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Research Paper

Molecular phylogenetic analyses support the validity of *Ceratiomyxa porioides* (Amoebozoa, Eumycetozoa) at species level

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ABSTRACT

The frequently encountered macroscopic slime molds of the genus *Ceratiomyxa* have long been recognized by mycologists and protistologists for hundreds of years. These organisms are amoebozoan amoebae that live and grow inside and on the surface of decaying wood. When conditions are favorable, they form subaerial sporulating structures called fruiting bodies which take on a variety of forms. These forms are typically some arrangement of column and/or branches, but one is uniquely poroid, forming folds instead. Originally, this poroid morphology was designated as its own species. However, it was not always clear what significance fruiting body morphology held in determining species. Currently, *Ceratiomyxa fruticulosa* var. *porioides*, the poroid form, is considered a taxonomic variety of *Ceratiomyxa fruticulosa* based on morphological designation alone. Despite its long history of observation and study, the genus *Ceratiomyxa* has been paid little molecular attention to alleviate these morphological issues. We have obtained the first transcriptomes of the taxon *C. fruticulosa* var. *porioides* and found single gene phylogenetic and multigene phylogenomic support to separate it from *C. fruticulosa.* This provides molecular evidence that fruiting body morphology does correspond to species level diversity. Therefore, we formally raise *Ceratiomyxa porioides* to species level.

1. Introduction

The species of the genus *Ceratiomyxa,* and specifically the *C. fruticulosa* complex, exhibit worldwide distribution and are among the most observed macroscopic slime molds (Rojas et al., 2008; Scheetz, 1972). These species of *Ceratiomyxa* present visually striking and unique sporulating structures, termed fruiting bodies. These fruiting bodies develop from a multinucleate plasmodium that inhabits the interior space of decaying wood (Gilbert, 1935). During favorable weather, usually heavy rainfall, the plasmodium emerges from the decaying wood and begins to secrete small mounds of extracellular gelatinous matrix which serves as the foundation of the fruiting bodies. As a fruiting body develops, the plasmodium continues to secrete the gelatinous matrix and shapes it into distinctive forms and eventually spreads itself as a thin film over the structure. In the late stages of maturation, the plasmodium undergoes a round of mitosis, then cleaves its cytoplasm into unicellular prespore cells containing a single nucleus. Each prespore cell then develops into a protosteloid sporocarp, bearing a single spore mass on a thin secreted stalk in which meiosis finally occurs (Olive and Stoianovitch, 1979).

The extracellular structure of the fruiting body varies considerably within what is currently considered the *C. fruticulosa* complex, and includes simple columns, clusters of columns, thin branching columns, an arbuscular form, and a poroid form. Naturally, these forms were used as morphological traits to describe taxa, but historically there has been uncertainty in the importance of fruiting body structure in species delimitation (Gilbert, 1935).

Since its inception, the genus has undergone an extremely convoluted taxonomical history with at least seven name changes. While the validity of the genus name has been contested numerous times (see Lado et al., 2005), the name *Ceratiomyxa* has become ubiquitous among mycologist and protistologists alike and keeping the genus is most desirable to avoid confusion. The first species of the genus seems to have been first described, with polynomials, by Micheli (1729) under *Puccinia*

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Table 1
Summary of isolate information, including species, isolate ID, location, approximate coordinates, habitat, and collection date.

Species	Isolate ID	Location	Coordinates	Habitat	Collection date
Ceratiomyxa fruticulosa var. *porioides*	CpMBa	Starkville, MS, USA	33.494399° N, −88.752658° E	Rotting wood	September 2022
Ceratiomyxa fruticulosa var. *porioides*	CpMBb	Starkville, MS, USA	33.494399°N, −88.752658° E	Rotting wood	September 2022
Ceratiomyxa fruticulosa var. *porioides*	CpMBco	Starkville, MS, USA	33.494399°N, −88.752658° E	Rotting wood	September 2022
Ceratiomyxa fruticulosa var. *porioides*	FB1	Ava, MO, USA	37.027348° N, −92.630253° E	Rotting wood	May 2019
Ceratiomyxa fruticulosa var. *porioides*	FB2	Ava, MO, USA	37.027348° N, −92.630253° E	Rotting wood	May 2019
Ceratiomyxa fruticulosa	NRb	Brooksville, MS, USA	33.269693° N, −88.782188° E	Rotting wood	May 2022

in the historical mycological text first illustrating fungi develop from spores with the book "*Nova plantarum genera*", which is believed to be the birth of mycology as a field. In this text, the drawings of the fruiting bodies were highly stylized, but clearly resemble what we now attribute to *Ceratiomyxa*.

Ceratiomyxa is now known to be a eumycetozoan genus in the supergroup Amoebozoa and contains several species of slime mold amoeboid organisms (Adl et al., 2019). In addition to the *C. fruticulosa* complex, several other species of *Ceratiomyxa* are recognized: *C. hemisphaerica*, *C. sphaerosperma*, *C. morchella*, *C. alba*, *C. lutea*, *C. oblonga*, *C. opojii*, *C. rattanii*, and *C. rosea* (Ejale, 2010; Martin and Alexopoulos, 1969; Olive and Stoianovitch, 1979), although the latter six species are tenuously described (pages from Ejale, 2010, available from Lado, 2005-2024).

The phylogenetic placement of the genus *Ceratiomyxa* has undergone several taxonomic changes, following the increasing understanding of its characteristics. As early as 1975, *Ceratiomyxa* was considered a member of Protostelia (i.e., the protostelids of Eumycetozoa), a now obsolete grouping of sporocarpic amoebozoan slime molds (Olive, 1975). Concurrently, it was debatably included in Myxomycetes, but in a group (Exosporeae) sister to all other Myxomycetes (Martin et al., 1983). Shortly after, Spiegel and colleagues suggested that *Ceratiomyxa* grouped with two other genera of protosteloid amoebae, *Protosporangium* and *Clastostelium*, in a group sister to Myxogastria (endosporous Myxomycetes) based on similarities in life stages and ultrastructure (Spiegel and Feldman, 1988; Spiegel et al., 1986). Despite this, Cavalier-Smith and co-authors assigned *Ceratiomyxa* to a new order of Myxogastria, Parastelida (Cavalier-Smith et al., 2004). Fiore-Donno and colleagues later confirmed that *Ceratiomyxa* was sister to the rest of Myxogastria with molecular data from two genes, the SSU rRNA and EF1-alpha genes (Fiore-Donno et al., 2010). An SSU rDNA gene tree generated by Shadwick et al. (2009) showed that *Protosporangium* and *Clastostelium* group together in Protosporangiida but lacked sequences of *Ceratiomyxa*. Finally, a phylogenomic analysis of Amoebozoa, including sequences of *Ceratiomyxa*, *Protosporangium*, and *Clastostelium*, was able to robustly place them all together (Kang et al., 2017). The taxonomic odyssey of *Ceratiomyxa* conclusively led to its inclusion in the well-supported group Protosporangiida, sister to Myxogastria.

Within the species complex of *C. fruticulosa*, several of the taxonomic varieties have different forms of fruiting bodies. Some of the current varieties were even originally conceptualized as species, but taxonomically reassigned based on observable behavior (Lister, 1911). For instance, the poroid form, *C. fruticulosa* var. *porioides* was originally described as a species under the name *Ceratium porioides* by Albertini

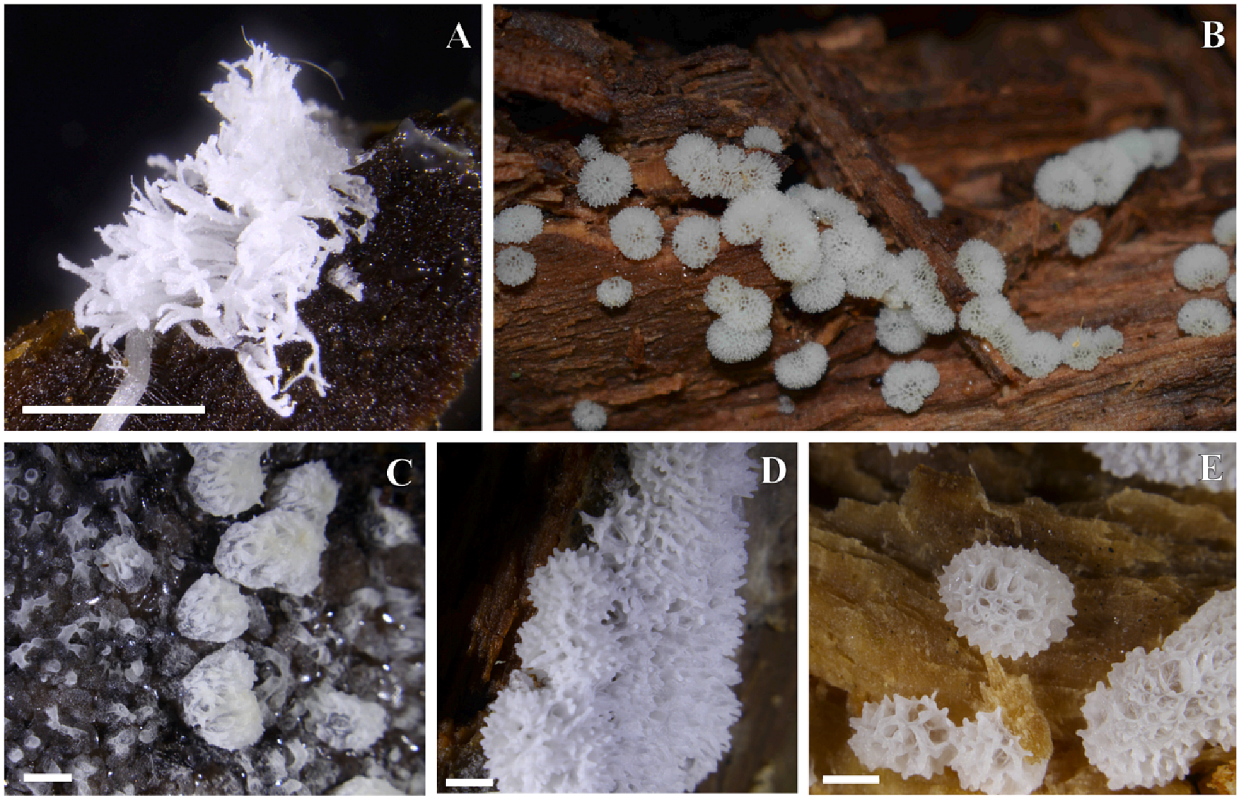

Fig. 1. Image plate of fruiting body morphology of each newly sequenced *Ceratiomyxa* isolate. *Ceratiomyxa fruticulosa* is shown in (A) and *C. fruticulosa* var. *porioides* in (B–E). Images captured under reflected light. (**A**) Isolate NRb, scale bar: 0.5 mm. (**B**) Isolates FB1 and FB2, no scale bar available. (**C**) Isolate CpMBco, scale bar: 1 mm. (**D**) Isolate CpMBb, scale bar: 1 mm. (**E**) Isolate CpMBa, scale bar: 1 mm.

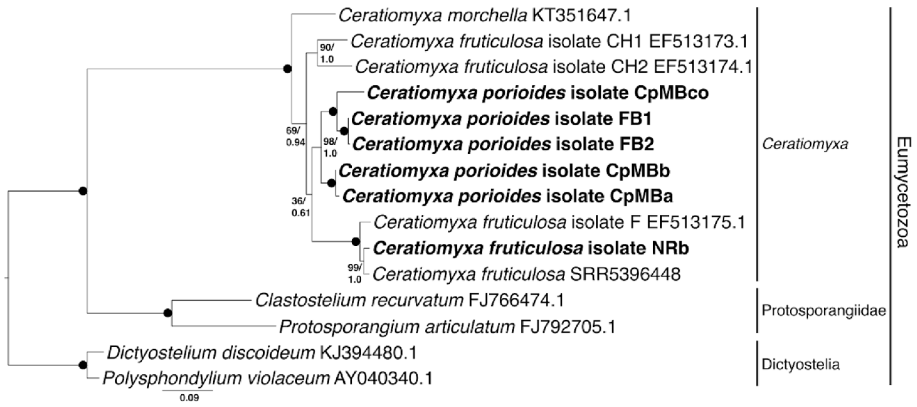

Fig. 2. Maximum likelihood (ML) SSU rRNA gene tree of Eumycetozoa, including all available Protosporangiida SSU rDNA sequences with Dictyostelia as an outgroup. The tree was constructed with RAxML using the GTRGAMMA model of evolution with 1969 sites. A black dot at a node indicates full ML bootstrap support and full support from Bayesian posterior probability. Lower values are indicated at their nodes. Novel sequences in bold.

and von Schweinitz based on its unique morphology (Albertini and Schweinitz, 1805). They describe the fruiting bodies as always porous with angled honeycomb-like pores, which starkly contrasts the typical branching forms of other *Ceratiomyxa* species (Albertini and Schweinitz, 1805). Schröter (1897) then combined the taxon to *Ceratiomyxa porioides* (Alb. & Schwein.) J. Schröt.

Lister deemed this unique morphology insufficient to remain a separate species and reassigned it as *C. fruticulosa* var. *porioides* (Lister, 1911). He explained that because poroid forms were frequently observed fruiting intermediately with branching forms, the poroid form was only a variety of the branching one, i.e., *C. fruticulosa*. In this project, we collected several new individuals of *C. fruticulosa* var. *porioides,* summarized in Table 1, and characterized them with morphological, molecular, and phylogenomic approaches to determine whether this unique fruiting body morphology corresponds to a separate species level taxon.

2. Materials and methods

2.1. Collection and photography

Pieces of rotting wood from various species of trees containing *Ceratiomyxa* samples were cut from logs in the field and brought into the lab for photography. A summary of collected isolates is presented in Table 1. Samples were viewed under reflected light with a dissecting microscope (Leica M205 C), and pictures taken with a Canon 650D camera (Fig. 1). The samples of *Ceratiomyxa fruticulosa* var. *porioides* "FB1" and "FB2" were photographed separately by a citizen scientist with an unknown camera.

2.2. RNA extraction and reverse transcription

Spores from each isolate were picked with a platinum needle and deposited into 2.3 μL cell lysis buffer (0.2 μL Superase-IN, 1.8 μL 0.2 %-TritonX-100, 0.3 μL water). The spores then underwent six freeze/thaw cycles between a –80 °C block of lead and a warm water bath to ensure that spore walls were broken. We added 1 μL of oligo-dT's and 1 μL of dNTP's to the extracted mRNA and incubated at 72 °C for 3 min. We then followed Smart-Seq2 (Picelli et al., 2014) starting at the reverse transcription step, using mRNA extracted by our above methodology, to reverse transcribe mRNA to cDNA.

We added 5.7 μL of reverse transcriptase master mix to each sample, which contains: 0.5 μL Superscript IV reverse transcriptase (200U/μL), 0.5 μL Superscript IV buffer (5x), 2.0 μL Rnase-IN Rnase inhibitor (20U/μL), 2.0 μL betaine (5 M), 0.06 μL $MgCl_2$ (1 M), 0.5 μL dithiothreitol (DTT; 100 mM), and 0.1 μL template switching oligo (TSO; 100 μM). Afterwards, all samples were run in a thermal cycler to reverse transcribe RNA into cDNA following protocols from Picelli et al. (2014). After reverse transcription, we added an ISPCR master mix (12.5 μL KAPA HiFi HotStart ReadyMix (2x), 0.25 μL ISPCR primers (10 μM), and 2.25 μL nuclease-free water). 15 μL of this master mix was added to each sample, and cDNA was amplified with PCR following protocols from Picelli et al. (2014).

Following amplification, cDNA was purified using Mag-Bind Totalpure NGS magnetic beads. 25 μL of the magnetic bead solution was added to each sample, a 1:1 volume ratio to the cDNA sample. Samples were then incubated at room temperature for 7 min to allow cDNA to bind to the magnetic beads. Following binding, beads were pelleted on a magnetic stand and the supernatant was removed. Beads were then washed twice with 200 μL of 80 % ethanol. After removal of all ethanol, cDNA was eluted from the beads with 15 μL of elution buffer (EB) off the magnetic stand for 2 min, and then the beads were pelleted on the magnetic stand and the supernatant containing the eluted purified cDNA was transferred to a new permanent tube.

2.3. Illumina library preparation

The cDNA libraries were prepared for sequencing on the Illumina platform using a Nextera XT DNA Library Preparation Kit following manufacturer protocols. The Nextera XT libraries were pooled with other independent libraries from organisms of unrelated studies and sequenced on an Illumina HiSeq 4000 instrument at Génome Québec (Montréal, Canada) or Psomagen (Rockville, MD, USA).

2.4. Transcriptome assembly

Adaptors, primer sequences, and low-quality bases were removed from the raw sequencing reads using Trimmomatic ver. 0.36 with the options ILLUMINACLIP 2:30:10 SLIDINGWINDOW:4:5 LEADING:5 TRAILING:5 MINLEN:25 (Bolger et al., 2014). The remaining reads were assembled using Trinity ver. 2.8.5 (Grabherr et al., 2011).

2.5. Molecular phylogenetics

We bioinformatically extracted small subunit (SSU) rRNA nucleotide gene sequences from the transcriptomes of the isolates to build a SSU rRNA single gene tree. To do so, we created a BLAST (Altschul et al., 1990) database from each assembled transcriptome of the isolates and queried a known *Ceratiomyxa morchella* (KT351647.1) SSU rRNA gene sequence against them. This produced SSU rRNA gene sequence hits from the isolates that we used in further analysis.

Small subunit rRNA gene sequences from our six samples were

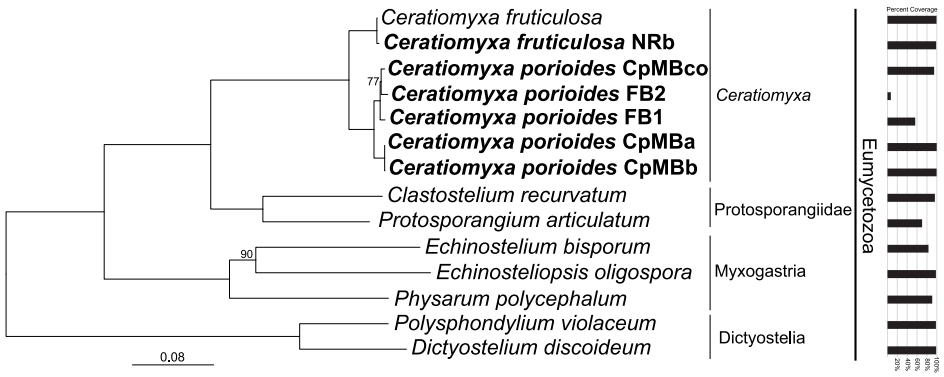

Fig. 3. Maximum likelihood phylogenomic amino acid tree of Eumycetozoa, using Dictyostelia as an outgroup, built with IQTree2 under LG + Γ4 + C60 model with 100 real bootstrap replicates. It is constructed from a multi-gene concatenated matrix of 129 proteins covering 33,874 total sites generated by PhyloFisher. The numbers at each node represent bootstrap support values, and blank nodes represent full bootstrap support. Novel sequences in bold, each other are from the public PhyloFisher database. Histogram on the right side denotes the percent coverage of sites in the phylogenomic matrix.

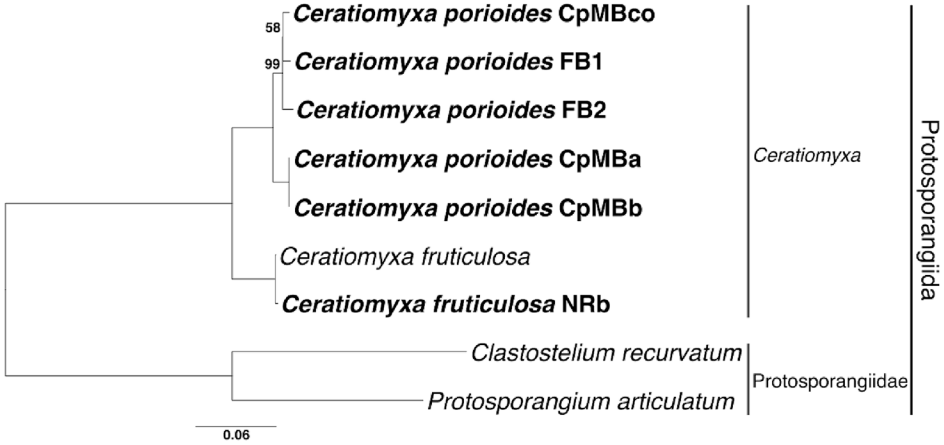

Fig. 4. Maximum likelihood phylogenomic amino acid tree of Protosporangiida, using Protosporangiida as an outgroup, built with IQTree2 under LG + Γ4 + C60 model with 100 real bootstrap replicates. It is constructed from a multi-gene concatenated matrix of 158 proteins covering 50,474 total sites generated by PhyloFisher. The numbers at each node represent bootstrap values generated under the LG + G4 + C60 model, and blank nodes represent full bootstrap support. Novel sequences in bold, all others are from the public PhyloFisher database.

included with nine other eumycetozoan SSU rRNA gene sequences available from GenBank (Clark et al., 2015) and were aligned using MAFFT with the L-INS-i algorithm (Katoh and Standley, 2013). Two Dictyostelia SSU rRNA sequences from GenBank were used as an outgroup. The aligned sequences were then trimmed with BMGE software with the global entropy parameter (−g) of 0.7. The final dataset contained 1969 unambiguously aligned characters. The maximum likelihood tree of SSU rRNA gene sequences was built with RAxML ver. 8.2.12 (Stamatakis, 2014) with general time reversible model across lineages along with four discrete gamma rate categories (+Γ) distributed rates across sites (GTRGAMMA).

We performed Bayesian analysis on the final dataset to corroborate the maximum likelihood (ML) tree using MrBayes ver. 3.2.7a under the GTRGAMMA model of substitution (Ronquist et al., 2012) as in the ML analyses. The analysis ran for 20 million generations with a burnin of 1,650,000 generations as the average standard deviation of split frequencies stabilized below 0.025.

2.6. Molecular phylogenomics

First, proteins were predicted from the assembled transcriptomes using TransDecoder ver. 5.0.0 (https://github.com/TransDecoder/TransDecoder/). From these predicted protein sequences, we queried 240 housekeeping genes using PhyloFisher (https://thebrownlab.github.io/phylofisher-pages). PhyloFisher allowed us to assemble a phylogenomic dataset from our samples and a curated database using these 240 housekeeping genes, and to identify and remove probable paralogs and contaminants by manual parsing of single homolog trees (Jones et al., 2024; Tice et al., 2021). The curated PhyloFisher dataset includes taxa representing the full known diversity of eukaryotes, from which we used all available amoebozoan taxa. We produced an amino acid supermatrix with which we built two phylogenomic trees.

From the amino acid supermatrix, we used the PhyloFisher utility "*select_orthologs.py*—percent_complete" to filter out genes with less than 75 % occupancy in the taxa we selected for the two trees (Tice et al., 2021). Occupancy filtering helped reduce large gaps of uninformative difference that produced erroneous divergence rates. For the first phylogenomic tree we covered the group Eumycetozoa. We included all our *Ceratiomyxa* sequences and representative taxa from Protosporangiidae, Myxogastria, and with Dictyostelia as an outgroup (Fig. 3). This eumycetozoan tree's matrix was comprised of 129 genes covering 33,874 amino acid sites. The second phylogenomic tree covered the group Protosporangiida and included only our *Ceratiomyxa* sequences with taxa from Protosporangiidae as an outgroup (Fig. 4). The Protosporangiida tree's matrix was comprised of 158 genes and covered 50,474 amino acid sites. Each of these matrices were then used as input to generate the respective phylogenomic trees with IQTree2 under the LG + Γ4 + C60 site heterogeneous model of evolution with 100 real

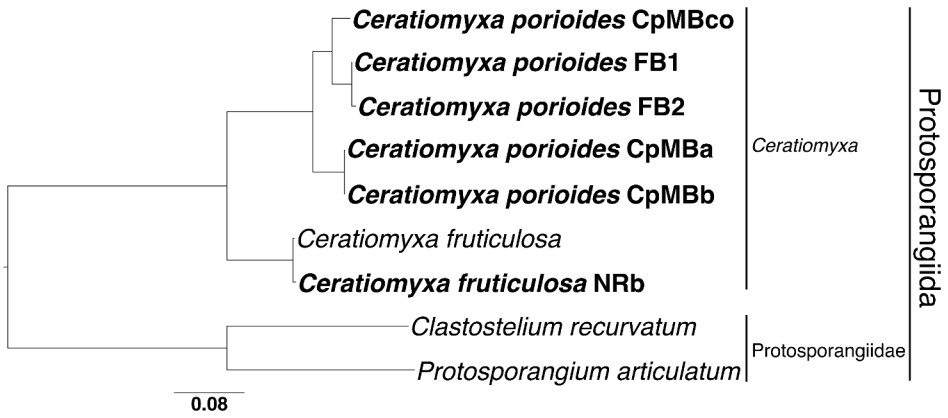

Fig. 5. Maximum likelihood phylogenetic nucleotide tree of Protosporangiida, using Protosporangiidae as an outgroup, built with IQTree2 under GTR + Γ4 + F model with 1000 ultrafast bootstrap replicates. It is constructed from a multi-gene nucleotide matrix of 158 protein-coding genes and the SSU rRNA gene covering 160,074 sites. Blank nodes represent full bootstrap support, and every node is fully supported. Novel sequences in bold, each other are from the public PhyloFisher database.

bootstrap replicates (Minh et al., 2020).

We continued to analyze the shallow species relationships of our *Ceratiomyxa* sequences by using another PhyloFisher utility, "*nucl_matrix_constructor.py*," to build a nucleotide matrix from the amino acid matrix (Jones et al., 2023) for the Protosporangiida tree (Fig. 5). To this nucleotide matrix we appended aligned sequences of the SSU rRNA genes from each organism for additional phylogenomic signal. The final matrix contained 159 genes and covered 160,074 nucleotide sites. We used this matrix as input to generate a phylogenomic tree with IQTree2 under the general time reversible (GTR) model across lineages along with four discrete gamma rate categories (+Γ4) distributed rates across sites with empirical base frequencies (F) model of evolution with 1000 ultrafast bootstrap replicates (Minh et al., 2020).

3. Results

3.1. SSU rRNA gene phylogenetic analysis

The SSU rRNA single gene phylogenetic tree, consisting of eumycetozoan taxa with Dictyostelia as an outgroup, shows our *Ceratiomyxa* spp. isolates forming a monophyletic group with other publicly available *Ceratiomyxa* spp. sequences within Protosporangiida with full support from both maximum likelihood and Bayesian analyses. The bootstrap support for a monophyletic grouping of *C. fruticulosa* var. *porioides* is high, 98 %, and is fully supported by Bayesian analysis (Fig. 2). As suspected, the other *C. fruticulosa* isolates are genetically diverse (Fiore-Donno et al., 2010). However, due to low bootstrap support values, the exact relationship between other clades of *C. fruticulosa* is unresolved in our analyses.

3.2. Phylogenomic analysis

Our phylogenomic trees of Eumycetozoa and Protosporangiida were built from amino acid matrices and constructed under LG + Γ4 + C60 site heterogeneous model of evolution. From the eumycetozoan tree we demonstrate the expected placement of the genus *Ceratiomyxa* in the group Eumycetozoa and show that *Ceratiomyxa fruticulosa* var. *porioides* group apart from *Ceratiomyxa fruticulosa* with full support (Fig. 3). As expected, the deeper relationships between the groups within Eumycetozoa are also fully supported.

The Protosporangiida amino acid tree focuses on the shallow relationships between the *Ceratiomyxa* sequences. It similarly shows that the grouping of *C. fruticulosa* var. *porioides* has full statistical support (Fig. 4). The Protosporangiida nucleotide tree adds further support for the differentiation of *C. fruticulosa* var. *porioides* from *C. fruticulosa*, also showing full statistical support (Fig. 5).

4. Discussion

Six new isolates of *Ceratiomyxa* including both *C. fruticulosa* and *C. fruticulosa* var. *porioides* were collected and sequenced for this study. These sequences include the first transcriptomes of *C. fruticulosa* var. *porioides* and fill a conspicuous gap in molecular data on a genus that has been often observed and recorded in publication for over 200 years (Albertini and Schweinitz, 1805).

Ceratiomyxa fruticulosa var. *porioides* is easily visually distinguishable from other *Ceratiomyxa* species and varieties based on its shape. Its fruiting bodies form pores between folds – poroid, hence the epithet – (Fig. 1B–E) instead of branches like *C. fruticulosa* (Fig. 1A). The *C. porioides* samples we sequenced match the historical morphological descriptions (Albertini and Schweinitz, 1805). We believe that they represent the same organisms, however we recognize that we cannot rule out cryptic speciation because this has not been investigated in detail but it is an active topic of research we aim to address in the near future with further deep sampling of all morphotypes. It is worth noting that the fruiting bodies of the sample "CpMBco" (Fig. 1C) were slightly depreciated and lacked the sharp definition of pores seen in samples "CpMBa" and "CpMBb" (Fig. 3D–E), but still displayed clear poroid shape.

The obvious morphological differences of *C. fruticulosa* var. *porioides* from *C. fruticulosa* are macroscopically apparent as well as phylogenetically valid. In the SSU rRNA gene tree, the *C. fruticulosa* var. *porioides* sequences group together separately from the other publicly available *C. fruticulosa* (Fig. 2) and our *C. fruticulosa* sample "NRb". The bootstrap support for the *C. fruticulosa* var. *porioides* clade was high (98 %), and the corroborating Bayesian analysis showed full support for this clade. We built multiple phylogenomic trees to further clarify the branching order and validate support for this group. The resulting phylogenomic trees show similar topology to our SSU rRNA single gene tree, and each phylogenomic tree shows full support for the monophyletic grouping of *C. fruticulosa* var. *porioides* (Figs. 3–5).

The phylogenomic data show clear monophyly of the *C. fruticulosa* var. *porioides* clade. However, due to the low support of the other *C. fruticulosa* clades in the SSU rRNA gene tree and the lack of conclusive phylogenomic data from both clades, we cannot yet build a full picture of the species diversity of *Ceratiomyxa.* Subsequently, it is currently difficult to completely rule out poroid morphology as phenotypic plasticity of *C. fruticulosa*. However, the deep phylogenetic structure of both SSU rDNA and phylogenomic trees both with robust clades within the genus, illustrate that there are undoubtedly more species with the

Table 2
Full list of accession numbers for SSU rDNA sequences and RNAseq of new isolates.

Isolate ID	SSU rRNA gene accession number	RNAseq accession number
NRb	PP493426	SRR27175282
CpMBco	PP493427	SRR27175285
CpMBa	PP493428	SRR27175287
CpMBb	PP493429	SRR27175286
FB1	PP493430	SRR27175284
FB2	PP493431	SRR27175283

classically held variations of *C. fruticulosa*. Unfortunately, the exact morphological details of the source fruiting bodies for the publicly available SSU rDNA sequences are sparse or simply not available, requiring further sampling with careful morphological vouchering.

We are reassured of the taxonomic reassignment of *C. fruticulosa* var. *porioides* by the fact that each of the other *C. fruticulosa* clades in our phylogenetic trees bear branching or columnar morphology, rather than poroid, but acknowledge this may not be absolute. Regardless of which clade of *Ceratiomyxa* is best attributed to *C. fruticulosa*, none of the historical descriptions of the taxon *C. fruticulosa* include description of poroid variation.

Regarding the *C. fruticulosa* isolates included in our SSU rDNA tree, the branching structure suggests a paraphyletic relationship (Fig. 2). Support values for this situation of paraphyly are only slightly supported by maximum likelihood but highly supported by Bayesian analysis (69/0.94). We find these results intriguing, especially considering previous recognition of genetic diversity within species recognized as *C. fruticulosa* (Fiore-Donno et al., 2010); however, to obtain conclusive evidence of paraphyly and subsequent taxonomic restructuring of these isolates and their forms requires additional phylogenomic data.

At this moment we are limited by the lack of morphological data linked to the molecular data available. We can discern our new strain presented herein, NRb, has a tree like (arbuscular) morphology (Fig. 1A) and the same is true of the strain from Kang et al. (2017) (SRR5396448, see Supplemental Fig. 1). Further, based on personal communications with Fiore-Donno, we understand the strain F (EF513175, Fiore-Donno et al., 2010) displayed similar arbuscular morphology. This arbuscular morphology is consistent with the original *Puccinia* description and stylized drawings of Micheli (1729) (see Tab. 92 and Fig. 2 therein). This taxon was later validly published as *Byssus fruticulosa* by O.F. Müller, *Flora Danica* 4(12):6, tab. 718 (1777). Müller's description shows a similar branching tree-like fruiting body structure (see Tab. DCCXVII). Macbride (1899) synonymized all other generic and species names (nine in total) as *Ceratiomyxa fruticulosa* (see MacBride, 1899 for the full taxonomic history). It is important to note that both Micheli (1729) and Müller (1777) show arbuscular morphology much like our strain NRb as what they observed.

Alternatively, the strains CH1 (EF513173) and CH2 (EF513174) (Fiore-Donno et al., 2010) were columnar in appearance (Fiore-Donno, personal communication), this more consistent with filiform and simple pillar morphologies, which almost certainly represent another or multiple species given the phylogenetic structure and the molecular variation. The genus is undoubtedly in need of more careful taxonomic works.

At this time, sampling other described morphological variations will be necessary to clarify these other instances of unresolved genetic diversity and reveal whether they also represent valid species. Historically, it has been unclear what significance the form of a fruiting body has for determining separate species level taxa in *Ceratiomyxa* (Gilbert, 1935). Our work here provides evidence that the form of the fruiting body does, in fact, correspond to species level diversity. Based on our current molecular data and the apparent morphological synapomorphy of our isolates, we propose to raise again the variety *Ceratiomyxa fruticulosa* var. *porioides* to the species *Ceratiomyxa porioides*.

5. Taxonomic summary

Ceratium porioides Albertini and Schweinitz, 1805
Ceratiomyxa porioides (Albertini and Schweinitz) J. Schröter, 1897
Ceratiomyxa fruticulosa var. *porioides* (Albertini and Schweinitz) G. Lister, 1911
Ceratiomyxa porioides (Albertini and Schweinitz) J. Schröter, 1897 *sensu* Fry and Brown, 2024

Taxonomy of *Ceratiomyxa porioides*

Diagnosis. From the original description by Albertini and Schweinitz, and evident in the samples photographed here (Fig. 1B–E): fruiting bodies of *Ceratiomyxa porioides* are consistently porous with five to eight angled honeycomb-shaped pores. The corners of these pores are punctuated by short horns (Albertini and Schweinitz, 1805). Fruiting bodies of this species have been exclusively found growing on rotting wood.

Gene sequence data. The transcriptomic data and SSU rRNA gene sequences for isolates of this species have been deposited to GenBank under the BioProject PRJNA1048962 and nucleotide accession numbers PP493427, PP493428, PP493429, PP493430, and PP493431 (Table 2).

CRediT authorship contribution statement

Nicholas W. Fry: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Robert E. Jones:** Writing – review & editing, Software, Data curation. **Quentin Blandenier:** Writing – review & editing, Investigation, Conceptualization. **Alexander K. Tice:** Writing – review & editing, Investigation. **Alfredo L. Porfirio-Sousa:** Writing – review & editing, Investigation, Funding acquisition. **Felicity Kleitz-Singleton:** Writing – review & editing, Investigation. **Tristan C. Henderson:** Writing – review & editing, Investigation. **Matthew W. Brown:** .

Data availability

All molecular data associated with this manuscript are available on FigShare (https://doi.org/10.6084/m9.figshare.25365364). This includes transcriptome assemblies, predicted proteomes, alignments (trimmed and untrimmed), as well as phylogenetic trees.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejop.2024.126083.

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