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Lyomyces denudatus, a new species with a disjunct distribution

Lyomyces denudatus, una especie nueva con distribución disyunta

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ABSTRACT

We describe a new corticioid species *Lyomyces denudatus* based on morphological and molecular data (nuc rDNA ITS). It occurs both in the extreme south of South America and New Zealand. Our phylogenetic analyses place it near *L. densiusculus* from Africa and *L. fimbriatus* from East Asia. In addition, we comment on the status of *L. leptocystidiatus* and *L. fumosus*.

Keywords — Schizoporaceae; Hymenochaetales; phylogeny; austral distribution.

RESUMEN

Describimos una nueva especie de hongo corticioide, *Lyomyces denudatus*, con base en datos morfológicos y moleculares (nuc rDNA ITS). La especie ocurre tanto en el extremo sur de Sudamérica como en Nueva Zelanda. Nuestros análisis filogenéticos la ubican cerca de *L. densiusculus* de África y *L. fimbriatus* del este de Asia. Además, comentamos el estado de *L. leptocystidiatus* y *L. fumosus*.

Palabras clave — Schizoporaceae; Hymenochaetales; filogenia; distribución austral.

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INTRODUCTION

Lyomyces P. Karst. had been treated in *Hyphodontia* J. Erikss. for a couple of decades until Hjortstam & Ryvar den (2009) re-introduced it for its generic type *Lyomyces sambuci* (Pers.) P. Karst and four similar species. Subsequently, the concept of *Lyomyces* has been expanded based on DNA data with a number of clades considered by Hjortstam & Ryvar den (2009) *Xylodon* (Pers.) Gray, the closest relative of *Lyomyces*. All contemporary authors working on the group keep maintaining *Lyomyces* as a genus separate from *Xylodon*, which we follow in this paper. We describe here a new *Lyomyces* species based on four collections made by Leif Ryvar den in February 1982 in the extreme south of Argentina near Ushuaia. We also discuss the status of its closest relatives: *L. fimbriatus* (Sheng H. Wu) Riebesehl & Yurchenko, *L. fumosus* C.L. Zhao, and *L. leptocystidiatus* Xue W. Wang & L.W. Zhou.

MATERIALS AND METHODS

Morphological methods.— Collections from H were studied. Microscopic methods were described in Miettinen *et al.* (2006). All measurements were made in Cotton Blue (CB, Merck 1275; Kenilworth, New Jersey) with phase contrast illumination (1250 \times). The following abbreviations were used in microscopic descriptions: L — mean spore length; W — mean spore width; Q — mean L/W ratio; n — number of elements (basidiospores, basidia, cystidia, and hyphae) measured, which are followed by the number of specimens studied. We excluded 5% of measurements from each end of the range representing variation of basidiospores and cystidia. Excluded extreme values were indicated in parentheses when they strongly differed from the lower or higher 95% percentile.

DNA extraction and sequencing.— Total genomic DNA was extracted from herbarium specimens using a CTAB-chloroform extraction protocol (Kutuzova *et al.*, 2017). We used standard primers to amplify complete nuc rDNA ITS1-5.8S-ITS2 (ITS) (Table 1). After amplification, PCR products were run on a 1.5 % agarose gel stained with GelRed staining (Biotium, Fremont, California) and visualized under UV light. PCR products were purified from agarose gels using a Fermentas Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, Massachusetts). Sequencing reactions were performed on an ABI 3730XL DNA analyzer (Applied Biosystems) by Macrogen (Amsterdam, the Netherlands). All newly produced sequences used in this study have been deposited in GenBank (Table 2).

Phylogenetic analyses.— High variability of ITS sequences in *Lyomyces* does not allow construction of a reliable all-encompassing alignment. Therefore, we constructed an ITS alignment to fit all available sequences that could be reliably aligned to the newly described species. To this end, we retrieved sequences from GenBank (Benson *et al.*, 2018) identified through BLAST as of 29 June 2022. Only sequences that could be reliably aligned were used in the ITS analyses. This corresponded to 91.6% threshold of pairwise similarity to our newly produced sequences and an outgroup (*L. aff. orientalis*) with 89% pairwise similarity.

Table 1. Primers used in this study.**Tabla 1.** Primers usados en este trabajo.

Primer name	Sequence	Target DNA locus	Binding site	Direction	Reference
ITS5	GGAAGTAAAAGTCGTAACAAGG	ITS, ITS1	18S	fwd	White <i>et al.</i> , 1990
ITS2	GCTGCGTTCTTCATCGATGC	ITS1	5.8S	rev	White <i>et al.</i> , 1990
58A1F	GCATCGATGAAGAACGC	ITS2	5.8S	fwd	Martin & Rygielwicz, 2005
ALR0.2	GATATGCTTAAGTTCAGCGGG	ITS, ITS2	28S	rev	Riebesehl & Langer, 2017

Table 2. GenBank accession numbers for DNA sequences used in this study.**Tabla 2.** Números de Acceso de GenBank para las secuencias de ADN usadas en este trabajo.

Species	Specimen	ITS	Reference
<i>Lyomyces aff. orientalis</i>	Boidin 383	MH857295	Unpublished
<i>Lyomyces densiusculus</i>	Ryvarde 44818	OK273853	Viner <i>et al.</i> (2021)
<i>Lyomyces denudatus</i>	Burdsall 19410	MW740296	Unpublished
<i>Lyomyces denudatus</i>	Ryvarde 19256	ON980759	This study
<i>Lyomyces denudatus</i>	Ryvarde 19436	ON980760	This study
<i>Lyomyces fimbriatus</i>	Wu 910620-7	MK575209	Yurchenko <i>et al.</i> (2020)
<i>Lyomyces fimbriatus</i>	Wu 911204-4	MK575210	Yurchenko <i>et al.</i> (2020)
<i>Lyomyces fumosus</i>	Zhao 8188	MW713744	Luo <i>et al.</i> (2021)
<i>Lyomyces fumosus</i>	Zhao 17855	MW713745	Luo <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170814-14	MT319429	Wang <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170814-8	MT319432	Wang <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170815-2	MT319430	Wang <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170815-30	MT319427	Wang <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170815-43	MT319428	Wang <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170818-1	MT319431	Wang <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170818-8	MT319433	Wang <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170818-9	MT319435	Wang <i>et al.</i> (2021)
<i>Lyomyces leptocystidiatus</i>	Zhou 20170908-14	MT319434	Wang <i>et al.</i> (2021)
<i>Lyomyces sp.</i>	Burdsall 19323	MW740297	Unpublished

Alignments were calculated through the MAFFT 7.429 online server (<https://mafft.cbrc.jp/alignment/server/>) using the L-INS-I strategy (Katoh *et al.*, 2017). After removing unalignable fragments, the length of the alignment and the number of parsimony informative characters were correspondingly 565 and 52 bp.

We inferred rooted phylogenetic trees with maximum likelihood (ML) and Bayesian Inference (BI). Nucleotide substitution models for BI were chosen with TOPALI 2.5 (Milne *et al.*, 2008) based on the Bayesian information criterion (BIC). We performed BI using MrBayes 3.2 (Ronquist *et al.*, 2012). In these analyses, three parallel runs with four chains each and other default parameters were run for one million generations. A burn-in of 25% was used in the final analyses, ensuring the average standard deviation of split frequencies had reached <0.01 for all data sets.

When depicting phylograms, the support for nodes is indicated when posterior probabilities are ≥ 0.90 . For ML analyses, IQ-TREE 1.2.2 (Nguyen *et al.*, 2015) with the best-fitted model option was used. Bootstrapping was performed using the standard nonparametric bootstrap algorithm with the number of replicates set to 1000. Support for nodes is indicated with bootstrap values ≥ 70 .

RESULTS

BI and ML resulted in similar topologies. Relevant support values were indicated at nodes in Fig. 1. Newly described *Lyomyces denudatus* forms a highly supported clade with a New Zealand sequence (*Harold H. Burdsall 19410*; MW740296). Morphological differences between species in *Lyomyces* are often small, but we have found reliable characters to distinguish newly described *L. denudatus* from all other *Lyomyces* known to us.

After the New Zealand sequence, the closest matches to *L. denudatus* are *L. densiusculus* Viner & Ryvarden and *L. fimbriatus*. *Lyomyces leptocystidiatus* and *L. fumosus* ended up in the same clade as *L. fimbriatus* (Fig. 1). All three are East Asian taxa. Despite being strongly structured, our phylogenetic analyses do not resolve these three species as separate.

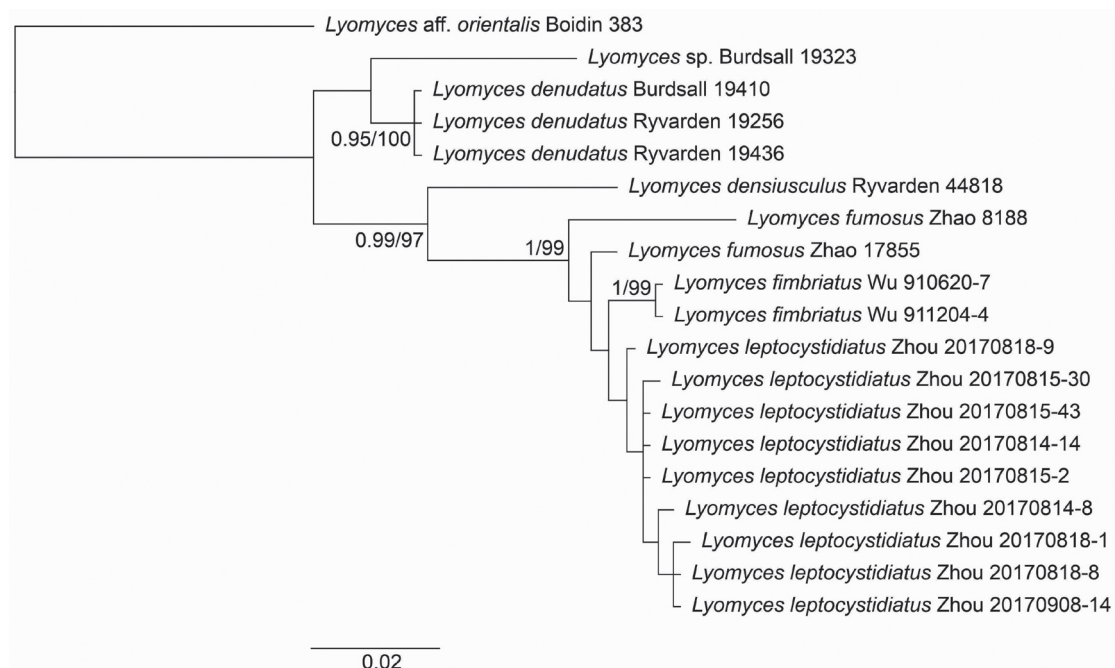


Fig. 1. Phylogenetic relationships of *Lyomyces denudatus* and allied taxa inferred from ITS sequences using BI analysis. Bayesian posterior probabilities followed by ML bootstrap values are shown at nodes; branch lengths reflect estimated number of changes per site.

Fig. 1. Relaciones filogenéticas de *Lyomyces denudatus* y taxones afines inferidos de secuencias ITS usando análisis de IB. En los nodos se muestran las probabilidades posteriores bayesianas seguidas de los valores de bootstrap de ML; las longitudes de las ramas reflejan el número estimado de cambios por sitio.

TAXONOMY

Lyomyces denudatus Viner, sp. nov. Fig. 2, 3
MycoBank MB 844866

Typification: ARGENTINA. Tierra del Fuego Province: Ushuaia, Monte Olivia, -54.7°: -68.2°, on *Nothofagus* bark, 23 Feb 1982, *L. Ryvarden 19436* (holotype H 7006820, isotype O). GenBank: ITS = ON980760.

Etymology: *denudatus* (Latin), denuded, in reference to the naked ends of cystidia, which in their proximal part are sheathed in thick solid crystalline matter.

Basidiocarps effused, 150–250 μm thick, up to 3 cm in the widest dimension. Margin distinct, hymenial surface cream-colored, smooth. Hyphal system monomitic; hyphae clamped, thin-walled, in subiculum also slightly thick-walled. Large clusters of crystalline matter (dissolved in 5% KOH) are sprinkled throughout the basidiocarp, which obscures the hyphal structure on some slides. If not covered by crystalline matter, individual hyphae can be observed. Subhymenial hyphae slightly cyanophilic, (1.1–)1.5–3.5 μm wide ($n = 50/4$). Subicular hyphae not cyanophilic, branched mostly at right angles, 2–4(–4.5) μm wide ($n = 50/4$). Cystidial elements mostly capitate rarely tapering, (21–)34.9–62 \times (3.5–)4–5.5(–7) μm ($n = 80/4$). Most of them are covered by thick solid crystalline matter in their proximal part while their ends lack any crystals. Single cystidia are almost naked, in this case, the proximal part of cystidia is clearly thick-walled. Basidia suburniform, 4-spored, 15–21.1(–25) \times 3.8–5.5 μm ($n = 40/4$). Basidiospores thin-walled, narrowly ellipsoid, slightly cyanophilic, (4.1–)4.8–7 \times 2.8–4.2(–4.7) μm ($n = 120/4$), $L = 5.74$, $W = 3.70$, $Q = 1.56$.

Ecology and distribution.— Our data suggest that *L. denudatus* occurs both in New Zealand and the extreme south of South America. All Argentinean collections studied by us grew on *Nothofagus*. Unfortunately, the substrate data for the New Zealand sequence of *L. denudatus* is limited to a “down branch” only.

Other specimens examined.— ARGENTINA. Tierra del Fuego Province: Ushuaia, estancia Harberton, on *Nothofagus* bark, 18 Feb 1982, *L. Ryvarden 19152* (O, duplicate H 7006819); the Tierra del Fuego National Park, 15 km west of Ushuaia, on *Nothofagus* wood, 19 Feb 1982, *L. Ryvarden 19233* (O, duplicate H 7006818); *L. Ryvarden 19256* (O, duplicate H 7006815).

Notes.— While making the decision to introduce *L. denudatus* as a new species, we were guided by the following considerations. Its characteristic cystidia allow separating this species from all described *Lyomyces* we know, which, in our opinion, makes distinguishing *L. denudatus* easy. According to our molecular analyses, *L. denudatus* is distant enough (the closest match is 92.7 %, or 41 bp difference in ITS) from any described *Lyomyces* in public databases, to not belong to some recently described species.

We studied the types of its relatives *L. fimbriatus* from Taiwan (*Wu 880729-13*) and *L. densiusculus* from Uganda (*Ryvarden 44818*). *Lyomyces fimbriatus* has grandini-

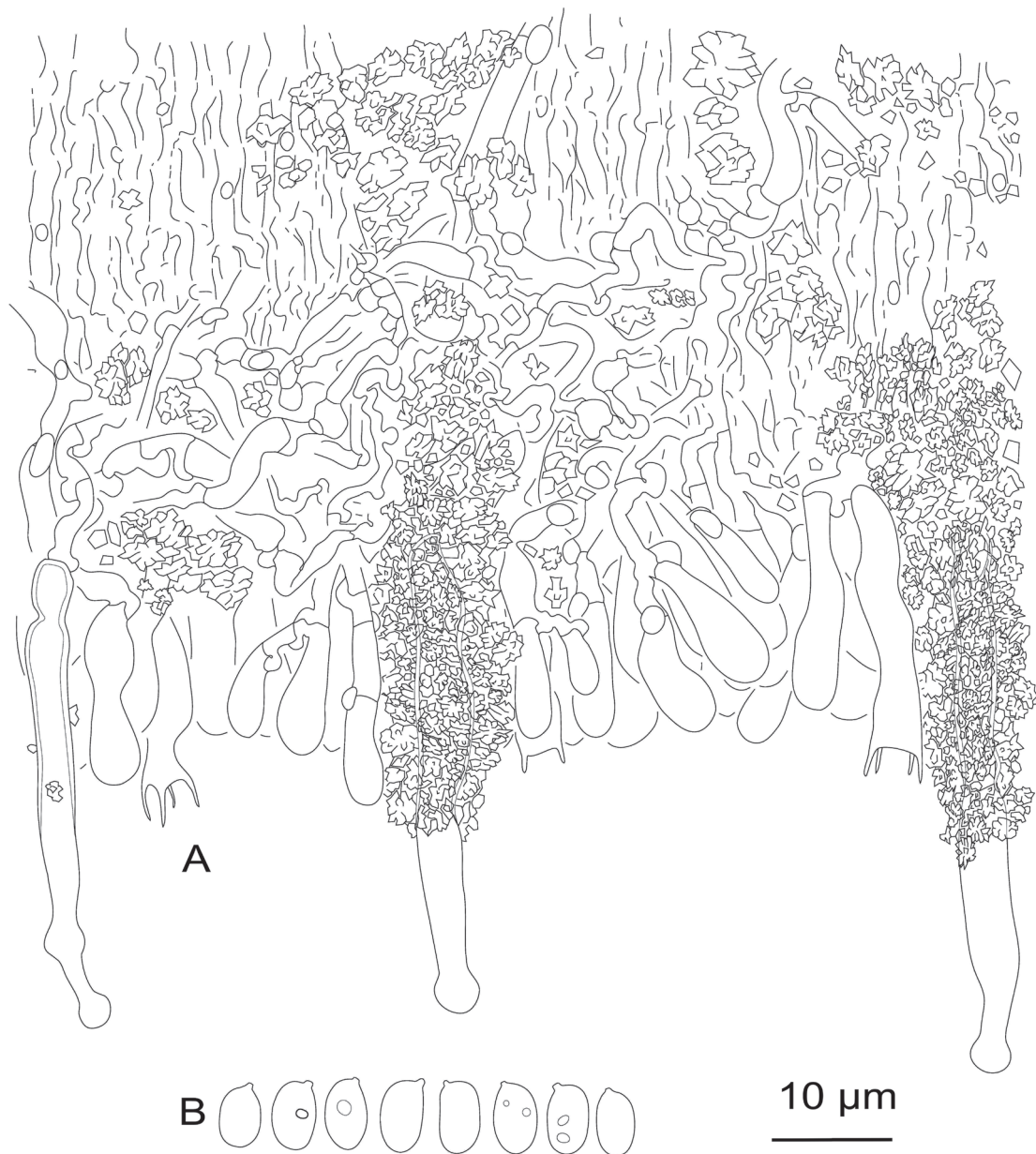


Fig. 2. *Lyomyces denudatus*. A) Section of the basidiocarp and subhymenium including cystidia. B) Spores. Ryvarden 19436 (holotype H).

Fig. 2. *Lyomyces denudatus*. A) Sección del basidiocarp y subhimenio incluyendo cistidios. B) Esporas. Ryvarden 19436 (holotipo H).

oid basidiocarps with fimbriate projections. Its cystidia vary more in shape, do not have thick crystalline sheath, and lack thickening of the cell wall in the proximal part of cystidia. *Lyomyces densiusculus* has less differentiated, shorter cystidia covered at most with single crystals, and its subhymenium consists of densely packed hyphae. In addition, both *L. densiusculus* and *L. fimbriatus* have smaller spores than *L. denudatus*.

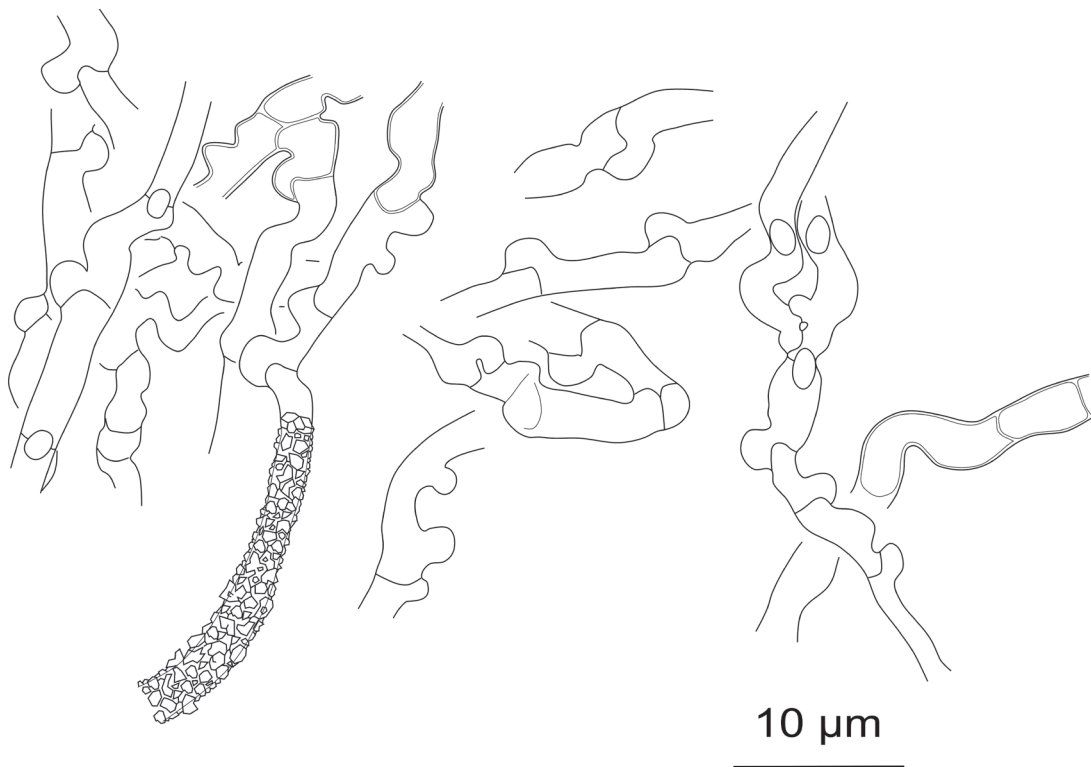


Fig. 3. *Lyomyces denudatus*. Subicular hyphae. Ryvarden 19436 (holotype H).

Fig. 3. *Lyomyces denudatus*. Hifas subiculares. Ryvarden 19436 (holotipo H).

DISCUSSION

As treated here, *Lyomyces denudatus* has a disjunct distribution, which is supported by the perfect match of a New Zealand sequence (0 bp difference) to our Argentinean material. Our finding goes in line with a number of austral wood-decaying fungi considered conspecific in New Zealand/Tasmania and Argentina (Rajchenberg, 1995, 2003; Greslebin *et al.*, 2000; Miettinen & Rajchenberg, 2012). If this interpretation is correct, one explanation for such a distribution pattern may be driftwood carried by the Antarctic Circumpolar Current. An alternative explanation includes a historical connection between these landmasses in the Mesozoic era, which seems less likely since it implies more than enough time for multiple speciation events in diverged populations. In any case, we are unwilling to make far-reaching conclusions. A denser gene sampling might split *L. denudatus*—similarly to other cases of presumable New Zealand/South American conspecificity—into more than one species but this is clearly out of the scope of this paper.

The closest relatives of *L. denudatus*, East Asian species, *L. fimbriatus*, *L. fumosus* and *L. leptocystidiatus*, belong to a well-supported clade but our analyses do not resolve these three species as separate. Thus, recognition of *L. fumosus* and *L. leptocystidiatus* as independent species is questionable. We refrain from reducing them to synonyms of *L. fimbriatus*, which has nomenclature priority. The strong phylogenetic structure of the clade in question (Fig. 1) indicates that *L. fumosus* and *L. leptocys-*

tidiatus might still pass muster. Verifying this will require producing a multigene dataset for all three species and studying their types.

We were not able to study the types of *L. fumosus* and *L. leptocystidiatus*, which is especially relevant in this case as the descriptions of these two species (Luo *et al.*, 2021; Wang *et al.*, 2021) do not exclude their conspecificity with *L. fimbriatus* (Wu, 1990). Moreover, microscopic drawings in Luo *et al.* (2021) and Wang *et al.* (2021) are misleading to the point of confusion with unrelated genera such as *Hyphoderma* Wallr. Viner *et al.* (submitted) pointed out that the LSU sequence of the *L. fumosus* paratype (Zhao 17855) is clustered in a clade distant from the corresponding holotype (Zhao 8188). However, published ITS shows a contrary pattern: ITS sequences of the holotype and paratype are close enough to end up in the same clade as affirmed by our analyses. Fast-evolving ITS and conservative LSU are adjoining regions, and the most likely explanation for the conflicting signal is sequence identity confusion, indicating an issue with data quality in Luo *et al.* (2021). A revision of their material is clearly needed.

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