



Freshwater ascomycetes: *Lophiostoma vaginatispora* comb. nov. (Dothideomycetes, Pleosporales, Lophiostomaceae) based on morphological and molecular data

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Abstract

Lophiostoma vaginatispora comb. nov. was collected from submerged wood in a stream in Northern Thailand. The species is characteristic by immersed to erumpent ascomata, with slot-like ostioles, with long, branched and numerous periphyses, an unequally thick peridium where the upper part is narrower than the lower part, and 1-septate, narrowly fusiform ascospores with a thick surrounding papilionaceous sheath. The placement of this species in *Lophiostoma* is based on morphological characters and phylogenetic analyses of the partial nuclear ribosomal 18S small subunit and 28S large subunit DNA sequence data. *Lophiostoma vaginatispora* is distinguished from other *Lophiostoma* species in possessing a wide papilionaceous sheath.

Key words: *Lindgomyces*, *Misturatosphaeria*, phylogeny, Pleosporales, sheath

Introduction

We are studying the freshwater fungi along a north to south gradient from Tibet through to Australia (Hyde *et al.* 1988, Ho *et al.* 2002, Cai *et al.* 2003, Lou *et al.* 2004, Zhang *et al.* 2011). The study involves collecting submerged wood in various types of streams and recording the fungi present. By compiling this data we hope to provide a biogeographical distribution of freshwater fungi from north to south and establish the likely effects of global warming. In this study we report on a collection of a freshwater species from Thailand. The taxa was initially identified as a new species of *Lophiostoma* (Tode) Cesati & De Notaris (1863: 219), but eventually linked to the genus *Vaginatispora* K.D. Hyde (1995: 235). *Vaginatispora aquatica* K. D. Hyde (1995: 235) was described from wood submersed in freshwater in north Queensland (Hyde 1995) and has also been reported from Hong Kong (Tsui *et al.* 2000). The genus and species is characterized by depressed globose ascomata, immersed beneath a blackened neck, with a slot-like ostiole, numerous and filamentous pseudoparaphyses, cylindrical to clavate asci and narrowly ellipsoidal, hyaline, 2-celled ascospores with a mucilaginous collar around its equator and a spreading papilionaceous sheath (Hyde 1995).

Lophiostoma is a speciose genus containing numerous species that were previously thought to occur in terrestrial (Tanaka & Harada 2003), marine (Hyde *et al.* 2002) and freshwater habitats (Luo *et al.* 2004, Zhang *et al.* 2009). It is typified by *L. macrostomum* (Tode) Cesati & De Notaris (1863: 219), which is the type species of the family Lophiostomaceae in the order Pleosporales (Hyde *et al.* 2013) and was recently epitypified by Zhang *et al.* (2009). The genus is presently characterized by immersed to erumpent ascomata with a crest-like papilla or a slit-like ostiole, an unequally thick peridium, clavate asci, and hyaline to deep brown, multi-septate or even muriform

ascospores with terminal appendages (Zhang *et al.* 2012b, Hyde *et al.* 2013). Kirk *et al.* (2008) estimated that this genus comprises 83 species, while there are 441 names epithets in Index Fungorum (2013). The large species numbers originate from the studies of Chesters & Bell (1970) that provided a key of 23 European species, while Holm & Holm (1988) provided a key of 28 Swedish species. More recently, Tanaka *et al.* (2003) reported seven species from Japan. The asexual states of *Lophiostoma caulium* (Fries) Cesati & De Notaris (1863: 221), *L. nucula* (Fries) Cesati & De Notaris (1863: 222) and *L. semiliberum* (Desmazières) Cesati & De Notaris (1863: 222) were related to *Pleurophomopsis* Petrak (1924: 156) (Leuchtman 1984, Hyde *et al.* 2011, Wijayawardene *et al.* 2012).

Most species of *Lophiostoma sensu lato* have been included in the genus based on morphology (Hyde & Aptroot 1998, Hyde *et al.* 2000, Zhang *et al.* 2009) and molecular phylogenetic analysis shows that it is certainly polyphyletic (Zhang *et al.* 2009, Shearer *et al.* 2009). Previous molecular studies on *Lophiostoma* are those of Zhang *et al.* (2009) who analysed 18S, 28S rDNA and RPB2 gene data. Zhang *et al.* (2009) showed *Lophiostoma* to be polyphyletic and separated into two clades. The first clade contained a few species in *Lophiostoma sensu stricto* including *L. rugulosum* Yin. Zhang, J. Fournier & K.D. Hyde (2009: 242), *L. glabrotunicatum* Yin. Zhang, J. Fournier & K.D. Hyde (2009: 245) and *L. macrostomum*. They possess crest-like ostioles or a wide, umbilicate pore surrounded by 4–6 radial ridges or small ostiolar pores. Species in the second clade contained 12 lophiostomataceous species with slit-like ostioles lacking raised crests. Zhang *et al.* (2009) tentatively concluded that the second clade represented a natural group at the family level. This result accepted by subsequent researches (Zhang *et al.* 2012b, Hyde *et al.* 2013). Therefore, several species need to be recollected and their placement in the genus needs to be reconfirmed based on molecular studies.

During investigations of freshwater ascomycetes in Thailand (Kurniawati *et al.* 2010, Zhang *et al.* 2012a), we collected *Vaginatispora aquatica* in a lotic freshwater habitat. Molecular analysis of SSU and LSU gene data showed it to cluster within the genus *Lophiostoma*. The purpose of the present study is to propose a new combination, *Lophiostoma vaginatispora*.

Materials and methods

Submerged wood was randomly collected from lotic freshwater habitats in Chiang Mai Province, Thailand, in November 2010, following the procedures described in Kurniawati *et al.* (2010) and Tsui *et al.* (2003). Observations and photomicrography were carried out following the procedures described in Zhang *et al.* (2012a). Isolations were made from single ascospores, following the methodology of Chomnunti *et al.* (2011), on 2% water agar (WA). The herbarium specimens are deposited at Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Isolates from this study are deposited at Mae Fah Luang University Culture Collection (MFLUCC), Thailand and International Fungal Research & Development Centre Culture Collection (IFRDCC), China.

DNA extraction, PCR amplification and sequencing follow the procedures described in Liu *et al.* (2012). Sequences obtained from forward and reverse primers (LROR and LR5, NS1, ITS4 and ITS5) were manually aligned. All additional sequences used in the analysis follow major phylogenies published in Schoch *et al.* (2009), Zhang *et al.* (2012 b) and Hyde *et al.* (2013) were obtained from GenBank (Table 1). Sequences were manually aligned to obtain a consensus sequence using Bioedit 7.0.9 (Hall, 1999). Maximum-parsimony (MP) and Bayesian analysis were performed, both following Zhang *et al.* (2012a). The generated phylogram was presented using Powerpoint.

TABLE 1. Species and sequences database accession numbers used in this study (newly generated sequences are indicated in bold).

Species	Voucher/Culture	GenBank accession no.	
		SSU	LSU
<i>Byssothecium circinans</i>	CBS675.92	GU205235	GU205217
<i>Decaisnella formosa</i>	BCC25616	GQ925833	GQ925846
<i>Decaisnella formosa</i>	BCC25617	GQ925834	GQ925847
<i>Dothidea sambuci</i>	DAOM231303	AY544722	AY544681
<i>Floricola striata</i>	JK56781	GU296149	GU301813
<i>Helicascus aegyptiacus</i>	FWCC99	KC894852	KC894853

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TABLE 1. (continued)

Species	Voucher/Culture	GenBank accession no.	
		SSU	LSU
<i>Helicascus aquaticus</i>	MFLUCC10-0918	KC886640	KC886638
<i>Helicascus elaterascus</i>	HKUCC7769	AY787934	NS
<i>Helicascus kanaloanus</i>	A237	AF053729	NS
<i>Helicascus nypae</i>	BCC36752	GU479755	GU479789
<i>Helicascus thalassioideus</i>	MFLUCC10-0911	KC886636	KC886637
<i>Lentithecium aquaticum</i>	CBS123099	GU296158	GU301825
<i>Lentithecium arundinaceum</i>	CBS619.86	GU296157	GU301824
<i>Lentithecium fluviatile</i>	CBS122367	GU301825	GU296158
<i>Lentithecium lineare</i>	IFRD2008	FJ795478	FJ795435
<i>Lindgomyces breviappendiculatus</i>	KT1399	AB521734	AB521749
<i>Lindgomyces ingoldianus</i>	ATCC200398	AB521719	AB521736
<i>Lindgomyces rotundatus</i>	KT966	AB521722	AB521739
<i>Lophiostoma arundinis</i>	CBS621.86	DQ782383	DQ782384
<i>Lophiostoma caulium</i>	CBS623.86	FJ795479	FJ795436
<i>Lophiostoma compressum</i>	IFRD2014	FJ795480	FJ795437
<i>Lophiostoma crenatum</i>	CBS629.86	DQ678017	DQ678069
<i>Lophiostoma fuckelii</i>	CBS101952	NS	DQ399531
<i>Lophiostoma fuckelii</i>	CBS113432	NS	EU552139
<i>Lophiostoma macrostomoides</i>	CBS123097	FJ795482	FJ795439
<i>Lohiostoma macrostomum</i>	KT635	AB521731	AB433273
<i>Lohiostoma macrostomum</i>	KT508	AB618691	AB619010
<i>Lohiostoma macrostomum</i>	KT709	AB521732	AB433274
<i>Lophiostoma quadrinucleatum</i>	GKM1233	NS	GU385184
<i>Lophiostoma scabridisporum</i>	BCC22836	GQ925832	GQ925845
<i>Lophiostoma semiliberum</i>	CBS626.86	FJ795484	FJ795441
<i>Lophiostoma vaginatispora</i>	MFLUCC11-0083	KJ591575	KJ591576 (ITS: KJ591577)
<i>Lophiostoma viridarium</i>	IFRDCC2090	FJ795486	FJ795443
<i>Lohiotrema neoarundinaria</i>	MAFF239461	AB524455	AB524596
<i>Lohiotrema neohysterioides</i>	KT756	AB618702	AB619020
<i>Lophiotrema nucula</i>	CBS627.86	GU296167	GU301837
<i>Lohiotrema vagabundum</i>	CBS628.86	FJ795485	FJ795442
<i>Lohiotrema vagabundum</i>	JCM17674	AB618704	AB619022
<i>Lohiotrema vagabundum</i>	JCM17675	AB618705	AB619023
<i>Massarina eburnean</i>	CBS473.64	GU296170	GU301840
<i>Massarina rubi</i>	CBS691.95	NS	FJ795453
<i>Misturatosphaeria aurantonotata</i>	GKM1280	NS	GU385174
<i>Misturatosphaeria claviformis</i>	GKM1210	NS	GU385212
<i>Misturatosphaeria kenyensis</i>	GKM1195	NS	GU385194
<i>Misturatosphaeria minima</i>	GKM169N	NS	GU385165
<i>Misturatosphaeria tennesseensis</i>	ANM911	NS	GU385207
<i>Misturatosphaeria uniseptata</i>	SMH4330	NS	GU385167
<i>Morosphaeria ramunculicola</i>	BCC18405	GQ925839	GQ925854
<i>Morosphaeria velatispora</i>	BCC17059	GQ925841	GQ925852
<i>Neottiosporina paspali</i>	CBS331.37	EU754073	EU754172

NS: no sequence available in GenBank.

Results

The combined SSU and LSU data set utilized 50 taxa with *Dothidea sambuci* as the outgroup taxon (Table 1). The maximum parsimony dataset consists of 1,797 characters; where 1,426 characters were constant, 105 variable characters were parsimony-uninformative and 234 characters were parsimony-informative. Kishino-Hasegawa (KH) test showed length = 846 steps, CI = 0.511, RI = 0.776, RC = 0.396 and HI = 0.489. All five trees were similar in topology and not significantly different (data not shown). The new collected species clusters at the base of the *Lophiostoma* clade with strong support (Fig. 1). We therefore introduce a new combination species *Lophiostoma vaginatispora* based on molecular analysis and morphological characters.

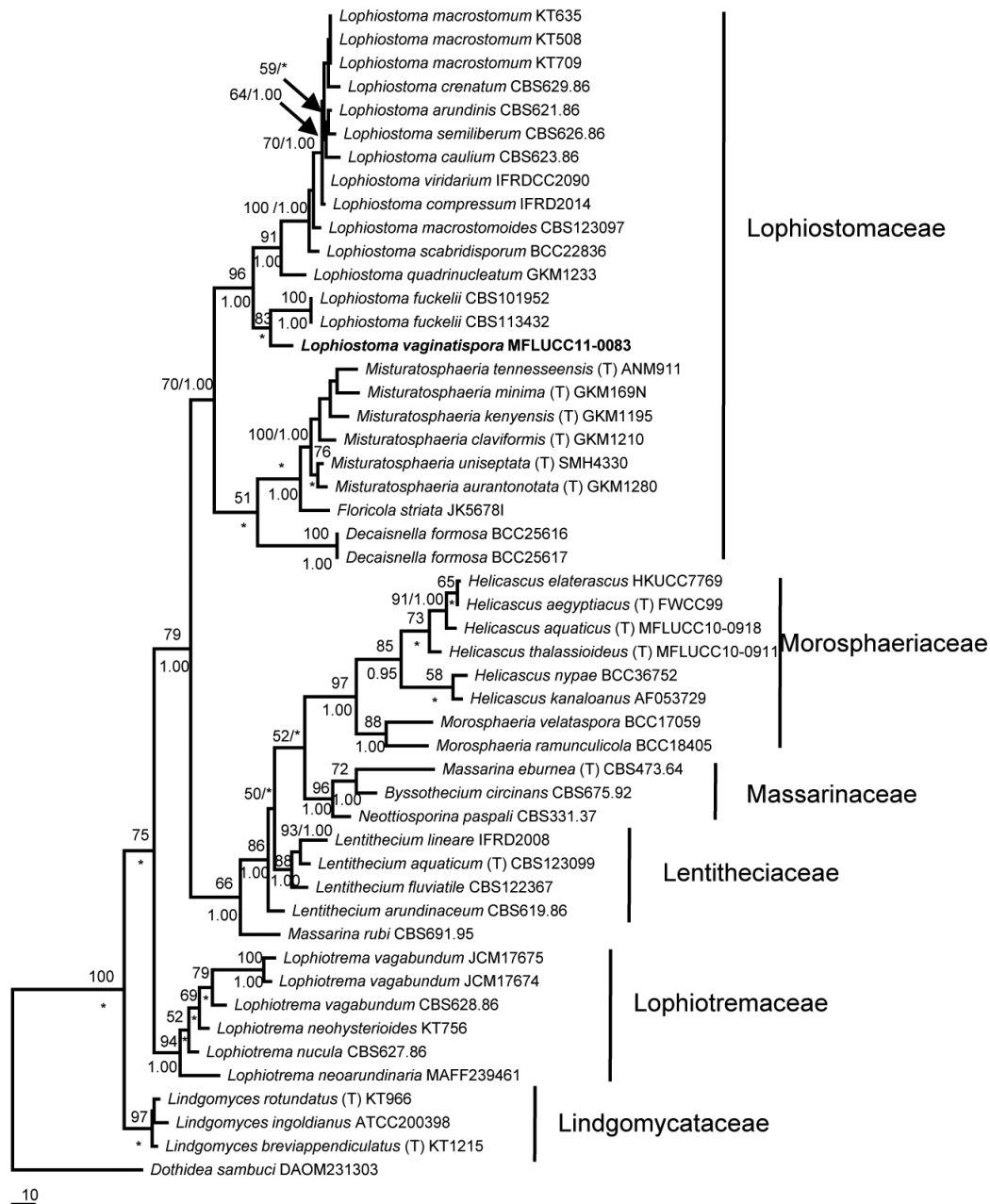


FIGURE 1. The most parsimonious tree generated from maximum parsimony (MP) analysis based on SSU and LSU rDNA sequences. The tree was rooted to *Dothidea sambuci*. Bootstrap support values for MP greater than 50% are above the nodes. Bayesian posterior probabilities greater than 0.95 are indicated under branches. Type strains are labelled as T.

Taxonomy

Lophiostoma vaginatispora (K.D. Hyde) Huang Zhang & K.D. Hyde, *comb. nov.* MycoBank 807462 (Fig. 2)

Basionym: *Vaginatispora aquatica* K.D. Hyde, *Nova Hedwigia* 61(1-2): 235 (1995)

Holotype:—THAILAND. Chiang Mai: Doi Inthanon, 16 Nov. 2010, *Huang Zhang* (MFLU 11-1115) – ex-living culture MFLUCC11-0083 = IFRDCC 2462.

Ascomata 400–500 µm high, 350–400 µm diameter (\bar{x} = 470 µm high × 390 µm diameter, n = 10), subglobose, immersed to erumpent, black, papillate, with a slot-like ostiole; papilla 150–170 µm long, 170–230 µm wide, black, papillate, with hyaline periphyses; *periphyses* 35–45 µm long, 2.5–3 µm wide, branched at the base, numerous. *Peridium* unequal in thickness, 20–60 µm thick at upper part, broader (up to 100 µm) near the base, composed of parallel rows of rectangular to polygonal brown cells of 5–8 × 2.5–5 µm. *Pseudoparaphyses* 1.8–2.5 µm thick (\bar{x} = 2.2 µm, n = 10), hypha-like, hyaline, septate, filamentous, numerous, anastomosing above the asci. *Asci* 140–160 × 20–30 µm (\bar{x} = 151 × 25 µm, n = 10), 8-spored, fissitunicate, cylindrical to clavate, with a relatively long pedicel (22–30 µm), apically round with a broad ocular chamber. *Ascospores* 33–45 × 11–12.5 µm (\bar{x} = 38.3 × 12 µm, n = 20), L/W 2.7–3.6 (mostly 3.2), mostly 2-seriate, narrowly ellipsoidal with acute ends, 1-septate, constricted at the septum, septum mostly median, upper cell slightly broader than lower cell, hyaline, with one large guttulate in each cell, smooth, thin-walled, with a mucilaginous collar around its equator, surrounded by a spreading papilionaceous sheath, 20–28 µm thick. Germ tube mainly formed from both end cells.

In culture: ascospores germinating on WA within 12–24 hours. Colonies on PDA, dense, at first brownish in the middle and white towards the edge, becoming dark grey after 17 days, reaching up to 2 cm diameter at 17 days at 25–28°C, raised, velvety, aerial mycelium, entire edge smooth, clear.

Habitat and distribution:—Saprobic on submerged wood in fresh water in Thailand.

Notes:—*Lophiostoma vaginatispora* is similar to *L. macrostomum*, the type species, in having immersed to erumpent ascomata with a slit-like ostiole, an unequally thick peridium, clavate asci, and hyaline ascospores. But the ascospores of *L. macrostomum* are narrowly fusiform (L/W = 5.5–7.7) and with terminal appendages, whereas the ones in *L. vaginatispora* are narrowly ellipsoidal (L/W = 2.7–3.6) and surrounded by a spreading papilionaceous sheath. Eleven species of *Lophiostoma* have been described from freshwater habitats, including *L. amphibium* (Magnes & Hafellner) Aptroot & K.D. Hyde (2002: 106), *L. appendiculatum* Fuckel (1874: 29), *L. aquaticum* (J. Webster) Aptroot & K.D. Hyde (2002: 106), *L. arundinis* (Fries) Cesati & De Notaris (1863: 222), *L. armatisporum* (K.D. Hyde, Vrijmoed *et al.*) E.C.Y. Liew *et al.* (2002: 812), *L. corticola* (Fuckel) E.C.Y. Liew *et al.* (2002: 812), *L. glabrotunicatum*, *L. maquilangense* K.D. Hyde & Aptroot (1998: 499), *L. proprietunicatum* (K.M. Tsui *et al.*) Aptroot & K.D. Hyde (2002: 107), *L. purpurascens* (K.D. Hyde & Aptroot) Aptroot & K.D. Hyde (2002: 108), and *L. rugulosum*. None of these species are similar to *L. vaginatispora* in the shape of ascospore sheath. *Lophiostoma aquaticum* has ascospores with mucilaginous sheath which is also constricted in the centre. However, the dimensions of ascospores are smaller (22–34 × 3.5–5 vs. 33–45 × 11–12.5 µm) and the sheath is thinner. *Lophiostoma glabrotunicatum* and *L. rugulosum* differs from *L. vaginatispora* in having ascomata with small ostioles or wide, umbilicate pores surrounded by 4–6 radial ridges. Whether these species belong in *Lophiostoma* will need to be confirmed by molecular data analysis.

Combined molecular analysis of SSU and LSU genes show *L. vaginatispora* and *L. fuckelii* Saccardo (1878: 336) form a sister group at the base of the *Lophiostoma* Clade with high bootstrap support (Fig. 1). *Lophiostoma fuckelii* is distinct in having small ascomata (200–300 µm diam.) and small ascospores (15–20 × 4–5 µm) with short appendages (Hyde *et al.* 2000). *Misturatosphaeria* Mugambi & Huhndorf (2009: 108) clusters with *Lophiostoma* in Lophiostomataceae with strong support (Fig. 1), as shown in Mugambi & Huhndorf (2009) and Hyde *et al.* (2013).

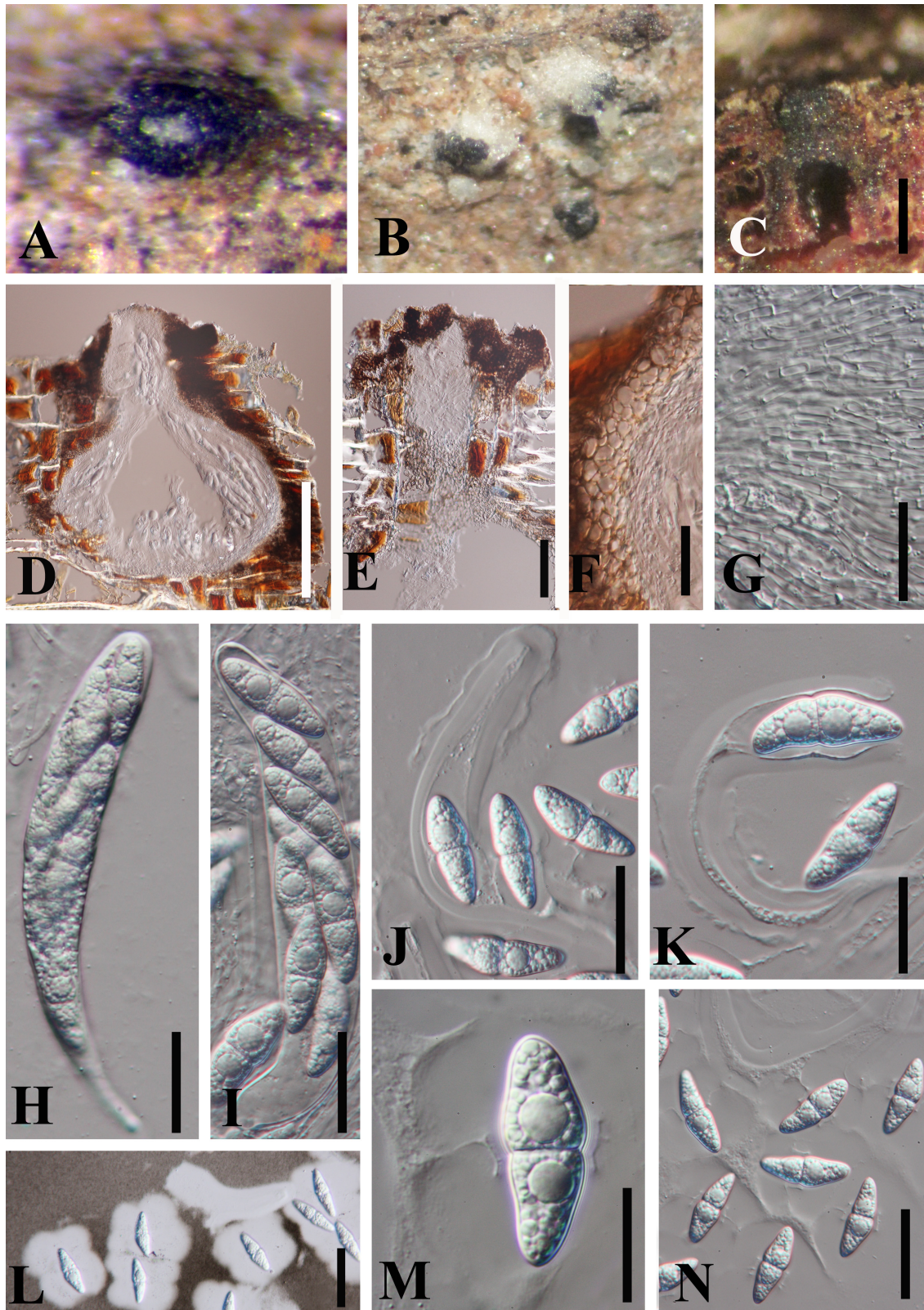


FIGURE 2. *Lophiostoma vaginatispora* (from holotype). A–B. Appearance of ascomata on wood. Note the oozed ascospores in B. C–D. Sections of ascomata. E. Papillate ascoma. F. Peridium. G. Wide cellular pseudoparaphyses. H–I. Asci. J–K. Asci releasing ascospores through fissitunicate dehiscence. L–N. Ascospores with wide papilionaceous sheath. L is in Indian ink. Scale bars: C = 300 μ m, D = 200 μ m, E = 50 μ m, F–G = 20 μ m, H–I = 30 μ m, J–L, N = 50 μ m, M = 20 μ m.

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