Phytotaxa 759 (1): 031–044
https://www.mapress.com/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.759.1.3

Strobilomyces pedireticulatus (Boletaceae, Boletales) a new species in section *Strobilomyces* from Central Mexico

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Abstract

The genus *Strobilomyces* constitutes a phylogenetically distinct lineage within the Boletaceae family, recognized for its significant biocultural, economic and forestry relevance. However, its diversity and distribution within Mexico remain notably understudied. Here in, *Strobilomyces pedireticulatus* is formally described based on specimens collected from mixed-conifer forest ecosystems in Tlaxcala State, Mexico. Morphological characteristics and molecular phylogenetic analyses provide the diagnostic evidence for this identification. Additionally, it has specific biocultural importance within this region. Comprehensive documentation includes detailed macro- and microscopic descriptions, photographic record of fresh basidiomata in situ and ex situ, and analytical illustrations of key diagnostic features. Descriptions, photographs and illustrations of *Strobilomyces pedireticulatus* are provided.

Key words: biocultural importance, *Pinus*, ectomycorrhizal, edible species

Introduction

The genus *Strobilomyces* Berk. (1851: 78) (Boletaceae, subfamily Boletoideae) (Wu *et al*. 2016) exhibits readily identifiable basidiomata presenting whitish, greyish, dark-brown, yellow-brown to blackish coloration. Distinctive morphological features include pileus surface ornamentation ranging from fine to pyramidal scales and basidiospores displaying reticulate, semireticulate, and echinate to verrucose ornamentation (Singer, 1986; Han *et al*. 2018). Based on morphological and molecular characters, the genus is divided into two sections: Section *Echinati* L.H. Han, Zhu L. Yang & Ndolo Ebika is characterized by echinate ornamented basidiospores, exhibits a distribution restricted to tropical Africa, which is considered the region of origin for the genus (Han *et al*. 2018), and forms an ectomycorrhizal association with *Gilbertiodendron* J. Léonard (1952;22) (Fabaceae). In contrast, section *Strobilomyces* Berk., possesses basidiospores that are reticulate, semireticulate or echinate to verrucose as can be seen in Figure 1. This section demonstrates a broad distribution across Asia, Europe, and North America, though it is absent from South America. It establishes ectomycorrhizal symbioses primarily with members of Dipterocarpaceae, Fabaceae, Myrtaceae, Fagaceae, mainly *Quercus* (1753:10), and Pinaceae, mainly *Pinus* (1753:1000) (Sato *et al*. 2017; Han *et al*. 2018).

In Mexico, species occurred within temperate and subtropical regions, forming associations predominantly with

Accepted by Carlos Salvador: 5 May 2026; published: 27 May 2026

Quercus spp., and *Pinus* spp. (Ayala-Vásquez *et al*. 2018; Ayala-Vásquez, 2021). Globally, 45 species have been formally described utilizing both morphological and molecular data (Index Fungorum, Deng *et al*. 2023). In Mexico, *Strobilomyces confusus* Singer (García and Castillo 1981), *S. dryophilus* although this species has been invalidated (Roman-Sarabia *et al*. 2024), and *S. strobilaceus* (Heim and Perreau 1964) have been described; however, Petersen *et al*. (2012) mention that *S. strobilaceus* is only distributed in Europe. The genus *Strobilomyces* holds significant economic, biocultural and forestry importance (Ayala-Vásquez 2021; Han *et al*. 2020). Several species, including *S. confusus* Singer (1945:108), *S. coturnix* Bouriquet (1946:25), *S. glabriceps* W.F. Chiu (1948:40), *S. seminudus* Hongo (1983:23), *S. strobilaceus* (Scop.) Berk. (1851:3), and *S. velutipes* Cooke & Massee (1889:18) are recognized for their edibility and associated economic value (Li *et al*. 2021; Burrola-Aguilar *et al.* 2024).

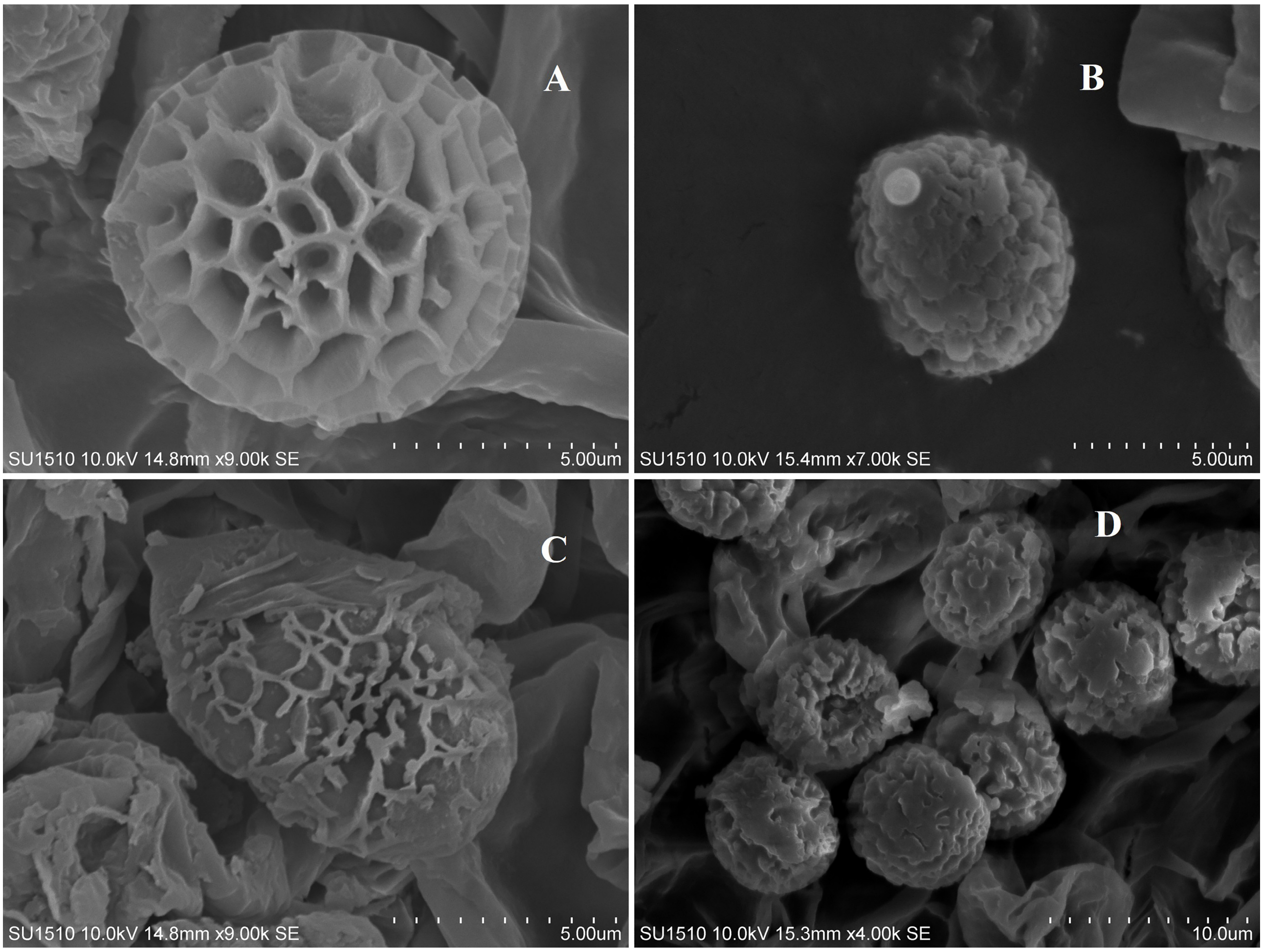

FIGURE 1. Type of basidiospores of *Strobilomyces* section on Scanning Electron Microscopy. A) Reticulate, B) Verrucose, C) Semi-reticulate, D) verrucose-echinate. (unpublished photographs AVO).

Within Mexico, species possess notable biocultural significance among several native cultures. For instance, the Tlahuica- *Pjiekakjoo* refer to one species as "Nchjo tyumendye" (grandfather's mushroom) (Ramírez-Carbajal *et al.* 2025). Significant cultural use is also documented in various communities within the state of Mexico (Burrola-Aguilar *et al.* 2024); among the Zapotec culture of the Sierra Juárez of Oaxaca (Garibay-Orijel *et al.* 2009), and within Nahuatl communities in Zaragoza, Puebla and Ojitos de Agua, Guerrero (pers. comm.).

Materials & methods

Sampling and morphological characterization—Specimen collection was conducted within the forested area of Piedra Canteada, Tlaxcala, during the period spanning August to September 2021. Morphological characterization followed the methodologies established by Lodge *et al*. (2004) and Rathnayaka *et al*. (2024). Colorimetric determinations for taxonomic descriptions were based on Kornerup and Wanscher (1978). Microscopic analysis employed an optical microscope (Leica DM3000, Germany) for quantitative measurements. Basidiospore dimensions are reported as follows: the quantity measured is denoted n/m, where n signifies the total number of spores measured and m the number of basidiomata sampled; Q denotes the quotient of mean spore length divided by mean width (Lm/Wm). Basidiospores ornamentation was examined using a ZEISS Sigma 300 Scanning Electron Microscope. Voucher specimens were accessioned into the National Herbarium of Mexico of the Institute of Biology of the National Autonomous University of Mexico (MEXU).

DNA Extraction, PCR amplification, and sequencing—We obtained the DNA from herbarium specimens. The CTAB protocol described by Martínez-González *et al.* (2017) was used to extract genomic DNA. Sequences of Internal Transcribed Spacer (ITS), nuclear large subunit ribosomal DNA (nLSU), translation factor 1-α (*tef*1-α), and polymerase II second largest subunit gene (*rpb2*) genes were amplified by polymerase chain reaction (PCR) with the primer pairs ITS4 + ITS5 (White *et al.* 1990), LROR + LR7 (White *et al.* 1990), EF1 + TEF1 (Carbone and Kohn, 1999), RPB2-B-F1 + RPB2-B-R (Wu *et al.* 2014), respectively.

The reaction mixture for PCR was prepared in a final volume of 15 µL containing: a buffer of the enzyme 1x Taq DNA polymerase, 0.8 mM deoxynucleoside trifosfate (0.2 mM of each one), 100 ng DNA, 20 pmol of each primer and 2 units of GoTaq DNA (Promega, USA). The PCR conditions were as follows: initial denaturation at 94 °C for 3 min, then denaturation at 94 °C for 30 s, annealing for 45 s with the corresponding temperatures (57 °C for ITS, 53 °C for nLSU, 56 °C for *tef*1-α, and 52 °C for *rpb2*), extension at 72 °C for 1 min, followed by 35 cycles, then a final extension at 72 °C for 10 min, using an Applied Biosystems S1000 ™ Thermal Cycler machine. The amplifications were verified through electrophoresis in an agarose gel at 1.2% prepared with 1× TAE buffer (Tris Acetate-EDTA) and run at 87 V cm^{-3} during for 1 hr. The gel was stained with GelRed (Biotium, USA) and the bands were visualized in an Infinity 3000 transilluminator (Vilber Lourmat, Germany). The amplified products were purified with the ExoSAP Purification kit (Affymetrix, USA), following the manufacturer's instructions. They were quantified and prepared for the sequence reaction using a BigDye Terminator v.3.1 (Applied Biosystem). These products were sequenced in both directions with an Applied Biosystem model 3730XL (Applied BioSystems, USA), at the Institute of Biology of the Universidad Nacional Autónoma de México (UNAM).

The sequences of both strands of each of the genes were analyzed, edited, and assembled using BioEdit version 7.0.5 (Hall 1999) to generate a consensus sequence. These consensus sequences were compared with those deposited in GenBank of the National Center for Biotechnology Information (NCBI), using the BLASTN 2.2.19 tool (Zhang *et al.* 2000).

Phylogenetic Analyses—Sequence data, retrieved from GenBank based on previous studies, are listed in Table 1. The sequences were subjected to standard BLASTn (http://www.blast.ncbi.nlm.nih.gov) searches in GenBank to determine the primary identity of the fungal isolates.

Each region was independently aligned using the online version of MAFFT v7 (Katoh *et al.* 2002, 2013). The alignments were revised in PhyDE v. 10.0 (Müller *et al.* 2005), followed by minor manual adjustments to ensure character homology between taxa. The matrices were formed for ITS by 60 taxa (651 characters), for nLSU by 32 taxa (521 characters), for *tef 1-α* region consisted of 127 taxa (710 characters); and the *rpb2* region comprised of 75 taxa (585 characters), and the matrix was formed for nLSU by 25 (719 characters). Eight partition schemes were established: one for the ITS, one for the nLSU, three for the *tef 1-α,* and three for the *rpb2* which were established using the option to minimize the stop codons with Mesquite v3.2 (Maddison & Maddison 2021). The data were analyzed using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). Maximum parsimony analyses were carried out in PAUP* 4.0b10 (Swofford 2002) using the heuristic search mode, 1000 random starting replicates, and TBR branch swapping, with MULTREES and Collapse on. Bootstrap values were estimated using 1000 bootstrap replicates under the heuristic search mode, each with 100 random starting replicates. Maximum likelihood analyses were carried out in RAxML v. 8.2.10 (Stamatakis, 2014) with a GTR + G model of nucleotide substitution. To assess branch support, 10000 rapid bootstrap replicates were run with the GTRGAMMA model. Bayesian inference was carried out in MrBayes v. 3.2.6 x64 (Huelsenbeck & Ronquist, 2001) with four chains and the best evolutionary model for alignment was sought using PartitionFinder (Frandsen *et al.* 2015; Lanfear *et al.* 2014, 2017). Phylogenetic analyses were performed using MrBayes v3.2.6 x64 (Huelsenbeck & Ronquist 2001). The information block for the matrix included two simultaneous runs of Montecarlo chains, temperature set to 0.2 and sampling 10 million generations (standard deviation ≤ 0.1). Chain convergence was visualized in Tracer v.1.6 (Rambaut *et al.* 2018). The remaining trees were used to calculate a 50% majority-rule consensus topology and posterior probabilities (PP). Trees were visualized and optimized in FigTree v. 1.1.4 (Rambaut, 2018), and then edited in Adobe Illustrator (Adobe Systems, Inc., San Jose, CA).

TABLE 1. GenBank accession numbers corresponding to the sequences used in the phylogenetic analyses. In bold the accessions of the new species.

Species	**Strain**	**Country**	**nLSU**	**ITS**	***tef*1-α**	***rpb2***
Afroboletus luteolus	PC0723573	Zambia	-	-	KX869290	KX869416
Anthracoporus holophaeus	HKAS59407	China	KT990708	-	KT990888	KT990506
Strobilomyces albidus	HKAS89104	China	-	-	KX869340	KX869469
Strobilomyces albidus	NY1393538	Thailand	-	-	KX869343	KX869472
Strobilomyces alpinus	HKAS77969	China	-	MG832045	KX869254	KX869380
Strobilomyces alpinus	HKAS57770	China	-	MG832044	-	KX869382
Strobilomyces alpinus	HKAS94051	China	-	-	KX869255	KX869381
Strobilomyces annulatus	NY1393525	New Guinea	-	-	KX869256	KX869383
Strobilomyces anthracinus	HKAS74532	China	-	MG832047	KX869330	KX869459
Strobilomyces anthracinus	HKKAS52570	China	-	-	KX869331	KX869460
Strobilomyces anthracinus	HKAS83740	China	-	-	KX869329	KX869458
Strobilomyces atrosquamosus	HKAS78563	China	KT990649	MG832048	KX869262	KX869388
Strobilomyces atrosquamosus	HMAS262149	China	-	-	KX869259	KX869385
Strobilomyces atrosquamosus	HKAS84682	China	-	MG832049	KX869261	KX869387
Strobilomyces baozhengii	FMMU1937	China	OR036230	OR035750	OR051644	OR051645
Strobilomyces baozhengii	FHMU3129	China	-	OR035751	-	OR051646
Strobilomyces brunneolepidotus	HKAS81935	China	-	MG832053	KX869265	KX869391
Strobilomyces brunneolepidotus	HKAS84683	China	-	-	KX869264	KX869390
Strobilomyces brunneolepidotus	HKAS80689	China	-	-	KX869263	KX869389
Strobilomyces brunneolepidotus	N.K. Zeng 2575	China	MT829135	MT822947	-	-
Strobilomyces calidus	HKAS87084	China	-	MG832085	KX869358	KX869487
Strobilomyces calidus	HKAS84700	China	-	MG832084	KX869359	KX869488
Strobilomyces cingulatus	HKAS73175	China	-	MG832050	KX869332	KX869461
Strobilomyces cingulatus	HKAS75582	China	-	MG832051	KX869334	KX869463
Strobilomyces cingulatus	HKAS91330	China	-	-	KX869335	KC869464
Strobilomyces conicus	N.K. Zeng 3116	China	OR036231	OR035752	OR051632	-
Strobilomyces confusus	WU17032	USA	-	MG832054	KX869267	KX869393
Strobilomyces confusus	WU17130	USA	-	-	KX869268	KX869394
Strobilomyces confusus	CFMR	Dominican Republic	MK601809	-	MK721163	MK766365
Strobilomyces densisquamosus	HKAS91250	China	-	-	KX869357	KX869486
Strobilomyces densisquamosus	HKAS82354	China	-	MG832055	KX869352	KX869481
Strobilomyces densisquamosus	HKAS83112	China	-	-	KX869354	KX869483
Strobilomyces douformis	HKAS87097	China	-	MG832058	KX869345	KX869474
Strobilomyces douformis	HKAS87134	China	-	MG832057	KX869346	KX869475
Strobilomyces echinatus	Sydney 1597	Congo	-	-	KX869269	KX869395
Strobilomyces dryophilus	72106	USA	KF030345	------	-	------
Strobilomyces echinocephalus	TO MG001	China	-	JX870649	-	--
Strobilomyces echinocephalus	HKAS59420	China	KF112463	MG832059	KX869274	KX869400
Strobilomyces echinocephalus	HKAS92153	China	-	-	KX869275	KX869401
Strobilomyces floccopus	Sf1	Germany	DQ534623	-	JQ327037	-
Strobilomyces floccopus	AFTOL-ID 716	-	AY684155	AY854068	AY883428	AY786065
Strobilomyces floccopus	RW103	Belgium	-	-	KT824044	KT824011
Strobilomyces foveatus	FRI62957	Malaysia	-	-	KX869277	KX869403
Strobilomyces foveatus	FRI69468	Malaysia	-	-	KX869276	KX869402
Strobilomyces giganteus	HKAS93250	China	-	MG832062	-	KX869454
Strobilomyces giganteus	HKAS77026	China	-	-	KX869328	KX869456
Strobilomyces giganteus	NY1393514	Thailand	-	-	KX869327	KX869455

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TABLE 1. (Continued)

Species	**Strain**	**Country**	**nLSU**	**ITS**	***tef*1-α**	***rpb2***
Strobilomyces giganteus	HKAS59637	China	KT990645	-	KT990838	KT990475
Strobilomyces glabellus	HKAS74887	China	-	MG832063	KX869278	KX869404
Strobilomyces glabriceps	HKAS78573	China	-	MG832065	KX869281	KX869407
Strobilomyces glabriceps	HKAS74964	China	-	MG832064	KX869280	KX869406
Strobilomyces glabriceps	Sharma S357	India	-	-	KX869279	KX869405
Strobilomyces hainanensis	N.K. Zeng 3210	China	OR036232	-	OR051633	-
Strobilomyces huangshanensis	HKAS 102613	China	-	-	MK329219	MK329217
Strobilomyces huangshanensis	HKAS 102612	China	-	-	MK329218	MK329216
Strobilomyces latirimosus	HKAS74865	China	KF112467	MG832066	KX869284	KX869410
Strobilomyces latirimosus	HKAS53348	China	KF112462	-	KX869289	KX869415
Strobilomyces latirimosus	HKAS77697	China	-	-	KX869288	KX869414
Strobilomyces latirimosus	HMAS250943	China	-	-	KX869287	KX869413
Strobilomyces microreticulatus	HKAS74863	China	-	MG832069	KX869337	KX869466
Strobilomyces microreticulatus	HKAS74963	China	-	MG832068	KX869336	KX869465
Strobilomyces mollis	HKAS59833	China	-	-	KX869295	KX869422
Strobilomyces montosus	HKAS74910	China	-	MG832072	KX869297	KX869424
Strobilomyces montosus	HKAS74809	China	KF112459	-	KX869296	KX869423
Strobilomyces minor	HKAS 101909	China	-	-	MK183829	MK183827
Strobilomyces minor	HKAS 101910	China	-	-	MK183830	MK183828
Strobilomyces mirandus	HKAS80364	China	-	MG832071	-	KX869420
Strobilomyces mirandus	HKAS59408	China	-	MG832070	KX869293	KX869419
Strobilomyces mirandus	OR115	Thailand	-	-	KT824038	KT824005
Strobilomyces pachycystidiatus	N.K. Zeng 2987	China	OR036233	OR035753	OR051634	OR051647
Strobilomyces pachycystidiatus	N.K. Zeng 2248	China	OR036234	OR035754	OR051635	OR051648
Strobilomyces pachycystidiatus	N.K. Zeng 2937	China	OR036235	OR035756	OR051636	-
Strobilomyces pachycystidiatus	N.K. Zeng 4427	China	OR036236	OR035757	OR051637	-
Strobilomyces pachycystidiatus	N.K. Zeng 4431	China	OR036237	OR035758	OR051638	-
Strobilomyces pachycystidiatus	N.K. Zeng 4434	China	OR036238	OR035759	OR051639	-
Strobilomyces pachycystidiatus	N.K. Zeng 2939	China	OR036239	OR035760	OR051640	OR051649
Strobilomyces pachycystidiatus	N.K. Zeng 4469	China	OR036240	OR035761	OR051641	OR051650
Strobilomyces pachycystidiatus	N.K. Zeng 2930	China	OR036241	OR035762	OR051642	-
Strobilomyces parvirimosus	HKAS74911	China	KF112460	-	KX869301	KX869428
Strobilomyces pinophilus	HKAS80300	China	-	MG832074	KX869350	KX869479
Strobilomyces pinophilus	HKAS94132	China	-	-	KX869351	KX869480
Strobilomyces pteroreticulosporus	HKAS80299	China	-	MG832076	KX869304	KX869431
Strobilomyces pteroreticulosporus	HKAS80350	China	-	MG832075	KX869303	KX869430
Strobilomyces pteroreticulosporus	HKAS91274	China	-	-	KX869306	KX869433
Strobilomyces pteroreticulosporus	HKAS80191	China	-	-	KX869305	KX869432
Strobilomyces pedireticulatus	**MEXU 30719**	**Mexico**	**PX088153**	**PX070275**	**MX52102**	**MX52154**
Strobilomyces pedireticulatus	**MEXU 30720**	**Mexico**	PX088154	**PX070276**	**MX52103**	**MX52155**
Strobilomyces pedireticulatus	**MEXU 30721**	**Mexico**	**PX088155**	**PX070277**	**MX52104**	**MX52156**
Strobilomyces rubrobrunneus	HKAS 101906	China	-	-	MH485372	MH485369
Strobilomyces sculptus	HKAS102606	China	-	-	MK241491	MK241488
Strobilomyces sculptus	HKAS101911	China	-	-	MK241490	MK241487
Strobilomyces sculptus	HKAS102610	China	-	-	MK241489	MK241486
Strobilomyces seminudus	HKAS59461	China	KF112479	-	KX869311	KX869438
Strobilomyces seminudus	NY1393550	Thailand	-	-	KX869310	KX869437
Strobilomyces seminudus	HKAS77085	China	-	-	KX869309	KX869436

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TABLE 1. (Continued)

Species	Strain	Country	nLSU	ITS	*tef*1-α	*rpb2*
Strobilomyces seminudus	HKAS83471	China	-	-	KX869307	KX869434
Strobilomyces seminudus	HKAS82848	China	-	-	KT990835	KT990472
Strobilomyces sp.	WU17057	USA	-	MG832080	KX869365	KX869494
Strobilomyces sp.	WU17033	Mexico	-	MG832081	KX869339	KX869468
Strobilomyces sp.	WU17052	Mexico	-	MG832082	KX869338	KX869467
Strobilomyces sp.	HKAS84055	USA	-	MG832083	KX869366	KX869495
Strobilomyces sp.	HKAS74893	China	-	-	KX869364	KX869493
Strobilomyces sp.	HKAS92326	China	-	-	KX869369	KX869498
Strobilomyces sp.	NY1393513	Australia	-	-	KX869367	KX869496
Strobilomyces sp.	PC0723581	Madagascar	-	-	KX869368	KX869497
Strobilomyces sp.	NY1034408	Australia	-	-	KX869348	KX869477
Strobilomyces sp.	NY2072541	Australia	-	-	KX869349	KX869478
Strobilomyces sp.	NY1491183	Australia	-	-	KX869347	KX869476
Strobilomyces sp.	MEL695356	Australia	-	-	KX869370	KX869499
Strobilomyces sp.	OR0778	Thailand	-	-	MG212610	MG212651
Strobilomyces sp.	CFMRDR555	Dominican Republic	MK601810	-	MK721164	MK766366
Strobilomyces sp.	OR0259	China	-	-	MG212609	MG212650
Strobilomyces sp.	OR0319	Thailand	-	-	MH614738	MH614785
Strobilomyces sp.	NY1193833	Australia	-	-	KX869361	KX869490
Strobilomyces sp.	NY1193834	Australia	-	-	KX869360	KX869489
Strobilomyces sp.	-	-	-	-	KX869324	KX869451
Strobilomyces sp.	OR1092	Thailand	-	-	MH614739	MH614786
Strobilomyces sp.	NY1034410	Australia	-	-	KX869324	KX869451
Strobilomyces sp.	NY1034411	Australia	-	-	KX869325	KX869452
Strobilomyces strobilaceus	WU17111	Mexico	-	MG832086	KX869316	KX869443
Strobilomyces strobilaceus	WU0016537	Austria	KT990647	-	KX869314	KX869441
Strobilomyces strobilaceus	MB001177	Germany	-	-	KX869313	KX869440
Strobilomyces subnudus	HKAS59435	China	KF112464	MG832088	KX869318	KX869445
Strobilomyces subnudus	HKAS82418	China	-	MG832087	KX869321	KX869448
Strobilomyces subnudus	HKAS844811	China	-	-	KX869320	KX869447
Strobilomyces velutinus	HKAS84755	China	-	MG832089	KX869322	KX869449
Strobilomyces velutinus	HKAS84776	China	-	-	KX869323	KX869450
Strobilomyces velutipes	NY1193952	Australia	-	-	KX869363	KX869492
Strobilomyces velutipes	NY2072516	Australia	-	-	KX869362	KX869491

Results

Phylogenetic analysis

The dataset of combined ITS, nLSU, *tef 1-α*, and *rpb2* comprised 127 taxa with 2467 characters including gaps. The three phylogenetic analyses, MP, ML, and BI, of the dataset recovered similar topologies (Fig. 2). No significant conflict (bootstrap value >80%) was detected among the topologies obtained via the separate phylogenetic analyses. The parsimony analysis of the alignment found 1021 trees of 185 steps (CI=0.1625, HI=0.1421, RI=0.2931, RC=0.1834). The best RAxML tree with a final likelihood value of -28063.003487 is presented. The matrix had 865 distinct alignment patterns, with 2.99% undetermined characters or gaps. Estimated base frequencies were as follows: A= 0.100244, C= 0.192375, G= 0.143012, T= 0.193177; substitution rates AC= 1.006155, AG= 1.903544, AT= 1.930000, CG= 1.803466, CT= 3.934015, GT= 1.0098635; gamma distribution shape parameter α= 0.000512. In the Bayesian analysis, the standard deviation between the chains stabilized at 0.004 after 7.8 million generations. No significant changes in tree topology trace or cumulative split frequencies of selected nodes were observed after about 0.31 million generations, which were discarded as 25% burn-in. The dataset of nLSU comprised 25 taxa with 719 characters. The three phylogenetic analyses, MP, ML, and BI, of the dataset recovered similar topologies (Fig. 3). The phyogram shows that *Strobilomyces pedireticulatus* belongs to the same clade as *Strobilomyces dryophilus*, with a support of 70MP/-ML/0.75IB; however, they are different species, with a support of 100MP/100ML/1IB.

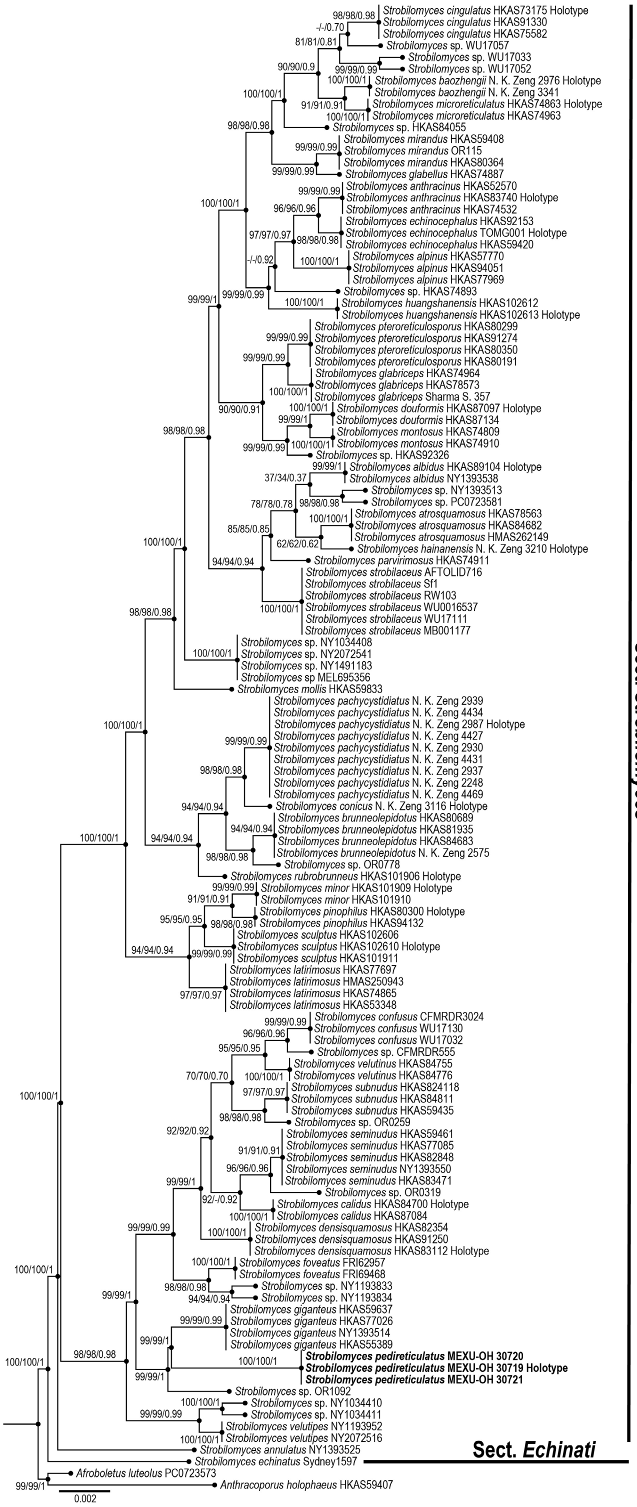

FIGURE 2. Maximum likelihood phylogeny based on the ITS + nLSU + *tef 1-α* + *rpb2* sequence data. Maximum parsimony and Bayesian analyses recovered identical topologies concerning the relationships among the main clades of *Strobilomyces*. For each node, the following values are provided: maximum parsimony bootstrap (%) / maximum likelihood bootstrap (%) / and posterior confidence (p-value). The scale bar represents the expected number of nucleotide substitutions per site. The newly proposed species of *Strobilomyces pedireticulatus* are shown in bold.

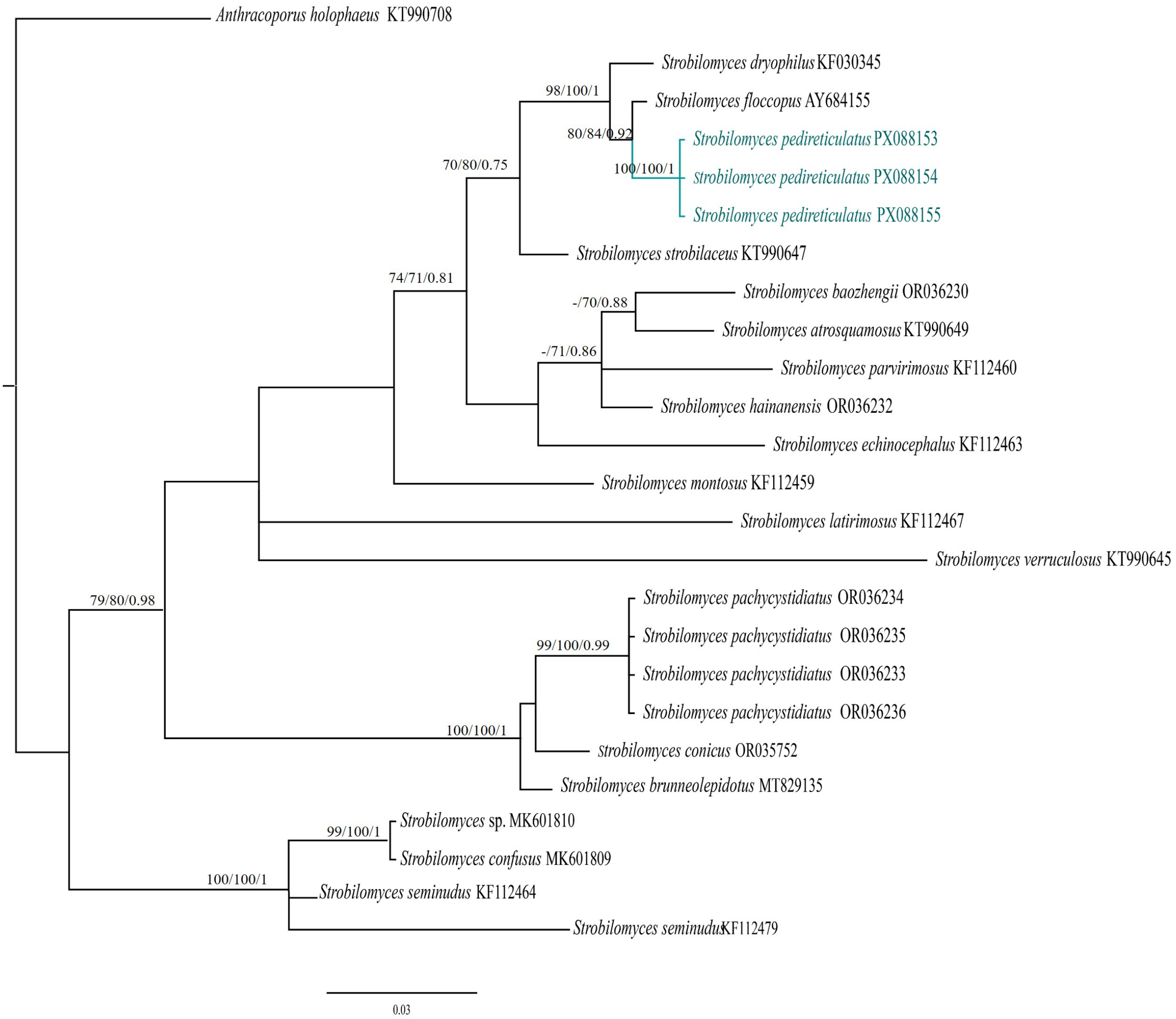

FIGURE 3. Bayesian analyses phylogeny (**p**-value) and Maximum likelihood bootstrap (%) based on the nLSU sequence data. The scale bar represents the expected number of nucleotide substitutions per site. The newly proposed species of *Strobilomyces pedireticulatus* are shown in green. *Anthracoporus holophaeus* is shown as the outgroup.

Taxonomy

Strobilomyces pedireticulatus Ayala-Vásquez, Balbuena-Carrasco & Pérez-Moreno *sp. nov.*
Figures 4–5
MycoBank No: MB860157

Diagnosis:—Basidiomata medium-sized. Stipe centrally to eccentrically positioned, cylindrical to subclavate at the top widened in some specimens; coloration ranging from whitish through pale grey to blackish brown. Surface exhibits pronounced reticulation to alveolation, predominantly from the upper third part to the apex; with a prominent, thickened annulus present at the stipe apex, and base markedly attenuated.

Basidiospores (7–) 8–10 (–12) × (7–)8–9 µm, broadly ellipsoid to subglobose. Episporial ornamentation verrucose to semireticulate. The germinative pore and the hilar appendix display reticulated-alveolate ornamentation.

Holotype:—MEXICO, Tlaxcala, Nanacamilpa, San Felipe Hidalgo, Piedra Canteada, 2818m, 19°27'23"N, 19°27'29"W, Ayala-Vásquez O., 6 de octubre del 2021, C174 (MEXU 30719).

Etymology:—The specific epithet is derived from the Latin word '*pedi*' (foot or stipe of the basidiomata), and '*reticulatus*' (netted), denoting the prominent reticulum present predominantly over the distal upper third part of the stipe.

Description:—Pileus 24–70 mm diameter, broadly convex to convex when young becoming plane-convex with age, brownish-grey (6E3), pale brown (5D3), brown-yellowish (5F4), brown (6F8) to blackish; margin persistently whitish, the pileus surface initially covered by a dense layer of semi-circular to irregular, whitish scales revealing a pale background; scales becoming brown at maturity, mature specimens frequently develop deep radial fissures exposing underlying hymenophore, partial veil remnants from a partial, persistent marginal zone 3–6 mm wide; initially whitish, darkening to rusty-black. Hymenophore broadly adnate to decurrent, pores initially occluded, becoming open at maturity, 0.4–1 mm in diameter. Color development of pores sequential: entirely whitish (2A1) when immature, progressing to greyish-white (1B1) or beige (2B2) at young stages, then beige (2B2), pale greyish (2C1), brown-yellowish (5E4), brown (6F8), to blackish in age, tubes 4–8mm long, concolorous with pores; context exhibiting immediate bruising reaction, staining brownish-orange (5C5–5C6) when cut. Context of the pileus 5–10 mm thick, spongy, whitish, upon sectioning, exhibits reddish-brown (8D7–8D8) coloration throughout, with central regions darkening to dark-brown (7F7–7F8) to blackish; context of the stipe whitish, spongy, when cut the apex turns dark-brown (7F7–7F8), the middle section reddish-brown (8D7–8D8) and the base dark-brown (7F7–7F8) to black. Stipe 108–115×21–40 mm, typically central, occasionally eccentric, cylindrical to subclavate at the top widened in some specimens; surface ornamentation reticulate-alveolate, with reticulum extending over most or all of the length, whitish background developing pale greyish (2C1) to brown (6E4) tones, maturing to predominantly dark-brown (7F7–7F8) basally. reticulum present and predominantly obvious over the stipe apex, forming cylindrical to angular alveolate ornamentation 2–4 mm deep and 4–6 mm in diameter. Conspicuous, thick, cottony-membranous annulus present in young specimens and, initially whitish, developing pale greyish (2C1), until becoming blackish with age. In immature specimens, this annulus envelops the developing hymenophore. Upon maturity, it detaches almost completely from the stipe, becoming displaced and typically persistent as fragmented remnants along the pileal margin.

FIGURE 4. A–B *Strobilomyces pedireticulatus* in different growth phases (MEXU 30719); C mature specimen showing the cracked pileus, revealing the tubes; D Stipe showing a clear reticulate-alveolate ornamentation.

Basidiospores (7–) 8–10 (–12) × 7–9 µm, 140/4, Q:1.12 µm, broadly ellipsoid to subglobose, episporium verrucose to semireticulate; in the episporium, germinative pore and hilar appendix reticulate-alveolate, pigmentation pale brown to brownish-yellow in KOH. Basidia 40–58× (8–)10–15 µm, clavate, tetrasporic, thick-walled, cytoplasm granular, hyaline to pale brown contents in KOH. Pleurocystidia (38–)45–50(–70) × (10–)11–14 µm, predominantly fusoid-mamillated to fusoid-ventricose, pale-yellow to brown in KOH, thick walled (≥1µm). Cheilocystidia (45–)50–71x10–13 µm, clavate to occasionally fusoid-ventricose, yellow to brown in KOH, thick walled (≥1µm). Hymenophoral trama divergent, with slightly intertwined central hyphae with gelatinous contents, 10–14 µm in diameter, lateral hyphae 2–10µm in diameter, hyaline to brown in KOH. Stipitipellis formed as a hymeniform layer of slightly interwoven hyphae with a vertical arrangement bearing caulocystidia (27–)30–51×(8–)10–16 µm, predominantly cylindrical with acute apex, subclavate to occasionally subfusoid, pale chocolate-brown to brown with a thick wall ≥1µm. Pileipellis formed by a trichoderm with terminal hyphae (16–)25–46 (–60)x (5–)6–11 µm, cylindrical to subclavate, pale brown to brown in KOH, clamp connection absent.

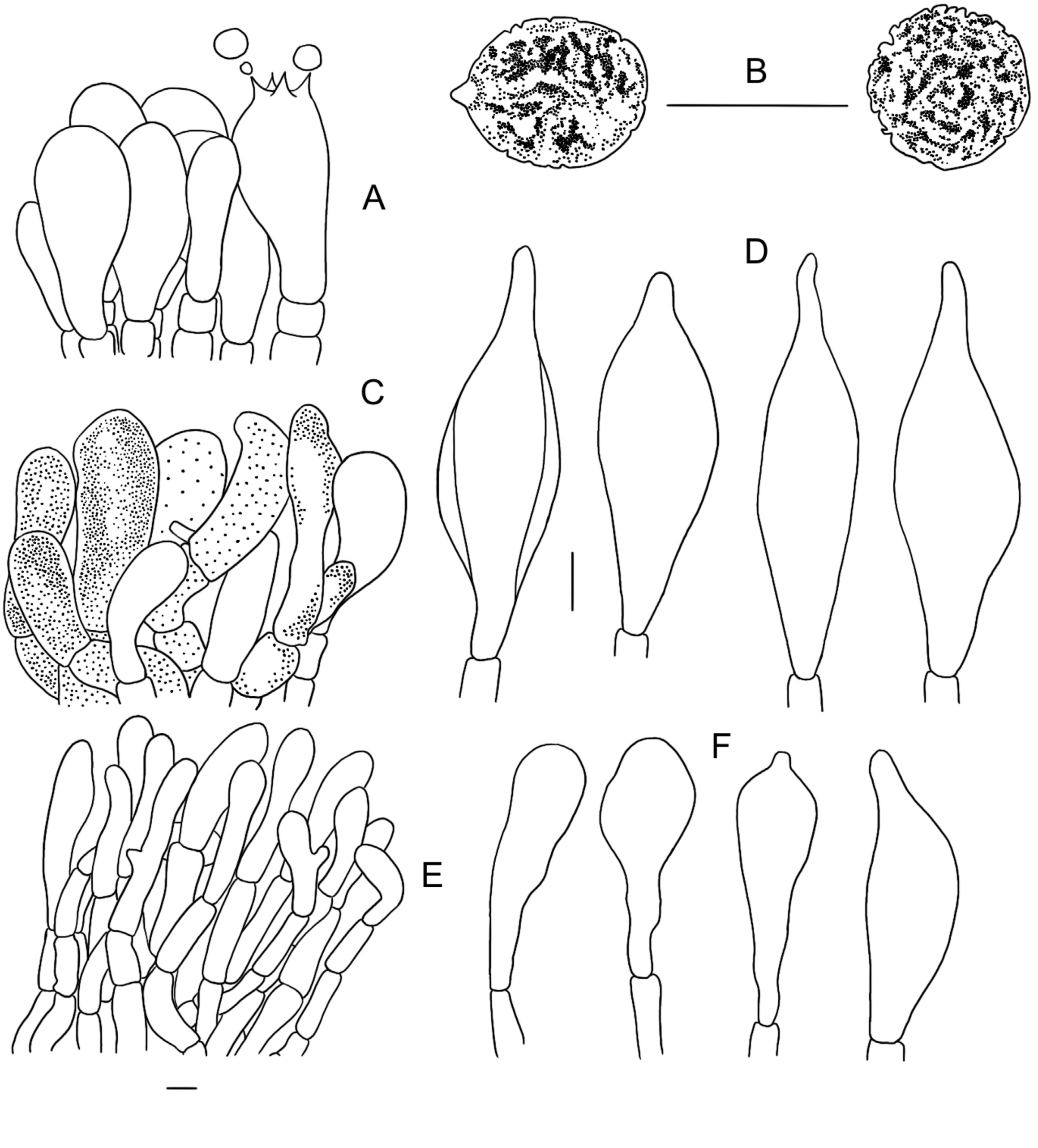

FIGURE 5. *Strobilomyces pedireticulatus* (MEXU-HO 30719); A. Basidia; B basidiospores; C caulocystidia; D pleurocystidia; E pileipellis; F cheilocystidia.

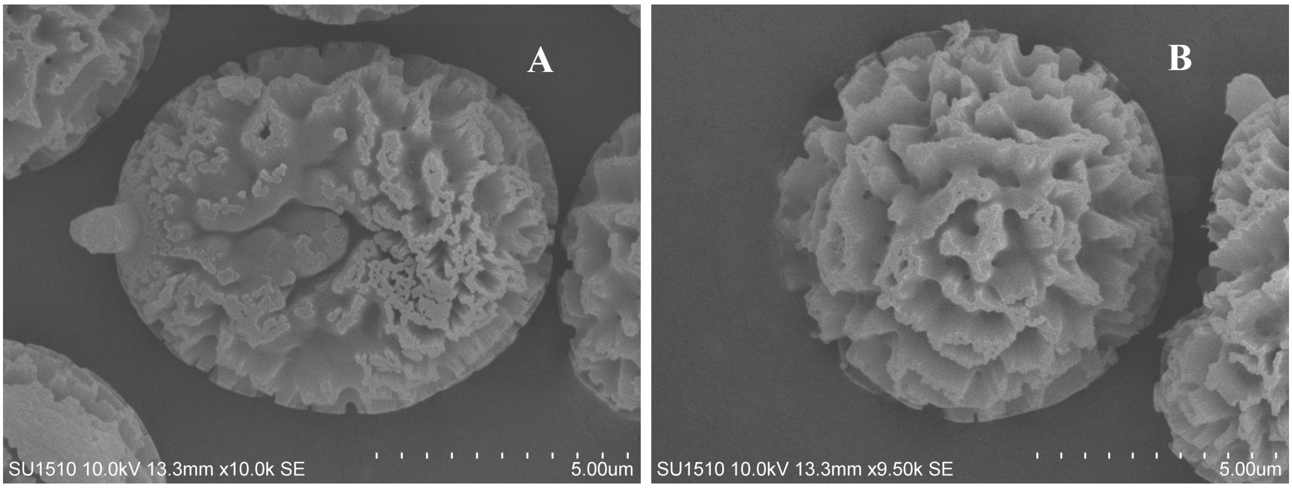

FIGURE 6. Basidiospores *Strobilomyces pedireticulatus* under scanning electron microscopy (MEXU 30719).

Habitat, habit, distribution:—solitary or scattered growing under mixed forest, (*Pinus, Quercus,* and *Arbutus* sp.) establishing ectomycorrhizal associations with *Pinus montezumae*, at altitudes between 2700 to 3000 m.

Additional material:—MEXICO, Tlaxcala, Nanacamilpa, San Felipe Hidalgo, Piedra Canteada, 2810m, 19°27'23"N, 19°27'28"W, Ayala-Vásquez O., 7 October 2021, C171 (MEXU 30721(HO); Piedra Canteada, 3000m, 19°27'22"N, 98°36'24"W Ayala-Vásquez O., 7 October 2021, C172 (MEXU 30720).

Discussion

Strobilomyces pedireticulatus is assigned to section *Strobilomyces* and phylogenetically clusters within the clade characterized by basidiospores exhibiting incomplete reticulate basidiospores. Our analysis resolve *S. giganteus* from China and *Strobilomyces* sp. from Thailand as sister species to *Strobilomyces pedireticulatus*. Morphologically, *S. pedireticulatus* shares with *S. giganteus* the character of semi-reticulate basidiospores. Nevertheless, *S. pedireticulatus* is distinguished by the following combination of features: a stipe surface that is prominently reticulate-alveolate, predominantly from the third upper part to the apex, with a very thick annulus positioned near the stipe apex, upper part widened in some specimens; a hymenium coloration ranging from whitish to greyish to brown; and pileus context pileus that is whitish initially upon sectioning but rapidly stains reddish-brown, progressing to brown-blackish. Its basidiospores measure (7–) 8–10 (–12)× 7–9 µm, are broadly ellipsoid to subglobose, and exhibit a verrucose to semireticulate episporium ornamentation, with a reticulate-alveolate pattern surrounding the germinative pore and hilar appendix. In contrast, S. *giganteus* has pileus of 100–150 mm, possesses a pileus surface densely covered with small hard erect, conical scales to rimmose-areolated; a stipe adorned with black minutely conical scales and fluffy fibrils ("floss"); and basidiospores 6–6.5 × 5–5.5 µm, ranging from subglobose to globose, with a semireticulate ornamentation (Zang, 1985).

In Mexico, *S. confusus* has only been reported with taxonomic data (Singer *et al*. 1991, García-Jiménez y Castillo 1981, Ayala-Vasquez *et al*. 2018). The second species is *S. strobilaceus*, which has been recorded from northern to southern Mexico (García-Jiménez y Castillo 1981, Ayala-Vasquez *et al*. 2018). However, Sato *et al*. (2007) mention that it is a cryptic species and that there are seven different species, *S. strobilaceus s. s.* is distributed in Europe. This is consistent with the phylogenetic analysis by Han *et al*. (2018), where *S. strobilaceus* can be seen in six different clades: the first clade is from European sequences, where the holotype specimen is located; the second clade is from Japan; the third clade is from the United States; the fourth clade is from Mexico, and the fifth and sixth clades are sequences from China. Our analysis suggests that Mexico has a high diversity of species of the genus, as the sequences MG832081 and MG832082 for *Strobilomyces* sp. from Nayarit, Mexico, are found in the reticulate basidiospore clade, while the sequences for *S. pedireticulatus* place it in the semi-reticulate clade, which is consistent with the phylogenetic analysis previously reported by Han *et al*. (2018) and Deng *et al*. (2023).

The *Strobilomyces pedireticulatus* is a sister species to *S. dryophilus* (100MP/100ML/1IB), as shown in figure 3 but differs by pileus surface cottony, woolly, appresed or erect, whithish, greyish-pink, pinkish-tan an matt brown;

hymenium white when young, soon becoming grey and finally black; stipe nearly equal, sometimes enlarget at the base, surface dry flocculose or reticulate, white when young, mainly at the apex and middle zone, brown and black. Basidiospores 9–12× 7–9 µm, complete reticulatum. This species was described in the United State and is distributed along the Gulf Coast, being most common in Texas and Florida and forms an ectomycorrhizal association with *Quercus* spp. (Weber & Smith, 1985; Bessette *et al.*, 2017). The species is currently invalidated according to the Index Fungorum.

The genus *Strobilomyces* is of biocultural importance, as several different species are consumed in different cultures in Mexico (Garibay-Orijel *et al.* 2009, Burrola-Aguilar *et al.* 2024, Ramírez-Carbajal *et al*. 2025), which has a name in the native languages but is unknown to science; making it very important to conduct a more exhaustive study of the genus soon.

Acknowledgments

Olivia Ayala-Vásquez thanks to SECIHTI-3129307 for postdoctoral funding. We acknowledge to M.Sc. María Berenit Mendoza-Garfias, head of the laboratory of Of the laboratory of scanning electron microscopy, of the Instituto de Biologia, UNAM. Jesús Pérez-Moreno acknowledges Colegio de Postgraduados for its support to undertake a research visit to the Kunming Institute of Botany-Chinese Academy of Sciences in Kunming, China. The authors express their agreement with the editor and the reviewers and extend their gratitude for the time and recommendations provided.

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