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A new heat-resistant species of *Leiothecium* Samson & Mouch. (Eurotiales, Ascomycota) from soil of Argentina

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ABSTRACT
Leiothecium Samson & Mouch. is a genus enclosed into the family Aspergillaceae Link (Eurotiales) including *L. ellipsoideum* Samson & Mouch. and *L. cristatum* Y. Marín, Stchigel & Cano, species exhibiting ascospore walls somewhat or irregularly reticulated and prominent crests. During a research on heat-resistant fungi from soil of the semi-arid region of Northern Argentina, several *Leiothecium* spp. isolates were obtained; morphological and molecular studies were carried out. Phylogenetics showed strong support for the monophyly of the *Leiothecium* clade and *L. ellipsoideum* as the sister taxon. Some isolates were identified as *Leiothecium ellipsoideum*, and a new species provided with

KEY WORDS
Aspergillaceae, Argentina, reticulated ascospores, cleistothecial ascomata, semi-arid region, new species.

reticulate-foveate ascospore ornamentation without prominent crests was described: *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. Molecular analysis and scanning electron microscopy (SEM) images support the description of *L. dictyophorum* S.M.Romero & Comerio, sp. nov. as a new species.

RÉSUMÉ

Une nouvelle espèce de Leiothecium *Samson & Mouch. résistante à la chaleur (Eurotiales, Ascomycota) issue du sol d'Argentine.*

Leiothecium Samson & Mouch. est un genre inclus dans la famille des Aspergillaceae Link (Eurotiales) qui comprend *L. ellipsoideum* Samson & Mouch. et *L. cristatum* Y.Marín, Stchigel & Cano, des espèces qui présentent des parois d'ascospores quelque peu ou irrégulièrement réticulées et des crêtes proéminentes. Lors d'une recherche sur les champignons résistants à la chaleur du sol de la région semi-aride du nord de l'Argentine, plusieurs isolats de *Leiothecium* spp. ont été obtenus; des études morphologiques et moléculaires ont été réalisées. La phylogénétique a montré un fort soutien à la monophylie du clade de *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. et de *L. ellipsoideum* comme taxon frère. Certains isolats ont été identifiés comme *Leiothecium ellipsoideum*, et une nouvelle espèce dotée d'une ornementation d'ascospores réticulées-fovéates sans crêtes proéminentes a été décrite : *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. L'analyse moléculaire et les images de microscopie électronique à balayage (MEB) soutiennent la description de *L. dictyophorum* S.M.Romero & Comerio, sp. nov. en tant que nouvelle espèce.

MOTS CLÉS
Aspergillaceae, Argentine, ascospores réticulées, ascomates cleistothéciques, région semi-aride, espèce nouvelle.

INTRODUCTION

The genus *Leiothecium* Samson & Mouch. was described in the course of a study on thermoresistant soil fungi (Samson & Mouchacca 1975). In that work, a single species, *Leiothecium ellipsoideum* Samson & Mouch., was described from a soil sample collected between rocks in Mystras, Greece. Almost thirty-five years later, Marin-Felix *et al.* (2014) described *Leiothecium cristatum* Y.Marín, Stchigel & Cano, from a rainforest soil sample collected in Iguazú National Park, Misiones province, Argentina.

Leiothecium is one of the fifteen genera accommodated in the family Aspergillaceae Link in Eurotiales (Houbraken *et al.* 2020); species in the genus presents dark cleistothecial ascomata, ascus with evanescent walls, and net-ornamented ascospores. *Leiothecium* includes, up to now, the only two species previously mentioned.

During broad research on heat-resistant fungi from the semi-arid region of Northern Argentina, more than two hundred isolates were obtained from soil. Most isolates were related to *Aspergillus* sect. *Fumigati*, but thirty-four isolates represented several interesting taxa (Romero *et al.* 2020). Nine isolates from different soil samples resembled *L. ellipsoideum*, but in some of them, morphological differences in the ascospores ornamentation were noted. The objective of this work was to present and describe a new *Leiothecium* species based on phylogenetics and morphological data.

MATERIAL AND METHODS

Isolation

Fifty 200 g soil samples were collected in the summer of 2009 and winter of 2011 from several sites in Catamarca and La Rioja provinces, Northern Argentina. A detailed description of the sampling area was done in Romero *et al.* (2020). About 200 g of soil per sample were collected using a sterile stainless-steel scoop and transferred aseptically into sterile self-sealing plastic bags of appropriate capacity. The samples were transported and stored at room temperature until processing in the laboratory. From each sample, *c.* 5 g of soil was placed into a conical flask, mixed with 100 mL of sterile melted Malt Extract Agar (MEA, Oxoid CM0059B, United Kingdom) plus 50 ppm of chloramphenicol, and heated at 75°C for 30 min. The suspension was plated into sterile Petri dishes (9.0 cm diam.) and, once gelled, incubated at 30°C for periods of less than thirty days (Samson *et al.* 2019). All colonies were transferred to individual Petri dishes (5.0 cm diam.) containing MEA to obtain pure cultures. The fungal strains were deposited in the culture collection of the Herbarium, *Universidad de Buenos Aires*, Argentina (BAFC) and the culture collection of the Faculty of Medicine, *Universitat Rovira i Virgili*, Reus, Catalonia, Spain (FMR).

Phenotypic characterization

Genus characterization was conducted according to Guarro *et al.* (2012). To carry out species identification, inoculations were made from spore suspensions prepared with agar 0.2 % w/v and Tween 80 0.05 % w/v solution. The isolates were inoculated on each plate in a three-point pattern using a micropipette with an inoculum size of 1 µl per spot. *Leiothecium* isolates utilized in this study are listed in Table 1. For standard macro-morphological observations cultures on MEA, Oatmeal Agar (OA), Creatine Sucrose Agar (CREA) and Potato Dextrose Agar (PDA) were performed according to Samson *et al.* (2019); all plates were incubated at 25°C in the dark, and examined at 7 and 14 days. Extra cultures on MEA were carried out and incubated at 15, 35 and 40°C for

TABLE 1. — Data used for phylogenetic analysis in this study and their corresponding GenBank accession numbers. Type species are denoted by (**T**). Sequences derived from this study are shown in **bold**. (**a**), Marin-Felix *et al.* 2014; (**b**), Vu *et al.* 2019; (**c**), Barbosa *et al.* 2017; (**d**), Houbraken & Samson 2011; (**e**), Gueidan *et al.* 2008; (**f**), Pettersson *et al.* 2011; (**g**), Peterson 2008; (**h**), Abarenkov *et al.* 2024.

		GenBank accession number			
Species name	**Strain number**	***BenA***	***rpb2***	***ITS***	***LSU***
Leiothecium cristatum Y.Marín, Stchigel & Cano (T)	FMR 11998T = CBS 134260T = NBRC 109843T	**PP187391**	HF954976.1(a)	NR_145180.1(a)	MH877551.1(b)
Leiothecium dictyophorum S.M.Romero & Comerio, sp. nov. (T)	BACF 53468 = BAFCcult 4595 = FMR 15063	**PP149064**	**PP149071**	**PQ359515**	**PQ359520**
Leiothecium dictyophorum S.M.Romero & Comerio, sp. nov.	BAFCcult 4592 = FMR 15065	**PP149067**	**PP149074**	**PQ359516**	**PQ359521**
Leiothecium dictyophorum S.M.Romero & Comerio, sp. nov.	BAFCcult 4608 = FMR 15066	**PP149065**	**PP149072**	**PQ359517**	**PQ359522**
Leiothecium dictyophorum S.M.Romero & Comerio, sp. nov.	BAFCcult 4597 = FMR 15067	**PP149066**	**PP149073**	**PQ359518**	**PQ359523**
Leiothecium ellipsoideum Samson & Mouch. (T)	CBS 607.74T	KY709178.1(c)	JN121541(d)	NR_144922.1(a)	NG_057811.1(e)
Leiothecium ellipsoideum Samson & Mouch.	BAFCcult 4598 = FMR 15064	**PP149063**	**PP149070**	**PQ359519**	**PQ359524**
Monascus argentinensis Stchigel & Guarro	CBS 109402	KY709174.1(c)	JN121423.1(d)	JF922046.1(f)	KY645974.1(d)
Monascus purpureus Went	NRRL 1596	EU014110.1(g)	EF669718.1(g)	OQ694412.1(h)	NG_069605.1(b)
Xeromyces bisporus L.R.Fraser	CBS 236.71	KY709179.1(c)	JN121466.1(c)	MH860089.1(b)	NG_057813.1(e)

7 days; fungal strains were also inoculated on Czapek Yeast Agar 20% Sucrose (CY20S) and Malt Extract Yeast Extract 50% Glucose Agar (MY50G) and incubated at 25°C for 7 days according to Pitt & Hocking (2009). The microscopic mounts to determine micro-morphological features, from MEA colonies, were made in lactic acid 85% w/w and lacto-fuchsin (Carmichael 1955; Samson *et al.* 2019). Preparations were observed through a Zeiss Axioscop microscope. Mature ascomata were crushed, coated with gold, and observed and photographed with a Zeiss Supra 40 (extra high tension = 3 Kv; working distance = 3.7-6 mm) scanning electron microscope.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Total DNA was extracted directly from colonies on PDA after 7-10 days incubation at 25°C in darkness, following the Fast DNA kit protocol (Bio 101, Inc., Vista, Canada, United States) with the homogenization step repeated three times with a FastPrep FP120 instrument (Thermo Savant, Holbrook, New York, United States). After each homogenization, the sample was kept in ice for 10 min. DNA was quantified with GeneQuant pro (Amersham Pharmacia Biotech, Cambridge, England) (Marimon *et al.* 2006). Extracted DNA was used to amplify a fragment of beta-tubulin (*BenA*) (T10/Bt2b primers; Glass & Donaldson 1995), a fragment of the RNA polymerase II subunit 2 gene (*rpb2*) (RPB2-5F/RPB2-7cR primers; Liu *et al.* 1999), a fragment of 28S nrRNA gene (*LSU*) (LR0R/LR5 primers: Rehner & Samuels 1994; Vilgalys & Hester 1990), and the internal transcribed spacer (*ITS*) (ITS5/ITS4 primers; White *et al.* 1990). The PCR amplifications were made in a total volume of 25 µL containing 5 µL 10x PCR Buffer (Invitrogen, CA, United States), 0.2 µM dNTPs, 0.5 µM of each primer, 1 U Taq DNA polymerase, and 1-10 ng nuclear DNA. PCR conditions were set as follows: for *BenA* and *ITS* regions, initial denaturation at 95°C for 5 min, followed by 35 cycles of (denaturation during 30 s at 95°C, annealing during 1 min at 55°C, and extension during 90 s at 72°C), and a final extension step at 72°C for 10 min. For *rpb2* and *LSU* regions, initial denaturation at 95°C for 5 min, followed by 35 cycles of (45 s at 95°C, 1 min at 56°C, and 90 s at 72°C) and a final extension step at 72°C for 10 min. Single-band PCR products were purified from agarose gels and sequenced at Macrogen Europe, which uses large-scale sequencing developed by "Applied Biosystem", which works using the Sanger method (Macrogen Inc., Madrid, Spain). Sequence assembly and editing were carried out using SeqMan software v.7.0 (DNAStar Lasergene, Madison, WI, United States).

PHYLOGENETIC ANALYSIS

We used a total of 10 taxa including: two representative taxa of *Monascus*, *Monascus purpureus* Went and *Monascus argentinensis* Stchigel & Guarro, one representative taxa of *L. cristatum*, two representative taxa of *L. ellipsoideum*, and four representative taxa of *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov.; *Xeromyces bisporus* L.R.Fraser, was used as outgroup. We generate twenty-one new sequences which sixteen correspond to *Leiothecium* S.M.Romero & Comerio, sp. nov., four correspond to *L. ellipsoideum* and one corresponds to *L. cristatum*. GenBank accession numbers for the newly generated sequences in this study and others corresponding to reference or ex-type strains are listed in Table 1.

DNA sequences were initially aligned with Muscle ver. 3.6 (Edgar 2004a, b), followed by manual alignment in the data editor of BioEdit ver. 7.0.1 (Hall 1999). The alignment was

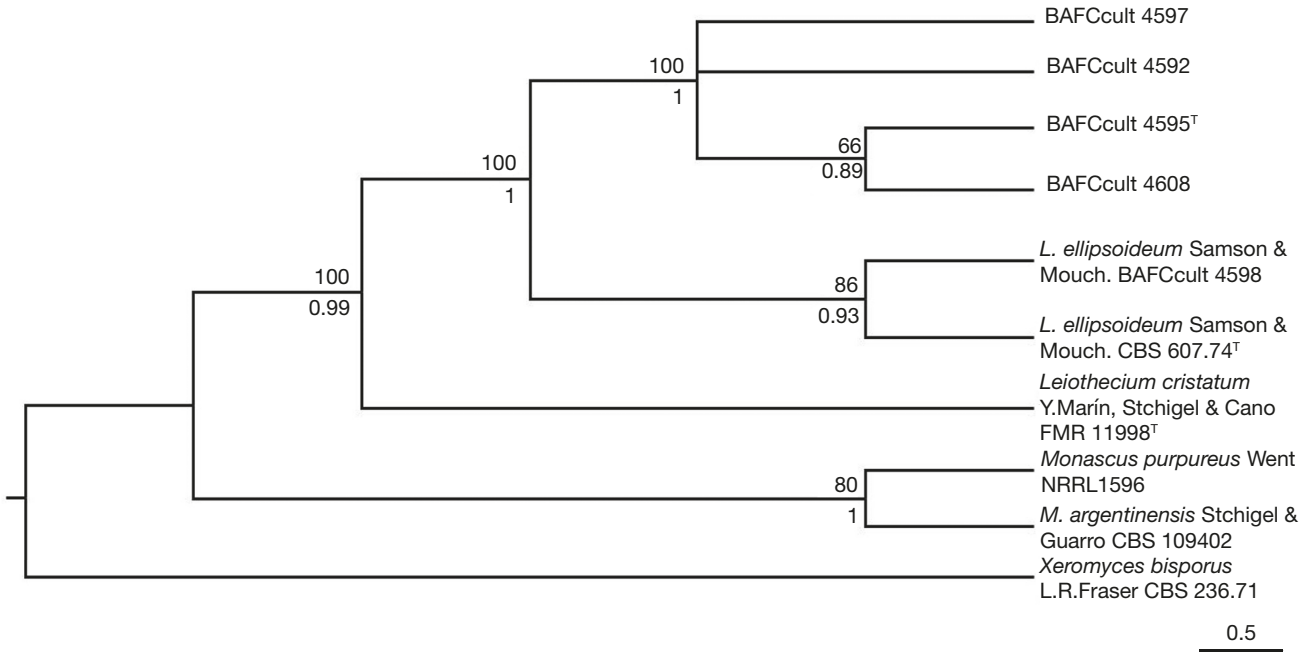

Fig. 1. — Fifty percent majority-rule Bayesian consensus of the molecular phylogenetic analyses of the combined dataset. Support values above the branches represent Bayesian posterior probabilities (PP>0.5). Support values below the branches represent bootstrap values (BS>50). Scale bar: 0.5 substitutions per site.

performed separately for each marker. To address the level of congruence among data partitions and their influence on combined datasets, the incongruence length difference (ILD) test (Farris *et al.* 1994) was performed. The ILD test with 1000 permutations and a significance level of P <0.01 was conducted in TNT (ILD.run script). Phylogenetic hypotheses were congruent between partitions according to the ILD test (P = 0.14). Based on the results of these incongruence tests, we determined that the different marker matrices can be combined. Maximum parsimony (MP) analysis was done with TNT ver. 1.1 (Goloboff *et al.* 2008). "Traditional" heuristic searches were carried out with a total of 1000 random addition sequences and submitted to tree-bisection reconnection (TBR) branch swapping, holding 50 trees, followed by a more extensive TBR holding 50 000 trees. The most parsimonious trees found were collected and a strict consensus was calculated using the "Nelsen" option in TNT. Support was estimated by bootstrap as implemented in TNT, resampling 1000 times with TBR set to 100 replications holding 20 trees, followed by a more extensive TBR holding 5000 trees, and saving the consensus for each resampling matrix.

Bayesian inference analyses were employed to infer the phylogeny using each dataset separately and subsequently using a combined dataset that included all sequences. Bayesian inference was performed using MrBayes ver. 3.2.7 (Ronquist *et al.* 2012). Models for each molecular dataset, as well as the combined dataset, were evaluated using ModelTest version 3.06 (Posada & Crandall 1998) to identify the best fit according to the Akaike Information Criterion (Akaike 1974). Bayesian analyses were run with 20 million Metropoliscoupled Markov chain Monte Carlo (MCMCMC) generations with four chains, sampling trees every 100th generation. Stationarity was determined based on the convergence of likelihood scores using TRACER ver. 1.5 (Rambaut & Drummond 2009), and sample points generated before stationarity were eliminated as burn-in (25%). The posterior probabilities (PP) of the clades were determined by a 50% majority-rule consensus of the trees retained.

RESULTS

Phylogenetic analyses

The alignment of the 10 accession dataset consisted of 2729 aligned positions (Table 2). Summary of the four datasets of DNA regions and the combined dataset, along with an overview of the parameters used for phylogenetic estimation and the statistical results of parsimony analyses, including the consistency index (CI), retention index (RI), number of parsimony-informative sites, tree length, best-fit models of evolution, and estimated log-likelihood are provided in Table 2. The strict consensus tree resulting from parsimony analyses of each dataset and the combined dataset (not shown) presented the same topology as the 50% majority rule consensus tree obtained from Bayesian analyses of the same datasets. For each dataset, both individually and combined, the Bayesian analysis shows that the average standard deviation of split frequencies reaches 0.005 after 20 million generations, and the four runs converged with a potential scale reduction factor (PSRF) tending to 1.001. The 50% majority-rule consensus tree from the Bayesian analysis of the combined dataset is shown in Fig. 1, whereas the trees derived from each individual dataset are shown in Fig. 4. Both parsimony and Bayesian analyses based on the combined dataset showed similar topologies throughout the general phylogeny, as

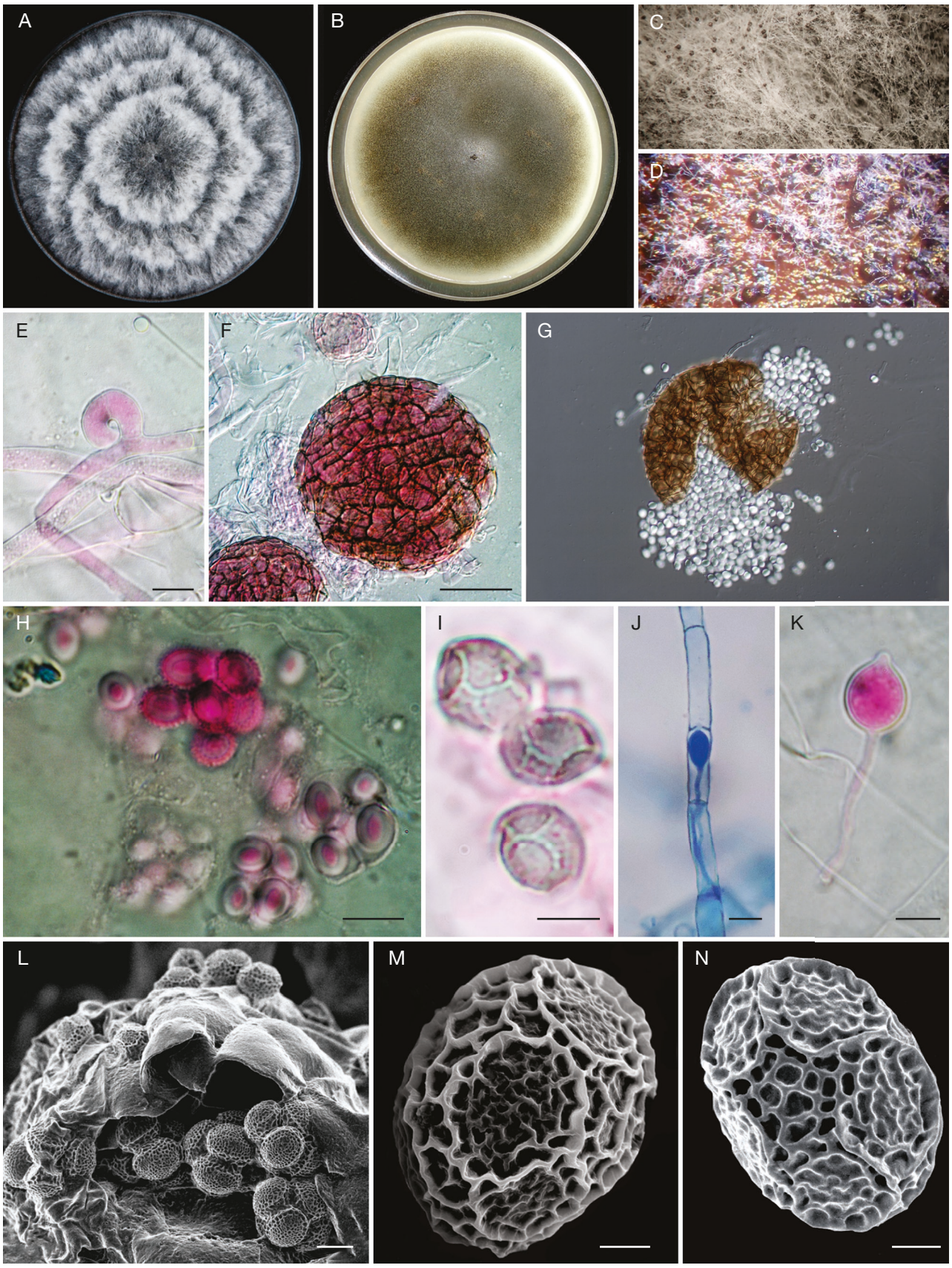

FIG. 2. — *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov.: **A**, **B**, colony on MEA, OA, 14 days, 25 at *c.* 1°C; **C**, ascomata from aerial mycelium; **D**, ascomata immersed in the culture media; **E**, initials; **F**, ascoma *Textura angularis* wall; **G**-**I**, ascospores; **J**, **K**, chlamydospores; **L**, broken ascoma showing asci and ascospores (SEM); **M**, **N**, ascospores (SEM). Scale bars: E, H, J, 10 µm; F, 20 µm; I, K, L, 5 µm; M, N, 1 µm.

TABLE 2. — Summary of the four datasets of DNA regions, along with an overview of the parameters used for phylogenetic estimation and the statistical results of parsimony analyses. To establish the nucleotide similarity percentage, a BLAST (Highly similar sequences -megablast- from NCBI) was performed, using sequences obtained from type strains of *Leiothecium ellipsoideum* Samson & Mouch. and *L.dictyophorum* S.M.Romero & Comerio, sp. nov.

	BenA	***rpb2***	***LSU***	***ITS***	**ALL**
Number of taxa	10	10	10	10	10
Average sequence length	358	848	849	540	—
% of nucleotide similarity	96	98.9	99.7	99.5	—
Aligned sequence length	437	850	854	588	2729
N° sites parsimony informative	101	123	22	38	284
Consistency index	0.84	0.74	0.71	0.7	0.76
Retention index	0.82	0.69	0.64	0.65	0.78
Tree length (steps)	299	373	79	163	917
Best-fit model of evolution, Akaike information criterion (AIC)	K2 + G	K2G	T92 + G	K2 + G	K2 + G
Estimated log-likelihood	–1767.2	–2747.6	–1610.8	–1568.4	–7915.3

DICHOTOMOUS KEY FOR THE KNOWN *LEIOTHECIUM* SAMSON & MOUCH. SPECIES

1. Ascospores reticulate, without prominent crests ..... *L. dictyophorum* S.M.Romero & Comerio, sp. nov.
1'. Ascospore wall reticulated, with prominent crests 2

2. Ascospores presenting several crests ..... *L. ellipsoideum* Samson & Mouch.
2'. Ascospores with only two crests ..... *L. cristatum* Y.Marín, Stchigel & Cano

well as consistent relationships between, *Leiothecium* clade and *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. clade (Fig. 1). Both parsimony and Bayesian analyses showed strong support for the monophyly of the *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. clade and *L. ellipsoideum* as the sister taxa. Thus, we present only the consensus tree resulting from Bayesian analyses and on their branches are included posterior probabilities (PP) and bootstrap support (BS).

TAXONOMY

Order EUROTIALES
G.W.Martin ex Benny & Kimbr.
Family ASPERGILLACEAE Link
Genus *Leiothecium* Samson & Mouch.

Leiothecium dictyophorum
S.M.Romero & Comerio, sp. nov.
(Fig. 2)

DIAGNOSIS. — *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov.; ex affinitate *L. ellipsoidei* Samson & Mouch. et *L. cristati* Y.Marín, Stchigel & Cano, ab utroque ascosporarum ornamento reticulato-foveato sine manifestis cristis distinctum.

Leiothecium dictyophorum S.M. Romero & Comerio sp. nov.; of the affinity of *L. ellipsoideum* Samson & Mouch. and *L. cristatum* Y. Marín, Stchigel & Cano, distinct from both by the ascospores' ornament reticulate-foveate without prominent crests.

TYPE MATERIAL. — **Argentina** • Catamarca, RN 60 km 1016; 29°30'4"S, 65°37'57"W; 237 m alt.; 22.VIII.2011; *S.M. Romero* leg.; Holotype: BAFC 53468 (dried culture); BAFCcult 4595 = FMR≈15063, culture ex type; GenBank *BenA* sequence PP149064, *rpb2* sequence PP149071.

ADDITIONAL SPECIMENS STUDIED. — **Argentina** • Catamarca; RN 60 km 1102; 28°55'15"S, 66°08'46"W; 338 m alt.; 5.I.2009; BAFCcult 4599 = FMR 16764 • RN 38 km 563; 28°35'5"S, 65°52'27"W; 522 m alt.; 12.I.2009; BAFCcult 4592 = FMR 15065 • RN 60 km 1016; 29°30'4"S, 65°37'57"W; 237 m alt.; 2.I.2010; BAFCcult 4608 = FMR 15066 • RN 38 km 563; 28°35'5"S, 65°52'27"W; 522 m alt.; 23.VIII.2011; BAFCcult 4597 = FMR 15067, FMR 16766, BAFCcult 4596 = FMR 16767.

MYCOBANK. — MB857727.

ETYMOLOGY. — Dictyon (δικτυον, net) and phorum (φορος, bearing) were combined to coin *dictyophorum* (adj.): net-bearing; regarding the distinctly net-like ornamentation of ascospores.

LATIN DESCRIPTION

Coloniae in agaro malto confecto, 25°C, post 7 dies, 75-79 mm diam., fuscae, albis annulis mycelialibus praeditae; pars aversa fusca. In agaro farina avenae confecto, 25°C, post 7 dies, 60-64 mm diam., fuscae, quasi continuum stratum ascomatum efficientes, mycelium aerium vix effectum.

Hyphae hyalinae, laeves, 3-11 µm diam. Cleistothecia globosa, glabra, atro-brunnea, 40-125 µm diam., inter mycelium aerium disposita vel in substratum semiimmersa; parietes cleistotheciorum persistentes, 5-8 µm lati, texturam angularem praebentes, e cellulis atro-brunneis crassitunicatis 15-25 µm diam. compositi. Asci globosi vel subglobosi, 13-15 × 14-18 µm, octospori, evanescentes. Ascosporae unicelulares, ellipsoideae, hyalinae, ornamentum reticulatum-foveatum ostendentes, 5.5-7 × 7-9 µm. Chlamydosporae subglobosae vel ellipsoideae,

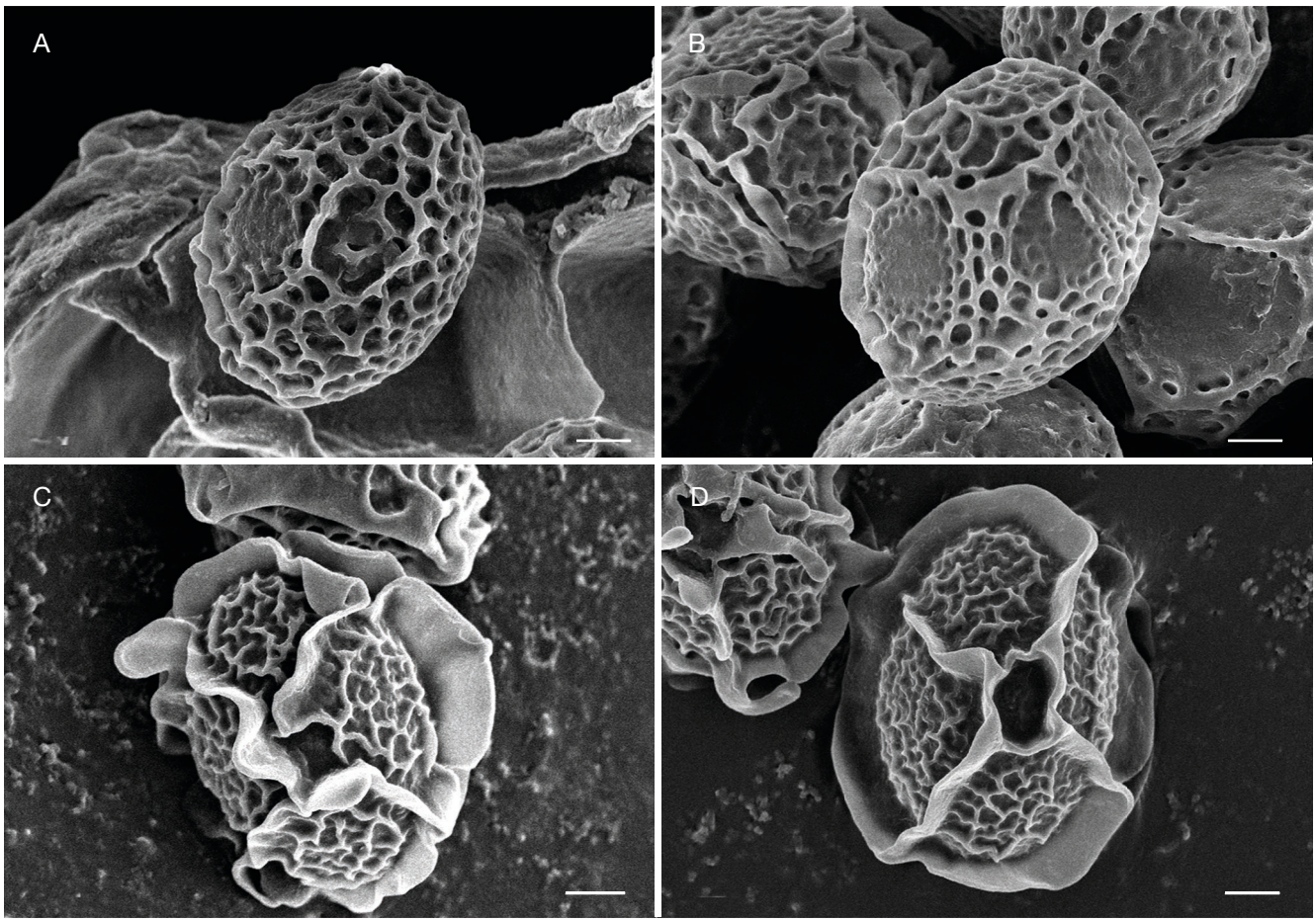

Fig. 3. — Ascospores (SEM): **A**, **B**, *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. : **A**, BAFCcult 4608; **B**, BAFCcult 4599; **C**, **D**, *Leiothecium ellipsoideum* Samson & Mouch.: **C**, BAFCcult 4598; **D**, BAFCcult 4604. Scale bars: 1 µm.

hyalinae, plerumque terminales umbonataeque, nonnumquam intercalares, etiam endogenae, crassitunicatae, 6-18 × 5-12.5 µm diam.

English description

Colonies on MEA, 25ºC, 7 days, 75-79 mm diam., dark, with aerial white mycelium in concentric circles; reverse dark (Fig. 2A). At 35°C, covering the whole plate before 7 days; reverse dark due to numerous ascomata. At 15°C, 7 days, 0-8 mm diam.; at 40°C, 7 days, 21-31 mm diam. On PDA, 25°C, covering the whole plate at 7 days, dark, with scarce aerial white mycelium, reverse dark. On OA, 25°C, 7 days, 60-64 mm, dark colonies forming an almost continuous layer of ascomata, aerial mycelium scarce (Fig. 2B). At 35°C, spreading all over the plate before 7 days; development in subtle circles, aerial mycelium less floccose than in MEA. On CY20S, 25°C, 7 days, 83 mm diam. On MY50G, 25°C, 7 days, 42 mm diam.

Mycelium composed of hyaline, smooth-walled, hyphae, 3-11 µm wide. Cleistothecial ascomata, globose, glabrous, dark-brown 40-125 µm diam., both intermixed within the white mycelium and semi-submerged in agar (Fig. 2C, D); with a persistent wall, textura angularis, 5-8 µm thick, composed of dark brown thick-walled cells of 15-25 µm diam. (Fig. 2F, G). Asci globose to subglobose, 13-15 × 14-18 µm, eight-spored, evanescent. Ascospores one-celled, ellipsoidal, hyaline, 5.5-7 × 7-9 µm, loosely reticulated (Fig. 2I); SEM shows a reticulated-foveate ornamentation in mature ascospores. Chlamydospores present, subglobose to ellipsoidal, hyaline, usually terminal but sometimes intercalary, endogenously generated present as well, smooth- and thick-walled, 6-18 × 5-12.5 µm.

Notes

Leiothecium dictyophorum S.M.Romero & Comerio, sp. nov. displayed a distinctive ascospore ornamentation which set it morphologically apart from *L. ellipsoideum* and *L. cristatum*, consisting in a reticulate-foveate-net-ascospores without prominent crests (Fig. 2N). Some ascospores of *L. dictyophorum* S.M.Romero & Comerio, sp. nov. have shown areas where the net was not completely developed, as it is shown at the central region of the ascospore illustrated in the Fig. 2M. Probably, this particular feature, present in several ascospores, reflects some unripeness degree. *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. ascospores has not exhibited prominent

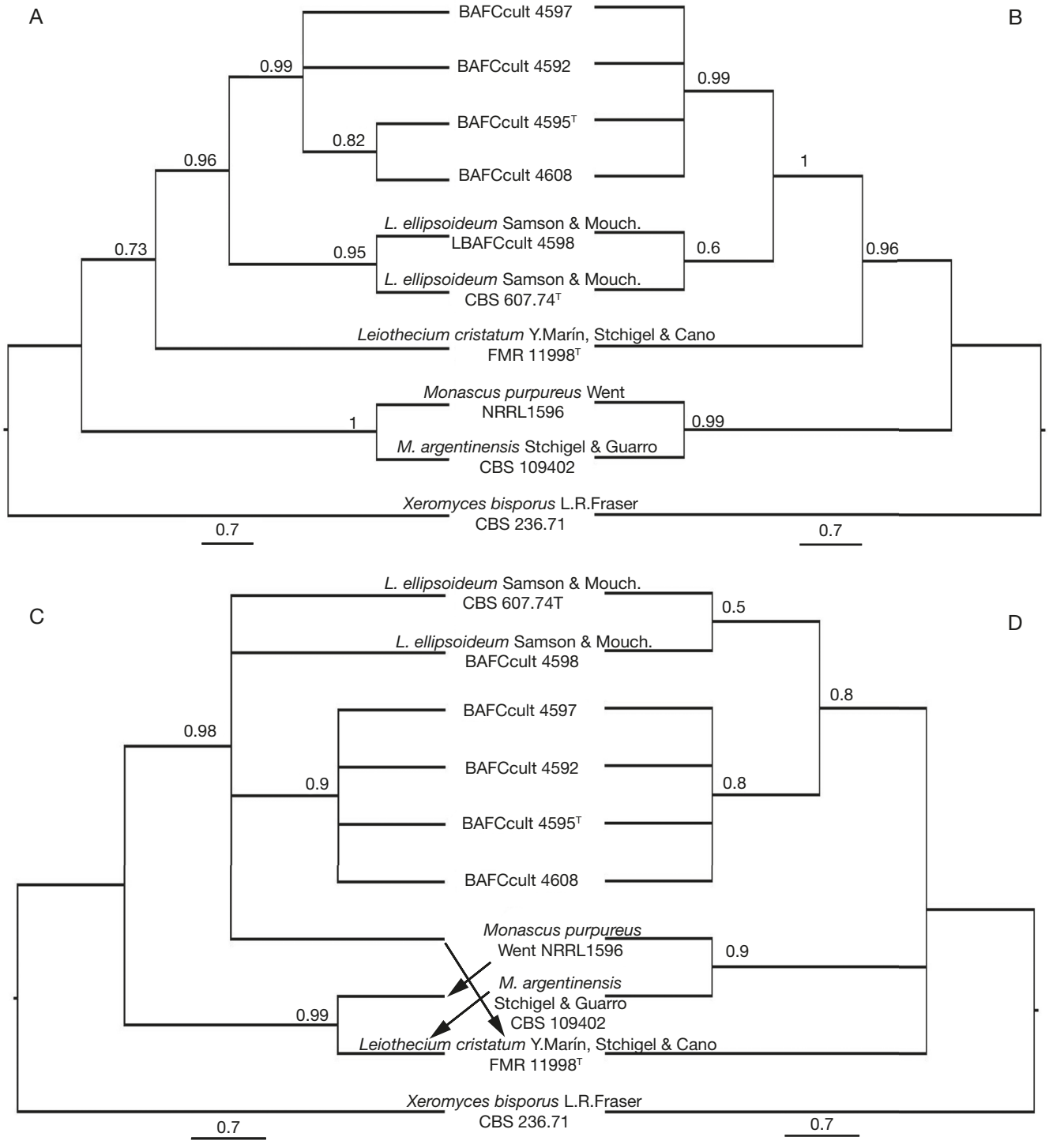

FIG. 4. — Fifty percent majority-rule Bayesian consensus trees from the molecular phylogenetic analyses of each dataset separately, including: **A**, *BenA*; **B,** *rpb2*; **C**, *LSU*; **D**, *ITS*. Support values above the branches represent Bayesian posterior probabilities (PP>0.5). Scale bar: 0.7 substitutions per site. **Arrows** indicate topological conflicts between ITS – and LSU – based phylogenies for *Leiothecium cristatum* Y.Marín, Stchigel & Cano, *Monascus purpureus* Went, and *Monascus argentinensis* Stchigel & Cano.

crests as *L. ellipsoideum* (Fig. 3C, D) and *L. cristatum* clearly do. The referred difference between ascospores of *L. dictyophorum* S.M.Romero & Comerio, sp. nov. and *L. ellipsoideum* is illustrated in Fig. 3.

DISCUSSION

The growth of *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. strains on the culture media studied was similar

to the Argentinean ones of *L. ellipsoideum*. In addition, the new species has shown good growth at 35°C as was observed for *L. ellipsoideum* (Samson & Mouchacca 1975). All the Argentinean strains of both *L. dictyophorum* S.M.Romero & Comerio, sp. nov. and *L. ellipsoideum,* exhibited a xerophilic behaviour (development on high-content-sugar media); as far as we know no data has been published up to now regarding the xerophilic character within the genus *Leiothecium*.

Our phylogenetic hypothesis, presented here, showed a similar topology to that obtained by Marin-Felix *et al.* (2014) for a similar clade (except for *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov.). Our results indicate strong support for the monophyly of the *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. clade and *L. ellipsoideum* as sister taxa. We report for the first time the phylogenetic position of *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. within the *Leiothecium* clade. Morphological features and phylogenetic analysis support the description of *Leiothecium dictyophorum* S.M.Romero & Comerio, sp. nov. as a new species.

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