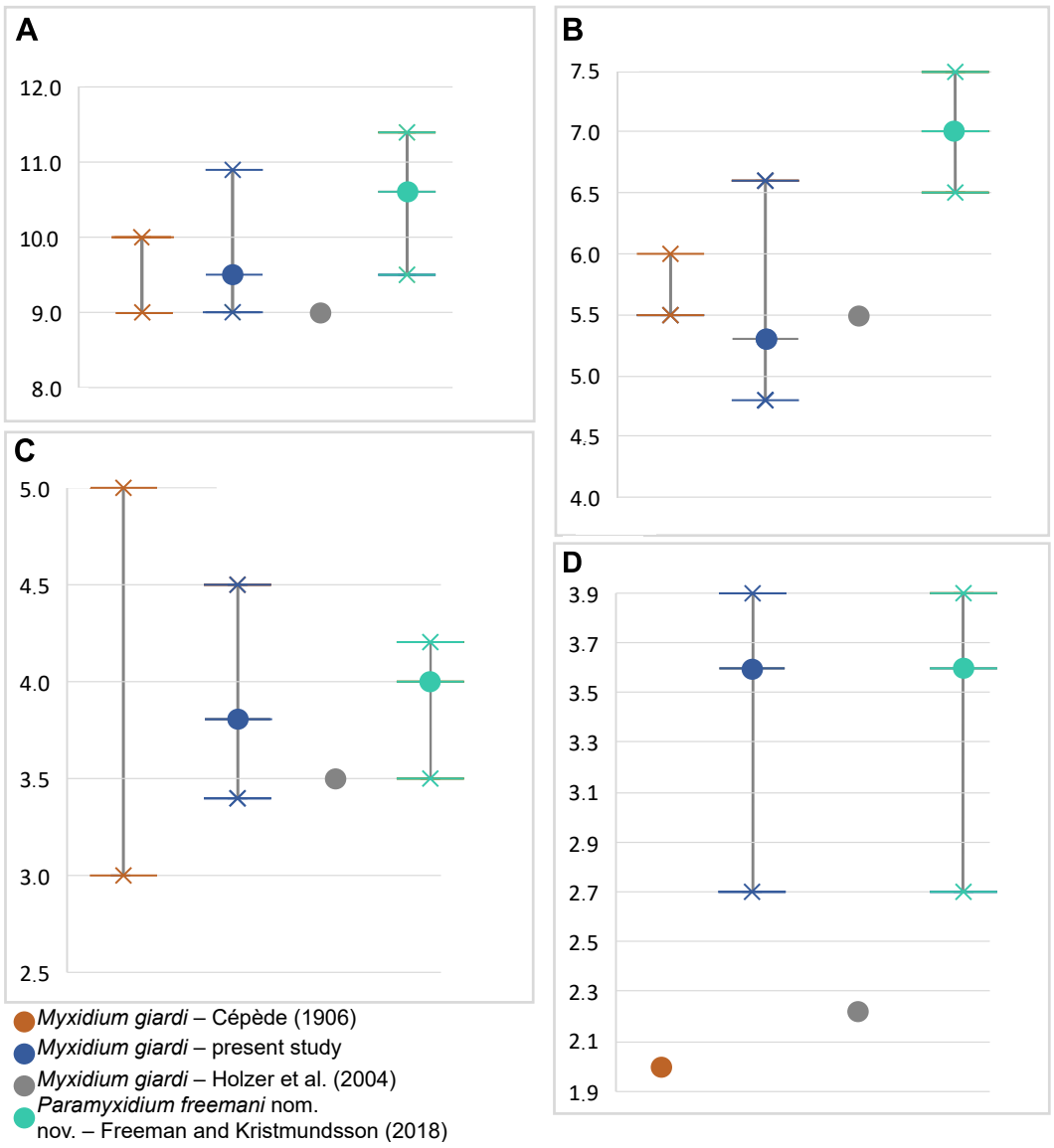

Fig. 2. Graphic representation of the measured values (maximum, minimum, average) for spores and polar capsules of selected *Myxidium* and *Paramyxidium* spp., colour-coded by species according to the legend. **A** – spore length (in µm); **B** – spore width (in µm); **C** – polar capsule length (in µm), **D** – polar capsule width (in µm).

our two Czech European eel-infecting isolates was 0.2% and the sequence identities of our isolates to phylogenetically closely related sequences were as follows: 99.7% across 908 bp with *Chloromyxum schurovi* Shulman et Ieshko, 2003 (GenBank: AJ581917), 99.1% across 908 bp with *M. giardi* (GenBank: AJ582213), 99.6% across 1,565 bp with Neoactinomyxum eiseniellae (GenBank: AJ582007), and 96.3%, across 1,590 bp with *Zschokkella* sp. (GenBank: AJ581918). The sequence identity of our *M. giardi* isolates and *P. giardi* was only 72.3–72.4% across 1,918 bp.

Resurrection, redescription and renaming the species

Based on the overlap of the morphological and morphometrical data of the type species description and our Czech European eel isolates, we assign the sequences obtained in this study as belonging to *M. giardi* (Fig. 2). We confirm that the AJ582213 sequence produced by Holzer et al. (2004) also corresponds to *M. giardi*, as demonstrated by the comparative analysis of both sequence and morphological data (Figs. 2, 3).

We additionally assume that the sequences AJ581917 and AJ582007 differing from *M. giardi* by less than 1% also represent the sequence data belonging to this parasite species. Based on the overlap of spore morphology/morphometrics, host affinity, and tissue tropism of the original *M. giardi* description (Cépède 1906) and of our isolates from Czech European eel, that additionally differ from *P. giardi* by the 18S rDNA sequence, we propose a resurrection of *M. giardi* species and provide a species redescription.

Moreover, *Paramyxidium freemani* nomen novum is proposed as a new name for *P. giardi* of Freeman and Kristmundsson (2018) which differs from *M. giardi* in i) spore shape; ii) spore length, width, polar capsule length, polar capsule width (Fig. 2A–D); iii) number of striations; and iv) 18S rDNA sequence. *Paramyxidium freemani* nom. nov. is designated as the type species of the genus *Paramyxidium*.

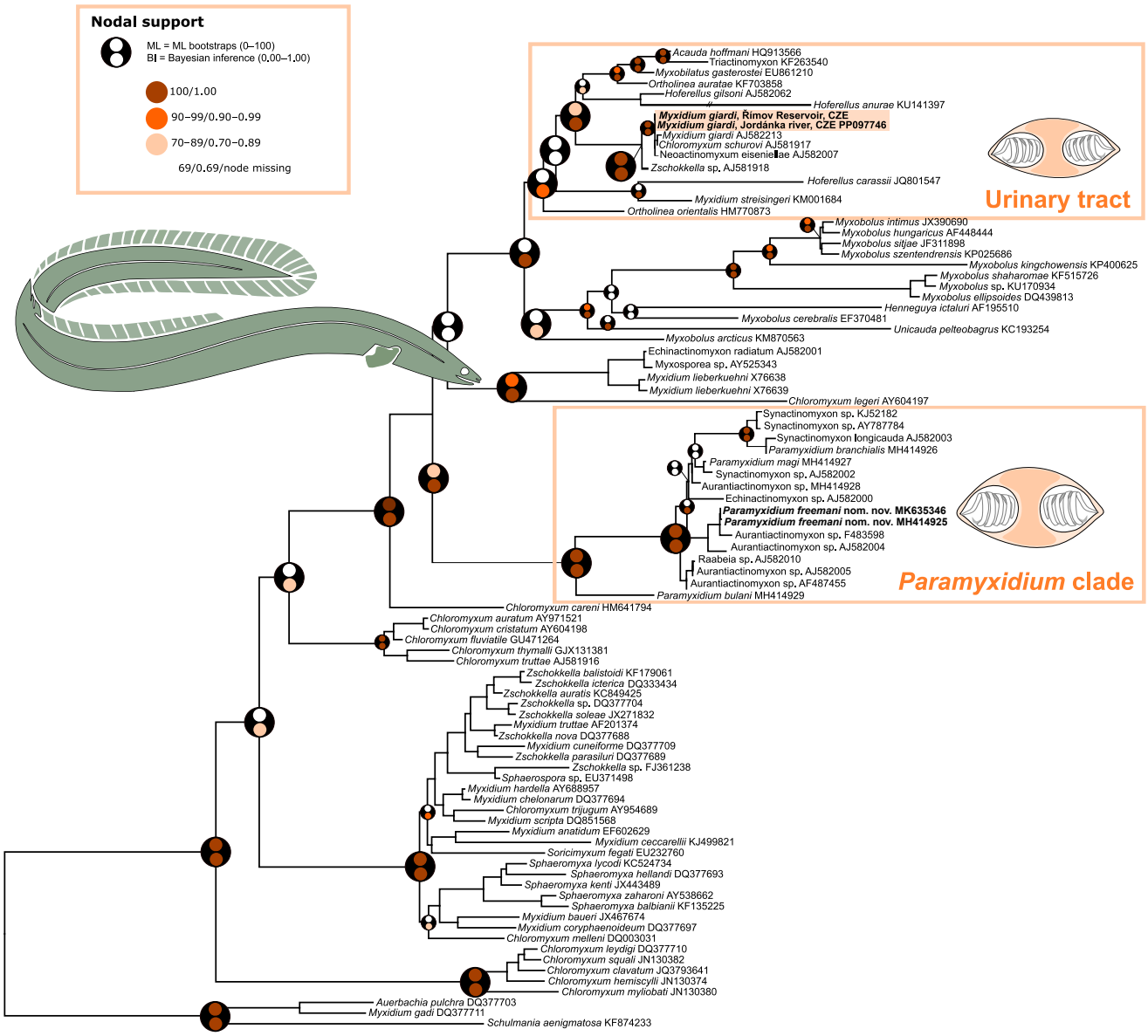

Fig. 3. 18S rDNA phylogenetic tree, including sequences of *Myxidium giardi* and *Paramyxidium* spp. The sequences of *Auerbachia pulchra* Lom, Noble et Laird, 1975, *Myxidium gadi* Georgévitch, 1916 and *Schulmania aenigmatosa* Kovaljova, Zubtchenko et Krasin, 1983 were used as outgroup. Newly identified sequences are highlighted in bold and with an orange box. Maximum likelihood/Bayesian inference nodal supports are shown at every node by colour circles according to scale shown in the figure. The branch of *Hoferellus anurae* Mutschmann, 2004 is shortened to 50% of its original length. Schematic drawings depict shape differences between spores of *M. giardi* Cépède, 1906 and *Paramyxidium* spp.

Family Myxidiidae Thélohan, 1892

Genus *Myxidium* Thélohan, 1892

Species: ***Myxidium giardi*** Cépède, 1906

Type and only host: *Anguilla anguilla* (Linnaeus) (Anguilliformes: Anguillidae), European eel.

Type locality: France (more precise locality not specified by Cépède 1906).

Other localities: Jordánka River, Czech Republic (50.5166N, 15.2012E); Římov Resevoir, Czech Republic (48.8329N, 14.4836E); Northern Scotland, United Kingdom (Holzer et al. 2004).

Site of tissue development: Coelozoic within kidney tubules.

Prevalence of infection: 13% (3/24).

Materials deposited: DNA material stored at the Protistological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences (BC CAS), České Budějovice, Czech Republic (code: IPCAS Pro 83); 18S rDNA sequence (1,889 bp, GenBank Acc. Number PP097746).

Description of sporogonic stages: Polysporic plasmodia ranging 800–900 µm in length (Cépède 1906).

Description of myxospore: Mature spores of spindle shape, longer than wide, typically tapering on both sides, 9.6 ± 1.0 µm (9.0–12.0 µm) long and 5.4 ± 0.6 µm (4.8–6.6 µm) wide. Two subspherical polar capsules opening to opposite sides, one at either end of spore, measuring 3.8 ± 0.3 µm (3.4–4.5 µm) in length and 3.6 ± 0.6 µm (2.7–3.9 µm) in width (n = 15; one host) (Fig. 1A–E). Polar tubules forming 4–5 turns (n = 3; one individual host). One binucleated sporoplasm (Fig. 1C, E). Two valve cells, each with 10–11 longitudinal surface ridges (Fig. 1B).

Remarks: Only *M. giardi* and the morphologically similar *P. giardi* have been identified in the kidney of European eel (Cépède 1906, Freeman and Kristmundsson 2018). The morphology of the mature spores examined in the present study is consistent with the characteristics typical for the genus *Myxidium*. In comparison to *P. freemani* nom. nov., the spores of *M. giardi* are narrower (Fig. 2B) and shorter (Fig. 2A), rendering them more elongated and spindle-shaped. Although the number of longitudinal surface ridges and turns of the polar tubules was not specified for *P. freemani* nom. nov., the line drawing provided by Freeman and Kristmundsson (2018) shows six turns of the polar tubules and eight valves ridges, thus not matching the morphology of *M. giardi* features as described by Cépède (1906).

Additional *Myxidium* species have been reported from various organs of European eel and other anguillid species (Hine 1975, Hine 1980, Chen and Ma 1998, Eiras et al. 2011, Nagasawa et al. 2007; Table 2). Some of these myxidiids, i.e., *Myxidium anguillae* (Ishii, 1915), *M. enchelypterygii* Hoshina, 1952, *M. illinoisense* Meglitsch, 1937, *M. serum* Hine, 1975 and *M. zealandicum* Hine, 1975, were later synonymised with *M. giardi* by Hine (1980), although these synonyms were not accepted in a later review of the genus *Myxidium* by Eiras et al. (2011). They differ in spore morphology and morphometry, host species spectrum and tissue tropism from *M. giardi* (Table 2).

DISCUSSION

Traditionally, the identification and description of eel-infecting *Myxidium* has relied on morphological and morphometric approaches, mainly based on spore and polar capsule shape and size (Eiras et al. 2011). However, further studies have highlighted the importance of molecular data for species discrimination, often leading to the deciphering of cryptic myxozoan species/species complexes (Fiala 2006, Bartošová and Fiala 2011, Bartošová-Sojková et al. 2018, Freeman and Kristmundson 2018, Lisnerová et al. 2020, Alama-Bermejo et al. 2023). We employ a comparative analysis of morphological, biological and molecular features to elucidate the species status of *Myxidium giardi* in European eel originally described by Cépède (1906) and later detected and sequenced by Holzer et al. (2004). This multifaceted approach has previously proven effective in achieving more accurate taxonomic discrimination of myxozoans at the species level (Bartošová-Sojková et al. 2018, Lisnerová et al. 2020).

As certain myxozoan genera are morphologically similar, discerning the subtle differences between them can be highly challenging (Fiala et al. 2015). The differences between *Paramyxidium* and *Myxidium* were addressed by Freeman and Kristmundson (2018), when the newly established *Paramyxidium* genus was defined as having lemon-shaped spores in valvular view and being oval or bluntly-rounded in sutural view. In comparison, the spores of *Myxidium* are spindle-shaped, slightly crescent, or even sigmoid, with more or less pointed ends (Lom and Dyková 2006). Our morphological comparison of *M. giardi* from its original description (Cépède 1906) to the species *P. freemani* nom. nov. (Freeman and Kristmundson 2018) revealed that *M. giardi* possesses slightly pointed, spindle-shaped mature spores, which differ from the less elongated spores of *P. freemani* nom. nov. These species supposedly also differ in the number of turns of polar tubules and surface ridges. We showed that our *M. giardi* isolates from the kidney of Czech European eel do not morphologically and genetically match the isolates of *P. freemani* nom. nov. from the kidney of Icelandic European eel.

We demonstrate that *Myxidium* species from the Czech European eel is unequivocally conspecific with *M. giardi* originally described from the same fish tissue and host species from France (Cépède 1906) and sequenced from Scottish European eel (Holzer et al. 2004). Given these findings, we resurrect *M. giardi* and redescribe the species by including additional data, especially the 18S rDNA sequence. We additionally rename the type species of the genus *Paramyxidium*, previously designated as *Paramyxidium giardi*, to *P. freemani* nom. nov. Regarding the phylogenetic clustering, the existence of distantly related, albeit superficially morphologically similar myxosporean genera infecting the European eel is probably the outcome of convergent evolution of ancestrally different lineages within this host, as previously demonstrated by Fiala and Bartošová (2010).

Myxidium giardi was originally described from the kidney of European eel (Cépède 1906), and later reported as a common eel kidney parasite with 26% prevalence (Holzer et al. 2004). Although other reports have indicated *M. giardi* presence in additional organs of multiple anguillid species (Ishii 1915, Jacob and Bremen 1953, Ghittino et al. 1974, Copland 1981), no sequence data have been made available to ascertain the conspecificity of these findings. In our study, *M. giardi* was found in kidneys of 13% of the examined European eel individuals. All gill and bile samples were microscopically and molecularly negative.

Although our study did not include the examination of all other organs and tissues previously reported as sites of infection for *M. giardi*, it is likely that this species is a kidney-specific parasite of the European eel, and that other isolates reported from distinct organs of anguillids represent infections by other *Myxidium* or *Paramyxidium* species. *Paramyxidium* spp. appear to be relatively common in European eel, with an overall 83% prevalence in gills and 32% prevalence in European eel kidneys (Freeman and Kristmundson 2018). A more comprehensive sampling effort is needed in future studies to provide more data into the tissue preference of these parasites.

Of note is the more than 99% similarity found among the 18S rDNA sequences of *M. giardi* (GenBank: PP097746, AJ582213; present study, Holzer et al. 2004), *Chloromyxum schurovi* (GenBank: AJ581917; Holzer et al. 2004) and Neoactinomyxum eiseniellae (GenBank: AJ582007; Holzer et al. 2006). The previously published sequences (Holzer et al. 2004, 2006) originate from European eel, Atlantic salmon and the annelid *Eiseniella tetraedra* (Savigny) captured in the same riverine system in the Scottish Highlands (Holzer 2004, Holzer et al. 2004). Interspecific 18S rDNA variation in the myxozoans is typically > 2%, and when myxozoan species differ by < 1%, they can safe-

ly be considered conspecific in most cases (Fiala 2006, Bartošová and Fiala 2011, Atkinson et al. 2015, Lisnerová et al. 2020).

Therefore, the *C. schurovi* and N. eiseniellae sequences (Holzer et al. 2004, 2006) are considered to belong to *M. giardi*. While we suggest that N. eiseniellae sequence belongs to an actinospore stage of *M. giardi* as an inherent part of the parasite life cycle, the "*C. schurovi"* sequence record is probably incorrect, and the original sequenced sample did not contain any amplification from the observed *Chloromyxum* species. Incorrect sequencing was likely caused by laboratory contamination or by the presence of blood stages of *M. giardi* in the kidney of examined salmon. In fact, the blood stages of various myxozoan species have frequently been observed in various organs of their fish hosts. These stages likely serve for vascular system transport to reach the final site of parasite sporogonic development (Holzer et al. 2006, 2013, 2014).

PCR is a highly sensitive method capable of detecting low amounts of parasite DNA, namely in the cases of young parasite stages, such as proliferative blood stages, that might pass unnoticed on microscopic observations (Grossel et al. 2005). Unfortunately, no eel blood sampling was done in our study. Such investigation would facilitate the assessment of potential existence of *M. giardi* blood stages. Notably, blood stages have been molecularly detected in non-target fish hosts in which the parasite is not able to complete its development into the spore stage (Holzer et al. 2013), raising concerns about potential misidentification of viable host-parasite pairs (Atkinson et al. 2015). Although we assume that the life cycle of *M. giardi* probably includes European eel as the intermediate host and the freshwater oligochaete *E. tetraedra* as a definitive host, further experimental evidence is needed. Additionally, future sequencing of *C. schurovi* is necessary to reveal the true genetic identity of this parasite.

Unfortunately, our eel sampling only included European eel as the only native species in European habitats (Froese and Pauly 2023), and no other anguillid species have been available for additional fish host screening. Such host enrichment would be very useful to evaluate the possible host generalist nature of *M. giardi* and, more importantly, to explore the potential existence of other so far undiscovered myxidiids in these fish.

In summary, the morphologically similar but phylogenetically distant *Myxidium* and *Paramyxidium* species detected in European eels represent an interesting group of parasites from the biodiversity and evolutionary point of view but also in terms of animal protection. These parasites are widely distributed among European eel populations and some of them have been reported to cause pathology to both the farmed and wild European eel hosts (Copland 1981, Freeman and Kristmundsson 2018). Thus, they can have a significant impact on the health status of critically endangered European eel (IUCN 2023) and monitoring of such parasite species is important. The diagnosis of these parasites should be conducted carefully, incorporating molecular analyses to prevent confusion in taxonomy and the misattribution of sequences to incorrect species.

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