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ORIGINAL ARTICLE

Investigation of CZEUM cultures reveals new genera and species of *Cladochytriales* (*Cladochytriomycetes*, *Chytridiomycota*)

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Abstract

Cladochytriales comprises a taxonomic order and lineage of chytrid fungi that are primarily saprobes of organic matter in aquatic and terrestrial ecosystems. The order contains species from 12 genera, though molecular confirmations of almost all type species are lacking, and many genera are considered polyphyletic. We conducted phylogenetic analyses of ribosomal RNA genes and developmental morphology of *Cladochytriales* isolates from the Collection of Zoosporic Eufungi at the University of Michigan and noted some strains that were distinct from described genera. In our phylogenetic analysis, *Endochytriaceae* represented by only one strain of *Endochytrium ramosum* was sister to *Septochytriaceae* while the *incertae sedis* lineage formed by the new species *Thomasia carolinae* was sister to *Nowakowskiellaceae*. Additionally, the phylogenetic relatedness of the other two new species, *Dogmamyces elongatus* and *Allochytrium aureum*, was also highly supported. A thorough revision of the order is needed because some genera, such as *Catenochytridium* and *Nephrochytrium*, remain polyphyletic or paraphyletic. In addition to indicating these problems, our updated phylogeny supports the description of two new genera and three new species and thereby begins to bring the knowledge of the *Cladochytriales* up to date.

Keywords Chytrid fungi · Three new taxa · Molecular phylogeny · Morphology

Introduction

The *Cladochytriales* is a lineage of chytrid fungi that primarily comprises saprobes of organic matter, especially plants materials in aquatic and terrestrial ecosystems. This order was previously considered the family *Cladochytriaceae* in the *Chytridiales* (Sparrow 1960; Karling 1977). Comprehensive molecular phylogenies of the *Chytridiomycota* grouped representatives of eight genera in a cohesive group with minimal ("*Nowakowskiella* clade"; James et al. 2000) or strong support ("*Cladochytrium* clade"; James et al. 2006). Mozley-Standridge et al. (2009) cited these phylogenies and previous ultrastructural studies (Lucarotti 1981; Barr 1986; Barr and Désaulniers 1987) as evidence to elevate the *Cladochytrium* clade to ordinal status as the *Cladochytriales*. Since the ordinal description, few taxonomic revisions or novelties have been proposed (Steiger et al. 2011; Jerônimo et al. 2019, 2022), though these studies led to the inclusion of four additional genera and the typification of the order by Wijayawardene et al. (2018). Several morphologically based genera are polyphyletic or lack molecular confirmation of their type species (Jerônimo et al. 2022).

As with other orders of the *Chytridiomycota*, the traditional classification system was based on morphological similarities of taxa on natural substrates. However, we now know that morphological characters are often homoplasious. With the innovation of molecular phylogenetics and phylogenomics, investigators have revised the classification of the *Chytridiomycota* (e.g., James et al. 2006; Letcher et al. 2006,

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2008; Simmons et al. 2009; Longcore and Simmons 2012; Amses et al. 2022), and both expanded (Steiger et al. 2011; Jerônimo et al. 2019, 2022) and limited (Vélez et al. 2011; Longcore and Simmons 2012) the bounds of the *Cladochytriales*. As examples of the latter, Sparrow (1960) grouped polycentric genera, such as *Cladochytrium*, *Physocladia*, and *Polychytrium*, in *Cladochytriaceae*. However, under molecular phylogenetic hypotheses, *Physocladia* is now considered to be closely related to several monocentric lineages in the *Chytridiales* (Vélez et al. 2011), and *Polychytrium* is the type genus of the more recently described *Polychytriales* (Longcore and Simmons 2012), an order containing genera exhibiting both monocentric and polycentric development. Assumptions of relatedness based on gross morphological comparisons become less accurate as molecular phylogenies illustrate the convergence of these traits across the phylum, making molecular analyses crucial for exploring the biodiversity and describing the taxonomic novelties of chytrid fungi.

Toward an increasingly resolved phylogeny of the *Cladochytriales*, we examined isolates deposited in the Collection of Zoosporic Eufungi at the University of Michigan (CZEUM) to chronicle the diversity of this lineage from materials in-hand, collected over more than 30 years from a variety of locations, mostly in northeastern USA. Using phylogenetic analyses of ribosomal RNA genes and studies of morphological development, we concluded that some strains, already included in the phylogeny of Jerônimo et al. (2022) as unknown taxa, were distinct from all described genera in *Cladochytriales*. Herein, we expand knowledge of the *Cladochytriales* by presenting an updated phylogeny for the order and describing two new genera and three new species.

Material and methods

Studied cultures

We revitalized strains from CZEUM that had been annotated as members of the *Cladochytriales* (Simmons et al. 2020). In addition, we analyzed two strains isolated in streams or lakes from Brazil (Table 1). We produced stock cultures from these cultures by aseptically transferring a small piece of biological material (aggregated zoosporangia or rhizomycelium) to Erlenmeyer flasks containing 50 mL of mPmTG or PmTG liquid medium and incubated stocks for 10–25 days at 21 °C. Stocks were propagated via this transfer method over the course of the study.

DNA extraction, amplification, and sequencing

For DNA extraction, we followed a 2 × CTAB extraction protocol similar to James et al. (2008), without using phenol. Genomic DNA was quantified on a Qubit 4 fluorometer (Thermo Fisher Scientific), and a portion of this material was diluted to produce working stocks for PCR amplification (0.1–1 ng/μL). To amplify ribosomal units (nc SSU rDNA and nc LSU rDNA), we prepared an amplification reaction with TaKaRa ExTaq™ DNA Polymerase kits (Takara Bio USA, Inc.) using the primer pairs LR0R/LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1994) for partial nc LSU rDNA and NS1.5/NS6 (White et al. 1990) for nc SSU rDNA. Reagents were combined as follows: 2.9375 μL UV-radiated PCR water, 1.25 μL 10 × ExTaq™ buffer, 1.00 μL 25 mM $MgCl_2$, 1.00 μL 10 mM dNTPs, 0.0625 μL ExTaq polymerase, 0.625 μL each primer (10 μM), and 5.0 μL working stock DNA template (~ 1 ng/ μL). Thermocycling profiles followed protocol described by James et al. (2006). Amplicons were purified with ExoSap-IT™ PCR product cleanup reagent (Thermo Fisher Scientific) and sequenced at GENEWIZ, NJ, USA.

Sequences and phylogenetic analyses

For newly generated sequences, we assembled the contiguous sequences pairs (forward and reverse primers) with Sequencher 4.1.4. For phylogenetic reconstruction, we selected data from 53 isolates of *Cladochytriales*, and three of them (*Cladochytrium* JEL714, *Cladochytrium* sp. GHJ55, *Karlingiella elongata* GHJ56) were used as outgroup taxa (Fig. 1), with information from two new strains in this study (*Cladochytrium* sp. GHJ55, *Karlingiella elongata* GHJ56), 28 generated by Simmons et al. (2020), and 23 from additional GenBank submissions (Table 1). The separate ribosomal loci were aligned using the MAFFT version 7 web tool (http://mafft.cbrc.jp/ alignment/server/) (Katoh et al. 2017). The nc SSU rDNA and nc LSU rDNA alignments were then concatenated using SequenceMatrix 1.8 (Vaidya et al. 2010), resulting in a final length of 3059 base pairs. Maximum likelihood (ML) phylogenetic analyses were conducted in RaxML-HPC v.8 on XSEDE (Stamatakis 2014) and Bayesian inference (BI) in MrBayes 3.2.6 (Ronquist et al. 2012), on the CIPRES Science Gateway platform (https://phylo.org). In both cases, partition models were generated by jModelTest 0.1.1 (Posada 2008). We performed the ML analysis with 1000 bootstrap replicates and the BI using the Markov chain Monte Carlo (MCMC) methodology to calculate posterior probabilities (PPs). The parameters for BI were 5 million generations, with the first 10% of iterations discarded as burn-in, then sampled every 1000th iteration from the remainder. Values < 70% (ML) or < 0.70 (BI) are omitted from the final tree. The character matrix (concatenated alignment), ML tree, and BI tree are deposited in TreeBase (Study TB2: 30430).

Table 1 Species, strains/vouchers, GenBank accession numbers, substrates, and country. Isolates highlighted in bold represent the new species and those marked with an asterisk were isolated in this study

Species	Voucher	GenBank accession number		Substrate	Country
		SSU	LSU		
		ONT rDNA (18S + 28S)			
Allochytridium luteum	ATCC60989	JN940948	AY439066	NA	NA
Allochytridium luteum	JEL324	AY635844	DQ273816	Onion skin	Canada
Allochytridium luteum	ALLO	AY349047	NA	NA	NA
Allochytridium sp.	JEL075	MT730664		*Eriocaulon* sp.	United States
Allochytridium sp.	JEL574	MT730749		*Eriocaulon* sp.	United States
Allochytridium sp.	JEL896	MT730876		*Eriocaulon* sp.	United States
Allochytridium aureum	JEL386	MT730712		*Nitella* sp.	United States
Unknown	JEL877	MT730862		Onion skin	United States
Catenochytridium sp.	JEL024	MT730657		Cellophane	United States
Catenochytridium sp.	JEL145	EU828475	EU828503	Onion skin	United States
Catenochytridium sp.	JEL775	MT730811		*Eriocaulon* sp.	United States
Catenochytridium sp.	JEL783	MT730812		Onion skin	United States
Catenochytridium sp.	JEL044	EU828478	EU828506	Detritus	United States
Cladochytrium replicatum	JEL714	MT730791		Onion skin	United States
Cladochytrium sp.*	GHJ55	OR297624	OR297685	Corn leaves	Brazil
Cylindrochytridium johnstonii	JEL596	JF796051	JF796052	Onion skin	United States
Dogmamyces elongatus	JEL050	MT730661		Onion skin	United States
Dogmamyces elongatus	JEL931	MT730898		Onion skin	United States
Unknown	JEL049	MT730660		*Typha* sp.	United States
Endochytrium ramosum	JEL402	MT730716		*Cladophora* sp.	United States
*Karlingiella elongata**	GHJ56	OR297625	OR297686	Onion skin	Brazil
Nephrochytrium aurantium	JEL907	MT730886		*Eriocaulon* sp.	United States
Nephrochytrium aurantium	JEL036	EU828468	EU828495	*Sparganium* sp.	United States
Nephrochytrium sp.	JEL125	AH009049	EU828511	Onion skin	United States
Nephrochytrium sp.	JEL327	EU828467	EU828494	*Nitella* sp.	United States
Nowakowskiella crenulata	CCIBt 4258	MH590092	MH590080	Onion skin	Brazil
Nowakowskiella crenulata	CCIBt 4259	MH590093	MH590081	Onion skin	Brazil
Nowakowskiella elegans	JEL046	MT730659		NA	United States
Nowakowskiella elegans	JEL157	EU828465	EU828492	Cotton fiber	United States
Nowakowskiella multispora	CCIBt3864	MH590089	MH590077	Cellophane	Brazil
Nowakowskiella multispora	CCIBt4015	KJ539147	KJ539149	Cellophane	Brazil
Nowakowskiella ramosa	CCIBt4294	MH590090	MH590078	Onion skin	Brazil
Nowakowskiella sp.	CCIBt4426	OR045412	OR046719	Onion skin	Brazil
Nowakowskiella sp.	CCIBt 4260	MH590094	MH590082		Brazil
Nowakowskiella sp.	JEL078	MT730665		Cellophane	United States
Nowakowskiella sp.	JEL154	MT730682		Cellophane	United States
Nowakowskiella sp.	JEL381	MT730711		Unknown algae	United States
Nowakowskiella sp.	JEL426	MT730718		NA	United States
Nowakowskiella sp.	JEL789	MT730814		Twine fiber	United States
Nowakowskiella sp.	JEL901	MT730881		*Eriocaulon* sp.	United States
Nowakowskiella sp.	JEL902	MT730882		*Eriocaulon* sp.	United States
Nowakowskiella sp.	JEL921	MT730895		Onion skin	United States
Nowakowskiella sp.	JH-Fr	EU828479	EU828507	NA	NA
Nowakowskiella sp.	JH-HBR	EU828469	EU828496	NA	NA
Phytochytrium stagnum	JEL1002	OL473637	OL473639	Onion skin	United States
Septochytrium sp.	JEL177	EU828474	EU828502	Onion skin	United States
Septochytrium variabile	JEL191	EU828483	EU828512	*Eriocaulon* sp.	United States

Table 1 (continued)

Species	Voucher	GenBank accession number		Substrate	Country
		SSU	LSU		
		ONT rDNA (18S + 28S)			
Sparrowiella insolita	JEL1004	OL473638	OL473640	Onion skin	United States
Thomasia carolinae	JEL818	MT730829		*Nitella* sp.	United States
Diplochytridium lagenaria	JEL072	MT730663		*Oedogonium* sp.	United States
Septochytrium sp.	JEL319	MT730696		Grass	United States
Septochytrium sp.	JEL785	MT730813		Onion skin	United States
Septochytrium sp.	JEL793	MT730815		Onion skin	United States

Morphological characterization of the isolates

We observed the development of the strains mentioned above on agar media (mPmTG, PmTG, Cd) (Barr 1986; Longcore 1992; Longcore and Simmons 2012) and/or sterilized onion skin. To assign species identity, we used the classic morphological characters (type of development, growth pattern, operculation, shape, size and ornamentation of zoosporangia and resting spores, catenulation, apophysis, and number and color of lipid globules in zoospores). The morphological structures were examined using a Leica DMLB2 compound microscope and photographed with a Leica MC170 HD camera using Leica Qwin 3.1 software. We used Sparrow (1960) and Karling (1977) monographs as references, deferring to original descriptions of the genera and species when necessary (e.g., Berdan 1939; Karling 1938; Sparrow 1933).

Results

Phylogenetic analysis

The most appropriate models of DNA substitution were TIM3 + G (nc SSU rDNA) and GTR + G (nc LSU rDNA), according to Akaike information criterion (AIC). In our phylogenetic analysis (ML boostrap/Bayesian posterior probability), *Endochytriaceae* represented by only one strain of *Endochytrium ramosum* was sister to *Septochytriaceae* while the *incertae sedis* lineage formed by the new species *Thomasia carolinae* was sister to *Nowakowskiellaceae*. Additionally, the phylogenetic relatedness of the other two new species, *Dogmamyces elongatus* and *Allochytrium aureum*, were also highly supported.

Taxonomy

We carefully analyzed the morphology and development of the study strains to determine if an isolate represented a previously described taxon, but we based the taxonomic novelties primarily on the phylogenetic analysis of ribosomal genes.

Septochytriaceae S.E. Mozl.-Standr., 2009.

MycoBank: MB512008

The circumscription of *Septochytriaceae* was recently expanded by Jerônimo et al. (2022) to include two new polycentric genera (*Sparrowiella* and *Phytochytrium*) with aseptate rhizomycelia. According to our molecular analysis (Fig. 1), the new proposed genus described below, *Dogmamyces*, belongs to *Septochytriaceae*, as does the new species *Allochytridium aureum*. *Septochytriaceae* now contains nine genera, with *Septochytrium* as the type of the family.

Allochytridium D.J.S. Barr & Désauln. 1987

Type: *Allochytridium expandens* Salkin ex. S.C. Jong & M.J. Edwards 1991

Description: Thallus monocentric and eucarpic. Zoosporangium operculate, formed as an expansion on the germ tube and fusing with the zoospore cyst by maturity. Zoospores posteriorly uniflagellate and rhizoidal system largely endobiotic.

Allochytridium aureum G.H. Jerônimo, C.L.A. Pires-Zottar., T.Y. James & J.E. Longcore, sp. nov. Fig. 2a–n

MycoBank: MB 848804

Typification: USA. New York, Adirondacks, Sep 2003, *J.E. Longcore*. Holotype (Fig. 2a–n), in this paper. Ex-type: Cryopreserved strain JEL386, deposited in CZEUM-MICH. GenBank (JEL386): 18S + ITS1 + 5.8S + ITS2 + 28S = MT 730712.

Etymology: The specific epithet "aureum" refers to the goldish-orange color of the lipid globule in zoospores.

Description: Fungus saprotrophic. Thallus monocentric with exogenous development. Zoosporangium smooth, spherical (10 to 15 µm), formed by the expansion of the germ tube and the fusion of the sporangium with the zoospore cyst, producing operculate discharge tubes which can reach up 30 µm. Rhizoids finely branched and smooth. Zoospore with an orange lipid globule. Resting spores not observed.

Notes: Strain JEL386 resembles representatives of *Endochytrium* but is not closely related to the type species *E. ramosum* JEL402. Carefully observing the morphology and development of JEL386, we noted important differences in the development of *Endochytrium* as described by Sparrow (1933). In JEL386, the zoosporangium forms by the expansion of the germ tube and the fusion of the sporangium with the zoospore cyst (Fig. 2b–d), similar to *Allochytridium* species (Salkin 1970; Barr 1986; Barr and Désaulniers 1987). In our phylogeny (Fig. 1), *A. aureum* is closely related to *Allochytridium luteum* (ALLO, JEL324 and ATCC60989), which is the single described species of the genus with orange lipid globule in zoospores. However, differently from *A. luteum* which forms a single rhizoidal system and a single discharge tube (Barr and Désaulniers 1987), *A. aureum* can produce multiple rhizoidal axes (Fig. 2e) and two discharge tubes (Fig. 2k). Besides that, JEL386 forms a distinctive branch, corroborating the morphological distinctions and indicating that it represents a new species of *Allochytridium* that also produces zoospores with carotenoids.

Dogmamyces G.H. Jerônimo, C.L.A. Pires-Zottar., T.Y. James & J.E. Longcore, gen. nov.

MycoBank: MB 848807

Type: *Dogmamyces elongatus* G.H. Jerônimo, C.L.A. Pires-Zottar., T.Y. James & J.E. Longcore.

Description: Fungus saprotrophic. Thallus monocentric with endogenous development. Zoosporangium apophysate, with operculate discharge tube. Multiple rhizoid axes with prominent constrictions. Zoospore with a single hyaline lipid globule. Resting spores not observed.

Etymology: The genus honors Irineo J. Dogma Jr., who described several species of chytrids. Prof. Dogma was a student at U. Michigan with Prof. Fred Sparrow and inspired generations of microbiologists in the Philippines.

Dogmamyces elongatus G.H. Jerônimo, C.L.A. Pires-Zottar., T.Y. James & J.E. Longcore, sp. nov. Fig. 3a–m

MycoBank: MB 848810

Description: Fungus saprotrophic. Thallus monocentric with endogenous development. Zoosporangium smooth, spherical (8 to 10 µm) or subspherical (8–10 to 12–15 µm), operculate, apophysate and releases zoospores through a long discharge tube (which can reach up 35 µm). In pure culture, additional discharge tubes are produced even when the first tube is already formed; multiple rhizoid axes with prominent constrictions. Zoospore with a single hyaline lipid globule. Resting spores not observed.

Etymology: The specific epithet "elongatus" refers to the length of discharge tubes.

Typification: USA. Maine: Orono, pond on Godfrey Drive, May 1989, *J.E. Longcore*. Holotype (Fig. 3a–m) in this paper. Ex-type: Cryopreserved strain JEL050, deposited in CZEUM-MICH. GenBank (JEL050): 18S + ITS1 + 5.8S + ITS2 + 28S = MT730661.

Other examined material: USA. Maine: Orono, Pond in Littlefield Ornamental Garden, April 2018, *J.E. Longcore*. Cryopreserved strain JEL931 deposited in CZEUM-MICH. GenBank (JEL931): 18S + ITS1 + 5.8S + ITS2 + 28S = MT 730898.

Notes: This strain exhibits endogenous development, dissimilar to *Endochytrium, Allochytridium*, and *Catenochytridium*, which develop exogenously. In addition, the rhizoids produce prominent constrictions, similar to catenulations observed in *Catenochytridium* (Fig. 3e, f). Based on these morphological characteristics and placement of these strains in our phylogenetic analysis, we propose a new genus and species.

Incertae sedis

Thomasia G.H. Jerônimo, gen. nov.

MycoBank: MB 848816

Type: *Thomasia carolinae* G.H. Jerônimo

Description: Fungus saprotrophic. Thallus monocentric with exogenous development. Zoosporangium smooth, producing operculate discharge tubes. Zoosporangium apophysate; extensive rhizoids with multiple axes. Zoospores contain a single hyaline lipid globule. Resting spores thick-walled with a central and prominent lipid globule.

Etymology: The genus honors Thomas C. Jerônimo Alves, the son of the first author.

Thomasia carolinae G.H. Jerônimo, sp. nov. Fig. 4a–p.

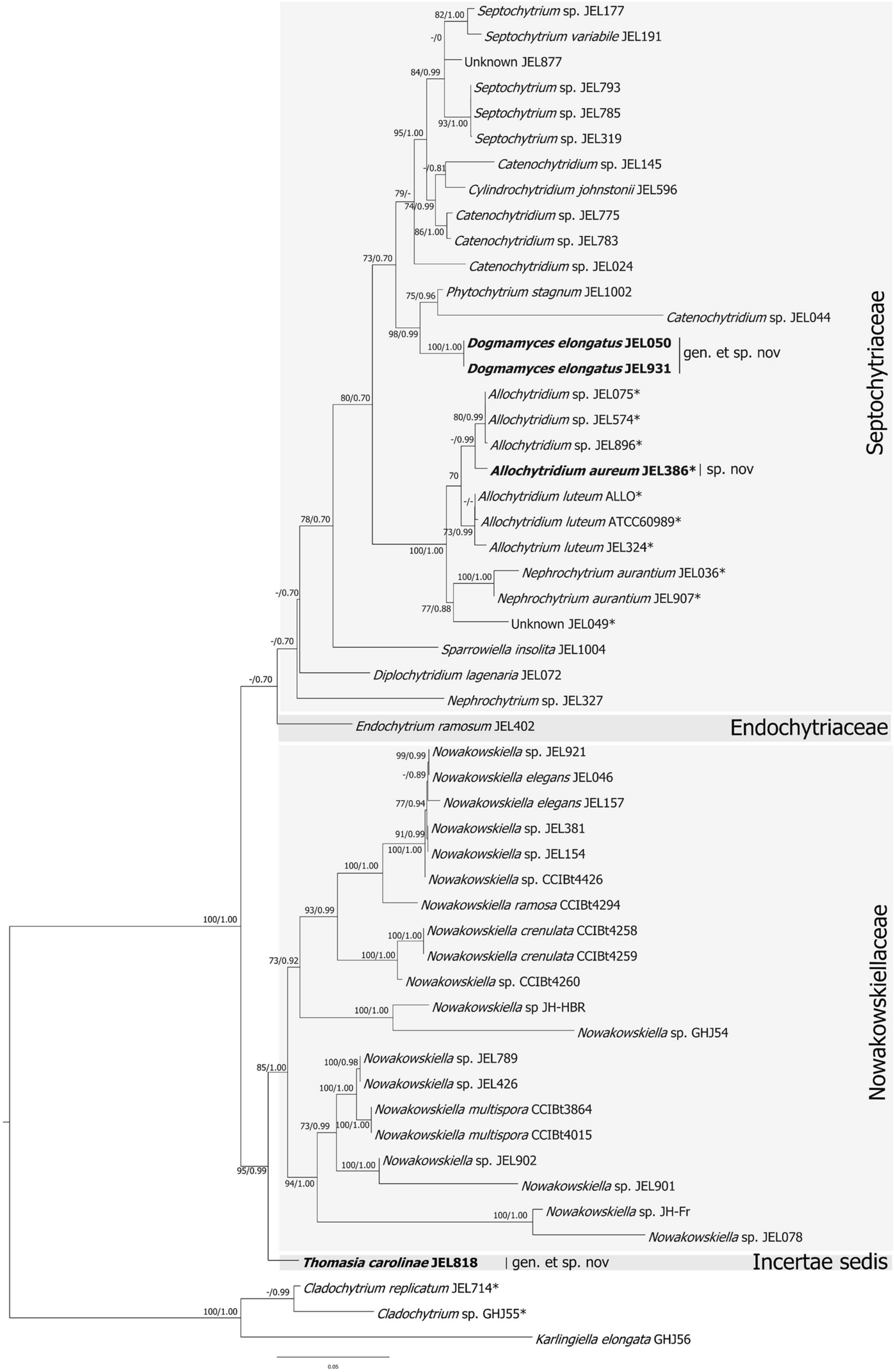
MycoBank: MB 848818

Description: Fungus saprotrophic. Thallus monocentric with an exogenous development. Zoosporangia smooth, spherical (15 to 18 µm), operculate, producing discharge tubes varying in length (8 to 20 µm). Zoosporangia apophysate, and rhizoids extensive, smooth, and with multiple axes. Zoospores contain a single hyaline lipid globule. Resting spores spherical (8 to 10 µm), thick-walled, with a central and prominent lipid globule, functioning as a prosporangium.

Typification: USA. Maine: Old Town, Pushaw Lake, Oct 2013, *J.E. Longcore*. Holotype (Fig. 4a–p) in this paper. GenBank (JEL818): 18S + ITS1 + 5.8S + ITS2 + 28S = MT 730829.

Etymology: The specific epithet "carolinae" honors Carolina C. Jerônimo Alves, the wife of the first author.

Notes: This monocentric strain is sister to the *Nowakowskiellaceae*, which exhibits polycentricity. The species develops exogenously, resulting in a non-persistent zoospore cyst (Fig. 4c–h) and an apophysate zoosporangium (Fig. 4i) with one or two operculate discharge tubes (Fig. 4j, l).


82/1.00
Septochytrium sp. JEL177
-/0
Septochytrium variabile JEL191
Unknown JEL877
84/0.99
Septochytrium sp. JEL793
Septochytrium sp. JEL785
93/1.00
95/1.00
Septochytrium sp. JEL319
-/0.81
Catenochytridium sp. JEL145
Cylindrochytridium johnstonii JEL596
79/-
74/0.99
Catenochytridium sp. JEL775
86/1.00
Catenochytridium sp. JEL783
73/0.70
Catenochytridium sp. JEL024
75/0.96
Phytochytrium stagnum JEL1002
Catenochytridium sp. JEL044
98/0.99
100/1.00
Dogmamyces elongatus JEL050
Dogmamyces elongatus JEL931
gen. et sp. nov
80/0.70
Allochytridium sp. JEL075*
80/0.99
Allochytridium sp. JEL574*
-/0.99
Allochytridium sp. JEL896*
Allochytridium aureum JEL386* | sp. nov
70
Allochytridium luteum ALLO*
-/-
78/0.70
Allochytridium luteum ATCC60989*
73/0.99
100/1.00
Allochytrium luteum JEL324*
100/1.00
Nephrochytrium aurantium JEL036*
-/0.70
Nephrochytrium aurantium JEL907*
77/0.88
Unknown JEL049*
-/0.70
Sparrowiella insolita JEL1004
Diplochytridium lagenaria JEL072
-/0.70
Nephrochytrium sp. JEL327
Septochytriaceae
Endochytrium ramosum JEL402
Endochytriaceae
99/0.99
Nowakowskiella sp. JEL921
-/0.89
Nowakowskiella elegans JEL046
77/0.94
Nowakowskiella elegans JEL157
91/0.99
Nowakowskiella sp. JEL381
100/1.00
Nowakowskiella sp. JEL154
100/1.00
Nowakowskiella sp. CCIBt4426
Nowakowskiella ramosa CCIBt4294
100/1.00
93/0.99
100/1.00
Nowakowskiella crenulata CCIBt4258
100/1.00
Nowakowskiella crenulata CCIBt4259
73/0.92
Nowakowskiella sp. CCIBt4260
100/1.00
Nowakowskiella sp JH-HBR
Nowakowskiella sp. GHJ54
100/0.98
Nowakowskiella sp. JEL789
85/1.00
100/1.00
Nowakowskiella sp. JEL426
Nowakowskiella multispora CCIBt3864
73/0.99
100/1.00
Nowakowskiella multispora CCIBt4015
100/1.00
Nowakowskiella sp. JEL902
95/0.99
94/1.00
Nowakowskiella sp. JEL901
100/1.00
Nowakowskiella sp. JH-Fr
Nowakowskiella sp. JEL078
Nowakowskiellaceae
Thomasia carolinae JEL818 | gen. et sp. nov
Incertae sedis
-/0.99
Cladochytrium replicatum JEL714*
100/1.00
Cladochytrium sp. GHJ55*
Karlingiella elongata GHJ56
0.05

◂**Fig. 1** Phylogram of the *Cladochytriales* inferred from Bayesian analysis of the combined rDNA alignment (18S + 28S). Only bootstrap support values and Bayesian posterior probability values > 70%/0.7 are labeled numerically. The scale bar indicates the average number of substitutions per site. Sequences from this study are indicated in boldface. Sequences marked with an asterisk produce zoospores with orange lipid globule

Resting spores observed on cellulosic substrates (Fig. 4n–p) have a thick wall and prominent hyaline lipid globule (Fig. 4n) and germinate as a prosporangium (Fig. 4p). Considering the phylogenetic placement and a distinctive morphology and development of the strain, we propose a new genus and species.

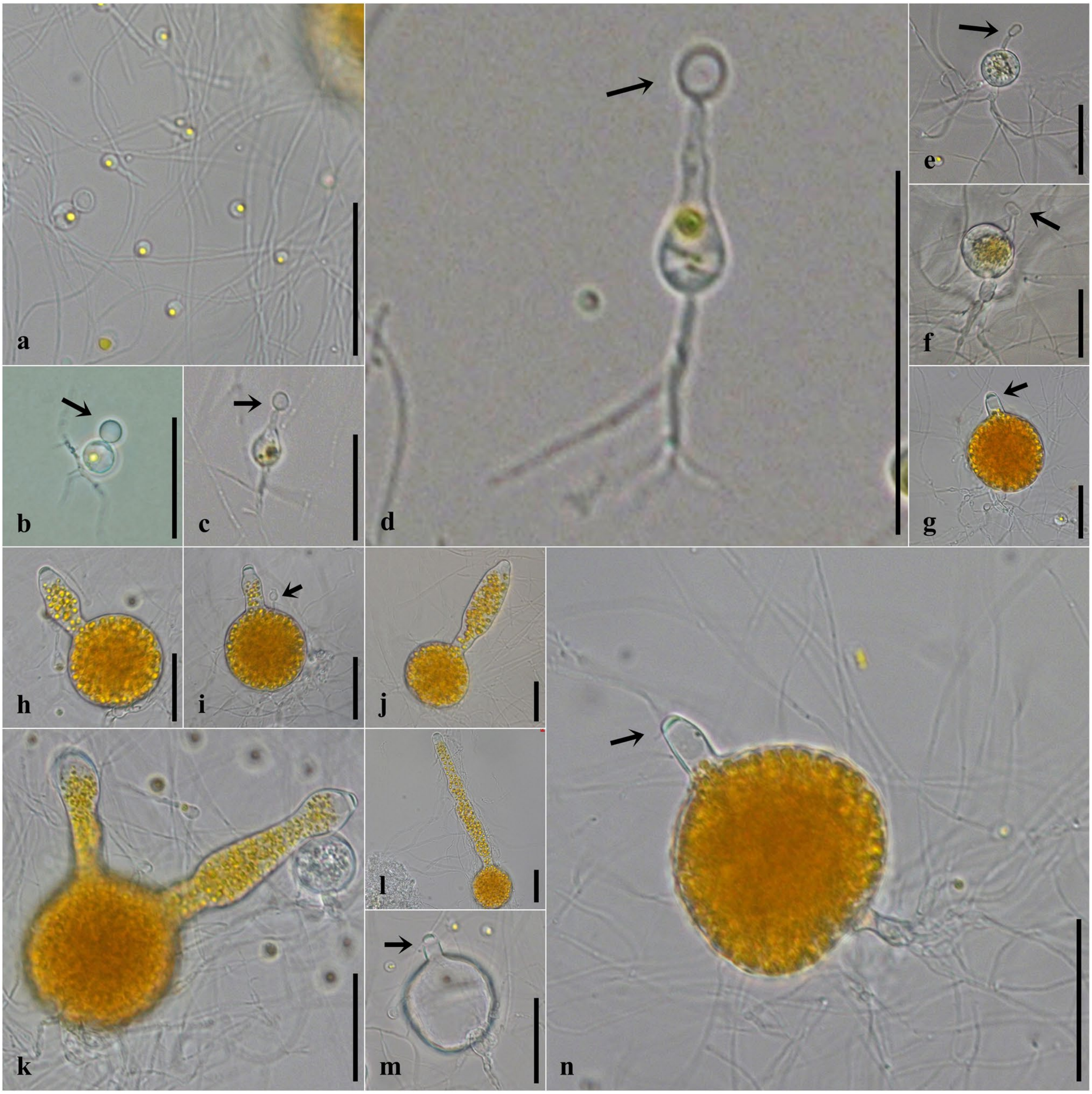


Fig. 2 **a**–**n** Development of *Allochytridium aureum* JEL386 in PmTG agar medium using differential interference contrast (DIC). **a** Zoospore germination. **b**–**d** Initial stages of exogenous development and details of the zoospore cyst (arrows). **e**–**f** Immature zoosporangium with zoospore cyst (arrows). **g**–**h** Mature zoosporangium with an operculate discharge tube (arrow). **i** Mature zoosporangium with a persistent cyst of zoospore (arrow). **j**–**l** Mature zoosporangium with operculate discharge tubes. **m** Empty zoosporangium and attached operculum (arrow). **n** General overview of a mature zoosporangium, showing operculate discharge tube (arrow) and rhizoidal system. Bars = 10 μm

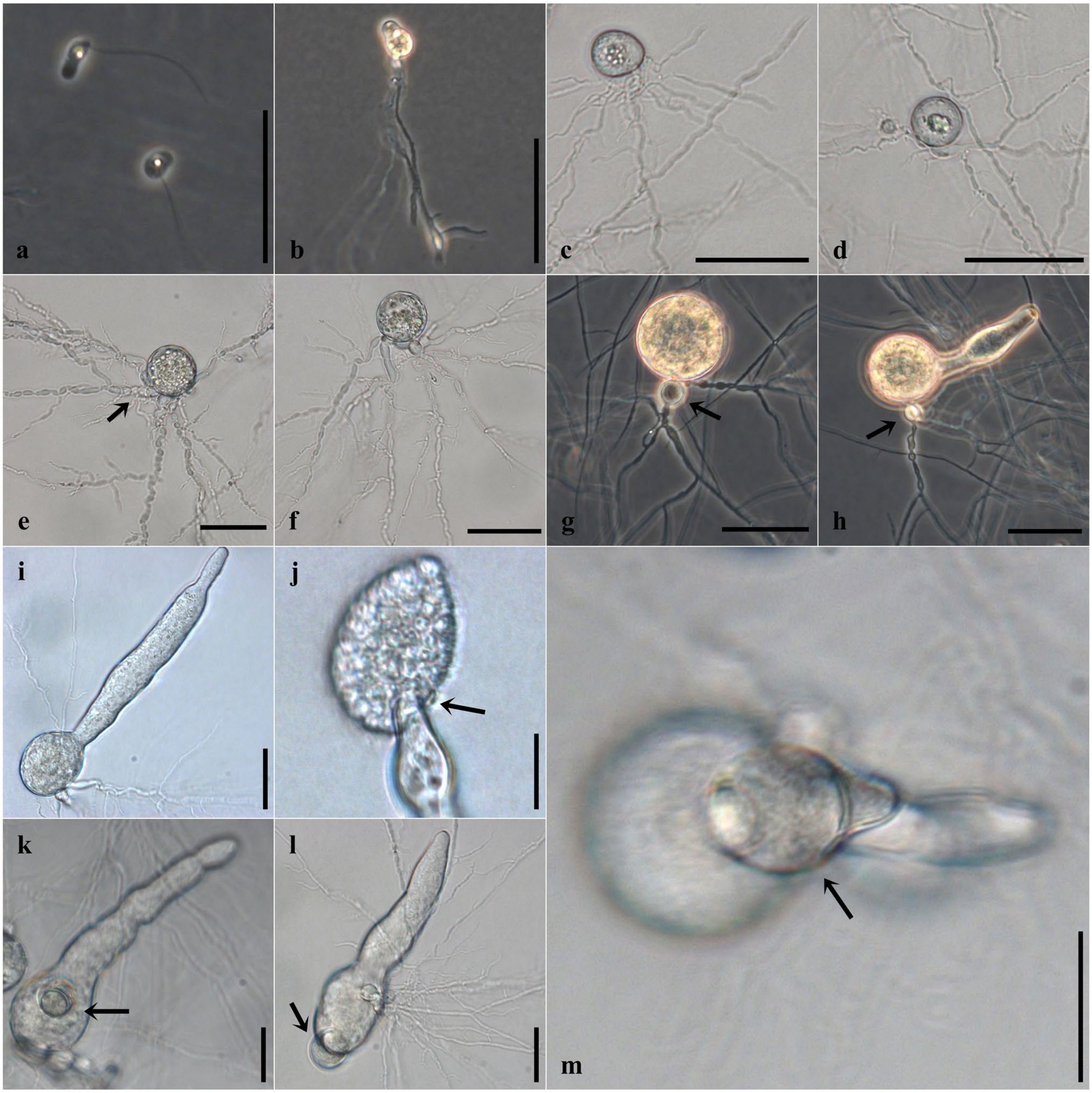


Fig. 3 **a**–**m** Development of *Dogmamyces elongatus* JEL050 in PmTG agar medium using differential interference contrast (DIC) and phase contrast (**a**, **b**, **g**, **h**). **a**–**b** Zoospore germination. **c**–**f** Initial stages of endogenous development and details of the constrictions in rhizoids (arrows). **e**–**f** Immature zoosporangium with zoospore cyst (arrows). **g**–**h** Zoosporangium with an apophysis (arrows). **i** Mature zoosporangium with a long discharge tube. **j** Detail of the operculum (arrow). **k**–**m** Development of a new discharge tube (arrows). Bars = 10 μm

Discussion

Our three new species, two of which belong to novel genera, illustrate the diversity of the *Cladochytriales*. Similar to many other fungal lineages, gross morphological similarity does not always indicate close phylogenetic relatedness. For example, the monocentric species *Dogmamyces elongatus* is sister to *Catenochytrium* sp. JEL044 and *Phytochytrium stagnum* JEL1002, the latter a polycentric species recently described by Jerônimo et al. (2022), further indicating that traditional classification systems reliant on developmental similarities do not reflect natural phylogenetic relationships. Finally, given the

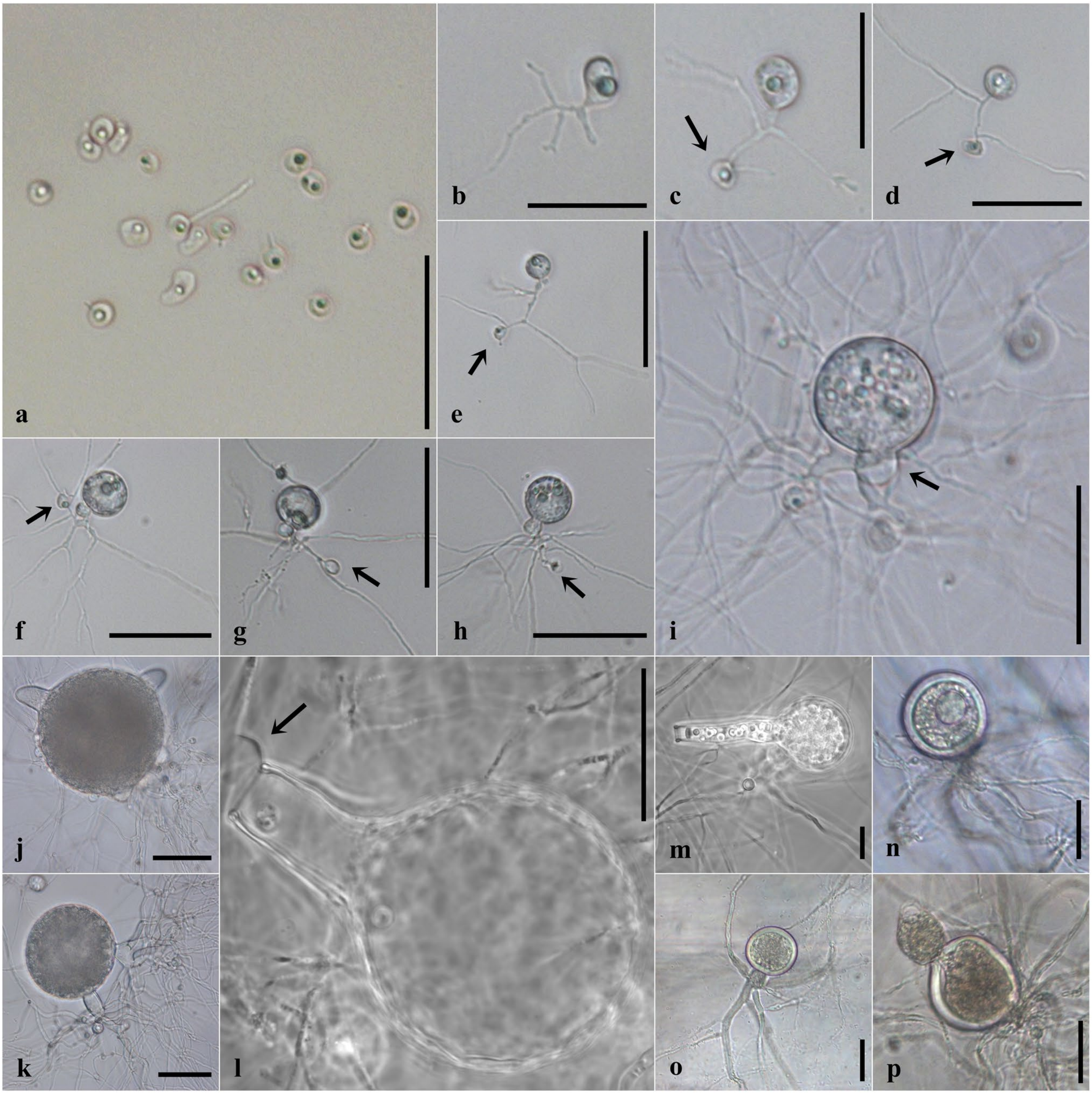


Fig. 4 **a**–**p** Development of *Thomasia carolinae* JEL818 in mPmTG (**a**–**k** and **n**–**p**) and Cd (**l**–**m**) agar media using differential interference contrast (DIC). **a**–**b** Zoospore germination. **c**–**e** Initial stages of exogenous development and details of the zoospore cyst (arrows). **f**–**h** Immature zoosporangium with cyst of zoospore (arrows). **i** Immature zoosporangium and detail of apophysis (arrow). **j** Mature zoosporangium and discharge tubes. **k** Mature zoosporangium with two rhizoidal axes. **l** Empty zoosporangium with hinged operculum (arrow). **m** Mature zoosporangium and a long discharge tube. **n**–**o** Resting spores with a prominent lipid globule. **p** Resting spore germinating as a prosporangium. Bars = 10 µm

lack of attention given to the study of *Cladochytriales*, further examination of the order is likely to lead to increasing amounts of higher-taxon diversity, as illustrated by our description of *Thomasia*, which potentially represents a new family.

Two clades within *Cladochytriales* (members marked with an asterisk on Fig. 1) produce zoospores with orange-pigmented lipid globule. Fuller and Tavares (1960) showed that lycopene, one of the carotenoids, is a major component of orange pigment in *C. replicatum*. The function of orange-pigmented lipid globules in these chytrids is unknown, but carotenoids in fungi are known to function as protection agents against oxidative stresses (Avalos and Limón 2015).

The carotenoids also act as precursors in the biosynthesis of physiologically active apocarotenoids. An example is retinal produced by oxidation of β-carotene (Avalos and Limón 2015). The retinal functions as a cofactor in the functionality of rhodopsin (Galindo et al. 2022), which is related to light sensitivity, especially that of the blue-green spectra.

The isolates in this study were the product of decades of isolation efforts by the few mycologists dedicated to chytrid diversity and systematics. This work exemplifies the potential of culture collections such as CZEUM to be used for the advancement of chytrid taxonomy and, potentially, for investigations into bioproducts, cell biology, physiology, and disease. Although *Cladochytriales* has been one of the most understudied orders in the *Chytridiomycota*, this study addresses that lack of attention by using previously unexamined, but readily available strains from CZEUM.

The taxonomic novelties proposed in this manuscript reflect the scarcity of researchers dedicated to understanding the systematics and evolution of *Cladochytriales*. The current classification of the order is still based on homoplasious morphological data; consequently, the order needs a thorough revision. Important taxonomic issues that need to be re-examined include the appropriateness of the *Catenochytridiaceae* (Doweld 2014), a family introduced to include operculate chytrids with catenulate apophyses, traditionally included in the genus *Catenochytrium*. The problem lies in the phylogenetic hypotheses that indicate *Catenochytrium* species do not form a monophyletic lineage (Mozley-Standridge et al. 2009; Steiger et al. 2011; Jerônimo et al. 2019, 2022; this study), and the type species (*C. carolinianum*) has not been sequenced, leaving the systematics of the genus unsolved. Another taxonomic uncertainty lies in the polyphyly of *Nephrochytrium*-like fungi (Mozley-Standridge et al. 2009; Steiger et al. 2011; Jerônimo et al. 2019, 2022; this study); the type species (*N. appendiculatum*) is not in culture and lacks molecular information, thus leaving the genus position in limbo. Although *Cladochytriales* taxonomy is still in need of significant revision, this manuscript expands our knowledge of generic and species diversity in the order, and our phylogenetic analysis highlights taxonomic issues that need attention.

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Author contribution All authors contributed to the study conception and design. Sample collection and strain isolation were performed by Joyce E. Longcore. Morphological characterization and description of new taxa were conducted by Joyce E. Longcore, Gustavo Henrique Jerônimo, and Carmen L.A. Pires Zottarelli. The DNA extraction and sequencing were performed by D. Rabern Simmons and Gustavo Henrique Jerônimo and phylogenetic analyses by Gustavo Henrique Jerônimo, Kevin R. Amses, Kensuke Seto, and Timothy Y. James. The first draft of the manuscript was written by Gustavo Henrique Jerônimo, D. Rabern Simmons, Joyce E. Longcore, and Carmen L.A. Pires Zottarelli, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability DNA sequences used in the present study are available in GenBank. Alignments and topologies were deposited in TreeBase (Study TB2: 30430). Fungal specimens are stored in the CZEUM collection culture (as indicated in specimens examined).

Declarations

Ethics approval Not applicable.

Consent to participate and consent for publication All authors have contributed to this work. They have also read and commented on the manuscript and accepted its publication in the Mycological Progress journal. This manuscript represents original work that is not being considered for publication, in whole or in part, in another journal, book, conference proceedings, or government publication with a substantial circulation.

Conflict of interest The authors declare no competing interests.

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