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Three new genera in Chytridiales from aquatic habitats in Argentina

Carlos G. Vélez

Departamento de Biodiversidad y Biología Experimental, Universidad de Buenos Aires, PRHIDEB-CONICET, C1428EHA Buenos Aires, Argentina

Peter M. Letcher[1]

Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487

Sabina Schultz

Universidad Nacional del Comahue, INIBIOMA-CONICET, Quintral 1250, San Carlos de Bariloche, 8400 Rio Negro Province, Argentina

Gabriela Mataloni

Grupo de Biodiversidad, Limnología y Biología de la Conservación 3iA, Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín Peatonal Belgrano 3563, San Martín1650 Buenos Aires Province, Argentina

Emilie Lefèvre

Martha J. Powell

Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487

Abstract: Sampling for chytrids in a variety of habitats has resulted in pure cultures that when analyzed have yielded hypotheses of relationships based on molecular and zoospore ultrastructural markers. To extend our understanding of diversity of Chytridiales in eastern Argentina and USA, we isolated and examined the morphology, ultrastructure and 28S and ITS1-5.8S-ITS2 rDNA sequences of numerous chytrids from aquatic habitats from these two regions. Three family-level lineages (Chytridiaceae, Chytriomycetaceae, family incertae sedis) are represented in our molecular phylogeny, and three new genera (*Avachytrium*, *Odontochytrium* in Chytriomycetaceae, *Delfinachytrium* in family incertae sedis) are described. These findings of new genera and species emphasize the potential for discovery of additional diversity.

Key words: chytrid, morphology, phylogeny, systematics, ultrastructure, zoospore

Introduction

Over the past decade, a major goal of establishing molecular-based monophyletic orders correlated with zoospore ultrastructure in Chytridiomycetes (= chytrids) (James et al. 2006) has been accomplished. Seven orders have been circumscribed or validated: Chytridiales (Vélez et al. 2011), Cladochytriales (Mozley-Standridge et al. 2009), Lobulomycetales (Simmons et al. 2009), Polychytriales (Longcore and Simmons 2012), Rhizophlyctidales (Letcher et al. 2008a), Rhizophydiales (Letcher et al. 2006) and Spizellomycetales (Wakefield et al. 2010). These revisions of chytrid systematics were based on molecular and ultrastructural analyses of pure cultures of isolates obtained from sampling for chytrids in diverse habitats and geographical locations. In concert, the revisions form hypotheses of relationships among chytrids, and as a derivation of these hypotheses, in many cases we can use specific molecular and ultrastructural markers to place organisms taxonomically.

Because chytrids cannot be detected macroscopically, sampling for these microscopic organisms is serendipitous, and processing each environmental sample is akin to ''opening a black box'' to find and isolate these organisms. Further, the morphology of chytrids is sufficiently conserved or converged that molecular or ultrastructural methods often are needed to determine the order to which an organism belongs. Analyses of a high number of environmental samples are necessary to recover a broad array of isolates. Only through intensive sampling has a sufficient number of isolates been recovered to characterize each clade. One conclusion of this intensive sampling is that relatively few chytrids are common to ubiquitous in the environment while many chytrids are scarce to rare (Letcher and Powell 2001; Letcher et al. 2004a, 2008a, b). When various geographical regions are intensively sampled, analyses reveal similar phylogenetic profiles but often with unique genetic diversity within specific habitats and/or regions (Letcher et al. 2004a, b, 2008b).

In surveys of the biodiversity of chytrids in Latin America, a wide range of species has been described and observed (e.g. Karling 1944, 1945, 1946; Letcher et al. 2008; Nascimento et al. 2011; Vélez et al. 2011; Steciow et al. 2012). In this report we include photographic illustrations of several previously described chytrids, which facilitate and confirm identification of taxa from disparate geographical regions. Despite the reported morphological diversity, the molecular phylogenetic diversity of chytrids in Latin America has not been explored extensively. However, a study focused on the comparative molecular and zoospore ultrastructural phylogenetic range of nu-

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[1] Corresponding author. E-mail: letch006@bama.ua.edu

merous isolates in the order Rhizophydiales from Argentina (38 isolates) and eastern North America (35 isolates) resulted in descriptions of seven new families and eight new genera (Letcher et al. 2008b). Thus, greater sampling coupled with molecular and ultrastructural analyses of isolates is required to adequately portray the range of chytrid diversity.

The goal of this study is to extend our understanding of morphological, ultrastructural and phylogenetic diversity of chytrids in Argentina and eastern North America in the order Chytridiales. Herein we analyze 38 isolates in the order, the majority from Argentina and North America and primarily from aquatic habitats. Three lineages in Chytridiales are represented in this sampling, and three new genera and a new zoospore morphology are described.

MATERIALS AND METHODS

Isolates.—We examined 38 ingroup isolates in Chytridiales (21 isolates from Argentina, 13 from North America, two from Australia, one from New Zealand and one from Scotland) and two outgroup isolates (TABLE I), JEL 222 *Rhizophydium globosum* (Rhizophydiales) and Barr 043 *Gaertneriomyces semiglobifer* (Spizellomycetales). Rhizophydiales + Spizellomycetales is a sister group of Chytridiales (James et al. 2006). The Australia isolate KP 013 *Rhizidium phycophilum* (Picard et al. 2009) and the New Zealand isolate JEL 378 *Rhizidium* sp. (Longcore 2005) were included to illustrate a relationship between a previously described clade (Picard et al. 2009) and a sister group of isolates revealed in this study; the Australia isolate PL AUS 026 *Polyphlyctis unispina* (Letcher et al. 2005) and the Scotland isolate KP 061 *Phlyctochytrium aureliae* (Letcher et al. 2012) were included to facilitate phylogenetic comparison among Chytridiaceae isolates. Water cultures were baited with pollen. Chytrids were isolated from colonized pine pollen bait into axenic cultures on PmTG agar (Barr 1986) using methods outlined in Fuller and Jaworski (1987). Pure cultures were maintained on agar slants at 5 C and held at the culture collection of the Facultad de Ciencias Exactas y Naturales (BAFCcult), University of Buenos Aires, and the University of Alabama chytrid culture collection.

DNA extraction, purification and amplification.—Sequences for ingroup and outgroup isolates were generated as described by Letcher and Powell (2005a) or obtained from GenBank. The LROR/LR5 primer pair (Vilgalys and Hester 1990, Reyner and Samuels 1994) was used for amplification of LSU (28S) nu-rDNA, and the ITS5/ITS4 primer pair (White et al. 1990) for the ITS1-5.8S-ITS2 region.

Phylogenetic analyses.—For molecular analyses we generated partial nucleotide sequences of the LSU rRNA gene (794–820 bp from the 5′ end) and complete sequences of the ITS1-5.8S-ITS2 region (550–658 bp). Sequences were assembled and aligned as described in Vélez et al. (2011). Maximum parsimony (MP) trees were generated with PAUPR at (Sikes and Lewis 2001), and bootstrap values were generated as heuristic searches with 500 replicates, each with 10 random-addition replicates. Maximum likelihood (ML) phylogenetic trees were constructed as described in Vélez et al. (2011). Modeltest 3.7 (Posada and Crandall 1998) was used to determine the best model of base substitution, and GARLI 0.951 (Zwikl 2006) was used to hypothesize maximum likelihood. Branch support was assessed with 500 bootstrapping replicates.

Morphology.—We examined ingroup isolates by light microscopy with either a Nikon Labophot-2 or a Zeiss Axioskop to assess range and variation in thallus morphological features, including size, shape and wall ornamentation of the sporangium, number and character of discharge pores, type of discharge and rhizoid morphology.

Zoospore ultrastructure.—Preparation and observations of zoospores for transmission electron microscopy was as described by Powell et al. (2013). Zoospore ultrastructural character states of characters typical of Chytridiales were analyzed (Barr and Hartmann 1976, Letcher et al. 2005, Picard et al. 2009, Vélez et al. 2011).

RESULTS

DNA extraction, purification and amplification.—Twenty-seven partial LSU sequences and 26 complete ITS1-5.8S-ITS2 sequences derived in this study were deposited at GenBank (TABLE I).

Phylogenetic analyses.—The combined dataset (partial LSU + complete ITS) had 1723 characters. For the MP analysis, 1139 parsimony informative sites remained after uninformative characters were excluded. Of the 1005 trees derived from PAUPRat, 658 were equally parsimonious (length [L] = 5246 steps, CI = 0.555, RI = 0.887) and were used to construct a majority rule consensus tree (>70% branch support). For the ML analysis, Modeltest indicated the most appropriate model of DNA substitution was the Hasegawa, Kishino and Yano model with rates of substitution among sites approximated by gamma substitution (HKY + G). ML log likelihood was −19511.35. MP and ML analyses produced similar trees with nodes supported by high bootstrap values. The MP phylogeny (FIG. 1) is presented with MP/ML bootstrap values indicated above branches. Three major clades (Chytridiaceae, Chytriomycetaceae, family incertae sedis), each with 100% support, occurred as a polytomy among ingroup isolates.

Chytridiaceae (FIG. 1, Clade A) contained four isolates from Argentina among eight isolates: ARG 100 *Chytridium olla* (FIG. 2A) (Braun 1851, 1855, 1856), ARG 066 and PL 167B the morphospecies *Chytridium lagenaria* (FIG. 2B) (Schenk 1858, Karling 1936, Sparrow 1936), ARG 109 and JEL 047 *Phlyctochytrium planicorne* (FIG. 2C, D) (Atkinson

TABLE I. Taxon sampling for phylogenetic analyses of 38 ingroup isolates in Chytridiales and two outgroup isolates

Isolate	GenBank 28S/ITS accession nos.		Habitat/substrate/location
ARG 012 Unidentified sp[a]	JX905504	JX905531	aquatic/pollen/Buenos Aires Prov., Argentina
ARG 037 *Chytriomyces hyalinus*[a]	JX905505	JX905532	aquatic/pollen/Corrientes Prov., Argentina
ARG 039 unidentified sp.	JX905506	JX905533	aquatic/pollen/Corrientes Prov., Argentina
ARG 041 *Chytriomyces hyalinus*	EF585631	EF585671	aquatic/pollen/Corrientes Prov., Argentina
ARG 043 *Rhizoclosmatium globosum*[a]	JX905507	JX905534	aquatic/pollen/Corrientes Prov., Argentina
ARG 050 *Avachytrium platense* n. gen. n. sp.	JX905508	JX905535	aquatic/pollen/Buenos Aires Prov., Argentina
ARG 053 *Avachytrium platense* n. gen. n. sp. (T)[a,b]	JX905509	JX905536	aquatic/pollen/Corrientes Prov., Argentina
ARG 062 *Chytriomyces hyalinus* clade	JX905510	JX905537	aquatic/pollen/Corrientes Prov., Argentina
ARG 066 *Chytridium lagenaria*[a]	FJ822969	FJ822969	aquatic/pollen/Mendoza Prov., Argentina
ARG 085 *Chytriomyces hyalinus*[a]	JX905511	JX905538	aquatic/pollen/Buenos Aires Prov., Argentina
ARG 095 *Odontochytrium milleri* n. gen. n. sp. (T)[a,b]	JX905512	JX905539	peat bog/pollen/Tierra del Fuego Prov., Argentina
ARG 097 *Chytriomyces hyalinus*	JX905513	JX905540	peat bog/pollen/Tierra del Fuego Prov., Argentina
ARG 100 *Chytridium olla*[c]	FJ822970	FJ822970	aquatic/*Oedogonium*/Buenos Aires Prov., Argentina
ARG 109 *Phlyctochytrium planicorne*[a]	JX905514	JX905541	aquatic/pollen/Entre Rios Prov., Argentina
ARG 112 *Phlyctochytrium bullatum*[a]	JX905515	JX905542	aquatic/pollen/Entre Rios Prov., Argentina
ARG 113 *Delfinachytrium mesopotamicum* n. gen. n. sp. (T)[a,b]	JX905516	JX905543	aquatic/pollen/Entre Rios Prov., Argentina
ARG 116 *Delfinachytrium mesopotamicum* n. gen. n. sp.[a]	JX905517	JX905544	aquatic/pollen/Entre Rios Prov., Argentina
ARG 117 *Delfinachytrium mesopotamicum* n. gen. n. sp.[a]	JX905518	JX905545	aquatic/pollen/Entre Rios Prov., Argentina
ARG 121 *Chytriomyces hyalinus*	JX905519	JX905546	aquatic/pollen/Entre Rios Prov., Argentina
ARG 122 *Chytriomyces hyalinus*	JX905520	JX905547	aquatic/pollen/Entre Rios Prov., Argentina
ARG 123 *Avachytrium platense* n. gen. n. sp.	JX905521	JX905548	aquatic/pollen/Entre Rios Prov., Argentina
JEL 006 *Rhizoclosmatium globosum*	AY988506	AY439061	aquatic/chitin/Maine, USA
JEL 047 *Phlyctochytrium planicorne*[c]	AY439028	AY439028	aquatic/*Oedogonium*/Maine, USA
JEL 103 *Odontochytrium milleri* n. gen. n. sp.[c]	AY439064	JX905549	aquatic/chitin/Maine, USA
JEL 221 *Rhizidium endosporangiatum*[c]	DQ273834	DQ273834	soil/pollen/Maine, USA
JEL 378 *Rhizidium phycophilum*[a]	DQ273832	FJ214804	tree-canopy detritus/pollen/South Is., New Zealand
KP 013 *Rhizidium phycophilum*[c]	FJ214802	FJ214803	soil/pollen/New South Wales, Australia
KP 061 *Phlyctochytrium aureliae*[c]	GU358607	GU358607	soil/pollen/Trossachs NP, Scotland
MP 004 *Chytriomyces hyalinus*[c]	DQ273836	AY349120	soil/chitin/Alabama, USA
MP 005 *Chytriomyces hyalinus*	AY988511	JX905550	peat moss/pollen/Michigan USA
MP 041 Unidentified sp.	JX905522	JX905551	aquatic/pollen/Alabama, USA
MP 059 Unidentified sp.	JX905525	JX905554	aquatic/pollen/Alabama, USA
MP 069 *Chytriomyces hyalinus*	JX905526	JX905555	aquatic/pollen/Alabama, USA
PL 115 *Chytriomyces hyalinus*[a]	AY988516	JX905556	soil/pollen/Alabama, USA
PL 167B *Chytridium lagenaria*[a]	FJ804156	FJ804156	aquatic/pollen/Louisiana, USA
PL AUS 026 *Polyphlyctis unispina*[c]	AY988518	FJ822973	soil/pollen/New South Wales, Australia
SL 001 *Chytriomyces hyalinus*	JX905527	JX905557	aquatic/pollen/Alabama, USA
WJD 131 *Chytriomyces hyalinus* Outgroup:	JX905530	JX905560	temporary pond/pollen/Alabama, USA
JEL 222 *Rhizophydium globosum*	DQ485551	DQ485616	
Barr 043 *Gaertneriomyces semiglobifer*	FJ827702	FJ827739	

[a] Zoospore ultrastructure examined for this study.

[b] T = type.[c] Zoospore ultrastructure examined in previous studies: ARG 100 (Vélez et al. 2012); JEL 047 (Letcher and Powell 2005b); JEL 221 (Powell et al. 2011); KP 013 (Picard et al. 2009); KP 061 (Letcher et al. 2012); JEL 103, MP 004, and PL AUS 026 (Letcher et al. 2005).

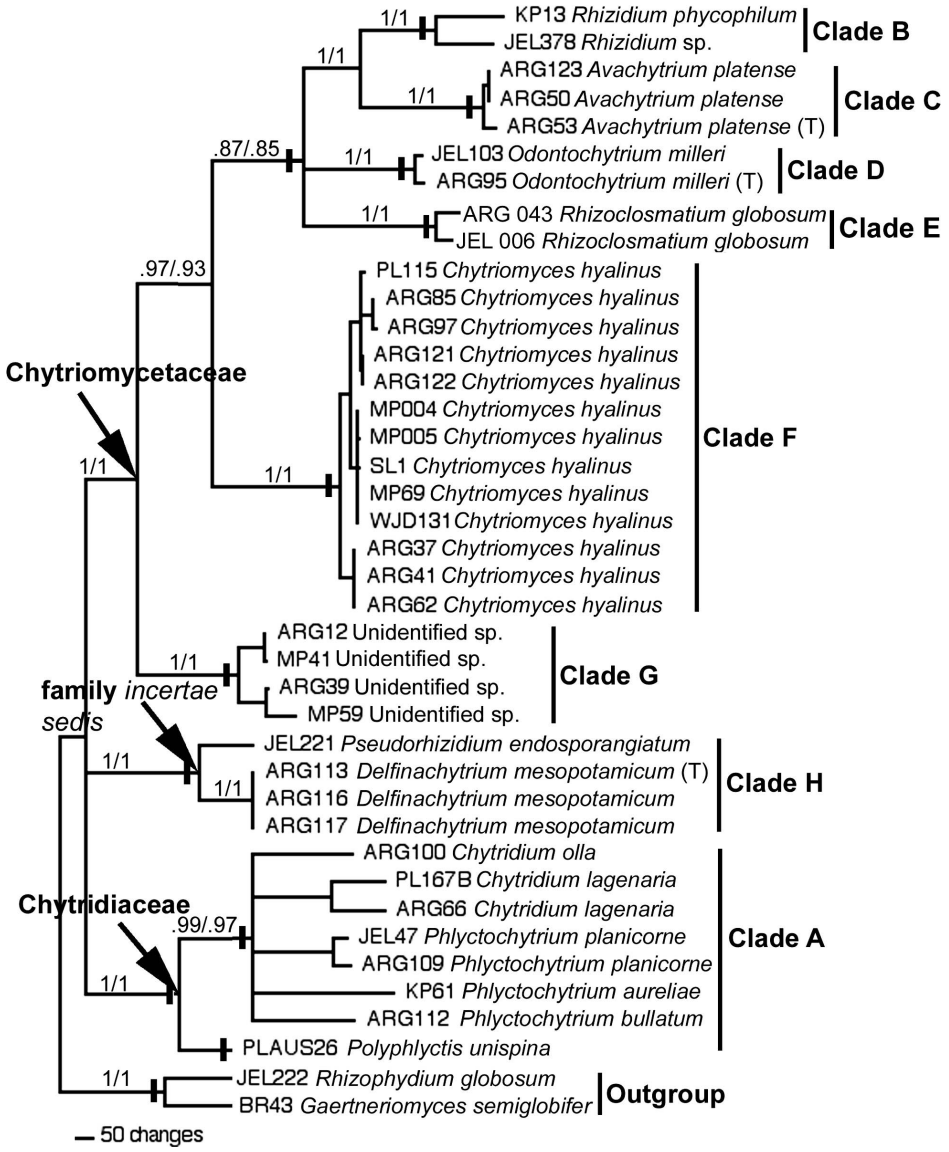

FIG. 1. Maximum parsimony majority rule consensus tree derived from analyses of combined ITS1-5.8S-ITS2 and partial large ribosomal subunit (28S) nuclear rDNA sequences of 38 isolates in Chytridiales. Isolates JEL 222 *Rhizophydium globosum* and BR 043 *Gaertneriomyces semiglobifer* were chosen as outgroup taxa for rooting purposes. Maximum parsimony/maximum likelihood bootstrapping values are indicated above branches. Branches with vertical hash marks have been shortened by half to aid viewing.

1909), ARG 112 *Phlyctochytrium bullatum* (FIG. 2E–J) (Sparrow 1937, 1938), KP 061 *Phlyctochytrium aureliae* (FIG. 2K) (Ajello 1945) and PL AUS 026 *Polyphlyctis unispina* (FIG. 2L–O) (Paterson 1956).

Chytriomycetaceae (FIG. 1, Clades B–G) included 14 isolates from Argentina among 29 isolates distributed in six clades. Clade B contained two isolates, KP 013 *Rhizidium phycophilum* and JEL 378 *Rhizidium* sp. A sister group of Clade B was Clade C with three isolates from Argentina, herein described in TAXONOMY as *Avachytrium platense* gen. et sp. nov. (FIG. 3A–F). A sister group of Clade B + C was Clade D containing two isolates, ARG 095 and JEL 103 ''Miller's dentate'' (Miller 1968), herein described in TAXONOMY as *Odontochytrium milleri* gen. et sp. nov. (FIG. 3G–N). A sister group of Clade B + C + D was Clade E containing two isolates, with one from Argentina and one from North America, identified as the morphospecies *Rhizoclosmatium globosum* (FIG. 3O–V) (Petersen 1903). A sister group of Clade B + C + D + E was Clade F containing 13 isolates, including seven from Argentina and six from North America, all putatively identified as the morphospecies *Chytriomyces hyalinus* (FIG. 4A–I) (Karling 1945).

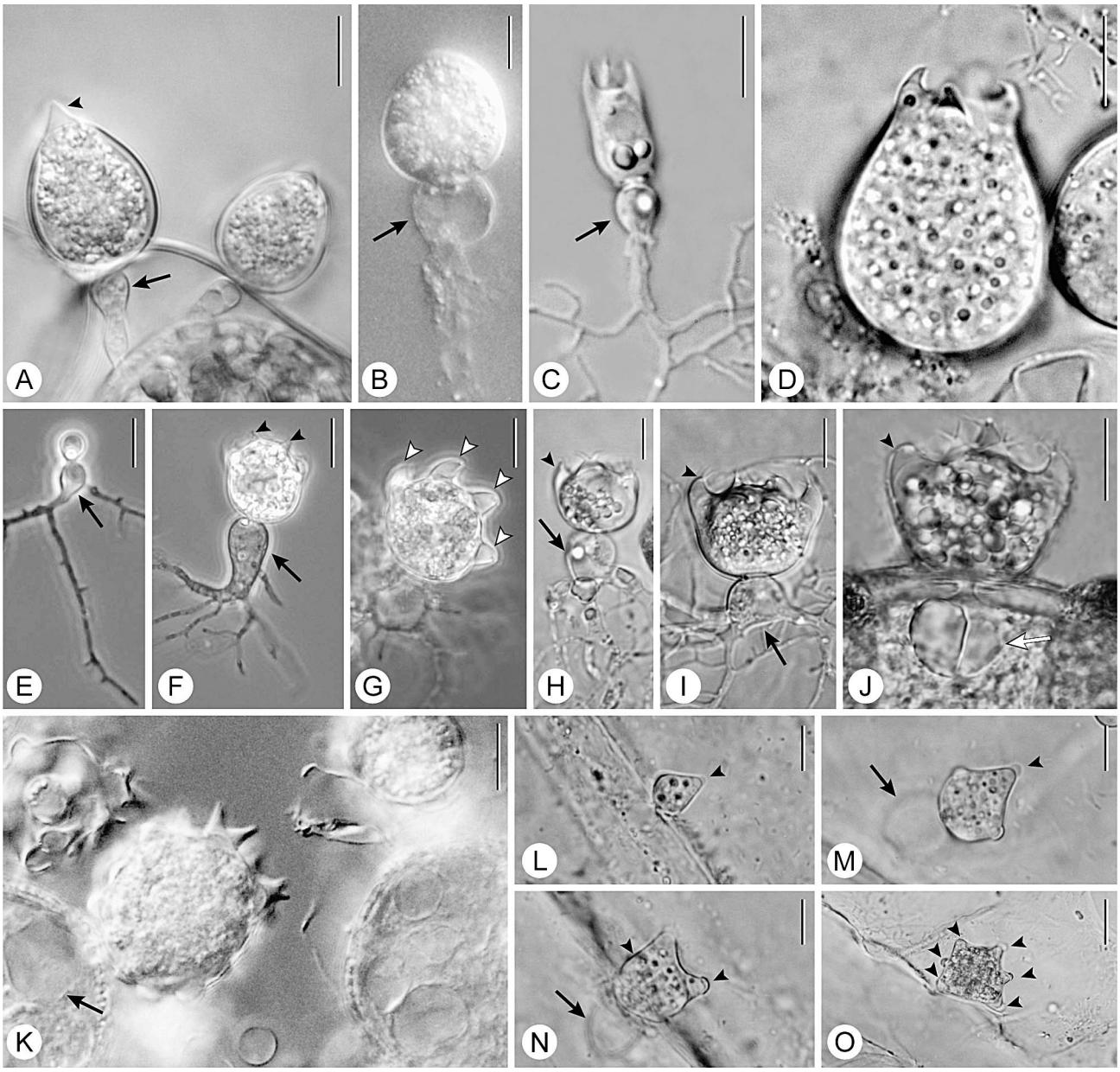

FIG. 2. Morphologies of five isolates in Chytridiaceae. A. Isolate ARG 100 *Chytridium olla* showing the rostrate operculum (arrowhead) and the well developed rhizoid (arrow); on oogonium of *Oedogonium capilliforme*. B. Isolate ARG 066 *Chytridium lagenaria*; maturing thallus with apophysis (arrow). C, D. Isolate ARG 109 *Phlyctochytrium planicorne*. C. Young thallus on agar; arrow indicates an apophysis. D. Mature thallus on pollen grain. E–J. Isolate ARG 112, *Phlyctochytrium bullatum*. The subsporangial apophysis is indicated by arrows and tooth-like sporangial ornamentation by arrowheads. E–G. On agar. E. Germling and early thallus development; arrow indicates apophysis. F, G. Immature thalli with the apical whorl of solid teeth (arrowheads) on the sporangium. H–J. On pollen grains; arrowheads indicate sporangial ornamentation, arrows indicate subsporangial apophysis. H, I. Immature interbiotic thallus. J. Immature epibiotic thallus. K. Isolate KP 061, *Phlyctochytrium aureliae*. Immature thallus on pollen grain; arrow indicates the endobiotic apophysis. L–O. Isolate PL AUS 026, *Polyphlyctis unispina* on cellulose. L. Early stage of development. M, N. Immature thalli with different numbers of discharge papillae (arrowheads); arrows indicate endobiotic apophysis. O. Mature thallus; arrowheads indicate discharge papillae. Bars: 10 μm.

A sister group of Clade B + C + D + E + F was Clade G containing four isolates, including two from Argentina and two from North America, herein considered an unidentified species (FIG. 4J–O).

Family incertae sedis (FIG. 1, Clade H) contained four isolates, with JEL 221 *Pseudorhizidium endosporangiatum* (Karling 1967a, Powell et al. 2013) sister of the group ARG 113, ARG 116 and ARG 117, herein

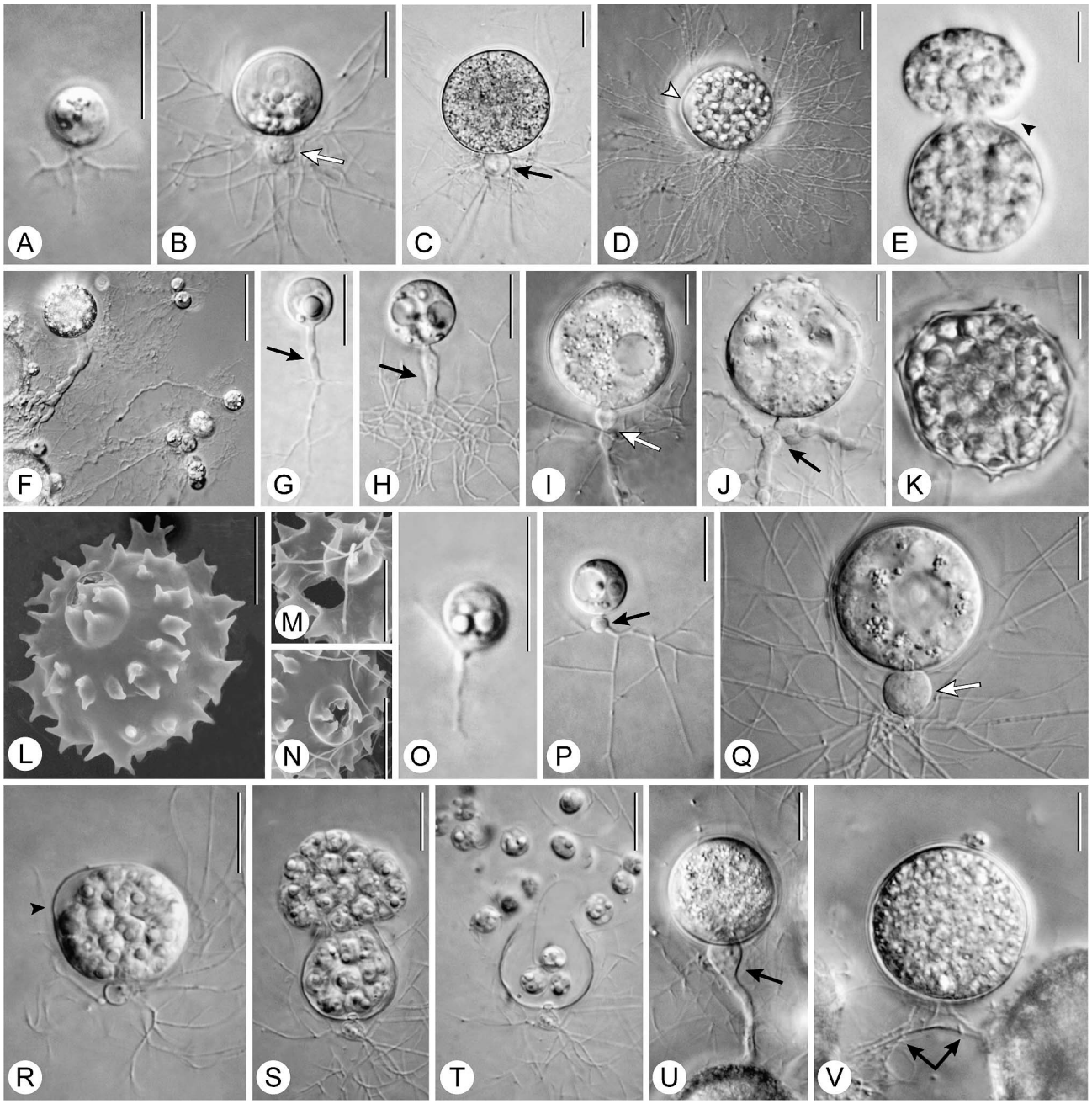

FIG. 3. Morphologies of three isolates in Chytriomycetaceae. A–F. Isolate ARG 053, *Avachytrium platense*. A–E. On agar. A. Germling and early thallus development. B, C. Immature thallus with a globose apophysis (arrow). D. Mature thallus just before zoospore release; the arrowhead indicates the presence of hyaline discharge plug below operculum. E. Zoospore releasing within a hyaline vesicle; note the delicate operculum (arrowhead). F. On pollen grains. Thalli lack apophysis and develop a rhizoidal system with a pronounced main axis. G–N. Isolate ARG 095, *Odontochytrium milleri*. G–K. On agar. G, H. Early thallus development. Note the distally branched, taproot-like main rhizoidal axis (arrow). I, J. Immature thalli with branched, tuberous main rhizoidal axes (arrows). K. Mature sporangium with wall ornamentation. L–N. On pollen grains; SEM micrographs. L. General view. M, N. Details of subapical and apical discharge pore respectively. O–V. Isolate ARG 043, *Rhizoclosmatium globosum*. O–T. On agar. O. Germling. P. Early thallus development, notice isodiametric rhizoids; arrow indicates symmetrical subsporangial apophysis. Q. Immature thallus with a conspicuous, almost spherical apophysis (arrow). R. Mature sporangium just before zoospore release; the arrowhead indicates the discharge papillae. S. Vesicle expansion during zoospore release. T. Inoperculate, almost empty sporangium and free-swimming zoospores. U, V. On pollen grains; interbiotic sporangia. The apophysis elongates into a single (U) or several (V) main rhizoidal axes, indicated by arrows. Bars: A–E, G–U = 10 μm; F = 30 μm; V = 20 μm.

described in TAXONOMY as *Delfinachytrium mesopotamicum* gen. et sp. nov. (FIGS. 4P–U, 5, 6).

Morphology.—Morphologies of taxa in Chytridiaceae (FIG. 1, Clade A) have been described, and our isolates conform to these descriptions and confirm their identity. Briefly, *Chytridium olla* (FIG. 2A) has an urceolate sporangium with a broad, umbonate operculum and a broad tubular rhizoid. *Chytridium lagenaria* (FIG. 2B) has an ovoid, operculate sporangium, a spherical subsporangial swelling, and stout branched rhizoids. *Phlyctochytrium planicorne* (FIG. 2C, D) has a narrow to broadly ovoid inoperculate sporangium with an apical collar of four converging plain teeth, a subsporangial apophysis and branched rhizoids. *Phlyctochytrium bullatum* (FIG. 2E–J) has a spherical or urceolate sporangium with two concentric whorls of solid, apical, converging teeth and a subsporangial swelling from which tapering rhizoids branch. *Phlyctochytrium aureliae* (FIG. 4K) has a spherical sporangium ornamented with generally short, bifurcate tooth-like enations, an epibiotic or endobiotic apophysis, and tapering, branched rhizoids. *Polyphlyctis unispina* (FIG. 2L–O) has an irregularly ellipsoid sporangium with multiple discharge papillae at maturity, an endobiotic apophysis and a single isodiametric rhizoid.

Morphologies of taxa in Chytriomycetaceae (FIG. 1, Clades B–G) that have been described are: *Rhizidium phycophilum*, *Rhizidium* sp., ''Miller's Dentate'' (FIG. 3G–N), *Rhizoclosmatium globosum* (FIG. 3O–V), and *Chytriomyces hyalinus* (FIG. 4A–I). Morphologies of *Pseudorhizidium endosporangiatum* in family incertae sedis (FIG. 1, Clade H) has been described.

In TAXONOMY, isolates ARG 050, ARG 053 (FIG. 3A–F), and ARG 123 (FIG. 1), Clade C are described as *Avachytrium platense* gen. et sp. nov.; isolates ARG 095 (FIG. 3G–N) and JEL 103 (FIG. 1), Clade D are described as *Odontochytrium milleri* gen. et sp. nov.; isolates ARG 113 (FIG. 4P–U), ARG 116, ARG 117 (FIG. 1), Clade H are described as *Delfinachytrium mesopotamicum* gen. et sp. nov.

Four isolates (FIG. 1), Clade G (ARG 012 [FIG. 4J–O], ARG 039, MP 041, MP 059) are ''Unidentified sp.'', pending further observations. For these isolates, the sporangium is spherical throughout development (FIG. 4J–M) and urceolate after zoospore discharge (FIG. 4N, O). The rhizoidal system is composed of a single, short, subsporangial axis and thin, branched, tapering rhizoids (FIG. 4J, K). At maturity, zoospores are released as a mass (FIG. 4N) from an operculate discharge pore (FIG. 4O).

Zoospore ultrastructure.—Isolates in Chytridiaceae (FIG. 1, Clade A) had a Group II-type zoospore (Barr 1980). Isolates examined in Chytriomycetaceae (FIG. 1, Clades B–G) had a Group I-type zoospore (Barr 1980, Picard et al. 2009). In family incertae sedis (FIG. 1, Clade H) the zoospore of *Pseudorhizidium endosporangiatum* recently was described (Powell et al. 2013); isolates ARG 113, ARG 116 and ARG 117 had undescribed zoospore morphology (FIGS. 5, 6), and that zoospore is described in TAXONOMY under *Delfinachytrium mesopotamicum* gen. et sp. nov.

TAXONOMY

In the following we delineate two new genera (*Avachytrium*, *Odontochytrium*) in family Chytriomycetaceae and one new genus (*Delfinachytrium*) in family incertae sedis in the order Chytridiales (Vélez et al. 2011) on the bases of thallus morphology, 28S + ITS1-5.8S-ITS2 sequence analyses, and zoospore ultrastructural features.

Avachytrium Vélez and Letcher, gen. nov.

FIG. 1, Clade C

MycoBank MB801481

Sporangium spherical with a single discharge pore. Rhizoidal system a spherical subsporangial apophysis and extensively branched fine rhizoids. Resting spore unknown. Group I-type zoospore (Barr 1980). Monophyletic in Chytriomycetaceae.

Etymology: The generic name honors the Ava (Guaraní), the original people from Paraguay, southwestern Brazil and northeastern Argentina.

Type: Avachytrium platense Vélez and Letcher

Avachytrium platense Vélez and Letcher, sp. nov.

FIG. 3A–F

MycoBank MB801482

On agar, germlings spherical with isodiametric, branching rhizoids. Mature sporangia spherical, 20–30 μm diam, with single, apical, operculate discharge pore. Rhizoidal system consisting of a spherical subsporangial apophysis 8–10 μm diam and profuse, thin, isodiametric, branched rhizoids. Zoospores released in vesicular mass. Group I-type zoospore (Barr 1980).

Etymology: The specific epithet *platense* acknowledges the Rio de la Plata basin, the geographic region in Argentina from which this isolate was collected.

Specimen examined: ARGENTINA. CORRIENTES PROVINCE: Iberá Lake, 28°32′16″S, 57°10′53″W, 64 m. Isolate ARG 053 collected Sep 2006 by Carlos G. Vélez and isolated on pollen.

Holoype: Isolate ARG 053, GenBank LSU rDNA sequence JX905509, ITS1-5.8S-ITS2 rDNA sequence JX905536, deposited with the Culture Collection of the Facultad de Ciencias Exactas y Naturales, University of Buenos Aires.

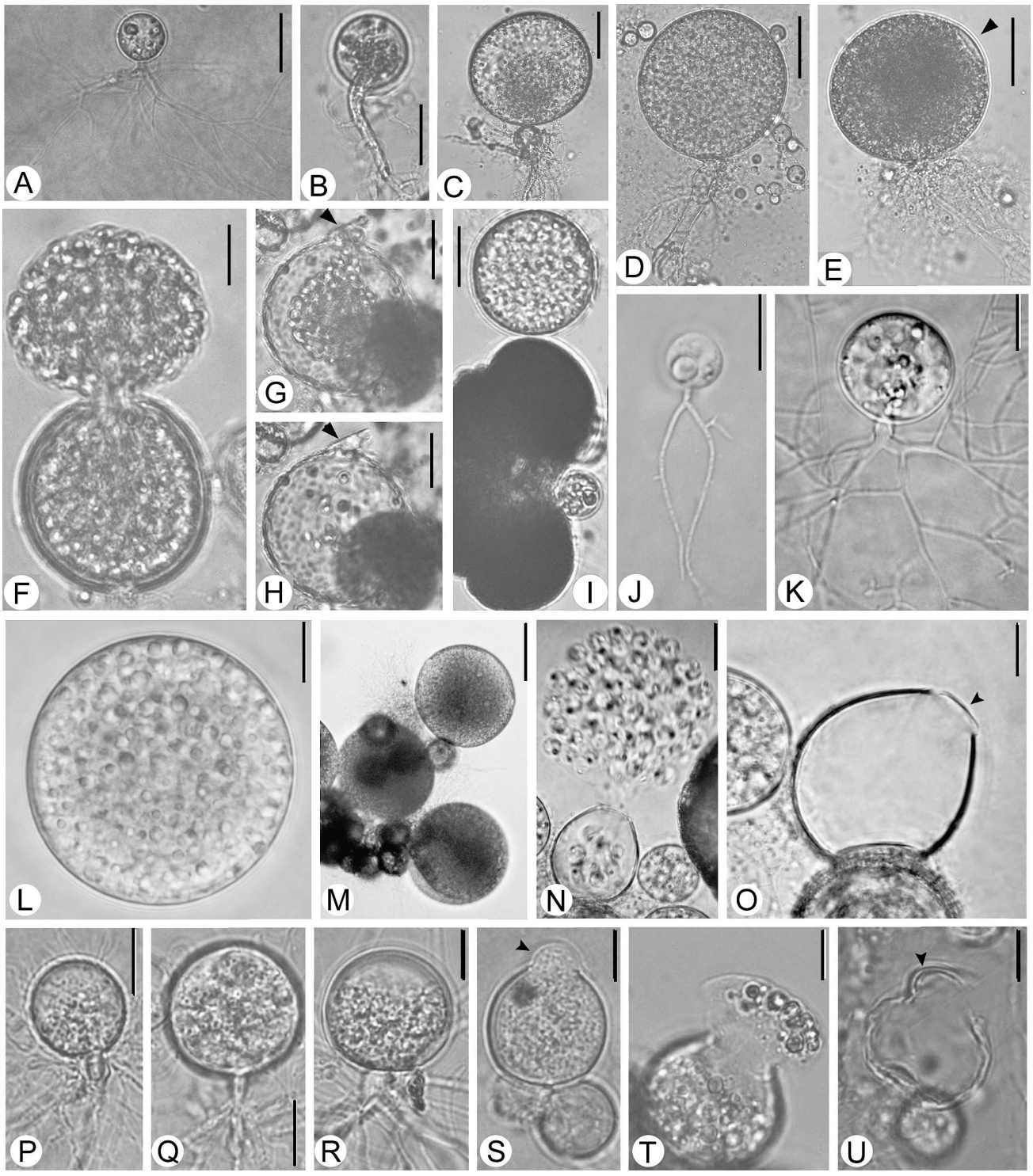

FIG. 4. Morphologies of two isolates in Chytriomycetaceae and one isolate in family incertae sedis. A–I. Isolate ARG 121, *Chytriomyces hyalinus*. A–H. On agar. A. Germling. B. Young thallus with taproot-like rhizoidal axis. C–E. Maturing sporangia. Arrow (E) indicates the presence of hyaline material below the operculum. F. Zoospore discharge in a hyaline vesicle. G, H. Near completion of zoospore discharge; arrows indicate operculum. I. Immature and mature sporangia on pollen grain. J–O. Isolate ARG 012, unidentified sp. J–L. On agar. J. Early thallus development. K. Immature thallus. L. Mature sporangium containing cleaved zoospores. M–O. On pollen grains. M. Large interbiotic thalli. N. Zoospores emerging within a hyaline vesicle. O. Empty, urceolate, operculate (arrowhead) sporangium. P–U. Isolate ARG 113, *Delfinachytrium mesopotamicum* on agar. P, Q. Immature sporangia, each with a short rhizoidal axis. R. Maturing sporangium; rhizoidal axis has thickened and

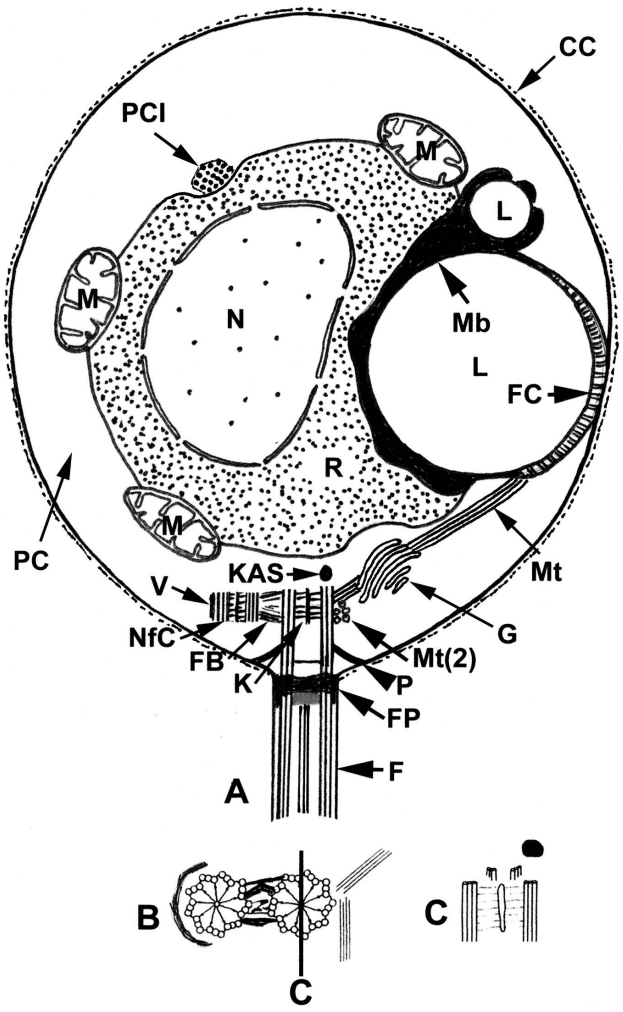

FIG. 5. Schematic drawing of sections through the zoospore of *Delfinachytrium mesopotamicum*. A. Longitudinal section. B. Cross section through kinetosome and non-flagellated centriole. C. Longitudinal section through kinetosome in plane illustrated in B; note position of KAS anterior to the kinetosome. 5, 6. Abbreviations: CC, cell coat; F, flagellum; FB, fibrillar bridge; FC, fenestrated MLC cisterna; FP, flagellar plug; G, Golgi apparatus; K, kinetosome; KAS, kinetosome-associated structure; L, lipid globule; M, mitochondrion; Mb, microbody; Mt, microtubular root; Mt(2), secondary set of microtubules; N, nucleus; NfC, non-flagellated centriole; P, flagellar prop; PC, peripheral cytoplasm; PCI, paracrystalline inclusion; R, ribosomes; TP, terminal plate; V, veil.

Notes: Avachytrium groups as sister to *Rhizidium* sp. and *R. phycophilum* but is erected as a new genus because neither its thallus characteristics nor its zoospore ultrastructure are compatible with those for *Rhizidium* spp. *Avachytrium* is operculate, apophysate, and has profuse, delicate rhizoids; *Rhizidium* is characterized as inoperculate, non-apophysate, and having a broad main rhizoidal axis that bears secondary branches (Sparrow 1960).

Odontochytrium Vélez and Letcher, gen. nov.
FIG. 1, Clade D

MycoBank MB801483

Sporangium extra-matrical, spherical with an apical or subapical, inoperculate discharge pore; sporangial wall ornamented with simple or bipartite enations. Epibiotic portion of rhizoid tubular, endobiotic portion slightly branched. Resting spore unknown. Group I-type zoospore (Barr 1980). Monophyletic in Chytriomycetaceae.

Etymology: The generic name prefix *Odonto-* reflects the dentate character of the sporangial ornamentation.

Type: Odontochytrium milleri Vélez and Letcher

Odontochytrium milleri Vélez and Letcher, sp. nov.
FIG. 3G–N

MycoBank MB801484

On agar, sporangia spherical, 20–30 μm diam, with single or bipartite tooth-like enations often in apical or subapical concentric whorls. Rhizoidal system a tapering, tubular-shaped extra-matrical structure with occasional bulges or an apophysis-like swelling near or far from the sporangium. Zoospores discharged as a loose mass, through an inoperculate aperture. Group I-type zoospore (Barr 1980).

Etymology: The specific epithet acknowledges Dr Charles Miller, the original observer of this taxon.

Specimen examined: ARGENTINA. TIERRA DEL FUEGO PROVINCE: Rancho Hambre peat bog, 54°44′49.52″S, 67°49′30.36″W, 120 m. Isolate ARG 095 collected by Carlos G. Vélez and Gabriela Mataloni, Feb 2010, and isolated by Carlos G. Vélez and Sabina Schultz on pollen.

Holotype: Isolate ARG 095, GenBank LSU rDNA sequence JX905512, ITS1-5.8S-ITS2 rDNA sequence JX905539, deposited with the Culture Collection of the Facultad de Ciencias Exactas y Naturales, University of Buenos Aires.

Notes: Miller (1968) described a spherical chytrid with tooth-like enations on the sporangium, observed

←

bifurcated. S. Initial phase of zoospore discharge, with endosporangial expansion (arrowhead) before zoospore release. T. Zoospore release. U. Empty, partially collapsed sporangium after zoospore discharge, with portion of the discharge pore or remnant of the endosporangium reminiscent of an operculum (arrowhead). Bars: A–K, N, P–U = 10 μm; C = 7.5 μm; L, M, O = 5 μm.

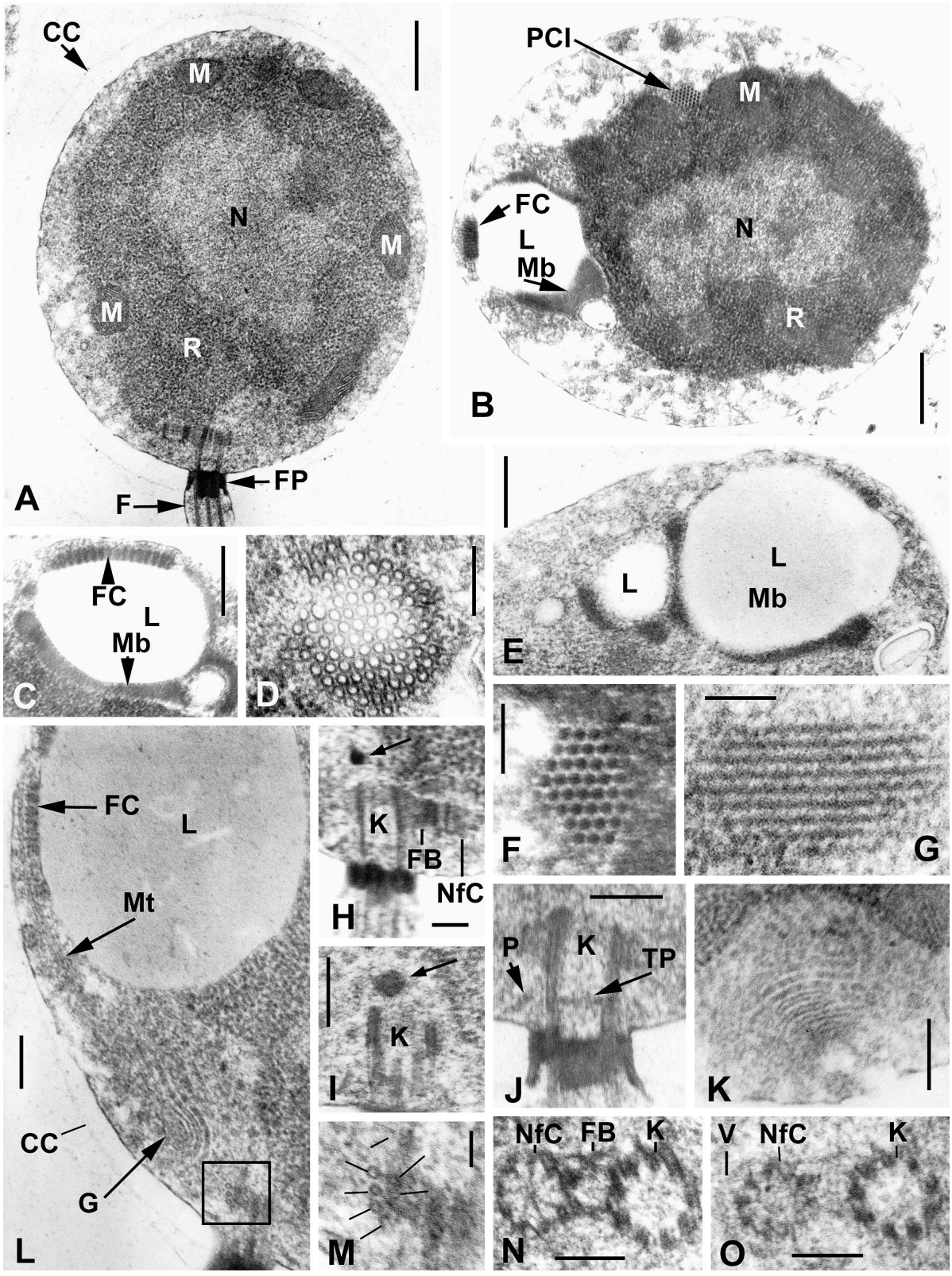

FIG. 6. Zoospore ultrastructure of *Delfinachytrium mesopotamicum*. A. Longitudinal section. B. Transverse section. C, D. Fenestrated MLC cisterna. C. Longitudinal section. D. Transverse section. E. Longitudinal section illustrating lobed microbody adjacent to two lipid globules. F, G. Paracrystalline inclusion. F. Transverse section. G. Longitudinal section. H. Longitudinal section through kinetosome; arrow indicates kinetosome-associated structure (KAS). I. Longitudinal section through

growing on sweet gum pollen (*Liquidambar styraciflua* L.) and chitin added to water collections from several aquatic habitats, which he colloquially named ''Dentate''. Although numerous chytrids have dentate sporangial ornamentation (e.g. Ajello 1945; Canter 1949; Sparrow 1938, 1939, 1960, 1966), the sporangium of ''Dentate'' is generally recognizable by the apical or subapical, laterally placed whorls of dentate ornamentation and the lack of an apophysis as is characteristic of *Phlyctochytrium aureliae* (Ajello 1945, Letcher et al. 2012). It also differs from *P. mucronatum* (Canter 1949), which has a single apical spine. Isolate ARG 095 demonstrated the form and habit of Miller's original description.

Molecular phylogenies (James et al. 2000, 2006; Letcher et al. 2005; Picard et al. 2009; Vélez et al. 2011) have included an isolate (JEL 103) variously referred to as ''Miller's Dentate'', ''*Chytriomyces* clade'' and ''Unidentified sp.'' that consistently nested in the Chytridiales. In Vélez et al. (2011), JEL 103 was included as a member of the Chytriomycetaceae. Isolates ARG 095 and JEL 103 have 28S sequences that are 99.5% similar and ITS1-5.8S-ITS2 sequences that are 93% similar, and thus these two isolates are considered to be the same taxon.

Ultrastructural examinations of JEL 103 (Letcher et al. 2005) and ARG 095 reveal that both isolates have a Group I-type zoospore. Thus, morphological, molecular and ultrastructural data indicate ARG 095 to be the same as the colloquial ''Miller's Dentate''. *Odontochytrium milleri* together with isolate ARG 097 (*Chytriomyces hyalinus* clade) constitute the first chytrids recorded from Tierra del Fuego (Steciow et al. 2012).

Delfinachytrium Vélez and Letcher, gen. nov.
FIG. 1, Clade H
MycoBank MB801577

Sporangium spherical, operculate. Rhizoidal system a short axis and profuse branched rhizoids. Resting spore unknown. Zoospore contains a single lipid globule partially covered with a fenestrated MLC cisterna, or occasionally two lipid globules, a microbody lobed around the globule or globules, a microtubular root between the kinetosome and MLC cisterna, a second microtubule root that extends from the kinetosome into the zoospore body, and a kinetosome-associated structure as a globule adjacent to the kinetosome.

Etymology: The generic name honors ''la Delfina'', a heroin of the Argentine War of Independence (1810–1818).

Type: Delfinachytrium mesopotamicum Vélez and Letcher

Delfinachytrium mesopotamicum Vélez and Letcher, sp. nov. FIGS. 4P–U, 5, 6
MycoBank MB801578

On agar, sporangia spherical, 25–30 μm diam with a single apical or subapical, discharge pore. Rhizoidal system composed of a single, short rhizoidal axis and profusely branched, tapering rhizoids. Discharge initiated with release of a portion of the protoplast through the discharge pore. Sporangial wall partially collapses after discharge. Zoospores spherical, 2.5–3.0 μm diam. Ribosomes aggregated in a central core, nucleus embedded in the ribosomal aggregation, and elongate, branched mitochondria enclose the aggregation. The microbody-lipid globule complex includes one large lipid globule and often a smaller, adjacent lipid globule, microbodies, and a fenestrated cisterna partially appressed to the larger globule. In the peripheral cytoplasm in the anterior portion of the cell, a paracrystalline inclusion is closely associated with the ribosomal core. Slightly extending into a pocket of the peripheral cytoplasm adjacent to the kinetosome, a Golgi apparatus occurs. Kinetosome and non-flagellated centriole are parallel, joined by dense, stacked fibrils (= fibrillar bridge), in cross section composed of strap-like bands. On the side of the non-flagellated centriole opposite the fibrillar bridge is a thin veil. A globular kinetosome-associated structure (KAS) is anterior to the kinetosome. In the flagellar base a two-layered flagellar plug is present, each layer approximately 125 nm thick, the anterior layer being more electron-dense than the posterior layer. A cord-like microtubular root radiates from the side of the kinetosome to the fenestrated MLC cisterna, and a second microtubular root composed of a bundle of microtubules extends from the kinetosome into the body of the zoospore.

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kinetosome, at right angle to H; arrow indicates KAS. J. Longitudinal section through kinetosome and two-layered flagellar plug (arrowhead). K. Golgi apparatus. L. Longitudinal section illustrating microtubular root to fenestrated MLC cisterna and secondary microtubular root (box). M. Enlargement of boxed secondary microtubular root in L. Lines indicate individual microtubules. N. Transverse section illustrating fibrillar bridge between kinetosome and non-flagellated centriole. O. Transverse section illustrating veil adjacent to non-flagellated centriole. Bars: A, B = 0.5 μm; H = 0.35 μm; C, E, I–L, N, O = 0.25 μm; D, F, G = 0.15 μm; M = 0.1 μm.

Etymology: The specific epithet recognizes the region of Argentina from which this chytrid was isolated: Entre Ríos = between rivers, also known as Mesopotamia (Región Mesopotámica).

Holoype: Isolate ARG 113, GenBank LSU rDNA sequence JX905516, ITS1-5.8S-ITS2 rDNA sequence JX905543, deposited with the Culture Collection of the Facultad de Ciencias Exactas y Naturales, University of Buenos Aires.

Specimens examined: ARGENTINA. ENTRE RIOS PROVINCE: marsh in semipermanent stream, tributary of Perucho Verna stream, 32°9′20.96″S, 58°20′5.03″W, 25 m. Isolate ARG 113 collected by Carlos G. Vélez and Sabina Schultz, Apr 2010, and isolated by Sabina Schultz on pollen. Isolates ARG 116 and ARG 117 collected at same location and on same date as isolate ARG 113.

Notes: Although superficially like *Chytriomyces* (being epibiotic and operculate), isolate ARG 113 is not like any existing *Chytriomyces* (Letcher and Powell 2002). The type of *Chytriomyces* is *C. hyalinus* (Karling 1945, Letcher and Powell 2002), and *C. hyalinus* is ultrastructurally defined by the Group I-type zoospore. Isolate ARG 113 is not monophyletic with the type and does not have a Group I-type zoospore and thus cannot be a member of that genus or of the family Chytriomycetaceae.

Morphologically isolates ARG 113, ARG 116 and ARG 117 *D. mesopotamicum* differ from their sister taxon, JEL 221 *Pseudorhizidium endosporangiatum*. *Delfinachytrium* has a single discharge pore, while *Pseudorhizidium* has multiple discharge pores. However, their rhizoidal systems are similar, in that a single, long, isodiametric germ tube becomes the primary rhizoidal axis, from which lateral branches extended at right or acute angles from the main rhizoidal axis. Also, their mode of zoospore discharge is similar, in which a portion of the protoplast is encapsulated by a layer of material (the ''endosporangium'' of Karling 1968). The cytoplasm then cleaved into zoospores, and after release either a portion of the endosporangium or a portion of the discharge pore remained, reminiscent of an operculum

Ultrastructurally isolates ARG 113, ARG 116 and ARG 117 *D. mesopotamicum* differ from their sister taxon, JEL 221 *Pseudorhizidium endosporangiatum*. *Delfinachytrium* has a fenestrated MLC cisterna and two microtubular roots, while *Pseudorhizidium* has a simple MLC cisterna, and although microtubules radiate from one side of the kinetosome they neither form an organized root nor connect with the MLC cisterna.

DISCUSSION

Morphology.—Chytrid morphology was the foundation for identification (Sparrow 1960, Karling 1977) before ultrastructural analysis and more recently molecular phylogenetic analyses. Much of the research in chytrid systematics over the past decade has revealed that many morphological features, such as an operculum, are convergent (Letcher et al. 2006, Powell et al. 2011), and thus in most instances morphological features alone are not reliable for taxon delineation. This does not negate the importance of thallus morphology but instead focuses its applicability more toward species than higher taxonomic levels.

Some chytrids, however, are readily identifiable by distinctive thallus morphological features. For example, in Chytridiaceae *Chytridium olla* is distinctive not only by its rostrate operculum and broad germ tube but also by its habit as an obligate parasite primarily of *Oedogonium*. *Phlyctochytrium planicorne*, *P. aureliae* and *P. bullatum* each has distinctive sporangial ornamentation that facilitates identification. In Chytriomycetaceae, *Odontochytrium milleri* is distinguishable from other ornamented chytrids by its pattern of sporangial ornamentation. *Rhizoclosmatium globosum* is recognizable by its thread-like rhizoids and symmetrical apophysis, and *Chytriomyces hyalinus* has a distinctive rhizoidal structure of one or more stout rhizoidal axes emanating from a single point of origin and taproot-like rhizoidal branches.

Molecular diversity.—Our sampling from a variety of aquatic habitats in Argentina and North America has revealed expanded diversity in the Chytridiales. Although many clades in our molecular phylogeny (clade A: Chytridiaceae; clade D: *Odontochytrium milleri*; clade E: *Rhizoclosmatium globosum*; clade F: *Chytriomyces hyalinus*; clade G: unidentified sp.) contain closely related isolates from both North America and Argentina, in few instances did related isolates have identical sequences. These results support two points: (i) many chytrid species have cosmopolitan distribution, occurring in geographically disjunct locations, but (ii) geographic isolation has resulted in genetic divergence in populations of these cosmopolitan taxa. For example, the morphospecies *Chytriomyces hyalinus* is among the more commonly reported taxa in broad-scale chytrid inventories (e.g. Australia: Willoughby 1965, Letcher et al. 2004b; Brazil: Nacimento et al. 2011; Great Britain: Willoughby 1964; Hawaii: Sparrow 1965; North America: Miller 1965, Letcher and Powell 2001; Oceania: Karling 1967b, 1968). In our phylogeny, *C. hyalinus* is represented by 13 isolates, evenly distributed between North America and South America. Great molecular diversity is evident within this morphospecies, and isolates from both locations are scattered within the clade. However, in our phylogenetic assembly isolates of *C. hyalinus* fall into

discreet subclades, either from North America or from South America, indicative of geographic isolation.

As a second example, in our phylogeny Chytridiaceae is a sparsely sampled family of eight isolates representing six taxa. Two of those taxa (*Chytridium lagenaria*, *Phlyctochytrium planicorne*) are morphologically distinctive organisms and thus readily identifiable with light microscopy. Two isolates of *C. lagenaria* (PL 167B from Louisiana, USA, and ARG 066 from Mendoza Province, Argentina) are 95% similar in their 28S sequences but only 65% similar in their ITS sequences. Two isolates identified as *P. planicorne* (JEL 047 from Maine, USA, and ARG 109 from Entre Ríos Province, Argentina) are almost the same (99% similar) in their 28S sequences and less similar (92%) in their ITS sequences. Thus, genetic diversity is apparent in identifiable morphospecies recovered from disjunct locations.

Ultrastructural diversity.—Diversity in zoospore ultrastructure has been revealed in our study. The zoospore of *Delfinachytrium mesopotamicum* is distinct from the Group I-type and Group II-type zoospores, as well as the zoospore of *P. endosporangiatum*, to which it is sister, although it shares certain character states with each of the three zoospores. Commonalities with the Group I-type zoospore (Chytriomycetaceae) are the position of the Golgi apparatus adjacent to the microtubular root between the kinetosome and fenestrated MLC cisterna, and the veil adjacent to the non-flagellated centriole. Commonalities with the Group II-type zoospore (Chytridiaceae) are the location of the nucleus inside the ribosomal aggregation and the globular KAS. Commonalities with the zoospore of *P. endosporangiatum* are the strap-like nature of the fibrillar bridge between the kinetosome and non-flagellated centriole, the veil adjacent to the non-flagellated centriole, the globular KAS and the two-layered plug in the base of the flagellum.

In our study, from an ultrastructural perspective there are two enigmatic groupings. The first grouping is composed of our clades B (*Rhizidium phycophilum* and *Rhizidium* sp.) and clade C (*Avachytrium platense*). These are sister groups with 100% support, yet their zoospores are remarkably different. The zoospore of *Avachytrium* is a characteristic Group I-type zoospore, while those of *R. phycophilum* and *Rhizidium* sp. (unpubl data) are considered a reduced Group I-type, lacking several Group I-type zoospore character states. The reduced zoospore lacks fenestrations in its MLC cisterna (= simple cisterna), a microtubular root and a kinetosome-associated structure, a constellation of features characteristic of the Group I-type zoospore. It also has a reduced paracrystalline inclusion. It may be that *R. phycophilum* and *Rhizidium* sp., composing a terminal clade, represent the most derived zoospore in Chytridiales, but further sampling will be necessary to better define character state reduction in the Chytridiales.

The second enigmatic grouping is in clade H, our family incertae sedis, with 100% support, composed of *Pseudorhizidium endosporangiatum* on one branch, and a sister group composed of three genetically identical isolates of *Delfinachytrium mesopotamicum*. Zoospores of these two lineages have different suites of character states. *Pseudorhizidium endosporangiatum* has a simple MLC cisterna and no organized microtubular root. Although microtubules are present, they do not connect with the MLC cisterna and their direction of radiation is not necessarily in the direction of the MLC. *Delfinachytrium mesopotamicum* has a fenestrated MLC cisterna and a microtubular root that extends from the vicinity of the kinetosome to the MLC cisterna, as well as a second assemblage of microtubules that extends from the kinetosome into the zoospore body. However, the zoospores have more character states in common than in contrast: (i) the zoospores of both lineages may have more than one lipid globule, (ii) the microbody associated with the lipid globule or globules is lobed, (iii) the paracrystalline inclusion in the peripheral cytoplasm is reduced in size compared with that of other Chytridiales zoospores (other than *R. phycophilum*, clade B, also reduced in size), (iv) the fibrillar bridge between the kinetosome and non-flagellated centriole is composed of strap-like bands, (v) the KAS is a globular structure adjacent to the kinetosome and (vi) there is a distinctive, prominent two-layered flagellar plug in the transition region of the flagellum. The zoospores of these isolates may well be considered as variants of either Group I-type or Group II-type zoospores, in that they have character states that identify with both those types, but it is the character state of the KAS that more closely identifies these ''bridge'' isolates with Chytridiaceae (Group II-type zoospore) than Chytriomycetaceae (Group I-type zoospore).

Our research has shown great diversity among isolates from a variety of aquatic habitats in Argentina and North America, resulting in delineation of three new genera in Chytridiales and emphasizing the potential for discovery of new species with additional sampling.

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LITERATURE CITED

Ajello L. 1945. *Phlyctochytrium aureliae* parasitized by *Rhizophydium chytriophagum*. Mycologia 37:109–119, doi:10.2307/3754853

Atkinson GF. 1909. Some fungus parasites of algae. Bot Gaz 48:321–338, doi:10.1086/330045

Barr DJS. 1980. An outline for the reclassification of the Chytridiales and for a new order, the Spizellomycetales. Can J Bot 58:2380–2394, doi:10.1139/b80-276

———. 1986. *Allochytridium expandens* rediscovered: morphology, physiology and zoospore ultrastructure. Mycologia 78:439–448, doi:10.2307/3793048

———, Hartmann VE. 1976. Zoospore ultrastructure of three *Chytridium* species and *Rhizoclosmatium*. Can J Bot 54:2000–2013, doi:10.1139/b76-214

Braun A. 1851. Betrachtungen über die Erscheinung der Verjüngung in der Natur, insbesondere in der Lebens- und Bildungsgeschichte der Pflanze. Leipzig, Germany, 363 p.

———. 1855. Über *Chytridium*, eine Gattung einzelmer Schmarotzergewächse auf Algen und Infusorien. Monatsber. Berlin Akad 1855:378–384.

———. 1856. Über *Chytridium*, eine Gattung einzelliger Schmarotzergewächse auf Algen und Infusorien. Abhandl. Berlin Akad 1855:21–83.

Canter HM. 1949. Studies on British chytrids. VII. On *Phlyctochytrium mucronatum* n. sp. Trans Brit Mycol Soc 32:236–240, doi:10.1016/S0007-1536(49)80011-8

Fuller MS, Jaworski A. 1987. Zoosporic fungi in teaching and research. Athens, Georgia: Southeastern Publishing. vii + 303 p.

James TY, Letcher PM, Longcore JE, Mozley-Standridge SE, Porter D, Powell MJ, Griffith GW, Vilgalys R. 2006. A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia 98:860–871, doi:10.3852/mycologia.98.6.860

———, Porter D, Leander CA, Vilgalys R, Longcore JE. 2000. Molecular phylogenetics of the Chytridiomycota support the utility of ultrastructural data in chytrid systematics. Can J Bot 78:336–350, doi:10.1139/cjb-78-3-336

Karling JS. 1936. The endo-exogenous method of growth and development of *Chytridium lagenaria*. Am J Bot 23:619–627, doi:10.2307/2436151

———. 1944. Brazilian chytrids II. New species of *Rhizidium*. Am J Bot 31:254–261, doi:10.2307/2437540

———. 1945. Brazilian chytrids VI. *Rhopalophlyctis* and *Chytriomyces*, two new chitinophilic operculate genera. Am J Bot 32:362–369, doi:10.2307/2437353

———. 1946. Brazilian chytrids IX. Species of *Rhizophydium*. Am J Bot 33:328–334, doi:10.2307/2437119

———. 1967a. Some zoosporic fungi of New Zealand IV. *Polyphlyctis* gen. nov., *Phlyctochytrium* and *Rhizidium*. Sydowia 20:86–95.

———. 1967b. Some zoosporic fungi of New Zealand VII. Additional monocentric operculate species. Sydowia 20:119–128.

———. 1968. Zoosporic fungi of Oceania IV. Additional monocentric chytrids. Mycopathol Mycol Appl 36:165–178, doi:10.1007/BF02049683

———. 1977. Chytridiomycetarum iconographia. Monticello, New York: Lubrecht & Kramer. xii + 414 p.

Letcher PM, Powell MJ. 2001. Distribution of zoosporic fungi in forest soils of the Blue Ridge and Appalachian Mountains of Virginia. Mycologia 93:1029–1041, doi:10.2307/3761665

———, ———. 2002. A taxonomic summary of *Chytriomyces* (Chytridiomycota). Mycotaxon 84:447–487.

———, ———. 2005a. *Kappamyces*, a new genus in the Chytridiales (Chytridiomycota). Nova Hedwigia 80:115–133, doi:10.1127/0029-5035/2005/0080-0115

———, ———. 2005b. Phylogenetic position of *Phlyctochytrium planicorne* (Chytridiales, Chytridiomycota) based on zoospore ultrastructure and partial nuclear LSU rRNA gene sequence analysis. Nova Hedwigia 80:135–146, doi:10.1127/0029-5035/2005/0080-0135

———, ———, Barr DJS, Churchill PF, Wakefield WS, Picard KT. 2008a. Rhizophlyctidales—a new order in Chytridiomycota. Mycol Res 112:1031–1048, doi:10.1016/j.mycres.2008.03.007

———, ———, Chambers JG, Holznagel WE. 2004a. Phylogenetic relationships among *Rhizophydium* isolates from North America and Australia. Mycologia 96:1339–1351, doi:10.2307/3762150

———, ———, ———, Longcore JE, Churchill PF, Harris PM. 2005. Ultrastructural and molecular delineation of the Chytridiaceae (Chytridiales). Can J Bot 83:1561–1573, doi:10.1139/b05-115

———, ———, Churchill PF, Chambers JG. 2006. Ultrastructural and phylogenetic delineation of a new order, the Rhizophydiales. Mycol Res 110:898–915, doi:10.1016/j.mycres.2006.06.011

———, ———, Picard KT. 2012. Zoospore ultrastructure and phylogenetic position of *Phlyctochytrium aureliae* Ajello is revealed. Mycologia 104:410–418, doi:10.3852/11-153

———, McGee PA, Powell MJ. 2004b. Distribution and diversity of zoosporic fungi from soils of four vegetation types in New South Wales, Australia. Can J Bot 82:1490–1500, doi:10.1139/b04-109

———, Vélez CG, Barrantes ME, Powell MJ, Churchill PF, Wakefield WS. 2008b. Ultrastructural and molecular analyses of Rhizophydiales (Chytridiomycota) isolates from North America and Argentina. Mycol Res 112:759–782, doi:10.1016/j.mycres.2008.01.025

Longcore JE. 2005. Zoosporic fungi from Australian and New Zealand tree-canopy detritus. Austral J Bot 53:259–272, doi:10.1071/BT04048

———, Simmons DR. 2012. The Polychytriales ord. nov. contains chitinophilic members of the rhizophlyctoid alliance. Mycologia 104:276–294, doi:10.3852/11-193

Miller CE. 1965. Annotated list of aquatic phycomycetes from Mountain Lake biological station, Virginia. Virginia J Sci 14:219–228.

———. 1968. Observations concerning taxonomic characteristics in chytridiaceous fungi. J Elisha Mitchell Sci Soc 84:100–107.

Mozley-Standridge SE, Letcher PM, Longcore JE, Porter D, Simmons DR. 2009. Cladochytriales—a new order in Chytridiomycota. Mycol Res 113:498–507, doi:10.1016/j.mycres.2008.12.004

Nascimento C, Gomes E, Pires-Zottarelli C. 2011. Occurrence and distribution of zoosporic organisms in water bodies from Brazilian Cerrado. Mycologia 103:261–272, doi:10.3852/10-139

Paterson RA. 1956. Additions to the phycomycete flora of the Douglas Lake region II. Mycologia 48:270–277, doi:10.2307/3755475

Petersen HE. 1903. Note sur les Phycomycètes observes dans les tèguments vides des nymphes de Phryganèes avec decription de trios especes nouvelles de Chytridinèes. J Bot 17:214–222.

Picard KT, Letcher PM. Powell MJ. 2009. *Rhizidium phycophilum*, a new species in Chytridiales. Mycologia 101:696–706, doi:10.3852/08-194

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818, doi:10.1093/bioinformatics/14.9.817

Powell MJ, Letcher PM, Longcore JE. 2011. *Operculomyces* is a new genus in the order Rhizophydiales. Mycologia 103:854–862, doi:10.3852/10-293

———, ———, ———. 2013. *Pseudorhizidium* is a new genus with distinct zoospore ultrastructure in the order Chytridiales. Mycologia 105:496–507, doi:10.3852/12-269

Reyner SA, Samuels GJ. 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res 98:625–634, doi:10.1016/S0953-7562(09)80409-7

Schenk A. 1858. Ueber das Vorkommen contractiler Zeller im Pflanzenreiche. Würzburg, Germany. 20 p.

Sikes DS, Lewis PO. 2001. Beta software. Version 1. PAUPRat: PAUP* implementation of the parsimony ratchet. Storrs: Department Ecology Evolutionary Biology, Univ Connecticut. Distributed by the authors.

Simmons DR, James TY, Meyer AF, Longcore JE. 2009. Lobulomycetales, a new order in the Chytridiomycota. Mycol Res 113:450–460, doi:10.1016/j.mycres.2008.11.019

Sparrow FK. 1936. A contribution to our knowledge of the aquatic phycomycetes of Great Britain. J Linn Soc London (Bot) 50:417–478, doi:10.1111/j.1095-8339.1936.tb02006.x

———. 1937. Some new species of chytrids. Occ Papers Boston Soc Nat Hist 8:295–296.

———. 1938. Chytridiaceous fungi with unusual sporangial ornamentation. Am J Bot 25:485–493, doi:10.2307/2436675

———. 1939. Unusual chytridiaceous fungi. Pap Michigan Acad Sci 24, Part I. p 121–126.

———. 1960. Aquatic Phycomycetes. Univ. Michigan Press, Ann Arbor. xxvi + 1187 p.

———. 1965. The occurrence of *Physoderma* in Hawaii, with notes on other Hawaiian phycomycetes. Mycopathologia 25:119–143. doi: 10.1007/BF02049618

———. 1966. A new bog chytrid. Arch Mikrobiol 53:178–180, doi:10.1007/BF00413042

Steciow MM, Milanez AI, Pires-Zottarelli CLA, Marano AV, Letcher PM, Vélez CG. 2012. Zoosporic true fungi, heterotrophic straminipiles and plasmodiophorids: status of knowledge in South America. Darwiniana 50:25–32.

Vélez CG, Letcher PM, Schultz S, Powell MJ, Churchill PF. 2011. Molecular phylogenetic and zoospore ultrastructural analyses of *Chytridium olla* establish the limits of a monophyletic Chytridiales. Mycologia 103:118–130, doi:10.3852/10-001

Vilgalys R, Hester M. 1990. Rapid identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol 172:4238–4246.

Wakefield WS, Powell MJ, Letcher PM, Barr DJS, Churchill PF, Longcore JE, Chen S-F. 2010. A molecular phylogenetic evaluation of the Spizellomycetales. Mycologia 102:596–604, doi:10.3852/09-120

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. New York: Academic Press. p 315–322.

Willoughby LG. 1964. A study of the distribution of some lower fungi in soil. Nova Hedwigia 7:133–150.

———. 1965. A study of Chytridiales from Victorian and other Australian soils. Arch Mikrobiol 52:101–131.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under maximum likelihood criterion [doctoral dissertation]. Austin: Univ Texas. 115 p.