

RESEARCH ARTICLE

Eelgrass (*Zostera* spp.) associated phytomyxids are host-specific congeneric parasites and predominant eukaryotes in the eelgrass rhizosphere on a global scale

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Abstract

Together with increasing environmental and anthropogenic pressures, pathogenic diseases are one of the important factors contributing to the ongoing decline of seagrass meadows worldwide; yet the diversity and ecology of the microorganisms acknowledged as seagrass parasites remain critically understudied. Here, we investigate phytomyxid parasites (Rhizaria: Endomyxa: Phytomyxea) of three different eelgrass (*Zostera* spp.) species found in the Northern hemisphere. We present molecular evidence that *Plasmodiophora bicaudata*, a long-recognized parasite of dwarf eelgrass taxa, is closely related to the novel phytomyxid recently discovered in root hairs of *Zostera marina*, and together they form a distinct clade within the order Phagomyxida, proposed here as *Feldmanniella* gen. nov. A full life cycle is systematically described in a phagomyxid representative for the first time, proving its conformity with the generalized phytomyxid life history, despite previous uncertainties. The presence of primary infection stages in nearly all collected eelgrass specimens, and subsequent analysis of amplicon sequences from a global *Z. marina* dataset, reveal phytomyxids to be ubiquitous and one of the predominant microeukaryotes associated with eelgrass roots on a global scale. Our discoveries challenge the current view of Phytomyxea as rare entities in seagrass meadows and suggest their generally low pathogenicity in natural ecosystems.

INTRODUCTION

Seagrasses of the genus *Zostera* (Alismatales: Zosteraceae), commonly referred to as ‘eelgrass’ and ‘dwarf eelgrass’, are a group of true marine angiosperms with ribbon-like leaves known to form vast underwater meadows in estuaries and intertidal to subtidal zones worldwide (den Hartog, 1970; Green & Short, 2003). They are considered important ecosystem engineers and provide a variety of essential ecosystem services such as habitat and feeding ground for numerous

animal assemblages (including commercially important fish and invertebrates), prevention of coastal erosion by reducing the hydrodynamic energy of waves, and stabilization of the seafloor sediment. (Bos et al., 2007; Kennedy et al., 2018; Meysick et al., 2022; Surugiu et al., 2021). With nine currently recognized species and a geographical range encompassing areas from the tropics to the subpolar regions, eelgrasses represent the most widely distributed and the second most diverse group of seagrasses altogether (Moore & Short, 2007).

Despite their extensive area coverage and tolerance of environmental conditions such as different light

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regimes, temperatures and salinities, eelgrass meadows (like other seagrasses) have been experiencing a rapid decline in the past 100 years, triggering an immense global initiative for their conservation (Boström et al., 2014; Eriander et al., 2016; Orth et al., 2006; Orth et al., 2010; Valle et al., 2015; Xu et al., 2021). Large-scale eelgrass losses are commonly attributed to several biotic and abiotic factors, with extreme climatological events, anthropogenic disturbance and pathogenic diseases suspected to be the leading causes (Orth et al., 2006). However, the diversity and complex dynamics of eelgrass pathosystems are still only poorly understood (Sullivan et al., 2018). There are three groups of microbial organisms currently recognized as parasites of eelgrass: labyrinthulids, the causative agents of eelgrass wasting disease (Short et al., 1987; Sullivan et al., 2013); the *Phytophthora/Halophytophthora* species complex, which is thought to negatively affect eelgrass seed germination and viability (Govers et al., 2016); and phytomyxids, obligate intracellular biotrophs causing morphological deformations in the infected plants (Neuhauser et al., 2011). Interestingly, while research focused on the first two groups seems to be gaining momentum, eelgrass-associated phytomyxids are perceived as rarely occurring in nature (den Hartog, 1989), and are generally overlooked in contemporary eelgrass pathogen studies (e.g., Menning et al., 2020, 2021).

Infamous for infecting economically significant crops, Phytomyxea (SAR: Rhizaria: Endomyxa) is a monophyletic group of protists known to parasitize a wide range of hosts, including numerous terrestrial plants, seagrasses, brown and green algae and oomycetes (Bulman & Neuhauser, 2017). It currently comprises nearly 20 established genera accommodated in two major orders—the terrestrial/freshwater Plasmodiophorida and the marine Phagomyxida, and a recently described deep-branching marine clade ‘TAGIRI-5/Marinomyxa’ (Bulman & Neuhauser, 2017; Hittorf et al., 2020; Kolátková et al., 2021). Similar to other endoparasites, the life cycle of phytomyxids is very complex and consists of numerous developmental stages typically taking place in three spatially separated environments: the ‘primary’ sporangial infection of root hairs, the ‘secondary’ sporogonic infection of cortical tissues, and the sediment-dwelling resting spores (Bulman & Braselton, 2014; Liu et al., 2020). This generalized scheme is, nevertheless, based mainly on the thoroughly studied representatives pathogenic to plants cultivated in agriculture, and may slightly differ in other phytomyxid taxa. In natural environments, the discoveries of novel phytomyxid species are sporadic and mostly tied to serendipitous observations of morphological changes in the host tissues. Typically, only a part of the life cycle is documented in the taxonomic descriptions, and a surprisingly large proportion of the recognized taxa has only been found on several occasions with old

drawings being the only evidence of their existence (Karling, 1968). In the marine-derived Phagomyxida, a complete life cycle has not been observed in any of the species described so far, leading to prior speculations that some of the developmental stages may be missing in this lineage (Bulman & Neuhauser, 2017; Maier et al., 2000; Parodi et al., 2010).

The first observation of phytomyxid parasites of the seagrass genus *Zostera* dates to 1938, when *Plasmodiophora bicaudata* was discovered in specimens of *Zostera noltei* from Tanouert, Mauritania (Feldmann, 1940). The parasite was reported to induce the formation of hypertrophies in the bases of the plant's shoots and suppress the growth of internodes, giving the host a tufted ‘*Isoetes*-like’ appearance (Feldmann, 1940; Feldmann, 1956). In a subsequent herbarium study, den Hartog (1989) also confirmed the presence of *P. bicaudata* in *Zostera capensis* from South Africa, *Zostera muelleri* from Victoria (Australia), and *Zostera japonica* from British Columbia (Canada) and concluded that the parasite is rare, but widely distributed throughout populations of different dwarf eelgrass species. Hundreds of *Z. marina* specimens were also examined for *P. bicaudata* infection, but no signs of galls and unusual hypertrophies were found (den Hartog, 1989). Despite its unknown pathogenic (and therefore ecological) potential, no further research on *P. bicaudata* was carried out, and secondary sporogonic plasmodia and resting spores with two characteristic appendages remain the only observed life stages of the organism so far (Feldmann, 1940, 1956). Furthermore, the lack of any molecular data has prevented verification of the morphometry-based taxonomic placement of the organism in the genus *Plasmodiophora*.

In 2015, a novel phytomyxid parasite was discovered in the roots of *Z. marina* in Puget Sound, WA (Elliott et al., 2019). This time, primary sporangial plasmodia and biflagellate zoospores were observed in the root hairs of infected plants, but no secondary infection or morphological deformations resembling *P. bicaudata* infection were detected. Phylogenetic analysis of the 18S rRNA gene placed the parasite within the phytomyxid order Phagomyxida comprising other marine representatives, with the closest relative being *Ostenfeldiella diplantherae* (86.9% nucleotide identity), a gall-forming parasite of the tropical seagrass *Halodule* (den Hartog, 1965; Ferdinandsen & Winge, 1914). However, no definite taxonomic treatment was given, since no genetic comparison to the previously described eelgrass parasite (*P. bicaudata*) could be performed. Since the observed developmental stages (primary sporangial plasmodia and biflagellate zoospores) conveniently complete the root hair component of the typical phytomyxid life cycle (as described above), we hypothesize that the organism investigated by Elliott et al. (2019) is either closely related to *P. bicaudata* or simply represents the so far overlooked life stages of the long-known phytomyxid species.

TABLE 1 List of seagrass specimens collected and examined in this study.

| Seagrass species | Date of collection | Locality | GPS | Collection method | Medium stored | Infection stage ^a |
|--------------------------------------|---------------------|---|-------------------------------|----------------------------|------------------------------|------------------------------|
| <i>Zostera noltei</i> ^b | July 14, 2018 | Zandkreek, the Netherlands | N 51.542335° E 3.897222° | Low tide | 70% EtOH | RHG + SG |
| <i>Zostera noltei</i> ^b | September 2, 2018 | Foros Bay, Burgas, Bulgaria | N 42.452311° E 27.460873° | Snorkelling (~0.3 m depth) | 70% EtOH | RHG + SG |
| <i>Zostera japonica</i> | Numerous, 2019–2021 | Dash Point, WA, USA | N 47.322412° W 122.409332° | Low tide | Seawater | RHG, no SG found |
| <i>Zostera japonica</i> | May 28, 2021 | Tsawwassen Beach, Tsawwassen, BC Canada | N 49.013233° W 123.119003° | Low tide | Seawater | RHG, no SG found |
| <i>Zostera japonica</i> ^b | June 25, 2021 | southern Roberts Bank, Delta, BC Canada | N 49.031271° W 123.147920° | Low tide | Seawater | RHG, no SG found |
| <i>Zostera japonica</i> ^b | August 6, 2021 | southern Roberts Bank, Delta, BC Canada | N 49.031271° W 123.147920° | Low tide | 70% EtOH, Karnovsky fixative | RHG + SG |
| <i>Zostera japonica</i> ^b | September 30, 2021 | Deep Cove, North Saanich, BC Canada | N 48.673176° W 123.481448° | Low tide | 70% EtOH, Karnovsky fixative | RHG + SG |
| <i>Zostera japonica</i> | September 2021 | Birch Bay, WA, USA | N 48.930603° W 122.754588° | Low tide | 70% EtOH | RHG + SG |
| <i>Zostera marina</i> | Numerous, 2019–2021 | Dash Point, WA, USA | N 47.322412° W 122.409332° | Low tide | Seawater | RHG |
| <i>Zostera marina</i> ^b | June 23, 2021 | Cowichan Bay, BC Canada | N 48.742744° W 123.624635° | Low tide | Seawater | RHG |
| <i>Zostera marina</i> ^b | August 6, 2021 | southern Roberts Bank, Delta, BC Canada | N 49.031271° W 123.147920° | Low tide | Seawater | RHG |
| <i>Zostera marina</i> | September 17, 2021 | Plumper Cove, Keats Island, BC Canada | N 49.404057° W 123.471639° | SCUBA (~3 m depth) | Seawater | RHG |
| <i>Zostera marina</i> | November 10, 2021 | Hope Bay, Pender Island, BC Canada | N 48.805845° W 123.278205° | SCUBA (~3 m depth) | Seawater | RHG |

^aRHG, root hair galls, SG, shoot galls.

^bSpecimens from these collections were used for molecular analysis.

To resolve the identity of the newly discovered phytomyxid parasite infecting *Z. marina* and to understand its phylogenetic relationships with *P. bicaudata* and other seagrass-associated phytomyxid parasites, we examined specimens of several eelgrass species from the Northern hemisphere susceptible to phytomyxid infection and, for the first time, analysed their parasites on a molecular level using rRNA-encoding phylogenetic markers. Moreover, we reviewed some previously published environmental sequencing amplicon datasets from eelgrass meadows around the world (Ettinger et al., 2021) to better understand the distribution of eelgrass-infecting phytomyxids on a global scale.

EXPERIMENTAL PROCEDURES

Seagrass tissue collection

Three different species of *Zostera* were collected for the purposes of this study. In the summer of 2018, a search for specimens of dwarf eelgrass, *Zostera noltei*, infected with *Plasmodiophora bicaudata* was

conducted at two distant localities in Europe (Table 1) at which the parasite's presence had been previously reported in the scientific literature (den Hartog, 1989; Hineva, 2017). In the mudflats of Zandkreek, Netherlands (Wadden Sea), a seagrass meadow was inspected during a low tide, and plants with a characteristic tufted appearance and bulbous shoot morphology (Figure 1A) were harvested and stored in 70% ethanol for future examinations and DNA analyses. In Foros Bay, Bulgaria (Black Sea), plants with similar deformations were collected from depths of 0.2–0.3 m while snorkelling and were treated identically.

In 2020–2021, numerous specimens of common eelgrass *Zostera marina* were collected during low tides from various sites throughout the Salish Sea, Northeast Pacific (Table 1) in search for root hair galls containing the novel phytomyxid parasite described by Elliott et al. (2019). Since the naked eye cannot detect the infection, random plants (including rhizomes and roots) were excavated from the surrounding sediment with a trowel, washed in ambient seawater, and stored in plastic zip-lock bags on ice in a cooler until transported to the laboratory. Within 2 h after collection from

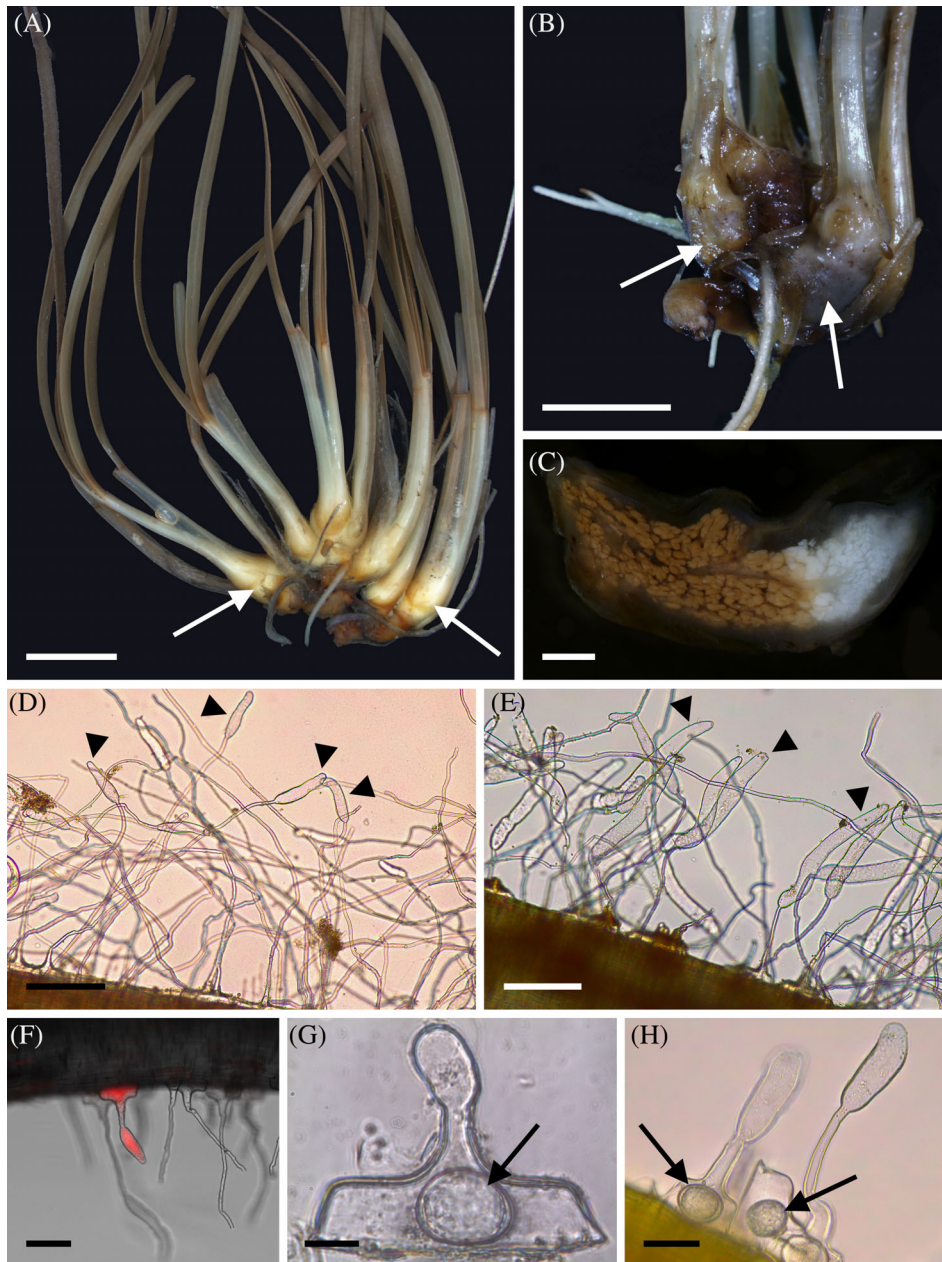


FIGURE 1 Morphology of seagrasses of the genus *Zostera* infested with phytomyxid parasites. (A) A specimen of *Zostera noltei* heavily infected with *Feldmanniella bicaudata*. The secondary infection stages of the parasite induce the formation of galls in the bases of the seagrass shoots (arrows) and suppress the development of internodes, giving the plant an *Isoetes*-like appearance. (B) Shoot galls in a specimen of *Zostera japonica* from the Salish Sea (Northeast Pacific) infected with *F. bicaudata*. (C) Cross-section of a *Z. japonica* shoot gall revealing hypertrophied host cells filled with secondary sporogenic plasmodia (white coloration) and mature phytomyxid resting spores (brown coloration). (D) Root hair galls (arrowheads) in *Zostera marina* containing primary developmental stages of *Feldmanniella radicapillae*. (E) Root hair galls (arrowheads) in *Z. japonica* containing primary developmental stages of *F. bicaudata*. (F) A root of *Z. marina* stained with Nile red showing the distribution of phytomyxid infection throughout the root hair. (G) Thick-walled ovoid structure of yet unknown function in the basal cell of an infected root hair of *Z. marina*. (H) Identical thick-walled ovoid structures in infected root hairs of *Z. japonica*. Scale bars: A, B = 5 mm; C = 1 mm; D, E = 200 μ m; F = 100 μ m; G = 20 μ m; H = 50 μ m.

the field, the rhizome and roots were carefully washed in artificial seawater (30 PSU, Instant Ocean®) to remove as much sediment as possible.

Alongside *Z. marina*, ***Zostera japonica*** (dwarf eelgrass invasive in Northeast Pacific) was also haphazardly collected at various localities in the Salish Sea

(Table 1) and treated as described above to verify whether root hair galls are also formed in this species. Furthermore, following the finding of *Zostera japonica* being infected with *Plasmodiophora bicaudata* near Roberts Bank (BC, Canada) in 1985 (den Hartog, 1989), several searches for the given

symbiosis were carried out at this locality during low tides in May–August 2021. Plants with signs of infection (Figure 1B) were placed in 70% ethanol for subsequent molecular analyses, and in Karnovsky fixative (Karnovsky, 1965) for electron microscopy.

Microscopy and measurements

Root hair galls

Individual roots of *Z. marina* and *Z. japonica* were cut off at their base with scissors and placed in a sonicator (FS 20, Fisher Scientific) filled with artificial seawater for approximately 10 s to further remove sediment and other material adhering to the roots. The roots were stained with trypan blue or Nile red and examined using an inverted Leica compound microscope (DMIL LED) or an upright Leica compound microscope (DM 750) at 200–1000 \times magnification. Images were taken with a Leica EC3 camera and software. The length and width of randomly chosen root hair galls were measured using ImageJ software (Schneider et al., 2012). For scanning electron microscopy (SEM) root tissue was fixed in 4% glutaraldehyde in sterile seawater for 2 h at 4°C and then dehydrated through an ethanol series (30%, 50%, 70%, 90% and 100%) and critical point drying using HMDS (hexamethyldisilazane) (Bray et al., 1993) Specimens were mounted on stubs and sputter coated with gold/palladium using a Denton Vacuum, Inc. sputter coater. The roots were then examined on a Hitachi S-3400 N scanning electron microscope.

Secondary zoospores

A total of 50 cm of root tissue was put into Petri dishes with 10 mL of sterile seawater (0.45 μ m filtered, 30 PSU) and incubated in the dark at 20°C for 24 h. Zoospores released from the roots were examined at 400–1000 \times magnification with a Leica inverted compound microscope and a Leica compound microscope. Measurements of zoospores were taken in ImageJ. Representative material was selected for further examination using scanning electron microscopy (SEM) and fixed in 4% glutaraldehyde in sterile seawater for 2 h at 4°C. The fixative was gently poured out of the Petri dishes, and the zoospores left on the bottom were subsequently dehydrated through an EtOH series (30%, 50%, 70%, 90% and 100%) and critical point drying using HMDS (Bray et al., 1993). The bottoms of the Petri dishes were mounted on stubs and sputter coated with gold/palladium using a Denton Vacuum, Inc. sputter coater. The galls were then examined on a Hitachi S-3400 N scanning electron microscope. Statistical analysis of all data was conducted using R (R Core Team, 2022).

Sporogenic plasmodia and resting spores

Bulb-like hypertrophies at the bases of *Z. noltei* and *Z. japonica* shoots (hereinafter referred to as ‘shoot galls’) were inspected with an Olympus SZX9 stereomicroscope equipped with an Olympus DP72 camera. Several galls were sliced with a razor blade to reveal their internal arrangement (Figure 1C). Plasmodial development was examined using transmission electron microscopy (TEM). *Z. japonica* gall tissue fixed in Karnovsky fixative (Karnovsky, 1965) was post-fixed in 1.0% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2), dehydrated through an ascending ethanol series (15 mins at 30, 40, 50, 70, 80, 90, 95 and 2 \times 100%), and embedded in EMBED 812 resin. Ultrathin sections (80–90 nm) were cut using a Reichert Ultracut E ultramicrotome, mounted on 300 mesh copper grids, post-stained with uranyl acetate and lead citrate and viewed with a Jeol JEM 1400 TEM equipped with a Gatan SC-1000 digital camera. For SEM, part of the dehydrated tissue was not embedded in plastic, but critical-point dried with liquid CO₂ in a Leica CPD300 critical point dryer, mounted on metal stubs, sputter-coated with gold and investigated using a Hitachi S-3500 N scanning electron microscope with Quartz PCI software.

Molecular methods

Roots of *Z. marina* and *Z. japonica* bearing root hair galls, as well as shoot galls found in *Z. japonica* and *Z. noltei* were used as source material for molecular analyses of phytomyxid DNA. For each host species, infected specimens from two different geographical locations were tested (Table 1). Plant tissue was homogenized in 1.5 mL microtubes with sterile plastic pestles and DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN Sciences Inc., Germantown, MD) following the manufacturer’s instructions. For each sample, partial 18S and 28S rRNA genes and a ribosomal ITS region (containing the ITS1, 5.8S and ITS2 genes) were amplified with three different sets of phytomyxid-specific primers (Table 2). PCR reaction mixtures (25 μ L) consisted of 12.5 μ L of MegaFi Fidelity 2X PCR MasterMix (ABM Inc., Richmond, Canada), 1.5 μ L of each primer (final concentration 0.6 μ M), 1 μ L of extracted DNA and 8.5 μ L of ultrapure distilled H₂O. To optimize the specificity of amplified fragments, touchdown PCR protocols were followed. For the amplification of the 18S and 28S rRNA genes, the protocol comprised an initial denaturation step at 98°C for 30 s, followed by 35 cycles of denaturation at 98°C for 10 s, primer annealing at 65°C for 30 s (in each cycle, annealing temperature was decreased by 1°C until 54°C was reached) and elongation at 72°C for 60 s, and a final elongation step at 72°C for 5 mins. The

TABLE 2 Oligonucleotide primer sequences used to amplify and sequence rRNA genes of examined phytomyxids.

| Name | Sequence | Orientation | Type | Specificity | position ^a | Reference |
|-------------------------|---------------------------------|-------------|-------------------------------|---------------------------|-----------------------|--------------------------------------|
| SSU_PHYT ^b | 5'-WCRGTGAAWC TGCRGATGGC-3' | Forward | Amplification | Phytomyxea | 76–95 | Kolátková et al. (2020), modified |
| SSU_PHYTr ^b | 5'-CKARTTGCA AGMSGCAAGCC-3' | Reverse | Amplification | Phytomyxea | 1550–1569 | Neuhauser et al. (2014), modified |
| 930F_PHYT ^c | 5'-GCATTCACCAAG GATGTCC-3' | Forward | Amplification | Phytomyxea | 951–969 | This study |
| ITS4_PHYT ^c | 5'-TCCTCCGCTTA ATTATATGCT-3' | Reverse | Amplification + sequencing | Phytomyxea | 2177–2197 | White et al. (1990), modified |
| 1570F_PHYT ^d | 5'-GCTTGCSKCT TGCAAYTMGC-3' | Forward | Amplification | Phytomyxea | 1550–1569 | Kolátková et al. (2020), modified |
| 21r ^d | 5'-GACGAGGCATT TGGCTACCTT-3' | Reverse | Amplification + sequencing | universal (Eukaryotes) | 4364–4384 | Schwelm et al. (2016) |
| 577F | 5'-GCCAGCAGCC GCGGT-3' | Forward | Sequencing | universal (Eukaryotes) | 559–573 | Elwood et al. (1985) |
| 577R | 5'-ACCGCGGCT GCTGG-3' | Reverse | Sequencing | universal (Eukaryotes) | 559–573 | Elwood et al. (1985) |
| 1055F | 5'-GGTGGTGCAT GGCCG-3' | Forward | Sequencing | universal (Eukaryotes) | 1272–1286 | Elwood et al. (1985) |
| 1055R | 5'-CGGCCATGCAC CACC-3' | Reverse | Sequencing | universal (Eukaryotes) | 1272–1286 | Elwood et al. (1985) |
| 1510R | 5'-GGGCATCACA GACCTG-3' | Reverse | Sequencing | universal (Eukaryotes) | 1434–1449 | Elwood et al. (1985) |
| ITS4_PHF | 5'-AGCATATAATT AAGCGGAGGA-3' | Forward | Sequencing | Phytomyxea | 2177–2197 | White et al. (1990), modified |
| LR3F | 5'-GTCTTGAACA CGGACC-3' | Forward | Sequencing | Fungi | 2844–2860 | Vilgalys and Hester (1990) |
| LR3 | 5'-CCGTGTTTCA AGACGGG-3' | Reverse | Sequencing | Fungi | 2844–2860 | Vilgalys and Hester (1990), modified |

^aBased on the sequence of *Maullinia ectocarpii* (Phytomyxea: Phagomyxida), GenBank accession number KX011113.

^bPrimer pair used for the amplification of the majority of the 18S rRNA gene sequence.

^cPrimer pair used for the amplification of a partial 18S and a complete ITS1 + 5.8S + ITS2 rRNA gene sequence.

^dPrimer pair used for the amplification of a partial 18S, a complete ITS1 + 5.8S + ITS2, and a partial 28S rRNA gene sequence.

same protocol was followed for the ITS region amplification, but the annealing temperature ranged from 62°C to 56°C. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN Sciences Inc.), sequenced via 'Sanger' sequencing in both directions by Sequetech Corp. (Mountain View, CA) using various internal primers (Table 2), and manually assembled in BioEdit 7.0.5.3. (Hall, 1999).

Approximately 4180 bp long consensus sequences covering a large part of the ribosomal operon were generated for the following phytomyxids: *Plasmodiophora bicaudata* in *Z. noltei* from Zandkreek, the Netherlands; *P. bicaudata* in *Z. noltei* from Faros Bay, Black Sea; *P. bicaudata* in *Z. japonica* from Roberts Bank and North Saanich, BC (sequence identical between both locations and types of galls); and the novel phytomyxid in root hairs of *Z. marina* from Tsawwassen and Cowichan Bay, BC (sequence identical between both locations). The sequences were deposited in GenBank under the accession numbers OP137117–OP137120.

Phylogenetic analyses

Phylogenetic analyses of the 18S and 28S rRNA genes were performed to determine the phylogenetic position of the examined protists within Phytomyxea. For the 18S rRNA gene phylogeny, a dataset containing the newly obtained sequences, a representative selection of 18S sequences of Phytomyxea, Endomyxa and affiliated environmental sequences was generated and aligned using MAFFT (Kato et al., 2002) on the MAFFT 7 server (<https://mafft.cbrc.jp/alignment/server/>) with the G-INS-i algorithm at default settings. The alignment was trimmed using trimAl v1.2 (Capella-Gutierrez et al., 2009) with '-gt 0.3 -st 0.001' parameters to a final dataset containing 1598 aligned sites. Trees were reconstructed by maximum likelihood (ML) and Bayesian inference (BI) methods. The ML analysis was performed with IQ-TREE (Nguyen et al., 2015) using the TIM2 + F + R4 model suggested as the best substitution model by ModelFinder (Kalyaanamoorthy et al., 2017), and 1000 nonparametric bootstrap

replicates. The BI analysis was performed with Mr. Bayes v3.2.7 (Ronquist et al., 2012) using the GTR + I + Γ model, with four rate categories for the gamma distribution. Four parallel runs of eight MCMCs were run for 20 million generations, with the first 5 million generations discarded as burn-in. The average standard deviation of split frequencies based on the remaining 15 million was <0.01. Trees were sampled every 500th generation.

For the 28S rRNA gene phylogeny, the newly obtained sequences were aligned with a dataset of LSU rRNA sequences from Schwelm et al. (2016) and trimmed as described above, generating a final alignment containing 1811 sites. The ML analysis was performed with IQ-TREE using the TIM3 + F + I + G4 model and 1000 nonparametric bootstrap replicates, and the Bayesian analysis was performed as described above.

Analysis of global amplicon sequencing data

To better estimate the global distribution of phytomyxid parasites in eelgrass meadows, 18S amplicon sequencing data from a large study of the *Z. marina* mycobiome by Ettinger et al. (2021) were analysed. Raw 2×300 bp paired-end Illumina V4 amplicon datasets, derived from 16 different geographical locations, were downloaded from the NCBI SRA repository (PRJNA667465). Adapter sequences 565F (CCAGCASCYGC GGTAATTCC) and 948R (ACTTTCGTTCTTGATYRA) were trimmed with CutAdapt v3.5 (Martin, 2011). Only trimmed reads pairs in which both adapters were identified were retained. Trimmed reads were imported into QIIME2 v2021.11 (Bolyen et al., 2019). Amplicon sequence variants (ASVs) were inferred with DADA2 (Callahan et al., 2016), with forward and reverse reads truncated after 260 bp, based on visual inspection of quality scores. All samples with a total frequency of less than 5000 reads were discarded from the analysis.

ASVs corresponding to *Zostera*-associated phytomyxid parasites were identified by comparison to the sequences obtained in this study and the previously published 18S gene sequence (MG847140.1; Elliott et al., 2019). To facilitate treatment of two 99% identical ASVs from the phytomyxid parasite of *Z. marina* as a single entity in downstream analyses, while excluding the 97% identical ASV from the phytomyxid parasite of *Z. nolteijaponica*, ASVs were collapsed into operational taxonomic units (OTUs) at a 98% identity threshold. The abundance of the *Z. marina* parasite in each dataset was calculated as the percentage of its OTU counts, after excluding counts derived from *Z. marina* itself (Table S1 and S2).

RESULTS

Prevalence of phytomyxid infection in eelgrass meadows

The prevalence of shoot galls in the *Zostera noltei* and *Zostera japonica* meadows was generally very low (<1% of infected plants) and appeared to be seasonal. Infected specimens of *Zostera noltei* were found in summer/early autumn at both European localities (Table 1). During periodic searches for *Plasmodiophora bicaudata* in *Z. japonica* near Roberts Bank, BC, no shoot galls were found during sampling trips in May-early July, whereas in early August, specimens with visible hypertrophies were discovered readily. By September 2021, the presence of shoot galls was confirmed at all the *Z. japonica* sampling sites (Table 1). Infected plants appeared to be distributed in patches and at times could be recognized by visibly denser and shorter leaves compared to the neighbouring plants with unaltered morphology. In *Zostera marina*, no shoot galls were observed at all.

In contrast, root hair galls were present in nearly all specimens of *Z. marina* and *Z. japonica* collected in the Salish Sea throughout 2020 and 2021, regardless of location and season. Root hair galls were also observed in the roots of *Z. noltei* specimens collected in Europe but were not further examined due to their storage in ethanol. Despite the omnipresence of phytomyxid parasites, the seagrass meadows did not seem to be under distress. Seagrass uprooting resulting from phytomyxid infection, as previously proposed by den Hartog (1989), was not recorded at any of the sampling sites.

Microscopy

Root hair galls

While the average diameter of root hairs in *Z. marina* ($8.4 \pm 2.38 \mu\text{m}$) and *Z. japonica* ($8.35 \pm 2.01 \mu\text{m}$) did not significantly differ (Wilcoxon, $W = 9792$, $p = 0.95$), the average length and diameter of *Z. marina* root hair galls (Figure 1D, length: $125 \pm 55 \mu\text{m}$, diameter: $29.6 \pm 5.27 \mu\text{m}$) were significantly smaller than those of *Z. japonica* (Figure 1E, length: $249 \pm 76.8 \mu\text{m}$, diameter: $36.3 \pm 4.61 \mu\text{m}$) (length: t -test, $t = 16.06$, $df = 295$, $p < 0.0001$, diameter: t -test, $t = 10.64$, $df = 295$, $p < 0.0001$). Staining of roots with Nile red revealed that the infection is not limited to the gall itself, but parasitic biomass can be found throughout the entire root hair, including the basal part of the cell (Figure 1F). Thick-walled structures located at the root hair bases previously reported by Elliott et al. (2019) were frequently observed in both seagrass species (Figure 1G, H), but their identity/role remains unclear.

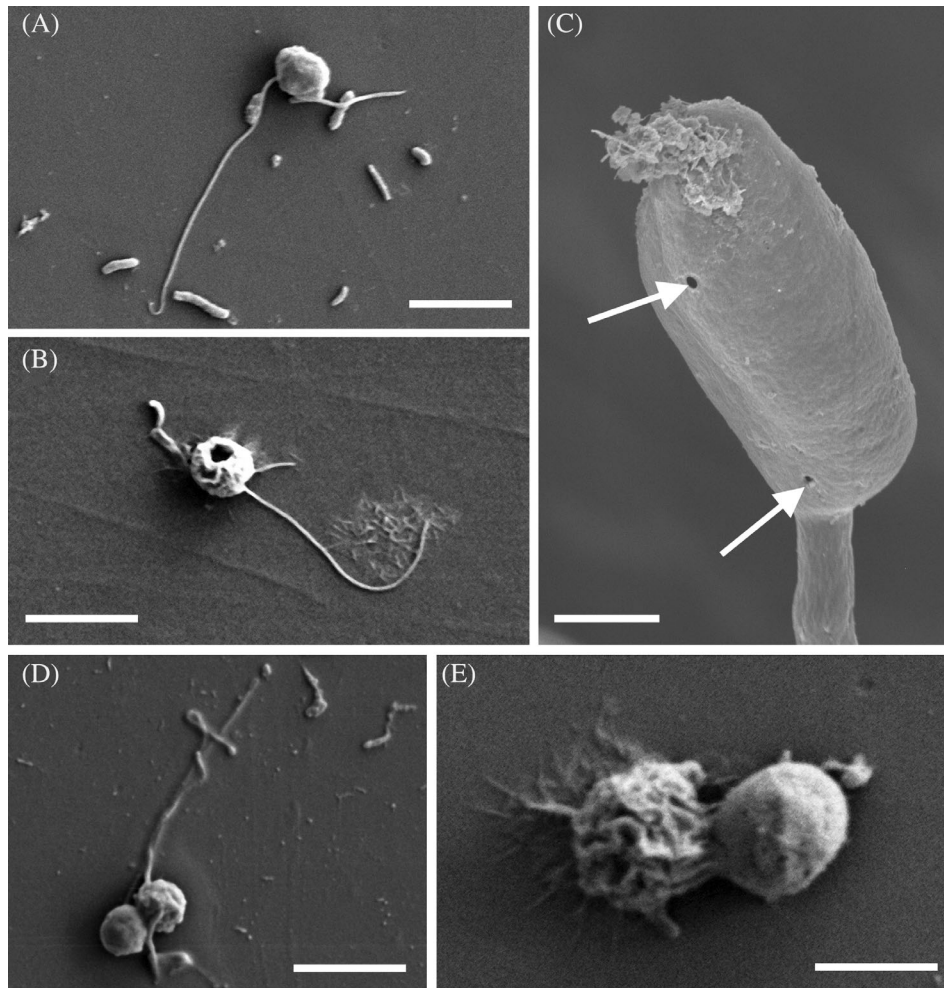


FIGURE 2 Scanning electron microscopy (SEM) micrographs of *Feldmanniella* spp. secondary zoospores. (A) Secondary zoospore of *Feldmanniella radicapillae* released from a root hair gall in *Zostera marina*. (B) Secondary zoospore of *F. bicaudata* released from a root hair gall in *Zostera japonica*. (C) Apertures in a *Z. marina* root hair gall (arrows) used for the release of phytomyxid secondary zoospores (see Movie S3). (D, E) Apparent conjugation in secondary zoospores of *F. radicapillae*. Scale bars: A, B, D = 5 μm ; C = 10 μm ; E = 2 μm .

Secondary zoospores

In measurements from light microscopy images, the average cell length of *Z. marina* zoospores ($5.77 \pm 0.38 \mu\text{m}$) was significantly greater than those of *Z. japonica* ($4.08 \pm 0.49 \mu\text{m}$) (t -test, $t = -12.66$, $df = 43$, $p < 0.0001$). The long posterior flagella of the zoospores were clearly visible in light microscopy videos, but the shorter anterior flagella were difficult to observe (see Movies S1 and S2). After fixation and dehydration for SEM, the cell body of the zoospores became smaller and more spherical in shape (Figure 2A, B). In measurements from SEM images, average cell length of *Z. marina* zoospores (Figure 2A; $3.02 \pm 0.21 \mu\text{m}$) was significantly greater than those of *Z. japonica* (Figure 2B; $2.57 \pm 0.29 \mu\text{m}$) (t -test, $t = -5.64$, $df = 36$, $p < 0.0001$). The flagella were clearly visible in SEM images, and those of *Z. marina* zoospores were longer (anterior flagella = $4.54 \pm 0.56 \mu\text{m}$, posterior = $14.44 \pm 1.45 \mu\text{m}$), than for

Z. japonica zoospores (anterior flagella = $2.45 \pm 0.96 \mu\text{m}$, posterior = $11.4 \pm 1.46 \mu\text{m}$). The anterior flagella were much shorter than the posterior flagella. The anterior flagella were on average 32% of the length of the posterior flagella in *Z. marina* and 18% of the length of the posterior flagella in *Z. japonica*. Fully developed secondary zoospores were observed leaving the root hair gall through small apertures in the gall's cell wall (Figure 2C, Movie S3). On several occasions, what appeared to be conjugating zoospores were captured both within (Movie S4) and outside of the root hair gall (Figure 2D, E).

Sporogenic plasmodia and resting spores

The sliced shoot galls of *Z. japonica* and *Z. noltei* revealed greatly hypertrophied host cells within the cortical tissue of the seagrass leaves (Figure 1C). The coloration of the host cells (white to dark brown) showed a

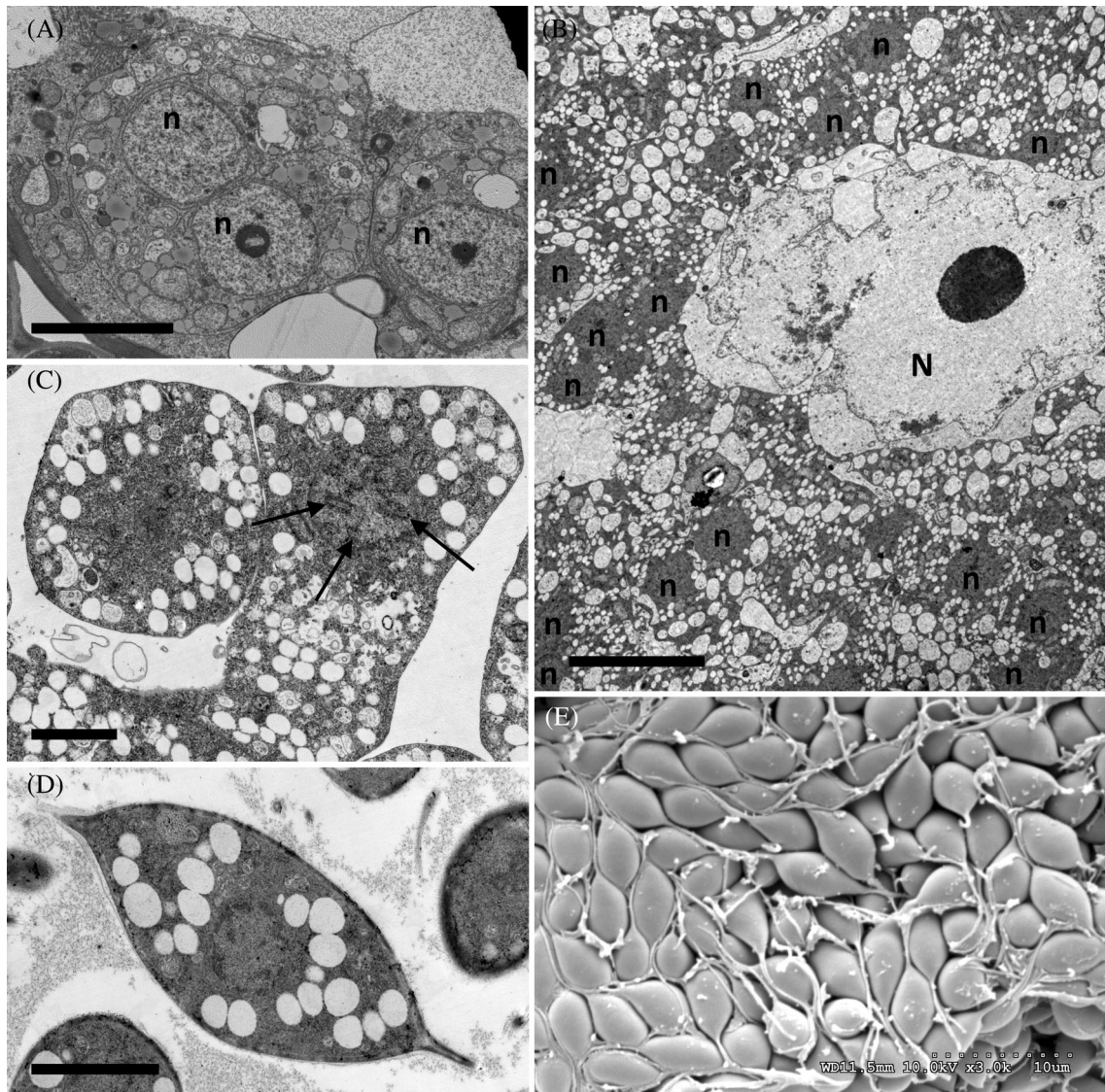


FIGURE 3 Secondary plasmodial development in *Feldmanniella bicaudata* (transmission electron microscopy [TEM] and Scanning electron microscopy [SEM]). (A) Young uninucleate and binucleate (N) sporogenic plasmodia dwelling near the host cell wall. (B) Multinucleate (n) sporogenic plasmodium surrounding a degrading host nucleus (N). (C) Cleaving transitional sporogenic plasmodium in an ‘acaryotic’ stage. Parts of synaptonemal complexes are visible in the nuclei (arrows). (D) TEM micrograph of a fully developed resting spore with two characteristic appendages. (E) SEM micrograph of resting spore arrangement within the host cell. Scale bars: A = 5 μm ; B = 10 μm ; C, D = 2 μm ; E = 10 μm .

distinct spatial distribution pattern of the parasite’s various developmental stages, with the youngest sporogenic plasmodia (white colour) located at the apical part of the gall and the mature resting spores located further down towards the rhizome (brown colour). Based on our TEM analysis, the secondary infection appeared to be induced by the presence of one or several uninucleate sporogenic plasmodia dwelling near the host cell wall (Figure 3A). The growing plasmodia eventually filled out the host cell until only a degraded host nucleus remained, surrounded by a single large multinucleate plasmodium (Figure 3B). At this phase, the plasmodium entered the ‘acaryotic’ transitional stage without visible nucleoli, during which meiosis occurs, and synaptonemal complexes can be observed

in the nuclei (Figure 3C). Cleavage into small uninucleate units followed, from which thick-walled spindle-shaped resting spores with the two characteristic appendages developed (Figure 3D). SEM micrographs of the host cells further uncovered that these appendages are much longer than previously anticipated and maintain a unique intertwined spore arrangement (Figure 3E).

Phylogenetic analyses and genetic diversity

The phylogenetic trees of Phytomyxea inferred from the 18S and 28S gene sequences are shown in

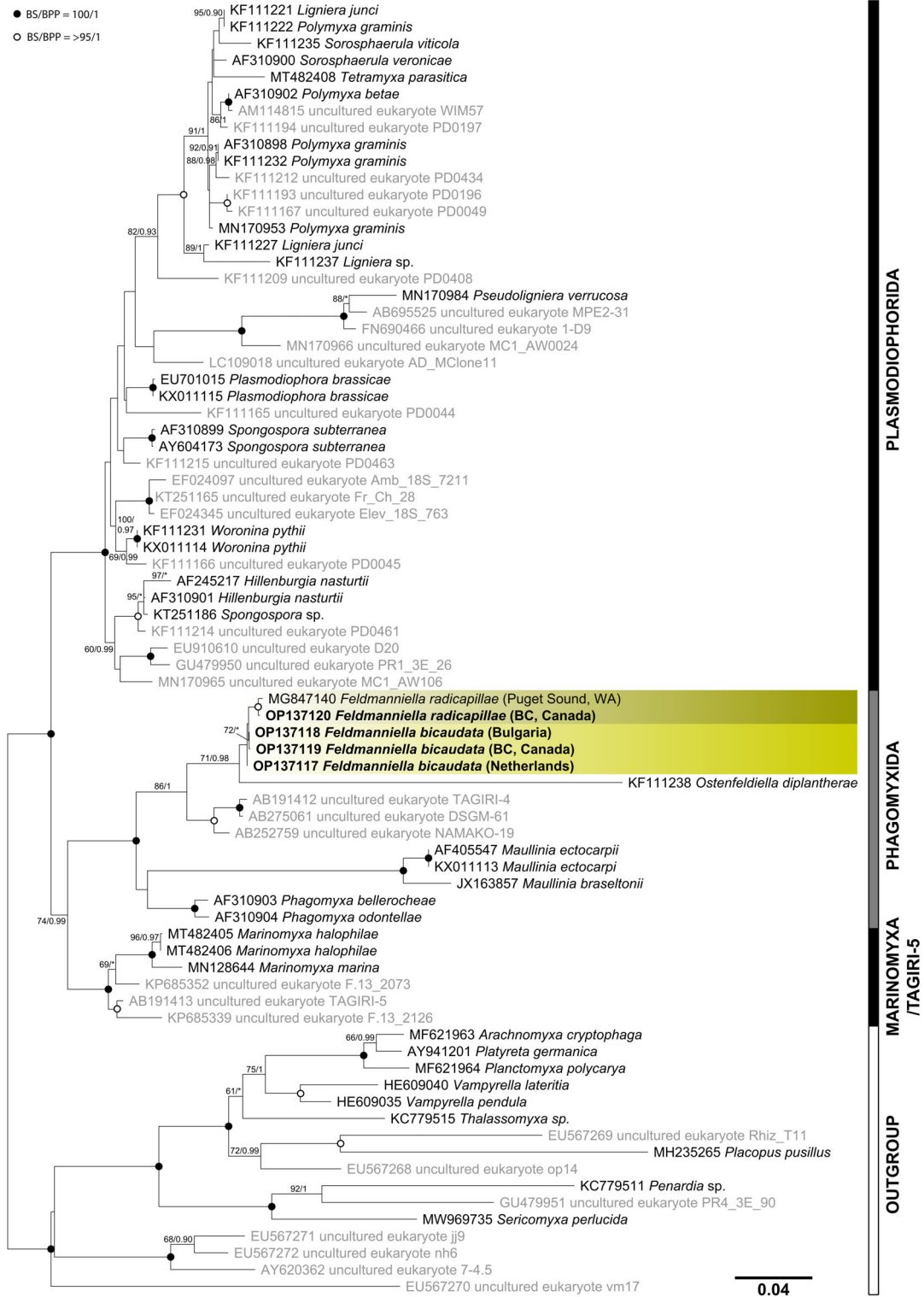


FIGURE 4 Phylogenetic tree of Phytomyxea based on maximum likelihood phylogenetic analysis of the 18S rRNA gene (1598 sites; 77 sequences). Yellow-green boxes highlight representatives of the newly established genus *Feldmanniella*. Sequences derived from isolates are in black, newly determined sequences of *Feldmanniella* spp. are in bold letters. Values at branches represent statistical support in bootstrap values (IQ-TREE)/posterior probabilities (MrBayes); support values below 60/90 are not shown or are represented by an asterisk.

Figure 4 and Figure 5, respectively. In the 18S tree, all three major phytomyxid lineages (i.e., Plasmodiophorida, Phagomyxida and Marinomyxa/TAGIRI-5) were

recovered with maximum support (bootstrap support 'BS' 100, Bayesian posterior probability 'BPP' 1). The newly obtained sequences of eelgrass-associated phytomyxids

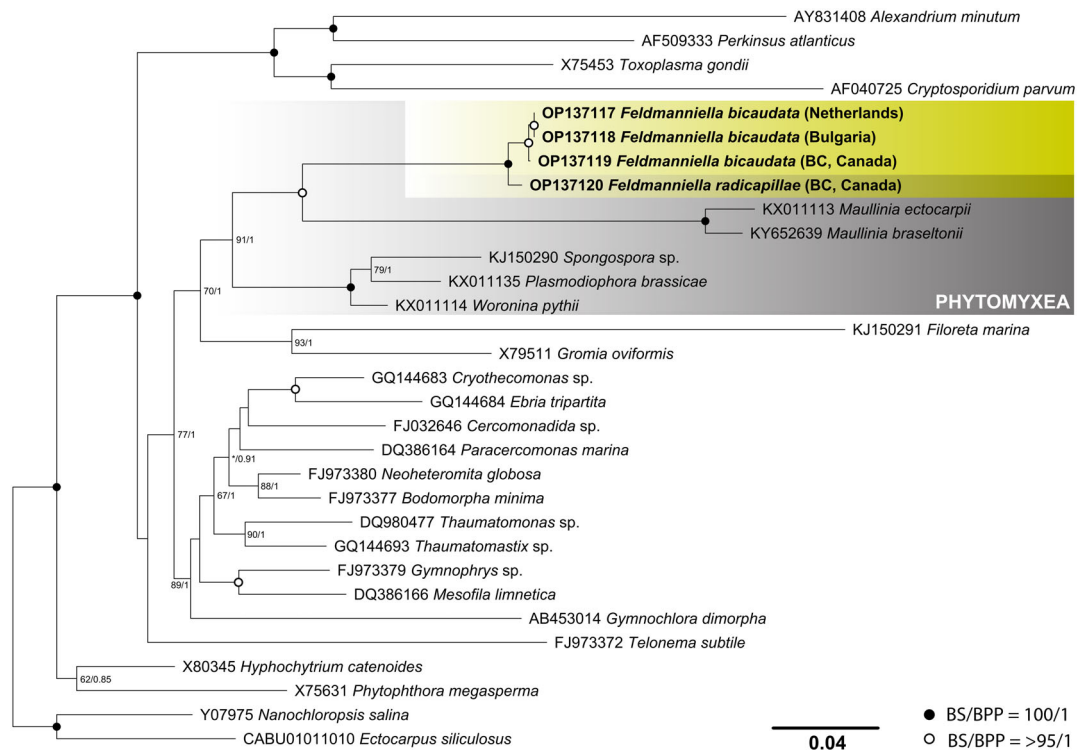


FIGURE 5 The 28S rRNA gene phylogenetic tree constructed by maximum likelihood analysis in IQ-TREE (1811 sites; 31 sequences chosen based on Schwelm et al. (2016)). Sequences of Phytomyxea are shaded in grey, yellow-green boxes highlight representatives of the newly established genus *Feldmanniella*. Sequences newly determined in this study are in bold letters. Values at branches represent statistical support in bootstrap values (IQ-TREE)/ posterior probabilities (MrBayes); support values below 60/80 are not shown or are represented by an asterisk.

formed a clearly defined clade within Phagomyxida and were closely related to another seagrass parasite *Ostenfeldiella diplantherae* and the ‘TAGIRI-4’ environmental sequences (BS 86, BPP 1). While the support for this clade was poor when the long-branching *O. diplantherae* was included in the analysis, it was robust (BS 100, BPP 1) when the *O. diplantherae* sequence was removed (Figure S1). Our 18S sequence of the parasite forming root hair galls in *Z. marina* was nearly identical (99.5% identity) with the previously published sequence MG847140.1 from Puget Sound, WA (Elliott et al., 2019) and together they formed a distinct, well-supported (BS 97, BPP 1) branch within the novel clade. 18S sequences of the parasites found in *Z. japonica* and *Z. noltei* were also highly similar to each other (>99.5% identity) but their exact phylogenetic positioning within the clade was not definitively resolved by the phylogenetic analyses.

The 28S gene tree further underlined these findings, with eelgrass-associated phytomyxids forming a robustly supported clade closely related to sequences of *Maullinia* spp. (Phagomyxida), parasites of brown algae. The newly determined sequences of the phytomyxids infecting dwarf eelgrass species (i.e., *Z. noltei* and *Z. japonica*) clustered together, whereas the phytomyxid from the

roots of *Z. marina* was once more recovered as a distinct branch within the clade.

Analysis of global amplicon sequencing data

The OTU of the phytomyxid parasite associated with *Z. marina* was found in datasets from *Z. marina* meadows from all 16 localities situated across the Northern hemisphere (Figure 6). More specifically, it was detected in 85/212 analysed leaf samples, 140/166 analysed root samples and 101/164 analysed sediment samples (Table S2). The phytomyxid was highly abundant primarily in the root samples, where it often represented one of the most abundant OTUs altogether. In root samples from southern California, southern Japan, and North Carolina, the parasite’s OTU reached the second-highest number of read counts, right after the OTU representing *Z. marina*. After the exclusion of *Z. marina* OTU from the dataset, the parasite’s OTU exceeded 10% of the root-derived read counts at 5 out of the 16 localities, with the maximum value reaching 35.28% of read counts in southern California. At the geographical locations where dwarf eelgrass species can be commonly found (such as

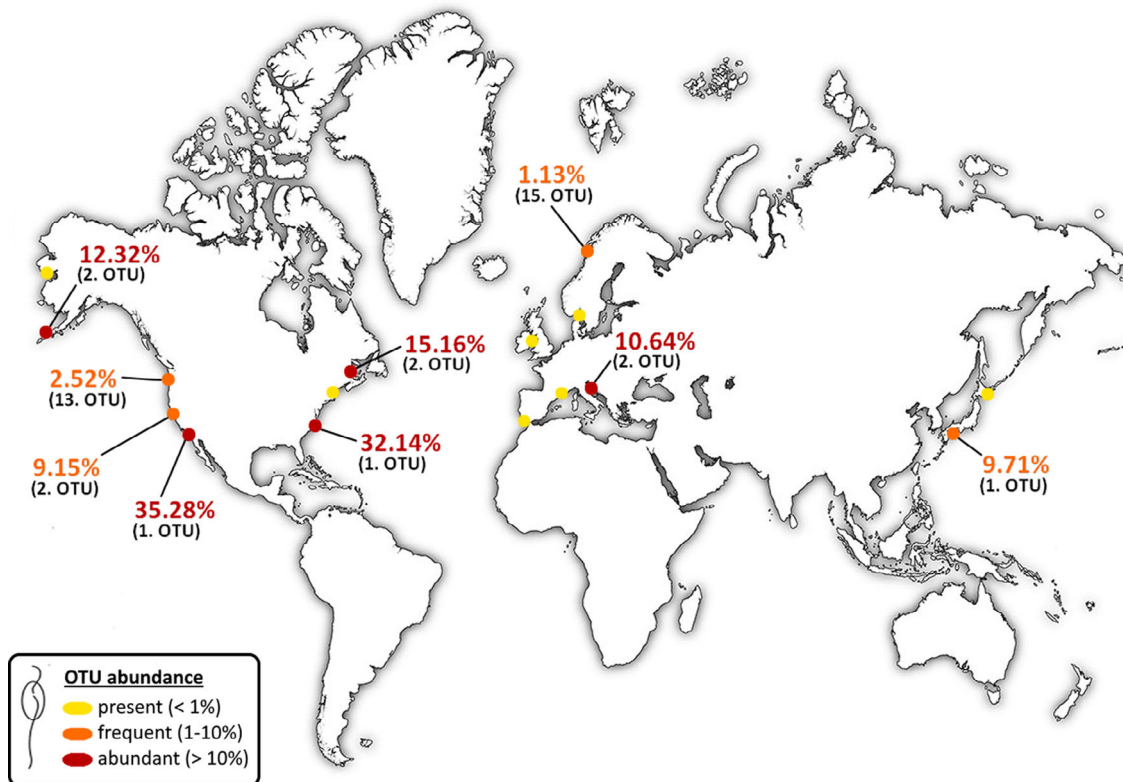


FIGURE 6 World map depicting 16 localities (circles) where specimens of *Zostera marina* leaves, roots and surrounding sediments were collected for an amplicon sequencing study conducted by Ettinger et al. (2021). Portrayed percentage values represent relative numbers of read counts belonging to the *Feldmanniella radicapillae* OTU in collected root samples at a given locality after removing *Z. marina* OTU counts from the dataset (values below 1% are not shown). Numbers in parentheses show the ranking of *F. radicapillae* OTU based on the total number of read counts from all root samples at a given locality; *Z. marina* OTU is not included in the ranking as it had the highest number of read counts at all sites.

Croatia, Portugal and Washington) the OTU representing *P. bicaudata* was also detected in numerous samples.

Taxonomic summary

Rhizaria: Endomyxa: Phytomyxea: Phagomyxida
***Feldmanniella* Kolátková, Mooney, Kelly,**
Hineva, Gawryluk et Elliott, gen. nov.

Type species: *Plasmodiophora bicaudata*
Feldmann, 1940

Included species: *Feldmanniella bicaudata*
(Feldmann, 1940) comb. nov., *Feldmanniella radicapillae*
(Kolátková et al., 2022) sp. nov.

Etymology: The name refers to Jean Feldmann (1905–1978), an exceptional aquatic botanist and the discoverer of the first representative of this genus; and his wife Geneviève (1910–1994) who first described the cytological development of the respective organism.

Description: Ubiquitous, globally distributed endoparasites of seagrasses of the genus *Zostera* (Alismatales: Zosteraceae). Primary multinucleate

plasmodia develop into zoosporangia within the host's root hairs where they induce formation of cylindrical root hair galls. Released secondary zoospores are of an oval to pyriform shape and have two smooth heterokont flagella. Secondary multinucleate plasmodia develop in the cortex of the basal parts of the seagrass shoots, undergo meiosis and cleave into unicellular units, which eventually mature into yellow, spindle-shaped resting spores with two rigid appendages.

***Feldmanniella bicaudata* (Feldmann) Kolátková, Mooney, Kelly, Hineva, Gawryluk et Elliott, comb. nov.**

Synonyms: *Plasmodiophora bicaudata* Feldmann, J., Bulletin de la Société d'Histoire Naturelle de l'Afrique du Nord 31: 171–177 (1940).

Original description: “Myxamoebae ignotae. Plasmodia plurinucleata, nucleis 3–4 μm longis, intra cellulas corticales eximie inflatas apicis caulis *Zosteræ nanae* Roth. parasitica. Sporae singulae, totam cellulam hospitis replentes, luteolae vel in cumulo fusciscentes (in speciminibus in alcoholo servatis), ovoideae, 7 μm longae 3–3.5 μm latae utrinque in setulam acutam productae” (Feldmann, 1940).

Note: In addition to Feldmann's observation (Feldmann, 1940), *F. bicaudata* was observed in herbarium specimens of *Zostera capensis*, *Zostera muelleri*, *Zostera japonica* and possibly also *Zostera capricorni* (den Hartog, 1989). In cytological study of infected specimens of *Zostera noltei* from southern France (Feldmann, 1956), the secondary plasmodia were interpreted as diploid with meiosis occurring prior to cleavage into resting spores. The fusion of zoospores or amoebae was hypothesized due to lack of evidence of karyogamy in the plasmodium (Feldmann, 1956). Furthermore, seasonality in the life cycle was observed with meiosis occurring in August and September, but not earlier in the year (Feldmann, 1956).

Addition to the description: Root hair galls containing primary sporangial plasmodia are ubiquitous and can be found year-round. In *Z. japonica*, the root hair galls are $249 \pm 76.8 \mu\text{m}$ long and $36.3 \pm 4.61 \mu\text{m}$ wide. Secondary zoospores are $3.5\text{--}4.5 \mu\text{m}$ long with posterior flagellum measuring $10\text{--}13 \mu\text{m}$ and anterior flagellum $\sim 2.5 \mu\text{m}$.

***Feldmanniella radicapillae* (Feldmann) Kolátková, Mooney, Kelly, Hineva, Gawryluk et Elliott, sp. nov.**

Description: Parasite of the seagrass *Zostera marina*. Root hair galls containing primary sporangial plasmodia are $125 \pm 55 \mu\text{m}$ long and 29.6 ± 5.27 wide. They are ubiquitous and can be found year-round. Secondary zoospores are $5.4\text{--}6.1 \mu\text{m}$ large with posterior flagellum measuring $13\text{--}16 \mu\text{m}$ and anterior flagellum $4\text{--}5 \mu\text{m}$. Secondary sporogenic plasmodia and resting spores have not been observed.

Etymology: The name indicates that the parasite was discovered in the root hairs of its host *Z. marina* (Elliott et al., 2019).

DISCUSSION

Our molecular analyses of phytomyxid parasites from three different species of seagrasses of the genus *Zostera* revealed that the newly discovered phytomyxid from Puget Sound, WA (Elliott et al., 2019) and *Feldmanniella* (formerly *Plasmodiophora*) *bicaudata* (Feldmann, 1940) are closely related, yet morphologically distinguishable organisms, which together form a novel clade within the order Phagomyxida, described here as *Feldmanniella* gen. nov. In agreement with conclusions made by den Hartog (1989), *F. bicaudata* was found only to parasitize dwarf eelgrass species (i.e., *Zostera noltei* and *Zostera japonica*) whereas the novel *Feldmanniella radicapillae* is a host-specific parasite of *Zostera marina*. This was true of all seagrass specimens obtained in the Salish Sea, including the ones from mixed meadows of *Z. japonica* and *Z. marina* near Roberts Bank, BC and Dash Point, WA

(see Table 1), where the two seagrasses grew concurrently. The strong host specificity and close phylogenetic relations of the examined protists correspond with an identical pattern previously observed in *Marinomyxa* spp.—parasites of the (sub)tropical seagrass genus *Halophila* (Kolátková et al., 2021), and further support the hypothesis of a long coevolutionary history between phytomyxids and their seagrass hosts (Kolátková et al., 2021).

Despite prior speculations that the life history of Phagomyxida may be different and possibly less complex than that of Plasmodiophorida (Bulman & Neuhauser, 2017; Maier et al., 2000; Parodi et al., 2010), all the developmental stages characteristic for the generalized plasmodiophorid life cycle (Bulman & Neuhauser, 2017) are definitely present in *Feldmanniella*. The observations given here and in the cytological study conducted by Feldmann (1956) are highly congruent with the life cycle of *Plasmodiophora brassicae* recently refined by Liu et al. (2020), although several life stages need further investigation to be fully understood. The frequent occurrence of the ovoid thick-walled structures (possibly mature primary zoosporangia) in the bases of infected root hairs (see Figure 1) suggests that they play a significant part in the infection process, which we have not uncovered yet. Similarly, Feldmann (1956) reported a vegetative fragmentation of sporogenic plasmodia occurring early in the growing season, during which diploid multinucleate reproductive elements ('schizonts') were formed. To our best knowledge, such a phenomenon has not been described in any other phytomyxid representative, and further investigation will be needed to clarify whether asexual reproduction happens at the secondary infection phase level.

As predicted by Feldmann (1956), a sexual (diploid) phase is likely initiated by conjugating secondary zoospores in *Feldmanniella*, and ends with meiosis in sporogenic plasmodium prior to its cleavage into resting spores. As usual for a sexual reproduction strategy, the secondary cortical infection resulting in the formation of shoot galls is relatively rare in nature and in this case apparently linked to summer and early autumn months (den Hartog, 1989; Feldmann, 1956). While the processes behind this seasonality are unclear, it has now been reported in several other phytomyxid representatives such as *Tetramyxa parasitica*, *Marinomyxa marina* and *Ligniera verrucosa* (den Hartog, 1963; Hittorf et al., 2020; Kolátková et al., 2020; Kolátková et al., 2022). For all the mentioned species, the occurrence of galls seems to somewhat correlate with the prominent blooming season of the hosts (den Hartog, 1963; Hittorf et al., 2020; Kolátková et al., 2022). In *Tetramyxa parasitica*, the lower salinity of the ambient water was also suggested as one of the potential factors correlated with the formation of shoot galls (den Hartog, 1963).

The secondary infection stages of *F. radicapillae* remain unknown for now. We have observed thousands of *Z. marina* shoots in nature and not once have we noticed anything resembling shoot galls. Considering the size of *Z. marina*, it is plausible that secondary plasmodia also develop within the host's cortical tissue but do not cause the evident morphological changes typical for dwarf eelgrass species and therefore remain overlooked. In addition, there might be lower environmental pressure on *F. radicapillae* to produce resting spores as populations of *Z. marina* are often perennial and grow in subtidal areas where the conditions are much more favourable for survival than in exposed intertidal flats where dwarf eelgrass species hosting *F. bicaudata* are found. On the other hand, life cycles of phytomyxids do not always follow the generalized scheme, and secondary infection may take place in non-cortical tissues of the host (Barr, 1979; Hittorf et al., 2022; Neuhauser & Kirchmair, 2009). There is a possibility that we have, for instance, simply failed to notice the later developmental stages of the *F. radicapillae* in the examined root tissue. However, we find this explanation to be rather unlikely given the number of investigated *Z. marina* specimens.

Although phytomyxids have been traditionally perceived as rare entities in seagrass meadows (den Hartog, 1965; den Hartog, 1989; Lipkin & Avidor, 1974), Elliott et al. (2019) demonstrated at four distant geographic locations in Puget Sound, WA, USA that they are actually highly prevalent in the roots of *Z. marina* and surrounding sediments. Our present analysis of a global dataset of *Z. marina* samples (Ettinger et al., 2021) further reveals that *Feldmanniella* spp. are ubiquitous and potentially one of the most abundant protists associated with eelgrass roots on a global scale. The omnipresence of phytomyxids in eelgrass beds together with their high host-specificity indicate that they are highly specialized members of coastal/estuarine ecosystems, but most likely not substantially harmful to their hosts. Recent observation of seeds in a specimen of an invasive seagrass, *Halophila stipulacea*, heavily infested with a phytomyxid parasite *Marinomyxa marina* proves that seagrasses are still capable of sexual reproduction even when infected by these protists (Kolátková et al., 2022). We hypothesize that the severe pathogenicity attributed to some of the representatives of Plasmodiophorida parasitizing agricultural crops is an outcome of the unstable character of agrocenoses where the plants (i.e., hosts) are artificially bred and cultivated in large monocultures, and that it does not correctly reflect the role of phytomyxids in natural habitats. Nevertheless, as intracellular parasites, phytomyxids undoubtedly count as a stressor in seagrass populations and should be considered in future health assessments and ecological studies concerning seagrass stress threshold. Given the number of environmental and anthropogenic pressures currently

posed on coastal ecosystems, it is not unlikely that naturally occurring diseases might eventually become one of the straws breaking the seagrass' back.

AUTHOR CONTRIBUTIONS

Viktorie Kolátková: Conceptualization (lead); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); visualization (lead); writing – original draft (lead). **Megan Mooney:** Investigation (equal). **Kate Kelly:** Investigation (equal). **Elitsa Hineva:** Investigation (supporting); writing – review and editing (supporting). **Ryan M.R. Gawryluk:** Conceptualization (supporting); data curation (equal); formal analysis (equal); funding acquisition (equal); resources (equal); supervision (equal); validation (equal); writing – review and editing (equal). **Joel Elliott:** Conceptualization (supporting); data curation (equal); funding acquisition (equal); investigation (equal); methodology (equal); resources (equal); supervision (equal); validation (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Sequences newly determined in this study are deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/) under the accession numbers OP137117-OP137120. Amplicon sequencing data originally published by Ettinger et al. (2021) and reanalyzed in this study can be accessed at the NCBI SRA repository (PRJNA667465). Any additional information required to reanalyze the data reported in this paper is available from Viktorie Kolátková and Ryan M. R. Gawryluk upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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