

Lanspora dorisauae, a new marine fungus from rocky shores in Taiwan (#83298)

1

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***Lanspora dorisauae*, a new marine fungus from rocky shores in Taiwan**

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Background. This paper reports a new marine fungus, *Lanspora dorisauae* (Sordariomycetes, Ascomycota), on trapped wood collected in coastal sites of Taiwan. *Lanspora dorisauae* is characterized by dark-coloured ascomata with a short neck, periphysate ostioles, subclavate, deliquescent asci without an apical ring, presence of wide paraphyses, striated wall ascospores with crown-like appendages on one pole of the ascospores. **Methods.** Phylogenetically, *L. dorisauae* grouped with *L. coronata* (type species) with strong support based on a combined analysis of the 18S, 28S, ITS rDNA, TEF1- α and RPB2 genes. **Results.** *Lanspora coronata* lacks paraphyses and ascospore appendages occur on both ends of the ascospores, which differs from *L. dorisauae*. *Lanspora cylindrospora* formed a sister clade with *L. coronata* and *L. dorisauae*, but it significantly differs in morphology with the latter two species in having cylindrical asci with an apical J-ring, smooth ascospore wall and no ascospore appendages, and may be better referred to a new genus. *Lanspora* belongs to the Phomatosporaceae, Phomatosporales with *Phomatosporea* and *Tenuimurus*, while *Phomatosporea berkeleyi* should be sequenced to test the validity of the order Phomatosporales and the family Phomatosporaceae.

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Abstract

Background. This paper reports a new marine fungus, *Lanspora dorisauae* (Sordariomycetes, Ascomycota), on trapped wood collected in coastal sites of Taiwan. *Lanspora dorisauae* is characterized by dark-coloured ascomata with a short neck, periphysate ostioles, subclavate, deliquescent asci without an apical ring, presence of wide paraphyses, striated wall ascospores with crown-like appendages on one pole of the ascospores.

Methods. Phylogenetically, *L. dorisauae* grouped with *L. coronata* (type species) with strong support based on a combined analysis of the 18S, 28S, ITS rDNA, TEF1- α and RPB2 genes.

Results. *Lanspora coronata* lacks paraphyses and ascospore appendages occur on both ends of the ascospores, which differs from *L. dorisauae*. *Lanspora cylindrospora* formed a sister clade with *L. coronata* and *L. dorisauae*, but it significantly differs in morphology with the latter two species in having cylindrical asci with an apical J- ring, smooth ascospore wall and no ascospore

appendages, and may be better referred to a new genus. *Lanspora* belongs to the Phomatosporaceae, Phomatosporales with *Phomatospora* and *Tenuimurus*, while *Phomatospora berkeleyi* should be sequenced to test the validity of the order Phomatosporales and the family Phomatosporaceae.

Introduction

The genus *Lanspora* was created to accommodate a marine fungus found on driftwood collected from rocky beaches in Seychelles (Hyde and Jones 1986). Originally placed in the Halosphaeriaceae (Microascales, Ascomycota) due to its perithecial ascomata, deliquescent asci and ascospores with crown-like appendages formed by fragmentation of the exosporium (Hyde and Jones 1986). Spatafora et al. (2006), in a phylogenetic analysis based on five genes (18S, 28S rDNA, EF1 α , RPB1, RPB2), showed that *L. coronata* grouped with *Ophiostoma piliferum* (Ophiostomataceae, Ophiostomatales), rather than in the Halosphaeriaceae as proposed by Hyde and Jones (1986). *Lanspora coronata* differs in having cylindrical or oblong-ventricose asci and periphyses from taxa of the Ophiostomatales which are characterized by globose to ovoid asci and the absence of periphyses (Alexopoulos et al. 1996).

In a 18S, 28S and ITS rDNA phylogeny, *Lanspora* (*L. coronata*) formed a generally well-supported clade with *Phomatospora* and *Tenuimurus* (*T. clematidis*), and consequently, a new order Phomatosporales and a new family Phomatosporaceae were established to accommodate these three genera (Senanayake et al. 2016). Hyde et al. (2020) described a new species *Lanspora cylindrospora* with a clypeus, presence of periphyses, cylindrical asci with a J- ring and cylindrical ascospores lacking appendages, characteristics not found in *L. coronata*. However, *L. cylindrospora* grouped with *L. coronata* in a phylogenetic tree based on sequences of the 18S, 28S, ITS rDNA, TEF1 α and RPB2 (Hyde et al. 2020).

Taiwan has a tropical/subtropical climate, but marine fungi of the temperate zone can be commonly found in northern Taiwan (Fryar et al. 2020, Tibell et al. 2020, Jones and Pang 2022), with many new marine fungi (e.g., *Pileomyces formosanus*, Pang and Jheng 2012; *Sclerococcum vrijmoediae* (as *Dactylospora vrijmoediae*), Pang et al. 2014). Recently, we discovered a marine fungus on driftwood/trapped wood in various rocky shores in Taiwan that is morphologically similar to *L. coronata*, and phylogenetically related to it using five different genes. In this paper, this new marine fungus is described.

Materials and Methods

Sample collection

The fungi from the dead wood were collected according to methods used in Pang et al. (2023). Trapped wood was collected at a rocky shore in Jin-shan, New Taipei City, Taiwan on 1 June 2022, placed on Ziplock plastic bag and transported to the laboratory at National Taiwan Ocean University, Keelung. The wood was cleaned briefly in running water, incubated on a tray and kept in a Ziplock plastic bag. The wood was checked periodically for the growth of marine fungi.

Morphological characterization

Fruiting bodies were observed using an Olympus SZ61 stereomicroscope (Tokyo, Japan), sectioned with a razor blade, grasped with fine forceps, and mounted on a slide in sterile seawater. Morphology of the asci and ascospores was observed using an Olympus BX51 microscope (Tokyo, Japan). Photographs were taken with an Olympus DP20 Microscope Camera (Tokyo, Japan).

Molecular analysis

For the molecular identification, single spore isolates were made and subsequently maintained on cornmeal seawater agar (CMAS; 17g cornmeal agar, 1L natural seawater). Aerial mycelia were collected from the agar plate, and ground into powder in a mortar and pestle, pre-cooled in a -80°C freezer overnight. DNeasy Plant Mini Kit (Qiagen, California, USA) was used for total DNA extraction according to the manufacturer's instructions. Extracted DNA (1 µL) was used directly for PCR reactions with the following ingredients: 0.2 µM of each primer (18S rDNA-NS1/NS2, ITS rDNA-ITS5/ITS4: White et al. 1990, 28S rDNA-LROR/LR6: Vilgalys and Hester 1990, Bunyard et al. 1994, EF1α-EF1-983F/EF1-2218R: Rehner and Buckley 2005, RPB2-fRPB2-5F/fRPB2-7cR: Liu et al. 1999), 0.5 volume of Gran Turismo PreMix (Ten Giga BioTech, Taiwan) and topped up to 25 µL with PCR water. The amplification cycle consisted of an initial denaturation step of 94°C for 5 min followed by 35 cycles of (i) denaturation (94°C for 0.5 min), (ii) annealing (55°C for 0.5 min) and (iii) elongation (72°C for 0.5 min) and a final 11 min elongation step at 72°C. The PCR products were shipped to Tri-I Biotech, Inc., Taiwan, for purification and direct sequencing with the same primers described above.

Returned sequences were checked for ambiguity, assembled and deposited in GenBank (accession nos. in Table 1). These sequences were aligned with the 18S, 28S, ITS, TEF1a and RPB2 genes of the taxa in the Phomatosporales and *Ophiostoma piliferum* and *O. stenoceras* (outgroup taxa) in the program MUSCLE (Edgar 2004) in MEGA11 (Tamura et al. 2021). The final dataset contained 16 sequences and a total of 7623 nucleotide positions. A maximum likelihood analysis including bootstrapping was performed in MEGA11 (Tamura et al. 2021) with the following settings: 500 bootstrap, General Time Reversible model (GTR), gamma distributed (G), number of discrete gamma categories set at 5, heuristic search with Nearest-Neighbor-Interchange, initial tree from NJ/BioNJ method, branch swapping strong. A maximum parsimony (MP) analysis including bootstrapping was also run in MEGA11 with the following settings: 500 bootstrap, Tree-Bisection-Reconnection (TBR), number of initial trees (random addition) = 5, MP search level = 1, maximum number of trees to retain = 100.

For Bayesian analysis, the alignment was entered into BEAUti v1.10.4 for prior settings and generation of XML files for Bayesian analysis in BEAST v1.10.4 and analyzed simultaneously (Suchard et al. 2018) with the following analytical settings: GTR, estimated base frequency, gamma distribution, number of gamma categories set at 5, a strict clock, Coalescent: Constant Size as the speciation model, running 10 million generations with parameters and trees sampled every 1000 generations. The first 10% of the trees were treated as the burn-in and discarded based on the effective sample size (ESS) of the parameter statistics in Tracer v1.7.2 (Rambaut et al. 2018). A summary tree was produced using TreeAnnotator v1.10.4 (Suchard et al. 2018) and viewed and edited in FigTree v1.4.4 (available at <https://github.com/rambaut/figtree/releases>).

Results

Based on the phylogenetic analysis of five genes (18S, 28S, ITS rDNA, EF1- α and RPB2), the new species *L. dorisauae* formed a strongly supported clade with *L. coronata* while *L. cylindrospora* formed a sister relationship with these two species (Figure 1). *Lanspora dorisauae* is similar to *L. coronata* in having dark-coloured, coriaceous ascomata, subclavate asci, elongate-ellipsoidal ascospores with striated wall and crown-like ascospore appendages (Hyde and Jones 1986). However, *L. dorisauae* differs from *L. coronata* in the striated ascospore wall and crown-like appendages at one end of the ascospores in the former species. Also, paraphyses are present

in *L. dorisauae*. *Lanspora dorisauae* was collected at several coastal locations in Taiwan, suggesting that it is a common marine fungus.

Taxonomy

Lanspora dorisauae K.L. Pang, Suetrong, M.W.L. Chiang and E.B.G. Jones, sp. nov.



Mycobank (MB#847455)

GeneBank (LSU=OQ130043, ITS=OQ130045, SSU=OQ130044, TEF1 α =OQ570968, RPB2=OQ570969)

Saprobic on trapped wood on a rocky shore. **Sexual morph** Ascomata 148–213 μm high, 240–347 μm diam. ($\bar{x}$ = 174 $\times$ 277 μm , n = 4), immersed, subglobose to ellipsoidal in side-view, solitary to gregarious, coriaceous, brown to black, papillate (Fig. 2a). Necks short, 44–55 μm long, 80–109 μm diam. ($\bar{x}$ = 48 $\times$ 99 μm , n = 4), cylindrical, brown to black (Figure 2a). Periphyses not observed. Peridium 13–24 μm ($\bar{x}$ = 19 μm , n = 4), equal in thickness, comprising one stratum of multilayers (over 5 layers) of brown cells of *textura angularis* with large lumina (Figure 2b). Paraphyses 74 $\times$ 9 μm (Figure 2c). Asci 41–51 $\times$ 16 μm ($\bar{x}$ = 44 $\times$ 16 μm , n = 4), 8-spored, unitunicate, thin-walled, clavate, pedunculated (Figure 2d). Ascospores 8–13 $\times$ 5–7 μm ($\bar{x}$ = 11 $\times$ 6 μm , n = 35), biseriate, overlapping, unicellular, broadly ellipsoidal with one half broader than the other, with longitudinal wall striations, guttulate, appendaged (Figures 2e-f). Appendages at one end of ascospores, five to seven in number, crown-like, sheet-like irregular and radiating, delicate and subgelatinous (Figure 2g). **Asexual morph** Undetermined.

Culture characteristics:

Holotype: TAIWAN: Jinshan. On a piece of unidentified trapped wood, 1 June 2022, S.Y. Guo and K.L. Pang, F26641 (National Museum of Natural Science Herbarium, Taichung, Taiwan), dried wood.

Ex-type culture: BCRC FU30316 (Bioresource Collection and Research Center, Hsinchu, Taiwan)

Etymology: In memory of Dr. Doris Wai-Ting Au, a marine zoologist/mycologist who taught me (K.L. Pang) the right way to do scientific research.

Distribution: Jin-shan, Li-lao, Ying-ke-shih (New Taipei City), Shih-Ti-Ping (Hualien County) (Taiwan).

Discussion

Hyde and Jones (1986) described *Lanspora coronata* from driftwood collected in the Seychelles. *Lanspora coronata* has perithecial ascomata, deliquescent asci and fusiform ascospores with crown-like appendages at both poles and was placed in the Halosphaeriaceae. However, Spatafora et al. (2006), based on the phylogenetic analysis of the 18S and 28S rDNA, found that *L. coronata* grouped with *Ophiostoma puliferum*. Senanayake et al. (2016) found that *L. coronata* grouped with *Phomatospora* based on 18S, 28S and ITS rDNA, and established a new order Phomatosporales and a new family Phomatosporaceae. Three genera are included in the Phomatosporaceae: *Lanspora*, *Tenuimurus* and *Phomatospora*, and they were monophyletic phylogenetically based on the 18S, 28S and ITS rDNA (Senanayake et al. 2016). Hyde et al. (2020) confirmed the placement of *L. coronata* in the Phomatosporaceae in a phylogenetic analysis of 18S, 28S, ITS, TEF1- α and RPB2, and described a new species *L. cylindrospora*.

Morphologically, *Phomatospora* (type species: *P. berkeleyi*) and *Tenuimurus* (type species: *T. clematidis*) are similar: with cylindrical, persistent asci with an apical J- ring, smooth-walled, ellipsoidal ascospores (Fallah and Shearer 1998, Senanayake et al. 2016). *Lanspora* (*L. coronata* and *L. dorisauae*) differs from these two genera in having subclavate, deliquescent asci and striated ascospores with crown-like appendages (Hyde and Jones 1986). Although *L. cylindrospora* phylogenetically is related to *L. coronata*, the former species morphologically differs significantly from the latter with cylindrical asci with a J- apical ring and ascospores without appendages (Hyde et al. 2020). In contrast, *L. coronata* and *L. dorisauae* both have clavate, deliquescent asci without an apical ring and ascospores with crown-like appendages (Hyde and Jones 1986, this study). *Lanspora cylindrospora* may be better referred to a new genus. More genes of the isolates of *L. cylindrospora* should be sequenced to provide a robust phylogeny to suggest this taxonomic change.

There are no sequences of *P. berkeleyi* in the GenBank and thus were not included in the phylogenetic analysis by Senanayake et al. (2016) when they introduced the Phomatosporales and Phomatosporaceae to include *Lanspora*, *Tenuimurus* and *Phomatospora*. It would be essential to isolate and sequence *P. berkeleyi* to evaluate the validity of Phomatosporales and Phomatosporaceae.

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Figure 1

The parsimonious tree produced from maximum parsimony analysis of a combined dataset of five genes (18S, 28S, ITS rDNA, EF1a, RPB2). The numbers at the nodes represent maximum parsimony bootstrap, maximum likelihood bootstrap and posterior probability 

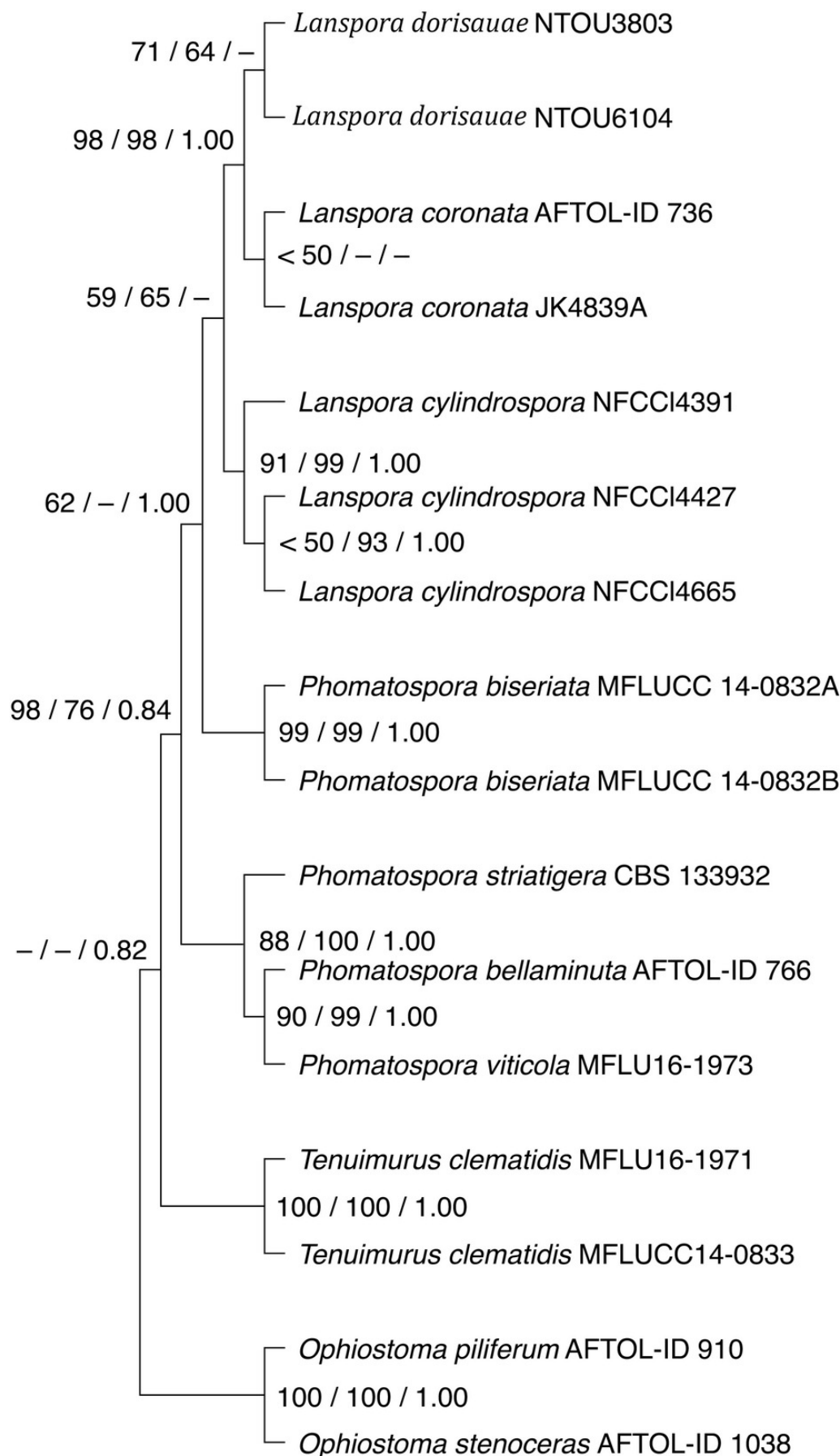


Figure 2

Figure 2. *Lanspora dorisauae*. a. Section of ascoma. b. Peridium made of rows of cells of textura angularis with large lumina. c. Paraphyses. d. Clavate ascus. e. Broadly ellipsoid

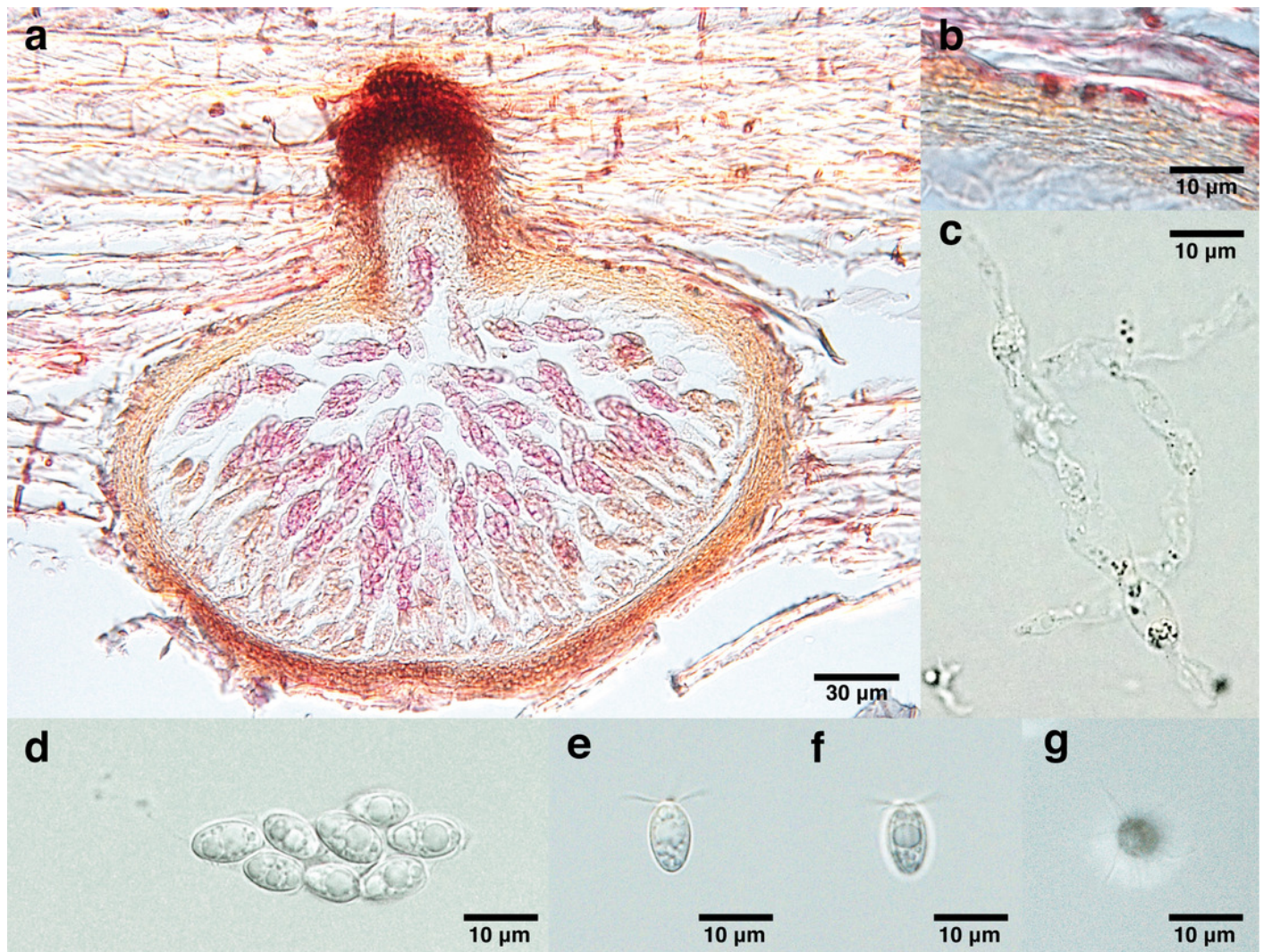


Table 1 (on next page)

Table 1

Table 1.

Species	Culture number	GenBank accession number				
		18S rDNA	28S rDNA	ITS rDNA	EF1 α	RPB2
<i>Lanspora audorisa</i> sp. nov.	NTOU3803					
<i>Lanspora audorisa</i> sp. nov.	NTOU6104					
<i>Lanspora coronata</i>	AFTOL-ID 736	DQ470996	—	—	DQ471067	DQ470899
<i>Lanspora coronata</i>	JK4839A	U48424	U46889	—	—	—
<i>Lanspora cylindrospora</i>	NFCCI4391	—	—	—	MN795088	MN795090
<i>Lanspora cylindrospora</i>	NFCCI4427	—	MN168892	MN168890	MN795089	—
<i>Lanspora cylindrospora</i>	NFCCI4665	MN169053	MN168891	MN168889	—	—
<i>Ophiostoma piliferum</i>	AFTOL-ID 910	DQ471003	DQ470955	—	DQ471074	DQ470905
<i>Ophiostoma stenoceras</i>	AFTOL-ID 1038	DQ836897	DQ836904	—	FJ190618	DQ836891
<i>Phomatospora bellaminuta</i>	AFTOL-ID 766	FJ176803	FJ176857	—	—	FJ238345
<i>Phomatospora biseriata</i>	MFLUCC 14-0832A	KX549458	KX549448	KX549453	—	—
<i>Phomatospora biseriata</i>	MFLUCC 14-0832B	KX549459	KX549449	KX549454	—	—
<i>Phomatospora striatigera</i>	CBS 133932	—	KM213618	KM213617	—	—
<i>Phomatospora viticola</i>	MFLU16-1973	—	KX549452	KX549457	—	—
<i>Tenuimurus clematidis</i>	MFLU16-1971	—	KX549451	KX549456	—	—
<i>Tenuimurus clematidis</i>	MFLUCC14-0833	—	KX549450	KX549455	—	—