Studies of *Myxidium giardi* Cépède, 1906 infections in Icelandic eels identifies a genetically diverse clade of myxosporeans that represents the *Paramyxidium* n. g. (Myxosporea: Myxidiidae)

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**Abstract**

**Background:** The myxosporean *Myxidium giardi* Cépède, 1906 was described infecting the kidney of the European eel, *Anguilla anguilla* (L.), having spindle-shaped myxospores and terminal sub-spherical polar capsules. Since then, numerous anguillid eels globally have been documented to have similar *Myxidium* infections. Many of these have been identified using the morphological features of myxospores or by the location of infection in the host, and some have been subsequently synonymised with *M. giardi*. Therefore, it is not clear whether *M. giardi* is a widely distributed parasite, infecting numerous species of eels, in multiple organs, or whether some infections represent other, morphologically similar but different species of myxosporeans. The aim of the present study was to assess the status of *M. giardi* infections in Icelandic eels, and related fish hosts in Malaysia and to use spore morphology and molecular techniques to evaluate the diversity of myxosporeans present.

**Results:** The morphologies of the myxospores from Icelandic eels were very similar but the overall dimensions were significantly different from the various tissue locations. Myxospores from the kidney of the Malaysian tarpon, *Megalops cyprinoids* (Broussonet), were noticeably smaller. However, the SSU rDNA sequences from the different tissues locations in eels, were all very distinct, with percentage similarities ranging from 92.93% to as low as 89.8%, with the sequence from Malaysia being even more dissimilar. Molecular phylogenies consistently placed these sequences together in a clade that we refer to as the *Paramyxidium* clade (sensu stricto). We erect the genus *Paramyxidium* n. g. (Myxidiidae) to accommodate these histozoic taxa, and transfer *Myxidium giardi* as *Paramyxidium giardi* Cépède, 1906 n. comb. as the type-species.

**Conclusions:** There is not a single species of *Myxidium* (*M. giardi*) causing systemic infections in eels in Iceland. There are three species, confirmed with a robust phylogeny, one of which represents *Paramyxidium giardi* n. comb. Additional species probably exist that infect different tissues in the eel and the site of infection in the host fish is an important diagnostic feature for this group (*Paramyxidium* n. g. clade). Myxospore morphology is generally conserved in the *Paramyxidium* clade, although actual spore dimensions can vary between some species. *Paramyxidium* spp. are currently only known to infect fishes from the Elopomorpha.

**Keywords:** *Paramyxidium* n. g., *Paramyxidium giardi* n. comb., Elopomorpha, Protacanthopterygii, Myxosporea, Chloromyxum

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Background
The importance of the shape of myxospores and its relative usefulness in myxosporean taxonomy has been scrutinised in recent years, and it has been unambiguously demonstrated that numerous genera are now polyphyletic in molecular phylogenetic analyses due to the use of spore morphology as the principal taxonomic measure [1–3]. However, myxospores remain the primary diagnostic feature of myxosporean infections in fish and other vertebrates [4] and their morphology, especially when combined with other important information from the host, can sometimes be sufficient to make preliminary identifications or diagnoses without the use of DNA analysis. The polyphyletic nature of some myxosporean genera in molecular analyses has occurred, in part, as our systematic framework for deciding which family and genus to place novel species has become less stringent over time, and gives priority to basic myxospore morphology over other characters [4]. This is combined with the fact that there also seems to be a general reluctance to establish new genera when necessary, with the historical preference being to further loosen the descriptive boundaries of existing genera [4]. This has led to artificially high numbers of species being placed in certain genera, which in some cases are clearly not closely related to each other, and has hence contributed to polyphyly in subsequent molecular phylogenies [2, 4].

Myxidium giardi Cépède, 1906 was originally described from the kidney of the European eel, Anguilla anguilla (L.), in France, with myxospores described as spindle-shaped with terminal sub-spherical polar capsules [5], but have since been reported in numerous sizes and described as both wide and slender, some with almost spherical polar capsules [6, 7]. Globally, numerous species of anguillid eels have been reported to have similar Myxidium spp. infections, many of which have been distinguished using morphological features such as spore size and number or lack of valvular striations [8, 9] or the site of infection in the eel (skin, gills, kidney, stomach, etc.) [10]. However, many descriptions of Myxidium spp. infecting eels have subsequently been synonymized with M. giardi, as less emphasis was placed on details such as the site of infection [11] and M. giardi was considered to have an almost worldwide distribution [12]. Currently, it is not clear whether M. giardi is a widely distributed parasite infecting numerous species of eels, in multiple organs, or whether some infections represent other, morphologically similar, species of myxosporeans. One short rDNA sequence exists for M. giardi from the urinary system of A. anguilla from Scotland, and phylogenetic analyses place this in the freshwater urinary clade, with numerous other myxosporeans found infecting the urinary systems of fish, but none with a similar spore morphology [13].

Anguillid eels (Order Anguilliformes) are catadromous fish consisting of 17 species in a single genus, Anguilla Garsault, distributed in all the world’s oceans. Two species inhabit the Atlantic Ocean, the European eel, A. anguilla, and the American eel, Anguilla rostrata (Lesueur) [14]. A dramatic decline has been experienced in populations of the European eel and diseases/parasites are considered to be one of several factors responsible. Consequently, since 2009, the European eel has been on the red list of the International Union for the Conservation of Nature (IUCN) and hence is listed as a ‘critically endangered’ species [15]. The Indo-Pacific tarpon, Megalops cyprinoides (Broussonet) (Order Elopiformes), inhabits tropical coastal and brackish waters of the Indo-Pacific, migrating between the open sea and inland rivers and mangroves [16]. Elopiformes are related to, but do not resemble anguillid eels, and like eels they spawn at sea producing leptocephalic larvae that later migrate inland [17]. Tarpon (Elopiformes) together with their sister group the eels (Anguilliformes) form the Elopomorpha which are one of the oldest major extant teleost lineages [18].

In the present study, we aimed to examine eels from Iceland and genetically characterise Myxidium giardi-like parasites from different infection sites in the hosts. In addition, we aim to compare these with morphologically similar parasites previously observed by one of us (MAF) in tarpon from Malaysia.

Methods
Thirty-one live European eels, A. anguilla, (growing yellow eels, total length range 42–68 cm) were collected from Lake Vifilsstadavatn in Iceland during May 2013 using fyke nets. The fish were transported to the laboratory, placed in tanks with freshwater and kept alive until examination. Prior to examination, the fish were euthanized in MS-222. All eels were dissected and examined for myxosporeans in the gills, kidney and stomach wall using dissecting and compound microscopes. Images were taken of twenty spores from each tissue location for size estimations using ImageJ. In addition, fourteen mature Pacific tarpon, Megalops cyprinoides total length range 22–34 cm, were caught with gill nets in Kilim mangroves on Langkawi Island, Malaysia. Tarpon were examined for gill and kidney myxosporeans, using dissecting and compound microscopes. In Malaysia, myxospores were photographed in the field using a portable compound microscope and a Dino-Eye eyepiece camera. Infected tissues from eels and tarpon were fixed in 10% buffered formalin and processed for standard histology, 3 μm thick sections prepared, stained with Giemsa and mounted in resin-based medium.

Oligochaetes (n = 100) were taken from sediment samples from the lake Vifilsstadavatn in Iceland, isolated and incubated at 10 °C in filtered (pore size 0.45 μm) lake water in 24-well tissue culture plates. Oligochaetes were examined daily using a dissecting microscope for the presence of actinospore production, typically characterized by
a cloudy secretion from the anus of the worms, and confirmed using a compound microscope. Every other day, the water in the wells was exchanged. The oligochaetes that produced actinospores were used for DNA analysis and images of the actinospores taken as detailed above.

Infected tissue (kidney, stomach wall) or spore-filled cysts (gills), equal to approximately 20–40 mg, were placed directly into DNA lysis buffer for molecular analysis. Total DNA was extracted using a GeneMATRIX DNA isolation kit (EURx, Gdansk, Poland) following the tissue protocol and used as templates in subsequent PCR reactions. Parasite small subunit ribosomal DNA (SSU rDNA) was amplified using the general myxosporean primers and methodology previously described [2] with a new reverse primer M-790r (5'-ACG ACC AAT TAA GGC TAT GC-3'), paired with 18e, utilising the PCR conditions as in [2]. In addition, the primer pair Mg-50f (5'-ACT AAG CCA TGC ATG TCT ATG T-3') and Mg-1170r (5'-TGA TCA ATC GAA ACG GTC TAG G-3') were designed to assist in further studies of these myxosporeans from anguilid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporean sequences identified from the BLAST lid eels and amplified a 1150 bp region of the SSU rDNA sequence alignments of the novel sequences to other related myxosporan

**Results**

All the fish examined in this study appeared in good condition and no gross signs of a disease were present. From the 31 eels examined, small myxosporean cysts were observed on the gills of 25 fish (prevalence of 83%) using a dissection stereoscope, ranging between 2–10 cysts per gill. Infections in the kidney and the stomach wall were only detected using a compound microscope. Spores were detected in the kidney of 10 fish (32%) and from stomach scrapings from 6 eels (19%). Infections were light in most cases, being more apparent in the gills. All myxospores detected had a similar morphology that conformed to the currently accepted shape and size range for *Myxidium giardi* (Table 1) [12]. No gill cysts were observed from 14 tarpon, examined in Malaysia, but the kidney of 4 fish (29%) were heavily infected with a myxosporean, observed in fresh tissue preparations. The myxospores from tarpon kidney had a similar morphology to the ones from the eels. However, they were noticeably, and significantly, smaller than all three species observed from the Icelandic eels (Tables 1 and 2).

The overall morphology and dimensions of the myxospores from Icelandic eels appeared similar (Fig. 1). However, taking average dimensions from 20 measured spores, they were significantly different with regard to spore length and/or width (Tables 1 and 2). Most fresh spores were typically observed in the sutural plane and had an oval to bluntly-rounded shape with almost spherical polar capsules opening sub-terminally (Fig. 1a-e). The sutural line, when visible, was sigmoidal and striations were visible on the surface of the spore valves (Fig. 1c, f), although these structures were not always clearly visible. In the valvar view, spores were more lemon-shaped than a true spindle or fusiform shape (Fig. 1e), but clearly resembled the original drawings.
by Cépède [5], however they did not have terminal capsular foramina. All myxospores in this study were between 62–70% as wide as they were long, which is similar to the original description of Myxidium giardi from eels in France (Table 1) [5]. The myxospores from tarpon in Malaysia, had a remarkably similar overall morphology to those from Icelandic eels (Fig. 1g-k), but they were significantly smaller in size (Tables 1 and 2). When myxospores were stained, it was clear that the sporoplasm contained two nuclei, in all cases, as originally described for M. giardi [5]. Only one oligochaete, morphologically identified as Lumbriculus variegatus, produced actinospores with an Aurantactinomyxon morphology (Fig. 1l, Table 3).

**Histological examination**

*Paramyxidium giardi* infecting the kidney of eels forms polysporous plasmodia which vary greatly in size (c.55–120 μm in this study). The plasmodia were most commonly found as intratubular, both in the proximal and distal kidney tubules, although they were also, to a lesser extent, observed in the renal interstitium. The associated histopathology was mostly due to mechanical disruption of the tissue as the large plasmodia often caused extensive widening of the kidney tubules followed by atrophy and degeneration of the tubular epithelial cells (Fig. 2a) which is in agreement with the pathology described by Copland [28]. Renal infections described in his study [28] were more extensive than in this study. Consequently, he observed varying degrees of pathology in other parts of the kidney, e.g. the Bowman capsule and the kidney interstitium.

*Paramyxidium magi* n. sp. is histozoic in the stomach wall. It forms polysporous plasmodia of variable sizes (c.60–95 μm) in the gastric gland interstitium (Fig. 2b). Large spore masses in the gastric glands cause focal compaction and disruption of the adjacent gastric glands similar to previous reports [28].

*Pararyxidium branchialis* n. sp. is histozoic in the gills. As observed by Copland [28], the great majority of infections were observed in the secondary lamellae. The plasmodia are polysporous and differ significantly in size (c.35–142 μm). Infections cause separation of the lamellar epithelial cells from the basement membrane and disruption of pillar cells (Fig. 2c) and in some cases oedema in the basal part of the secondary lamellae.

| Table 1 Measurements of twenty fresh myxospores of *Paramyxidium* spp., including dimensions from the original description of *P. giardi* |
|---------------------------------------------------------------|
| Species | Host species | Site of infection | Spore body Range (Mean) | Polar capsules Range (Mean) | Reference |
|---------|--------------|------------------|-------------------------|-----------------------------|-----------|
|         |              |                  | Length | Width | Length | Width |
| *P. giardi* | *A. anguilla* | Kidney | 9.0–10.0 | 5.5–6.0 | 3.0–5.0 | nd |
| | | | (nd) | (nd) | (nd) | (2.0) |
| *P. giardi* | *A. anguilla* | Kidney | 9.5–11.4 | 6.5–7.5 | 3.5–4.2 | 2.7–3.9 |
| | | | (10.6) | (7.0) | (4.0) | (3.6) |
| *P. magi* n. sp. | *A. anguilla* | Stomach wall | 10.8–12.9 | 7.0–8.4 | 3.6–4.6 | 3.1–4.0 |
| | | | (11.6) | (7.6) | (4.0) | (3.6) |
| *P. branchialis* n. sp. | *A. anguilla* | Gills | 10.7–12.3 | 6.6–7.8 | 3.9–4.5 | 3.2–4.2 |
| | | | (11.6) | (7.3) | (4.2) | (3.8) |
| *P. bulani* n. sp. | *M. cyprinoides* | Kidney | 6.1–6.9 | 4.2–5.1 | 2.1–2.5 | 1.5–2.0 |
| | | | (6.7) | (4.7) | (2.3) | (1.8) |

*Original description
Abbreviation: nd, no data

***Statistical comparison of spore length (above the diagonal) and width (below the diagonal) of the four species observed in the study***

| Species | *P. giardi* | *P. magi* n. sp. | *P. branchialis* n. sp. | *P. bulani* n. sp. |
|---------|-------------|------------------|-------------------------|------------------|
| *P. giardi* | *t*<sub>10</sub> = 6.53; *P* < 0.0001 | *t*<sub>10</sub> = 6.42; *P* < 0.0001 | *t*<sub>19</sub> = 30.64; *P* < 0.0001 |
| | ******** | ******** | ******** |
| *P. magi* n. sp. | *t*<sub>10</sub> = 6.22; *P* < 0.0001 | *t*<sub>10</sub> = -0.45; *P* = 0.6544 | *t*<sub>19</sub> = 51.94; *P* < 0.0001 |
| | ******** | **ns** | ******** |
| *P. branchialis* n. sp. | *t*<sub>10</sub> = -3.61; *P* = 0.0009 | *t*<sub>10</sub> = 3.18; *P* = 0.0029 | *t*<sub>19</sub> = 44.90; *P* < 0.0001 |
| | **** | **** | ******** |
| *P. bulani* n. sp. | *t*<sub>10</sub> = 29.38; *P* < 0.0001 | *t*<sub>10</sub> = 31.12; *P* < 0.0001 | *t*<sub>19</sub> = 33.70; *P* < 0.0001 |
| | ******** | ******** | ******** |

**Abbreviation: ns, not significant**

* *P* < 0.01, ** *P* < 0.001, **** *P* < 0.0001
Fig. 1 (See legend on next page.)
**Paramyxidium bulani** n. sp. infects the kidney. The plasmodia are polysporous, somewhat elongated (size range: length, 25–75 μm; width, 10–22 μm) and develop as intratubular, both in proximal and distal tubules. They are seen at various stages in development, some of which are fully mature, and commonly attached to the brush border of the proximal kidney tubule epithelium. In the distal kidney tubules, the proportion of mature plasmodia seem higher (Fig 2d). No pathology was associated with infections.

No host response, such as encapsulation by fibroblasts or infiltration of immune cells, was detected in association with any of these four different myxosporeans.

**Molecular analyses**

SSU rDNA sequences were successfully sequenced for *P. giardi* (2076 bp) and for the three species recognised here as new, i.e. *P. magi* n. sp. (2070 bp), *P. branchialis* n. sp. (2082 bp), *P. bulani* n. sp. (2056 bp) and the Aurantiactinomyxon (865 bp), which have been assigned accession numbers in GenBank: MH414925-MH414929. BLAST searches confirmed a myxosporean origin for all accession numbers in GenBank: MH414925-MH414929.

**Table 3**

| Actinospore type          | Host species | Diameter of spherical spore body (Mean) | Length of caudal process (Mean) | Width of caudal process (Mean) | Largest span between end of caudal processes (Mean) | Reference          |
|--------------------------|-------------|-----------------------------------------|-------------------------------|------------------|-----------------------------------------------|-------------------|
| Aurantiactinomyxon      | *L. variegatus* | 9.0–12.5 (10.4) | 14.5–16.3 (15.4) | 7.5–10 (8.5) | 29.9–325 (30.8) | Present study                               |

Molecular phylogenetic analyses produced trees with very similar topologies irrespective of the methodology used (Fig. 3). All *Paramyxidium* spp. were fully supported in a clade with numerous actinospore sequences (*Paramyxidium clade*) shown as the lilac box in Fig. 3. *Paramyxidium bulani* n. sp. from a tarpon in Malaysia, represented the basal sequence of the clade. The *Myxidium* clade (*sensu stricto*), that includes the type-species *M. lieberkuehni*, was also fully supported (red box, Fig. 3) and formed a fully supported sister group to the clade containing *Chloromyxum* spp. (green box, Fig. 3). Together these formed a robustly supported sister clade to the *Paramyxidium* clade. The Platysporina (blue box, Fig. 3) formed a fully supported sister clade to this *Paramyxidium / Myxidium* (s.s.) / *Chloromyxum* grouping. Basal to this is the hepatic biliary clade, a well-supported clade which contains numerous taxa currently assigned to the *Myxidiidae*, some of which infect vertebrates other than fish [22].

**Taxonomy**

Class Myxosporea Bütschli, 1881
Suborder Varisporina Lom & Noble, 1984
Family Myxidiidae Thélohan, 1892

**Genus Paramyxidium n. g.**

**Diagnosis**

Myxospore length width ratio between 1.4–1.6:1 resulting in a lemon-shaped form in valvular aspect and oval to bluntly-rounded form in sutural view. Valvular striations present, with sigmoidal sutural line. Polar capsules two, almost spherical. Two nuclei in the sporoplasm positioned between polar capsules. Capsular foramina open sub-terminally. Histozoic polysporous plasmodia develop in various host tissues with different species infecting different host tissues. **Type-species**: *Paramyxidium giardi* (Cépède, 1906) n. comb.
Other species: Paramyxidium branchialis n. sp.; Paramyxidium bulani n. sp.; Paramyxidium magi n. sp.

Etymology: The new genus is named Paramyxidium n. g. as it is placed next to the type-species of the genus Myxidium (s.s.) in phylogenetic analyses.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) [29], details of the new genus have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is: urn:lsid:zoobank.org:pub:716F81BB-DAFC-4-B33-89E5-A57684065765. The LSID for the new genus name Paramyxidium is: urn:lsid:zoobank.org:act:27B9FB C6-345A-4DD9-93D6-A13EACB82A18.

Remarks
Paramyxidium spp. are sufficiently different with respect to either myxospore morphology/characteristics, tissue location, host type or DNA sequences to necessitate the erection of a novel genus within the family

Table 4 Percentage identities of SSU rDNA sequences (above the diagonal) and number of bases compared (below the diagonal) for the new sequences in the Paramyxidium clade (bold), related actinospores and the type-species of the genus Myxidium, M. lieberkuehni

|   | 1   | 2      | 3       | 4     | 5   | 6      | 7      | 8     |
|---|-----|--------|---------|-------|-----|--------|--------|-------|
| 1 |     | Paramyxidium giardi | 92.93  | 89.80 | 98.29 | 89.91 | 94.79  | 81.75 | 77.98 |
| 2 |     | Paramyxidium magi n. sp. | 91.94  | 92.50 | 95.77 | 90.35 | 83.08  | 78.70 |
| 3 |     | Paramyxidium branchialis n. sp. | 2058  | 2055  | 2058  | 2055  | 2058  | 2055  | 91.94  | 83.08 | 78.70 |
| 4 |     | Aurantiactinomyxon (AF483598) | 1931  | 1933  | 1939  | 1939  | 1939  | 1939  | 94.07  | 81.27 | 77.60 |
| 5 |     | Synactinomyxon (AY787784) | 1605  | 1599  | 1606  | 1602  | 1602  | 1602  | 91.31  | 82.15 | 77.53 |
| 6 |     | Aurantiactinomyxon | 864  | 865  | 860  | 860  | 863  | 863  | 83.55  | 80.05 |
| 7 |     | Paramyxidium bulani n. sp. | 2044  | 2041  | 2039  | 1917  | 1585  | 863  | 863  | 78.70 |
| 8 |     | Myxidium lieberkuehni (K76638) | 2021  | 2014  | 2028  | 1893  | 1562  | 827  | 2009  |
Myxidiidae Thélohan, 1892 to accommodate them. The family Myxidiidae currently contains five genera: Myxidium Bütschli, 1882; Coccomyxa Léger & Hesse, 1907; Cystodiscus Lutz, 1889; Enteromyxum Palenzuela, Redondo & Alvarez-Pellitero, 2002; Soricomyxum Prunescu, Prunescu, Pucek & Lom, 2007; and

Fig. 3 Maximum likelihood topology of small subunit ribosomal DNA from 54 myxosporeans, rooted to Chloromyxum leydigi and C. riorajum (infecting cartilaginous fishes). Bootstrap support values and posterior probabilities are shown at the nodes; solid black dots indicate full support for that node. Novel sequences from this study are in bold and contained within the *Paramyxidium* Clade (lilac box). The *Paramyxidium* clade forms a robust sister clade relationship with *Myxidium* clade (*sensu stricto*) (red box) and the *Chloromyxum* clade (green box). The dashed green box within the *Chloromyxum* clade shows species that infect the gall-bladder (GB) of cypriniform fishes. Nodes with a red asterisk indicate that the common ancestor was likely to have been a renal-infecting myxosporean. The Platysporina (blue box) forms a fully supported sister clade to this *Paramyxidium* / *Myxidium* (*s.s*.) / *Chloromyxum* grouping and includes the Myxobilatidae (grey box). Highlighted within the Myxobilatidae, by a dashed black box, is the *Chloromyxum schurovi* sub-clade that contains other renal isolates from eels that have been reported as *Myxidium giardi* and *Zschokkella* sp. Basal to these main clades is the hepatic biliary clade, a well-supported clade which contains numerous taxa, some of which infect vertebrates other than fish.
Zschokkella Auerbach, 1910. Species of Cystodiscus and Soricimyxx are only found in non-fish hosts [22]. Enteromyxum spp. have very large and elongated polar capsules, spores that lack valvular striations, and are phylogenetically associated with morphologically similar forms from the gastrointestinal tract of marine fishes [4]. Coccomyxx spp. are found in the gall-bladder of marine fishes, are more ellipsoidal in morphology, and are phylogenetically distinctly placed in the marine gall-bladder (marine Myxidium) clade [2]. The genera Myxidium and Zschokkella are more complicated to decipher for as both are polyphyletic, with many species likely being wrongly attributed to each genus [1, 2, 4]. However, DNA sequence data convincingly placed all Paramyxidium taxa in a robust clade which is sister to the Myxidium clade (s.s.). In addition, host differences and tissue tropism are apparent, with Paramyxidium taxa infecting the Elopomorpha in numerous organs and Myxidium spp. (s.s.) infecting the renal system of members of the Protacanthopterygii.

Therefore, we erect the genus Paramyxidium to accommodate these myxosporeans, and transfer Myxidium giardi as Paramyxidium giardi Cépède, 1906 n. comb. as the type-species. The annelid hosts for Paramyxidium spp. are likely to be oligochaetes. Currently known species are restricted to freshwater environments and fish hosts from the Elopomorpha, presumably becoming infected during migratory periods into fresh water.

Description

**Spore.** Mature spores lemon-shaped in valvular aspect (Fig. 1a), oval to bluntly-rounded in sutural view with sigmoidal sutural line (Fig. 1b), measuring 10.8–12.9 × 7.0–8.4 (11.6 × 7.6) (L × W) (n = 20). Polar capsules almost spherical, 3.6–4.6 × 3.1–4.0 (4.0 × 3.6) (n = 20), opening sub-terminally (Fig. 1a-b). Fine spore striations visible across both spore valves (Fig. 1c).

**Remarks**

Histozoic in the stomach wall forming polysporous plasmodia of variable sizes (c.60–95 μm) in the gastric gland interstitium (Fig. 2b). Differs from other Paramyxidium spp. primarily on the site of infection and genetic distance.

**Paramyxidium bulani** n. sp.

**Type-host:** Megalops cyprinoides Broussonet (Elopiformes: Megalopidae).

**Type-locality:** Kilim mangroves, Langkawi Island, Malaysia.

**Other localities:** Pangkor Island, Malaysia.

**Type-specimens:** Hapantotypes (histological sections and phototypes) were deposited at the Natural History Museum, London, UK, under the accession number NHMUK 2018.237.

**Site in host:** Kidney.

**Prevalence:** 29% (4/14).

**Representative DNA sequences:** A partial SSU rDNA sequence (2059 bp) was deposited in the GenBank database under the accession number MH414929.

**ZooBank registration:** To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) [29], details of the new species have been submitted to ZooBank. The LSID for the new species name Paramyxidium bulani is: urn:lsid:zoobank.org:act:DA7B51B0-590F-45A5-88CF-32A015B99593.

**Etymology:** Specific name refers to the local Malay name for the fish host (ikan bulan).

**Description**

**Spore.** Mature spores oval to bluntly-rounded in sutural view, lemon-shaped in valvular aspect, 6.1–6.9 × 4.2–5.1 (6.7 × 4.7) (L × W), opening sub-terminally. Sutural line sigmoidal and inconspicuous (Fig. 1g-k), spore valves with fine striations.

**Remarks**

Plasmodia are elongated and polysporous (size range: length, 25–75 μm; width, 10–22 μm) and develop as intratubular, in both proximal and distal kidney tubules. Differs from other Paramyxidium spp. by having much...
smaller myxospores and infecting the kidney of Pacific tarpon. Stained spores have a sporoplasm containing two nuclei (Figs. 1i and 2d)

**Paramyxidium branchialis** n. sp.

**Type-host:** Anguilla anguilla (L.) (Anguilliformes: Anguillidae).

**Type-locality:** Lake Víflsstadavatn (64°4’47.39"N, 21°52’26.45"W), Iceland.

**Type-specimens:** Hapantotypes (histological sections and phototypes) were deposited at the Natural History Museum, London, UK, under the accession number NHMUK 2018.236.

**Site in host:** Gill.

**Prevalence:** 83% (25/31).

**Representative DNA sequence:** A partial SSU rDNA sequence (2082 bp) was deposited in the GenBank database under the accession number MH414926.

**ZooBank registration:** To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) [29], details of the new species have been submitted to ZooBank. The LSID for the new species name *Paramyxidium branchialis* is: urn:lsid:zoobank.org:act:2481F7F4-5B9F-42C8-B2EB-FED264FA7283.

**Etymology:** Specific name refers to site of infection in the host.

**Description**

**Spore.** Mature spores oval to bluntly-rounded in sutural view, lemon-shaped in valvular aspect, 10.7–12.3 × 6.6–7.8 (11.6 × 7.3) (L × W) (n = 20). Polar capsules almost spherical, 3.9-4.5 × 3.2–4.2 (4.2 × 3.8) (n = 20), opening sub-terminally. Sutural line sigmoidal and inconspicuous. Fine spore striations visible across both spore valves.

**Remarks**

Plasmodia are polysporous, differ significantly in size (c.35–142 μm), and develop in the gills (Fig. 2c), whereas other species in the genus develop in different host tissues. Differences from other *Paramyxidium* spp. primarily on the site of infection and genetic distance.

**Discussion**

Morphological features, such as myxospore size and number of valvular striation, have been used to identify or differentiate between species of *Myxidium* infecting eels in the past [6–10]. However, with such conserved myxospore morphology within the *Paramyxidium* n. g. clade, we do not find this to be a reliable feature to successfully differentiate between species. The location of the parasite within certain specific host tissues would appear to be a far more useful characteristic, with similar tissue tropism being reported for other myxosporean clades in the past [1, 2, 4]. The European eel and its parasites have been extensively studied [5–7, 14] and there have been numerous reports of *Myxidium giardi* infecting the European eel [7, 12, 30]. However, many of these reports of *M. giardi* infections are from tissues other than kidney and it is therefore highly likely that many of these diagnoses are in fact detailing a morphologically similar parasite from the genus *Paramyxidium* but not *M. giardi*. Numerous taxa that fit within the *Paramyxidium* may already be described [8–12], or have been synonymised with other species due to similar spore morphologies [7, 12]. Given the clear differences in SSU rDNA data that exist between the known members of this new genus, it is imperative that such data is supplied when validating or describing novel species within this group. The *Paramyxidium* clade currently contains sequences from myxospores that infect various tissues of fish from the superorder Elopomorpha (tarpon/ladyfish and eels). The clade is also comprised of numerous sequences generated from actinospores that probably represent a hidden diversity of unidentified myxosporeans infecting various tissues from anguillid eels; as the sequences have all been generated from European freshwater systems that lack elopiform fish other than eels and *Myxidium*-like infections have been noted in numerous tissues from the European eel [31, 32].

Our phylogenies show that the *Paramyxidium* group is robustly supported as a sister clade to the *Myxidium* clade (s.s.) / *Chloromyxum* grouping that form as fully supported subclades (Fig. 3). These three clades all demonstrate levels of fish host association and degrees of tissue tropism. All currently known members of the *Myxidium* clade (s.s.) are described from the renal systems of either pike or salmonids (*Protacanthopterygii*), whilst the adjacent *Chloromyxum* sister group are all described from Cypriniformes, when infecting the gall-bladder (dashed green box, Fig. 3), with the basal species from the clade, *Chloromyxum kurisis* (GenBank: KJ526212), being from the urinary tract of an Atheriniformes fish. This suggests that these two clades have evolved from a common ancestor that infected the renal system of fish potentially having a *Chloromyxum*-like form. The *Paramyxidium*, as a sister to these two groups, have only been described from the Elopomorpha, with the basal species in the clade again coming from a renal infection, further indicating that the common ancestor for the whole grouping was likely to have been a renal myxosporean. A deeper extension of this phenomenon can also be applied to our tree topology. The node from which the Platysporina clade forms as a sister to the *Paramyxidium/ Myxidium* (s.s.) clade, could also represent a common ancestor that infected the renal system of fish. Recently the Myxobilatidae (all renal-infecting) have been shown to be phylogenetically placed within the platysporinids [33],
therefore, a renal myxosporean ancestry for the Platysporina is plausible, which could also be associated with a Chloromyxum-form. This outcome is not unexpected, as it has been previously demonstrated that the Chloromyxum morphotype is important in phylogenetic studies and appears to represent a basal morphotype for numerous myxosporean clades [22, 23, 34]. Therefore, additional sequence data from this basal morphotype, in particular when infecting more ancient fish lineages (Atheriniformes, Cypriniformes, Elopomorpha and Protacanthopterygii), will assist in future phylogenetic studies of the Myxospora and help to better resolve the relationships between certain reproducible clades.

Certain anomalies do exist in our phylogenetic tree, which can be explained as follows. The Myxidium clade (s.s.) currently contains myxosporeans that infect the urinary system of fish from the Protacanthopterygii, a superorder of more basal teleosts, which includes the Salmoniformes (salmonids) and Esociformes (pikes). The sequence in the tree representing Sphaerospora oncorhynchi Kent, Whitaker & Margolis, 1993 (GenBank: AF201373) is, therefore, more likely to be from a fish that was co-infected with a Myxidium, likely related to the Myxidium sp. described as the CKX myxosporean from coho salmon in Canada [35]. Indeed, studies have found both S. oncorhynchi and Myxidium salvelini Konovalov & Shul’mann, 1966, in the renal systems from the same Oncorhynchus spp. [36, 37] and other striated Myxidium spp., have been reported from the urinary systems of salmonids: Myxidium minteri Yasutake & Wood, 1957; M. salvelini Konovalov et Shul’mann, 1966; Myxidium nobiei Konovalov, 1966; Myxidium sp. of Mavor & Strasser, 1918.

The positioning of the sequences Zschokkella sp. (GenBank: AJ581918), Chloromyxum schurovi (GenBank: AJ581917) and Myxidium giardi (GenBank: AJ582213) (dashed black box, Fig. 3), all generated from the same study [13], within a sub-clade of the Myxobilatidae (Platysporina) remains puzzling, especially the Myxidium/ Zschokkella spore forms that exclusively belong to the Variisporina and should not therefore be present in the Platysporina [33]. This concern is reinforced, as the sequence given for M. giardi in eels from Scotland is almost identical to that of C. schurovi from salmonids [13]. In his description of the genus Acauda, Whipp [38] demonstrated the clustering of the genera Acauda, Myxobilatus and Hofferellus in phylogenetic analyses and noted that the myxospores shared similar morphological features, all having longitudinally striated spore valves and a suture plane that bisects a pair of polar capsules. This combination of features justified the reuse of the family Myxobilatidae Shul’mann, 1953, to accommodate these three genera. In addition, it has been demonstrated that Ortholinea should be included in the family Myxobilatidae, as Ortholinea spp. share similar morphological features, group within the same clade, and collectively they are all parasitic in the renal system of fish [33]. Chloromyxum myxospores also share some synapomorphies with this group, having subspherical or elongated spores that may have caudal appendages, with a straight central suture. Chloromyxum is known to be polyphyletic genus and the retention of some features (4 polar capsules) in some derived species could explain why evolved chloromyxid forms are present in some clades, such as the Myxobilatidae clade (C. schurovi) and the hepatic biliary clade (Chloromyxum trijugum). Indeed, Holzer et. al. [39], reported that C. schurovi, whilst being genetically similar to members of the clade it occupied (Myxobilatidae), it shares less than 70% identity to other more basal Chloromyxum species and concluded it had evolved further than all other sequenced chloromyxids [39]. Therefore, the inclusion of C. schurovi with the Myxobilatidae can potentially be explained in terms of DNA sequence, spore morphology and tissue tropism. However, the presence of Myxidium giardi (GenBank: AJ582213) and Zschokkella sp. (GenBank: AJ581918) still cannot, as spores with these morphologies share no synapomorphies with any members of the clade or the predicted ancestral morphotypes, which are assumed to be chloromyxids. One possibility is that the sequences generated for M. giardi and Zschokkella sp. either do not correspond to the myxospores that were observed in the tissue samples, or that they were misidentified. They may represent variants of a sequence for Zschokkella stettinensis Wierzbicka, 1987, described from the urinary bladder of the European eel [40]. Indeed, the author of the sequences in question also indicated, in their PhD thesis, that Z. stettinensis was a possible identity for this myxosporean [41]. The myxospores of Z. stettinensis look superficially like those of M. giardi but are also very similar to the Neomyxobolus morphotype (known to infect the renal system of freshwater fishes), being described as spores having two delicate streaks running parallel to the suture [40], a feature almost unique to Neomyxobolus spp. [42]. Therefore, we suggest that these sequences represent variations of Z. stettinensis or genetically related forms, which are better placed in the genus Neomyxobolus Chen et Hsieh, 1960. Myxidium streisingeri Whipp, Murray & Kent, 2015 also groups in the Myxobilatidae and is another species showing several traits in common with the genus Neomyxobolus [33], indicating that it has been mistakenly placed as Myxidium. Furthermore, these species also have the tissue location (renal system) and spore characteristics to allow placement in the Myxobilatidae.

In the present study, coinfections with other myxosporeans were not observed in Icelandic eels, only Para-myxidium morphologies were observed. However, the Pacific tarpon is known to have other myxosporean parasites [4]. Therefore, it is possible when sampling tissues such as the kidney, that blood-borne stages of non-target
myxosporean could be amplified in error. This is considered to be unlikely in this case, as most of the myxosporean taxa from the Pacific tarpon from the type location have been identified [4, 43] (MAF, unpublished data). In addition, the fact that these distantly related parasites form a robust monophyletic clade, support the fact that the sequences were derived from the myxospore forms observed.

Conclusions
Paramyxidium giardi n. comb. (syn. Myxidium giardi) does not cause systemic infections in eels. In Iceland there are three species now confirmed, using morphological and molecular data. Additional species probably exist that infect different tissues, such as the skin, and a further member of this clade was identified here from an Aurantiactinomyxon sequence. Therefore, the site of infection in the host eels is an important diagnostic feature for this group (Paramyxidium clade). Myxospore morphology is highly conserved in the Paramyxidium clade, and although some myxospore dimensions are noted as significantly different between isolates, differentiating species based on spore dimension alone is not recommended. Despite very similar myxospore morphologies being present across the group there are relatively low genetic similarities between SSU rDNA sequences. The Paramyxidium clade is a well-supported sister to the Myxidium (s.s.) and Chloromyxum clades, each demonstrating a degree of fish host group specificity.

Abbreviations
BLAST: Basic Local Alignment Search Tool; LSID: Life Science Identifier; s.s.: sensu stricto; SEM: Scanning electron microscopy

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