

Towards a phylogenetic clarification of *Lophiostoma* / *Massarina* and morphologically similar genera in the *Pleosporales*

Zhang, Y.¹, Wang, H.K.², Fournier, J.³, Crous, P.W.⁴, Jeewon, R.¹, Pointing, S.B.¹ and Hyde, K.D.^{5,6*}

¹Division of Microbiology, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China

²Biotechnology Institute, Zhejiang University, 310029, P.R. China

³Las Muros, Rimont, Ariège, F 09420, France

⁴Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands

⁵School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand

⁶International Fungal Research & Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, Yunnan, P.R. China 650034

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Lophiostoma, *Lophiotrema* and *Massarina* are similar genera that are difficult to distinguish morphologically. In order to obtain a better understanding of these genera, lectotype material of the generic types, *Lophiostoma macrostomum*, *Lophiotrema nucula* and *Massarina eburnea* were examined and are re-described. The phylogeny of these genera is investigated based on the analysis of 26 *Lophiostoma*- and *Massarina*-like taxa and three genes – 18S, 28S rDNA and RPB2. These taxa formed five well-supported sub-clades in *Pleosporales*. This study confirms that both *Lophiostoma* and *Massarina* are polyphyletic. *Massarina*-like taxa can presently be differentiated into two groups – the *Lentithecium* group and the *Massarina* group. Of these, the type species *M. eburnea* together with the *Massarina* group represents *Massarina sensu stricto*. *Lophiostoma* taxa clustered in two groups – one group, including the type species *L. macrostomum*, is characterized by fusiform, hyaline one-septate ascospores which are pigmented and 3-septate when senescent, clavate asci, and apical structures which are highly variable, being crest-like in *L. macrostomum*, an umbilicate pore surrounded by 4-6 radial ridges in *L. rugulosum*, or papillate in *L. glabrotunicatum*. The second group comprises *Lophiostoma* species with heavily pigmented multi-septate ascospores and compressed crests. *Lophiotrema* species including the type species *L. nucula* form a monophyletic group. One new genus – *Lentithecium* with five new species – *Lentithecium aquaticum*, *Lophiostoma glabrotunicatum*, *L. rugulosum*, *Lophiotrema brunneosporum* and *L. lignicola* and three new combinations – *Lentithecium arundinaceum*, *L. fluviatile* and *L. lineare* are introduced in this paper.

Key words: *Lentithecium*, *Lophiostoma*, *Lophiotrema*, *Massarina*, phylogeny, type studies

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*Corresponding author: Kevin D. Hyde; e-mail: kdhyde2@gmail.com

Introduction

There is considerable confusion surrounding the genera *Lophiostoma*, *Lophiotrema* and *Massarina* (Scheuer, 1991; Hyde *et al.*, 2002; Liew *et al.*, 2002; Tanaka and Harada, 2003b; Tang *et al.*, 2003). Taxa in these genera mostly have immersed to erumpent ascomata, cellular pseudoparaphyses, and hyaline (or dark brown in *Lophiostoma*),

septate ascospores often with sheaths or appendages (Holm and Holm, 1988; Tanaka and Harada, 2003a,b). Delineation has been based on the fact that *Lophiostoma* has compressed crests, some with raised crests; the unequal thickness of the peridium, which is usually broader near the base; mostly clavate asci; and 1 to several septate, hyaline to dark brown ascospores with terminal appendages or mucilaginous sheaths (Holm and Holm, 1988;

Tanaka and Harada, 2003a). *Lophiotrema* differs in having a peridium (20-30 µm wide) of nearly equal thickness, composed of an outer *textura angularis* of uniformly pigmented cells, up to 12 µm, and an inner layer of small, hyaline cells, with somewhat thickened walls (Holm and Holm, 1988), and cylindrical or oblong asci (Holm and Holm, 1988; Tanaka and Harada, 2003b). As compared to the above two genera, the distinguishing morphological character of *Massarina* is its rounded papilla, and its exclusively hyaline ascospores (Holm and Holm, 1988; Aptroot, 1998).

The confusion between these genera was confounded when Hyde (1995) re-examined the generic type specimen of *Massarina eburnea* and found it comprising flattened ascomata immersed in bark of twigs of *Fagus sylvatica*, which had little resemblance to most species included in *Massarina*. This resulted in Aptroot (1998) monographing the genus with acceptance of 43 species. Aptroot (1998) placed much emphasis on the ascospore characters and only provided brief descriptions of hamathecium and ascoma structures. Further new species were introduced from aquatic (Hyde and Goh, 1998; Tsui *et al.*, 1999; van Ryckegem and Aptroot, 2001) or terrestrial (Poonyth *et al.*, 1999; Aptroot *et al.*, 2000) environments. Phylogenetic studies indicated the aquatic *Massarina* species having affinities towards *Lophiostoma* species (Liew *et al.*, 2002; Eriksson and Hawksworth, 2003). This led to the transfer of several *Massarina* species to *Lophiostoma* based on morphology and molecular data (Hyde *et al.*, 2002; Liew *et al.*, 2002).

Lophiostoma possesses typical characters of *Lophiostomataceae*. These include immersed to erumpent ascomata with a laterally compressed- or crest-like apex, mostly clavate asci, and 1- to several-septate, hyaline to dark brown ascospores with terminal appendages or mucilaginous sheaths. *Lophiostoma* is morphologically a well-studied genus (Chesters and Bell, 1970; Holm and Holm, 1988; Barr, 1990; Yuan and Zhao, 1994), and currently it comprises about 30 species (Tanaka and Harada, 2003a). The crest-like apex is not considered to be a stable character and varies considerably even in the same specimen (Chesters and Bell, 1970; Holm and Holm, 1988).

Lophiotrema is one of Saccardo's "sporological genera", introduced based on the oblong to fusiform, one to multi-septate, hyaline ascospores (Saccardo, 1878). Chesters and Bell (1970) regarded *Lophiotrema* as a synonym of *Lophiostoma*. *Lophiotrema* however, can also be distinguished from *Lophiostoma* by its peridium and ascal shape, i.e. the peridium of *Lophiotrema* is almost equal in thickness, while it is broader near the base for *Lophiostoma* (Holm and Holm, 1988; Tanaka and Harada, 2003b), and species of *Lophiotrema* have cylindrical or oblong asci, while those of *Lophiostoma* are clavate or oblong (Holm and Holm, 1988). Based on these characters, only the type species – *Lophiotrema nucula* and another two closely related taxa were included in *Lophiotrema* by Holm and Holm (1988). This concept has been widely accepted (Barr, 1990; Yuan and Zhao, 1994; Kirk *et al.*, 2008). Tang *et al.* (2003) introduced a new *Lophiotrema* species (*L. psychotriae*) with compressed apex and elongated slit-like ostiole, stating that the main difference between *Lophiotrema* and *Lophiostoma* were ascomatal dimensions, peridium structure, ascal shape, and ascospore sheaths.

Several *Lophiostoma* / *Massarina*-like taxa have been collected from France and Belgium by J. Fournier. We tried to assign these collections to genera (and species) but it was extremely difficult because the significance of various characters could not be determined. Practically, the taxonomic differences between these two genera are based on a single morphological character — the shape of the apex (*Lophiostoma* containing a compressed/crest-like apex and *Massarina* having a rounded papilla). The shape of the apex is however, highly variable, and taxa with a similar apical shape may be heterogeneous (Holm, 1957; Eriksson, 1981; Leuchtman, 1984; Holm and Holm, 1988).

Because of the difficulties in distinguishing these genera, we examined their generic types, and carried out a phylogenetic analysis in an attempt to better understand the taxonomy of this group. The aims of the present study are to (1) reevaluate the phylogeny and circumscription of *Lophiostoma*, *Lophiotrema* and *Massarina*; (2) evaluate

which morphological character such as ascospore (shape, colour, number or type of septum, sheath), ascoma or apical shape has phylogenetic significance.

Materials and methods

Sample collection and specimen examination

Eleven fresh specimens were collected from August to October 2006, from France and Belgium by J. Fournier, and returned to the laboratory in plastic bags. In most cases ascomata were collected directly on natural wood without incubation. The samples were processed and examined following the method described in Tsui *et al.* (2000). Colonies were sub-cultured onto 2% potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), 2% malt extract agar (MEA), and oatmeal agar (OA) (Gams *et al.*, 2007), and incubated under continuous near-ultraviolet (320-400 nm, mainly 340 nm) light at 25°C to promote sporulation. Observations and photographs were prepared from material mounted in water, Congo red, Cotton blue, chlorazol black, lactic acid or Indian ink. Other cultures used in this study were obtained from the Centraalbureau voor Schimmelcultures, the Netherlands (CBS). “Verified specimen / strain” used here means the specimen or voucher of particular strain was examined by authors, and compared with the type material or descriptions to make sure it is correctly identified.

Fungal isolates and DNA extraction

Isolates were on PDA and MEA, and total genomic DNA extracted from mycelia following the protocols as outlined by Cai *et al.* (2005, 2006). Forensic Kit (UltraClean™ Forensic Kit, Cambio) was used to extract DNA from specimens directly.

DNA amplification and sequencing

DNA amplification was performed by PCR. For partial LSU rDNA amplification, LROR and LR5 primers (Vilgalys and Hester, 1990) were used. Primer pairs NS1 and NS4 were used to amplify a region from the small subunit (18S) of the rDNA (White *et al.*, 1990). The fRPB2-5F and fRPB2-7cR primers were used for the amplification of the partial RPB2 (RNA polymerase subunit 2) gene (Liu *et al.*,

1999). The amplification reaction for partial LSU, SSU rDNA and partial RPB2 gene was performed in a 50 µl reaction volume as outlined by Jeewon *et al.* (2004) and Shenoy *et al.* (2007): 1 × PCR buffer, 0.2 mM dNTP, 0.3 µM of each primer; 1.5 mM MgCl₂, 0.8 units Taq Polymerase and 5-10 ng DNA. The PCR thermal cycle for partial LSU and SSU rDNA amplification was as follows: 95°C for 3 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 30 s and elongation at 72°C for 1 min, with a final extension step of 72°C for 10 min (Vilgalys and Hester, 1990). The PCR thermal cycle for partial RPB2 gene amplification consisted of 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 2 min and elongation at 72°C for 1.5 min, with a final extension step of 72°C for 10 min (Liu *et al.*, 1999). The PCR products, spanning approximately 900 bp (partial LSU rDNA) and 1200 bp (partial RPB2 and SSU rDNA), were checked on 1% agarose electrophoresis gels stained with ethidium bromide. The PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham Biosciences, Buckinghamshire, UK; product code: 27-9602-01). DNA sequencing was performed using the above-mentioned primers in an Applied Biosystem 3730 DNA analyser at the Genome Research Centre, The University of Hong Kong.

Sequence alignment and phylogenetic analyses

Sequences generated from different primers were analyzed with other sequences obtained from the GenBank. A Blast search was performed to find the possible sister groups of the newly sequenced taxa. In addition, fungal members from different families of the *Pleosporales* and related orders were also included in the analyses. Multiple alignment was done in BioEdit (Hall, 2005) and Clustal X (Thompson *et al.*, 1997) and analyses were performed in PAUP v. 4.0b10 (Swofford, 2002). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimize alignment. Maximum Parsimony

(MP) was conducted using heuristic searches as implemented in PAUP, with the default options method. Analyses were done under different parameters of maximum parsimony criteria as outlined in Jeewon *et al.* (2004). Clade stability was assessed in a bootstrap analysis with 1000 replicates, random sequence additions with maxtrees set to 1000 and other default parameters as implemented in PAUP. Independent Bayesian phylogenetic analysis was performed in MrBayes 3.1.2 using a uniform GRT+I+G model, as selected by hLRT in Mrmodeltest 2.2. The Metropolis-coupled Markov Chain Monte Carlo (MCMC) approach were used to calculate posterior probabilities. Chains were analyzed with random starting trees for 1,000,000 generations. Trees collected before the stable likelihood value points were discarded as “burn-in” (Kodsueb *et al.*, 2006). Trees were viewed in Treeview (Page, 1996). The nucleotide sequences reported in this paper have been deposited in GenBank (Table 1). A combined 18S rDNA and 28S rDNA dataset and an individual RPB2 dataset were analysed respectively in this study. The phylogenetic tree are colour coded following Zhang *et al.* (2008).

Results and Discussion

DNA sequencing - Combined LSU and SSU rDNA phylogenies

The combined 28S (LSU) and 18S (SSU) rDNA dataset consists of 58 strains. The dataset consists of 1920 characters after alignment, of which 1887 sites are included in the MP analysis. Of the included bases, 350 sites (18.5%) are parsimony-informative. A heuristic search with random addition of taxa (1000 replicates) and treating gaps as missing characters generates six equally parsimonious trees. All trees are similar in topology and not significantly different (figures not shown). A single parsimonious tree (TL = 1528, CI = 0.506, RI = 0.752, RC = 0.380, HI = 0.494) is shown in Fig. 1. Bootstrap support (BS) values (equal to or above 50% based on 1,000 replicates) are shown on the upper branches. Values of the Bayesian posterior probabilities (PP) (equal to or above 95% based on 1,000 replicates) from MCMC analysis are shown under branches in red.

DNA sequencing – RPB2 phylogenies

The RPB2 dataset consists of 44 taxa, with 731 sites after alignment, and all of them are included in the analyses. Of these, 398 sites (54.4%) are parsimony-informative. A heuristic search with random addition of taxa (1000 replicates) and treating gaps as missing characters generates three equally parsimonious trees. All trees are similar in topology and not significantly different (figures not shown). A single parsimonious tree (TL = 3037, CI = 0.285, RI = 0.573, RC = 0.163, HI = 0.715) is shown in Fig. 2. BS values (equal to or above 50% based on 1,000 replicates) are shown on the upper branches. Values of the PP (equal to or above 95% based on 1,000 replicates) from MCMC analysis are shown under branches in red.

Although fewer members are included in the RPB2 dataset, the phylogenetic investigation resulting from both rDNA and RPB2 datasets are almost consistent (Figs 1, 2). The *Lophiostoma*, *Lophiotrema* and *Massarina* taxa clustered in 5 well supported monophyletic clades in the tree based on the combined sequence of the 28S and 18S rDNA or RPB2 dataset (Figs. 1, 2, clades marked in red). In both of the analyses, the *Lophiostoma*- and *Massarina*-like taxa clustered throughout the tree and were polyphyletic, and they mainly grouped into four well-supported monophyletic clades. Members of *Lophiotrema*, including the generic type — *L. nucula*, form another well supported monophyletic group. All of these five clades are located in *Pleosporales*. Both Dothideomycetes and *Pleosporales* form a well supported monophyletic group with high MP and PP bootstrap values (Figs 1, 2).

Clade *Lophiostoma* I including the type species of *Lophiostoma* (*L. macrostomum*), consists of *L. glabrotunicatum*, *Lophiostoma macrostomum* and *L. rugulosum*, and receives high bootstrap support (Fig. 1). These three species are similar in producing 1-septate, hyaline ascospores which become pigmented with age. This group clustered in the *Melanomataceae* with high bootstrap support (Fig. 1). The sequence of *L. macrostomum* was obtained from GenBank, which was originally from a voucher specimen (Lundqvist 20504 deposited in Swedish Museum of Natural History (S)) from Finland. The identification of this

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

Species	Source of sequences	GenBank accession no.		
		LSU	SSU	RPB2
<i>Arthopyrenia salicis</i> A. Massal.	CBS 368.94	AY538339	AY538333	NS
<i>Bimuria novae-zelandiae</i> D. Hawksw., Chea & Sheridan	CBS 107.79	AY016356	AY016338	DQ470917
<i>Byssothecium circinans</i> Fuckel	CBS 675.92	AY016357	AY016339	DQ767646
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	CBS 170.54	DQ678057	DQ678004	DQ677952
<i>Cochliobolus sativus</i> (S. Ito & Kurib.) Drechsler ex Dastur	DAOM 226212	DQ678045	DQ677995	DQ677939
<i>Cochliobolus heterostrophus</i> (Drechsler) Drechsler	CBS 134.39	AY544645	AY544727	DQ247790
<i>Cucurbitaria elongata</i> (Fr.) Grev.	CBS 171.55	DQ678061	DQ678009	Dq677657
<i>Davidiella macrospora</i> (Kleb.) Crous & U. Braun	CBS 138.40	DQ008148	EU167591	NS
<i>Davidiella tassiana</i> (De Not.) Crous & U. Braun	CBS 399.80	DQ678074	DQ678022	DQ677971
<i>Diaporthe phaseolorum</i> (Cooke & Ellis) Sacc.	FAU458	AY346279	AY779278	AY780175
<i>Herpotrichia diffusa</i> (Schwein.) Ellis & Everh.	CBS 250.62	DQ678071	DQ678019	DQ677968
<i>Hydrocina chaetocladia</i> Scheuer	HME4375	AY789412	AY789411	NS
<i>Lentithecium aquaticum</i> Yin. Zhang, J. Fourn. & K.D. Hyde	CBS 123099	FJ795434	FJ795477	FJ795455
<i>Lentithecium arundinaceum</i> (Sowerby) K.D. Hyde, J. Fourn. & Yin. Zhang	CBS 619.86	DQ813509	DQ813513	FJ795473
<i>Lentithecium fluviatile</i> (Aptroot & Van Ryck.) K.D. Hyde, J. Fourn. & Yin. Zhang,	CBS 123090	FJ795450	FJ795492	FJ795467
<i>Lentithecium fluviatile</i> (Aptroot & Van Ryck.) K.D. Hyde, J. Fourn. & Yin. Zhang,	CBS 122367	FJ795451	FJ795493	NS
<i>Lentithecium lineare</i> (E. Müll. & Dennis) Yin. Zhang, J. Fourn. & K.D. Hyde	IFRD 2008	FJ795435	FJ795478	NS
<i>Lophiostoma arundinis</i> (Fr.) Ces. & De Not.	CBS 621.86	DQ782384	DQ782383	DQ782386
<i>Lophiostoma caulium</i> (Fr.) Ces. & De Not.	CBS 623.86	FJ795436	FJ795479	FJ795456
<i>Lophiostoma compressum</i> (Pers.) Ces. & De Not.	IFRD 2014	FJ795437	FJ795480	FJ795457
<i>Lophiostoma crenatum</i> (Pers.) Fuckel	CBS 629.86	DQ678069	DQ678017	DQ677965
<i>Lophiostoma fuckelii</i> Sacc.	CBS 101952	DQ399531	FJ795496	FJ795472
<i>Lophiostoma glabrotunicatum</i> Yin. Zhang, J. Fourn. & K.D. Hyde	IFRD 2012	FJ795438	FJ795481	NS
<i>Lophiostoma macrostomoides</i> De Not.	CBS 123097	FJ795439	FJ795482	FJ795458
<i>Lophiostoma macrostomum</i> (Tode) Ces. & De Not.	Lundqvist 20504 (S)	DQ384094	NS	NS
<i>Lophiostoma rugulosum</i> Yin. Zhang, J. Fourn. & K.D. Hyde	CBS 123093	FJ795440	FJ795483	FJ795459
<i>Lophiostoma semiliberum</i> (Desm.) Ces. & De Not.	CBS 626.86	FJ795441	FJ795484	FJ795460
<i>Lophiostoma viridarium</i> Cooke	IFRDCC2081	FJ795443	FJ795486	NS
<i>Lophiotrema brunneosporum</i> Yin. Zhang, J. Fourn. & K.D. Hyde	CBS 123095	FJ795444	FJ795487	NS
<i>Lophiotrema lignicola</i> Yin. Zhang, J. Fourn. & K.D. Hyde	CBS 122364	FJ795445	FJ795488	FJ795462
<i>Lophiotrema nucula</i> (Fr.) Sacc.	CBS 627.86	FJ795446	FJ795489	FJ795463
<i>Lophiotrema vagabundum</i> (Sacc.) Sacc.	CBS 628.86	FJ795442	FJ795485	FJ795461
<i>Massaria platani</i> Ces.	CBS 221.37	DQ678065	DQ678013	DQ677961
<i>Massarina phragmiticola</i> Poon & K.D. Hyde	CBS 110446	DQ813510	DQ813512	NS

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

Species	Source of sequences	GenBank accession no.		
		LSU	SSU	RPB2
<i>Massarina cisti</i> S.K. Bose	CBS 266.62	FJ795447	FJ795490	FJ795464
<i>Massarina corticola</i> (Fuckel) L. Holm	CBS 154.93	FJ795448	FJ795491	FJ795465
<i>Massarina eburnea</i> (Tul. & C. Tul.) Sacc.	CBS 473.64	FJ795449	AF164367	FJ795466
<i>Massarina igniaria</i> (C. Booth) Aptroot	CBS 845.96	FJ795452	FJ795494	FJ795469
<i>Massarina rubi</i> (Fuckel) Sacc.	CBS 691.95	FJ795453	NS	FJ795470
<i>Massarina walkeri</i> Shoemaker, C.E. Babc. & J.A.G. Irwin	CBS 257.93	FJ795454	FJ795495	FJ795471
<i>Melanomma pulvis-pyrius</i> (Pers.) Fuckel	IFRDCC 2044	FJ201984	FJ201985	FJ795474
<i>Melanomma pulvis-pyrius</i> (Pers.) Fuckel	CBS 371.75	FJ201988	FJ201989	FJ795475
<i>Neofabraea malicorticis</i> H.S. Jacks.	AFTOL-ID 149	AY544662	AY544706	DQ470885
<i>Ophiosphaerella herpotricha</i> (Fr.) J. Walker	CBS 620.86	DQ678062	DQ678010	DQ677958
<i>Ophiosphaerella herpotricha</i> (Fr.) J. Walker	CBS 240.31	DQ767656	DQ767650	DQ767645
<i>Phaeodothis winteri</i> (Niessl) Aptroot	CBS 182.58	DQ678073	DQ678021	DQ677970
<i>Phaeosphaeria avenaria</i> (G.F. Weber) O.E. Erikss.	AFTOL-ID 280	AY544684	AY544725	DQ677941
<i>Phaeosphaeria eustoma</i> (Fuckel) L. Holm	CBS 573.86	DQ678063	DQ678011	DQ677959
<i>Pleospora herbarum</i> (Pers.) Rabenh.	CBS 714.68	DQ678049	DQ767648	DQ677943
<i>Pleospora herbarum</i> (Pers.) Rabenh.	CBS 191.86	DQ247804	DQ247812	DQ247794
<i>Preussia terricola</i> Cain	AFTOL-ID 282	AY544686	AY544726	DQ470895
<i>Dendryphiella arenaria</i> Nicot	CBS 181.58	DQ470971	DQ471022	DQ470924
<i>Sporidesmium australiense</i> M.B. Ellis	HKUCC 10833	DQ408554	NS	DQ435080
<i>Cucurbitodithis pityophila</i> (J.C. Schmidt & Kunze) Petr.	CBS 149.32	DQ384102	DQ384072	NS
<i>Sporormiella minima</i> (Auersw.) S.I. Ahmed & Cain	CBS 524.50	DQ678056	DQ678003	DQ677950
<i>Trematosphaeria pertusa</i> (Pers.) Fuckel	CBS 122368	FJ201990	FJ201991	FJ795476
<i>Westerdykella cylindrica</i> (Malloch & Cain) Arx	CBS 454.72	AY004343	AY016355	NS

NS: no sequence available in GenBank.

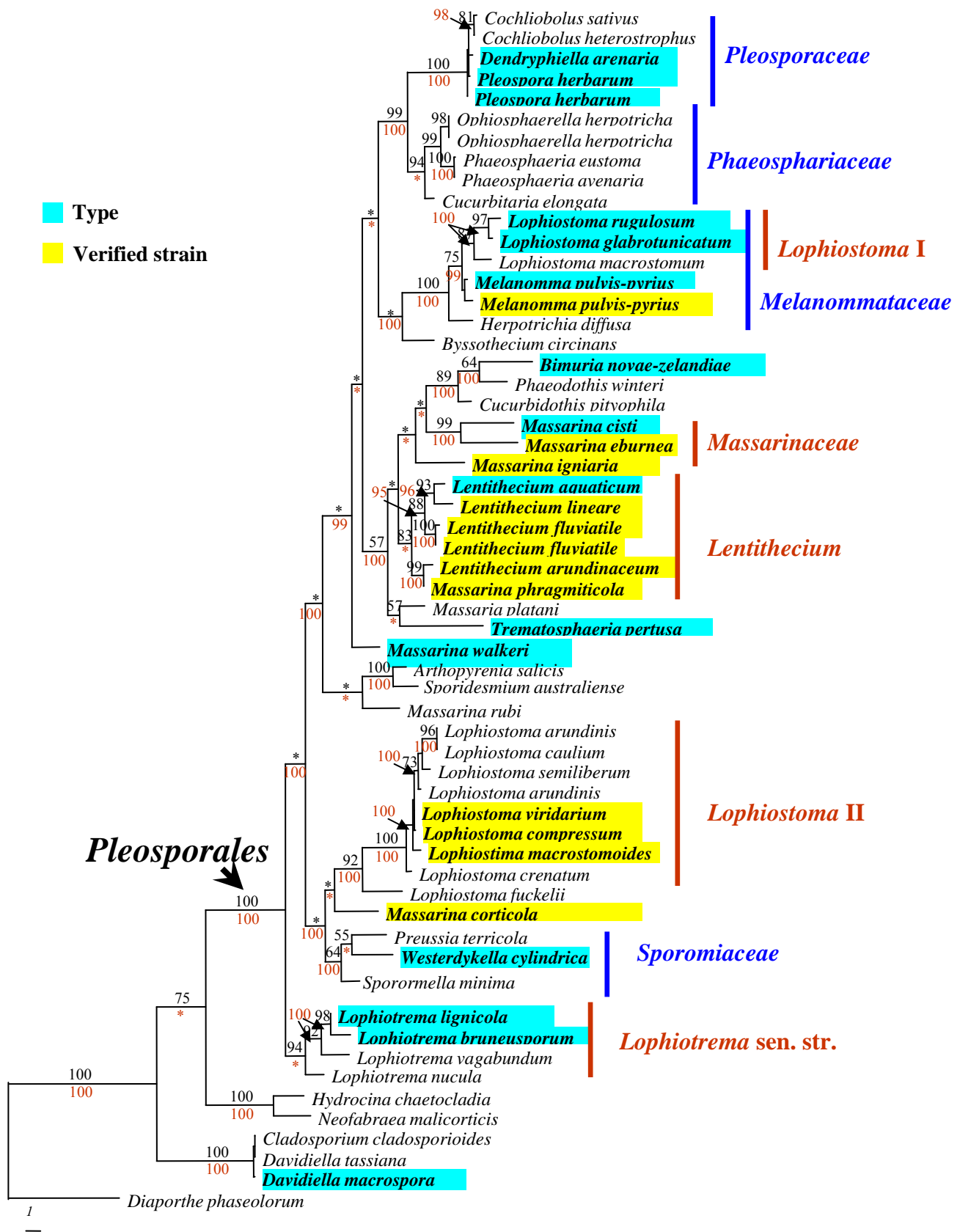
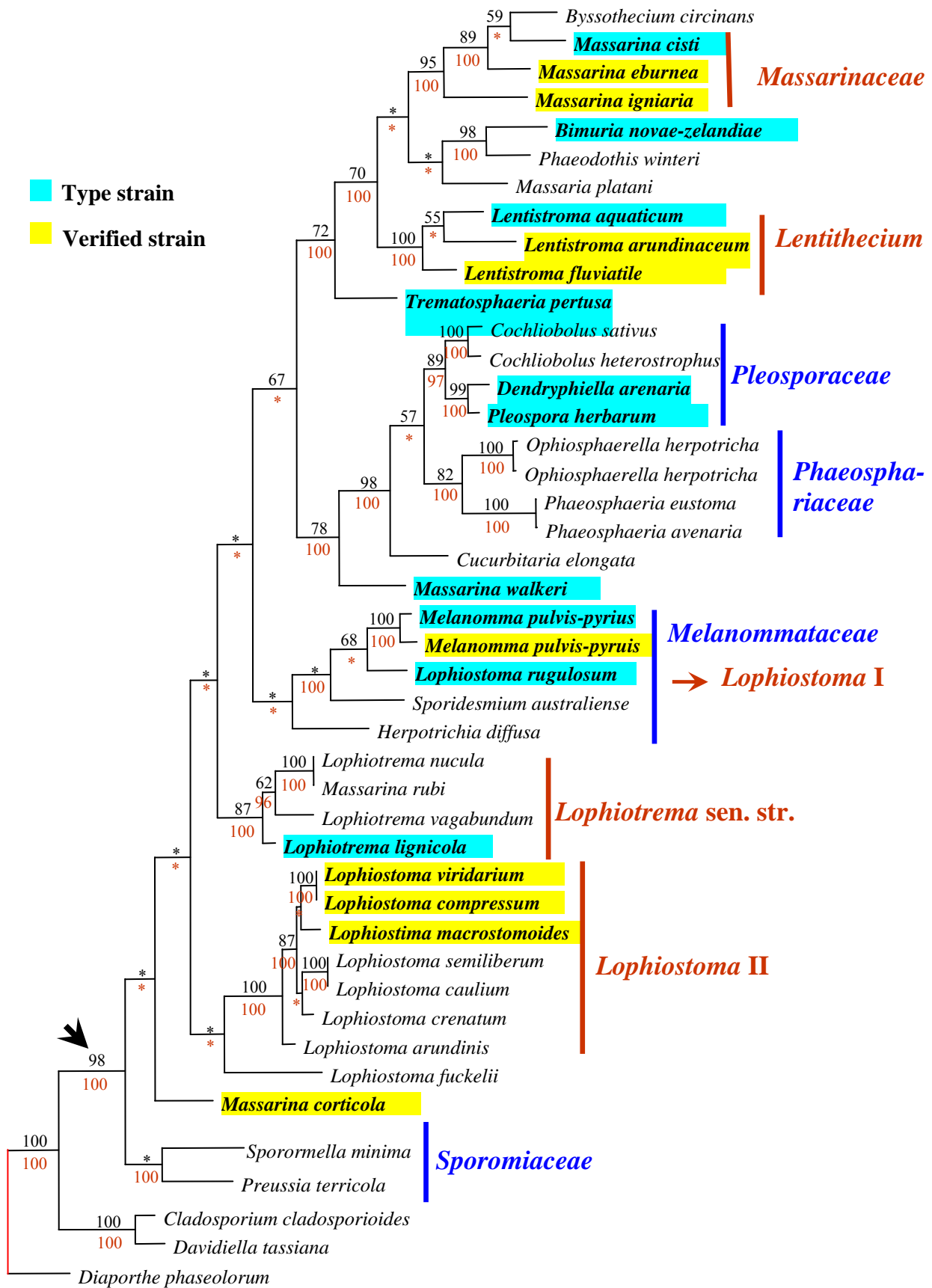


Fig. 1. The maximum parsimony tree generated based on sequence analysis of combined 28S and 18S rDNA dataset. Designated outgroup is *Diaporthe phaseolorum*. Maximum parsimony bootstrap support values above 50% shown at nodes are based on 1000 replicates. Bayesian support above 95% is shown under the branches in red.



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Fig. 2. The maximum parsimony tree generated based on sequence analysis of RPB2 dataset. Designated outgroup is *Diaporthe phaseolorum*. Maximum parsimony bootstrap support values above 50% shown at nodes are based on 1000 replicates. Bayesian support above 95% is shown under the branches in red.

specimen is not yet verified. The other two LSU sequences of *Lophiostoma macrostomum* in GenBank (Accession numbers: AB433273 and AB433274) are from the voucher specimens of HHUF:27290 and HHUF:27293 respectively, and they both located in Clade *Lophiostoma* II (result not shown). According to the hand drawing by Tanaka and Harada (2003a), HHUF:27293 might be wrongly identified as the apex is not crest-like. Thus the identification of these two specimens also needs to be verified. Therefore, it would be premature to separate *Lophiostoma sensu stricto* from the other *Lophiostoma* members in Clade *Lophiostoma* II as the specimens from which the gene sequences were obtained are not verified.

Clade *Massarinaceae*, from rDNA sequences, contains only two *Massarina* species (*M. cisti* and *M. eburnea*) and receives high bootstrap support (Fig. 1). *Massarina cisti* and *M. eburnea* share several morphological characteristics, including hyaline, broad to narrowly ellipsoidal ascospores and filamentous, broad, septate and hyaline cellular pseudoparaphyses, that lack or have anastomoses or branching (Hyde, 1995; Aptroot, 1998; Tanaka and Harada, 2003c). The identification of the two strains used here is verified (Zhang *et al.*, 2008). Liew *et al.* (2002) pointed out that species of *Massarina* with broad, ellipsoidal ascospores, together with *M. eburnea*, should remain in *Massarina sensu stricto*, and species with narrow, fusiform ascospores must be incorporated in *Lophiostoma*. This idea was accepted by other mycologists (Hyde *et al.*, 2002; Eriksson and Hawksworth, 2003). Thus here Clade *Massarinaceae* includes the generic type of *Massarina* (*M. eburnea*) and should represent *Massarina sensu stricto*. According to RPB2 sequence data, *Massarina eburnea* and *M. cisti* clustered together with *Byssothecium circinans* and *M. igniaria* (Fig. 2). *Byssothecium circinans* differs considerably from *M. eburnea* in morphology, such as the black, erumpent ascoma, fusiform ascospores with pigmented central cells and hyaline end cells. The unstable position of *B. circinans* might be because of the differences of sampling or gene number used in the analysis, and the later has been proved could influence the topology of the tree

(Rokas and Carroll, 2005), thus we are not sure if *B. circinans* has any relationship with *Massarina*. The identification of the *Massarina igniaria* strain (CBS 845.96) used here is verified by checking the voucher specimen (CBS H-006463). *Massarina eburnea* and *M. igniaria* share some common morphological characters, such as 3-septate fusiform ascospores and broad septate pseudoparaphyses. The rDNA sequence dataset however, does not support *M. igniaria* as belonging in *Massarina sensu stricto*.

Clade *Lentithecium* comprises four lignicolous species of *Lentithecium* including two transferred from *Massarina* (*M. arundinacea* and *M. fluvialis*) and one from *Keissleriella* (*K. linearis*). This Clade receives moderate bootstrap support from rDNA sequence dataset (Fig. 1), but high bootstrap support from RPB2 sequence dataset (Fig. 2). One well-supported subgroup in this Clade comprises *L. arundinaceum* and *Massarina phragmiticola* (Fig. 1). Morphological characters of these two species are also almost identical (Aptroot, 1998), which suggests these taxa are conspecific. *Keissleriella* was established by von Höhnelt (1919), and its most distinguishing characters are the black setae in and over the small apical papilla and the hyaline one-septate to rarely multi-septate ascospores (Barr, 1990). The familial placement of *Keissleriella* is debatable, such as in *Lophiostomataceae* (includes *Massarinaceae*) by Munk (1957), in *Pleosporaceae* by Arx and Müller (1975), in *Melanommataceae* by Barr (1990) and presently in *Massarinaceae* by Lumbsch and Huhndorf (2007). *Keissleriella linearis*, as the only taxon having setae in Clade *Lentithecium* (Fig. 11A), forms a robust Clade with other members, which might indicate that the presence of setae has little phylogenetic significance. Taxa in Clade *Lentithecium* share similar morphological characters in having immersed to erumpent lenticular ascomata, clavate asci, anastomosing cellular pseudoparaphyses and hyaline one-septate ascospores. Thus we introduce a new genus – *Lentithecium* to accommodate this group of fungi. The polyphyletic nature of *Massarina* has been noticed in previous studies (Hyde and Aptroot, 1998; Hyde *et al.*, 2002; Liew *et al.*, 2002). The lenticular ascoma is a common character

shared by all five members in Clade *Lentithecium*, but it is premature to draw the conclusion that ascomatal shape has any phylogenetic significance at the generic level classification.

Clade *Lophiostoma* II is a well supported clade containing seven lophiostomataceous species with *Lophiostoma fuckelii* clustering in a basal position (Fig. 1). The identification of three strains of this group, i.e. *L. viridarium*, *L. compressum* and *L. macrostomoides*, has been verified by carefully checking the fresh specimens we collected and referring to their detailed descriptions by Holm and Holm (1988). Members in this group share a compressed apex, mostly brown and multiseptate or even muriform ascospores. Ascospores of *L. semiliberum* are 1-septate, hyaline, becoming brown with additional septa when senescent. The ascomata with compressed apex are a striking common character possessed by all members of this group. Compared with the crest-like apex possessed by *L. macrostomum*, the compressed apex is a more stable and evolutionary older character state (Holm and Holm, 1988). This group of fungi seems to represent a natural group at family level but does not include the type of *Lophiostoma* — *L. macrostomum*. This conclusion can not be confirmed however, until the verified sequences of *L. macrostomum* (Clade *Melanommataceae*) are obtained.

The *Lophiotrema* group includes the type species — *L. nucula*, and contains four lophiostomataceous species (*L. brunneosporum*, *L. lignicola*, *L. nucula* and *L. vagabundum*) with consistent high bootstrap (Figs 1, 2). These four species share immersed to erumpent to nearly superficial ascomata, round or elongate ostioles, clavate to cylindrical asci, and 1-septate, hyaline to pigmented, deeply constricted ascospores (Holm and Holm, 1988; Tanaka and Harada, 2003b).

Clade *Sporomiaceae* contains four species which are moderately supported by parsimony analysis but well supported by MrBayes analyses with *M. corticola* in the basal position (Figs 1, 2). *Sporomiaceae* is a coprophilous group of fungi, and previous phylogenetic studies indicated *Sporomiaceae* to

be a monophyletic group (Kruys *et al.*, 2006; Schoch *et al.*, 2006). The circumscription of some genera in this family has, however, been based on some characters of weak phylogenetic significance (Arenal *et al.*, 2005; Nyberg Kruys, 2006).

Taxonomy

Lentithecium K.D. Hyde, J. Fourn. & Yin. Zhang, **gen. nov.**

Mycobank: 512790

Etymology: from the Latin “*lenti*” and “*thecium*”, in reference to the “lenticular” ascomata.

Ascomata immersa vel partim immersa, lenticular, solitarius vel disseminatus, nigra. Asci clavate vel oblongatus-cylindraco, pedicellati, bitunicati, fissitunicati. Ascospores cymbiforme, 1-septatae, aliquando 3-septatae cum supra-maturus, septum constrictae, hyaline.

Ascomata immersed to semi-immersed, lenticular. *Peridium* comprising a few layers of thin-walled cells. *Asci* bitunicate, fissitunicate, clavate-cylindrical to oblong-cylindrical. *Ascospores* hyaline, one-septate.

Type species — *Lentithecium fluviatile* (Aptroot & Van Ryck.) K.D. Hyde, J. Fourn. & Yin. Zhang, **comb. nov.** MB 512802

Basionym: *Massarina fluviatilis* Aptroot & Van Ryck., (2001), Nova Hedwigia 73: 162. (2001)

Lentithecium aquaticum Yin. Zhang, J. Fourn. & K.D. Hyde, **sp. nov.** (Fig. 3)

Mycobank: 512791

Etymology: from the Latin “*aquaticum*”, in reference to the freshwater habit.

Ascomata 130-160 μm alta, 240-320 μm longa, immersa, papillaris, lenticular, disseminatus, nigra. Asci 130-190 \times 17-23 μm , 8-spore, clavate, pedicellati, bitunicat. Ascospores 25-30 \times 8-12 μm , fusiformes, hyalinae.

Ascomata 130-160 μm high \times 240-320 μm diam., scattered, immersed, lenticular with a flattened base, papillate, the rounded papilla protruding slightly above the wood surface which is stained dark grey; ostioles round (Figs 3A, B). *Peridium* 13-25 μm thick, thin-walled, pigmented, *textura prismatica*, thicker and more pigmented in upper part (Figs 3B, C). *Hamathecium* of dense, long septate pseudoparaphyses, 2-3 μm broad, mixed in places with much thinner filaments, rarely anastomosing between and above the asci (Fig. 3G). *Asci*

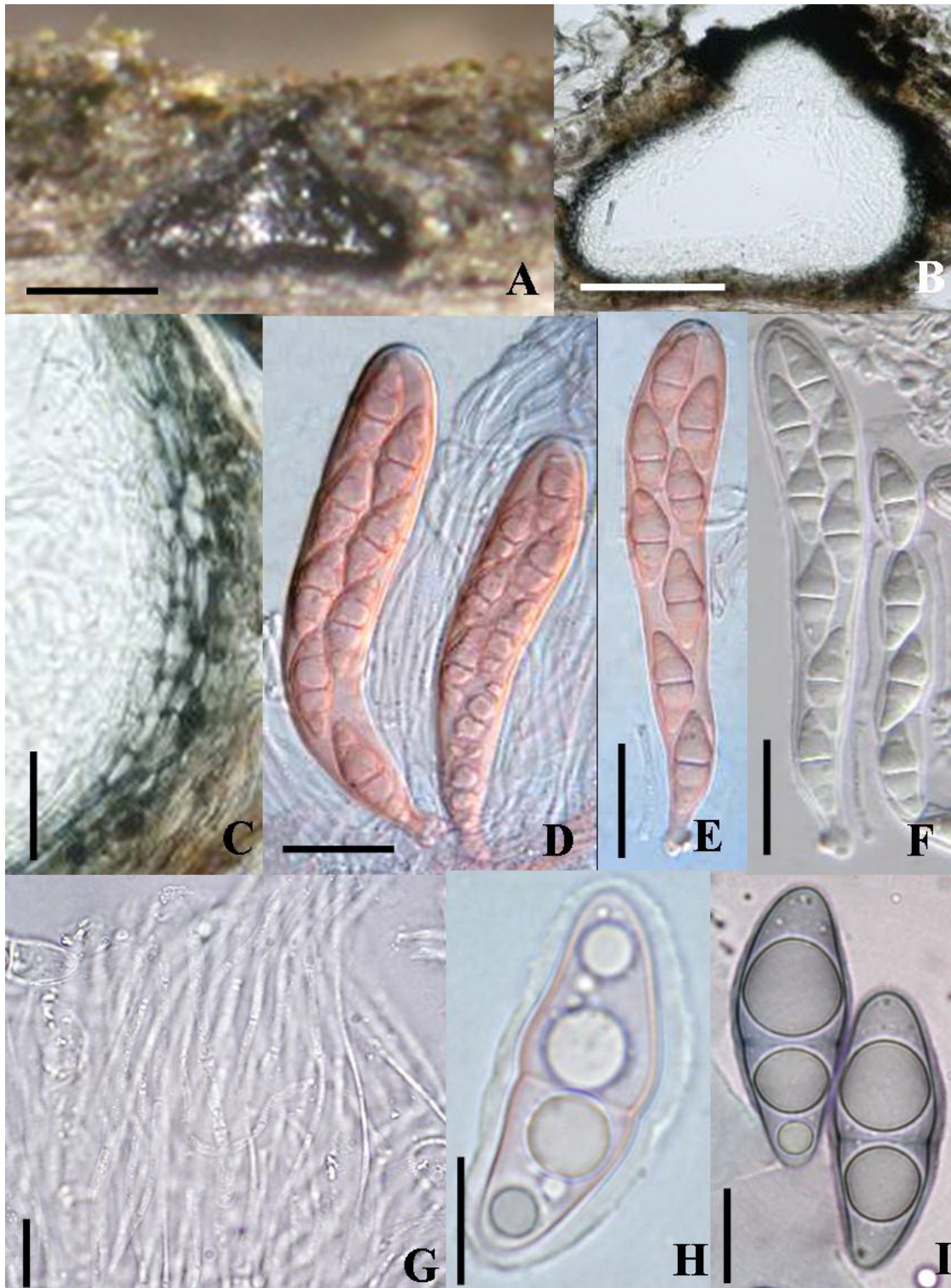


Fig. 3. *Lentithecium aquaticum* (from holotype IFRD 2007). **A.** Section of an ascoma. **B, C.** Section of the peridium comprising a few layers of cells of *angularis*. **D-F.** Asci with short pedicels. **G.** Pseudoparaphyses. **H, I.** Ascospores. Note the encompassing sheath. **B, C** in water; **D, E, H** in Congo Red; **F, G** in water; **I** in chlorazol black. Scale bars: **A, B** = 100 μ m, **C-F** = 20 μ m, **G-I** = 10 μ m.

130-190 \times 17-23 μ m (\bar{x} = 175 \times 21 μ m, n = 10), 8-spored, bitunicate, fissitunicate, pedicellate, with a small inconspicuous ocular chamber (Figs 3D-F). *Ascospores* 25-30 \times 8-12 μ m (\bar{x} = 28 \times 10.5 μ m, n = 10), biseriate, broadly fusiform with broadly rounded ends,

inequilateral in side view, hyaline, two-celled, turning four-celled with age, slightly constricted at the septum, smooth, containing four refractive globules, with a conspicuous encompassing sheath, 1.5-3 μ m thick (Figs 3H, I).

Culture characters (ex-holotype: CBS 123099)

On OA spreading, up to 15 mm diam., smoke-grey with woolly aerial mycelium; hyphae hyaline, smooth, 1.5-3 μm wide, forming hyphal strands with 15-20 hyphae that become dark brown, 4-5 μm wide. On PDA up to 15 mm diam.; central part iron-grey, with a wide cream border; aerial mycelium lacking, slimy or woolly, pale olivaceous-grey; grey-olivaceous in reverse, with cream margin. On MEA spreading, up to 15 mm diam., with moderate, woolly aerial mycelium and smooth, even margins; surface and reverse cream to smoke-grey.

Specimens examined: FRANCE, Ariège, Rimont, Peyrau, on submerged wood of *Fraxinus excelsior*, 400m, 09 Oct. 2006, leg., det. J. Fournier [holotype: CBS H-20220], ex-holotype living culture deposited in CBS (CBS 123099); 07 Nov. 2006, leg., det. J. Fournier [IFRD2021]; on submerged wood of *Alnus glutinosa*, 19 Oct. 2006, leg., det. J. Fournier [IFRD2024]; Le Baup brook, along D 18, on submerged wood of *Platanus* sp., 550m, 10 Nov. 2006, leg., det. J. Fournier, [IFRD 2022].

Notes: *Lentithecium aquaticum* can be distinguished from other members of *Lophiostoma*- and *Massarina*-like species by its minute, immersed ascomata, which are easily overlooked, and by the narrow conspicuous sheath around ascospores, which is easily visible in water and does not swell in water. *Massarina immersa* also has tiny immersed ascomata, but the ascomata of *M. immersa* are globose, and the broadly fusiform ascospores of *M. immersa* are distinctly smaller than those of *Lentithecium aquaticum* ((17-)19-22(-24) \times 6-8 μm vs. 25-30 \times 8-12 μm) (Aptroot, 1998). Ascospore shape is comparable with those of *Massarina appendiculata* and *M. moeszii*, but the ascomata of *M. moeszii* are much larger (500-900 μm), and the ascomata of *M. appendiculata* are round and larger (to 550 μm) (Aptroot, 1998). It is a commonly encountered species in all regions of France sampled, and is remarkably still present in its ascigenous state in winter, and occurs on various substrates.

Lentithecium arundinaceum (Sowerby) K.D. Hyde, J. Fourn. & Yin. Zhang, **comb. nov.**
MycoBank: 512819

Basionym: *Sphaeria arundinacea* Sowerby, Coloured Figures of English Fungi 3: t.336 (1803).

= *Massarina phragmiticola* Poon & K.D. Hyde, Botanica Marina 41: 145 (1998).

Specimens examined: DENMARK, Sjaeland, Frederikskilde, Suserup Skove, Tystrup Lake, on

submerged stems of *Phragmites* sp., 25 May 2007, leg., det. J. Fournier [IFRD2031]; FRANCE, Haute Garonne, Avignonet, Port Lauragais, Rosel artificial lake, on submerged stems of *Phragmites* sp., 15 Jul. 2007, leg., det. J. Fournier [IFRD2032]; Vendée, Vouvant, on submerged stems of *Phragmites* sp., Apr. 2005, leg., det. P. Leroy [IFRD2033].

Lentithecium lineare (E. Müll. & Dennis) K.D. Hyde, J. Fourn. & Yin. Zhang, **comb. nov.**
MycoBank: 512820

Basionym: *Keissleriella linearis* E. Müll. & Dennis (1964), Kew Bulletin 19: 120. (1964)

Specimen examined: FRANCE, Haute Garonne, Avignonet, Port Lauragais, Rosel artificial lake, on dead stems of *Phragmites* sp., 06 May 2006, leg., det. J. Fournier & P. Leroy [IFRD2008].

Lophiotrema

Lophiotrema nucula (Fr.) Sacc., *Michelia* 1: 338 (1878). (Fig. 4)

= *Sphaeria nucula* Fr., Kongliga Vetenskaps Academiens Handlingar 38: 266 (1817).

Ascomata 200-240 μm high \times 200-280 μm diam., scattered, erumpent to nearly superficial, with basal wall remaining immersed in host tissue, globose, subglobose, often laterally flattened, with a flattened base, black, roughened, often bearing remnants of wood fibres; with a cylindrical or laterally compressed papilla, to 120 μm long and 150 μm high (Fig. 4A). *Peridium* laterally 25-30 μm thick, very thin at the base, composed of heavily pigmented pseudoparenchymatous cells near the apex, cells 2-2 \times 6 μm diam., wall 1-3(-4) μm thick, near apex composed of pigmented cells of *textura angularis*, 3-5 μm diam., wall 0.8-1.5 μm thick, apical wall composed of heavily pigmented and thick walled small cells (Figs 4B, C). *Hamathecium* of dense, very long septate pseudoparaphyses, 1-2 μm broad, anastomosing and branching between and above asci, embedded in gel matrix (Fig. 4I). *Asci* 90-115 \times 9-11.5 μm (\bar{x} = 99.5 \times 11 μm , n = 10), 8-spored, bitunicate, fissitunicate, cylindrical, with a short, narrowed, furcate pedicel which is up to 10 μm long, with a small ocular chamber (Figs 4D-F, H). *Ascospores* 17-21(-25) \times (4-)5-6.5 μm (\bar{x} = 19.5 \times 5.5 μm , n = 10), obliquely uniseriate and partially overlapping to biseriata, broad-fusiform, fusiform to narrowly fusiform, with narrowly rounded ends, hyaline and lightly pigmented on very rare occasions when

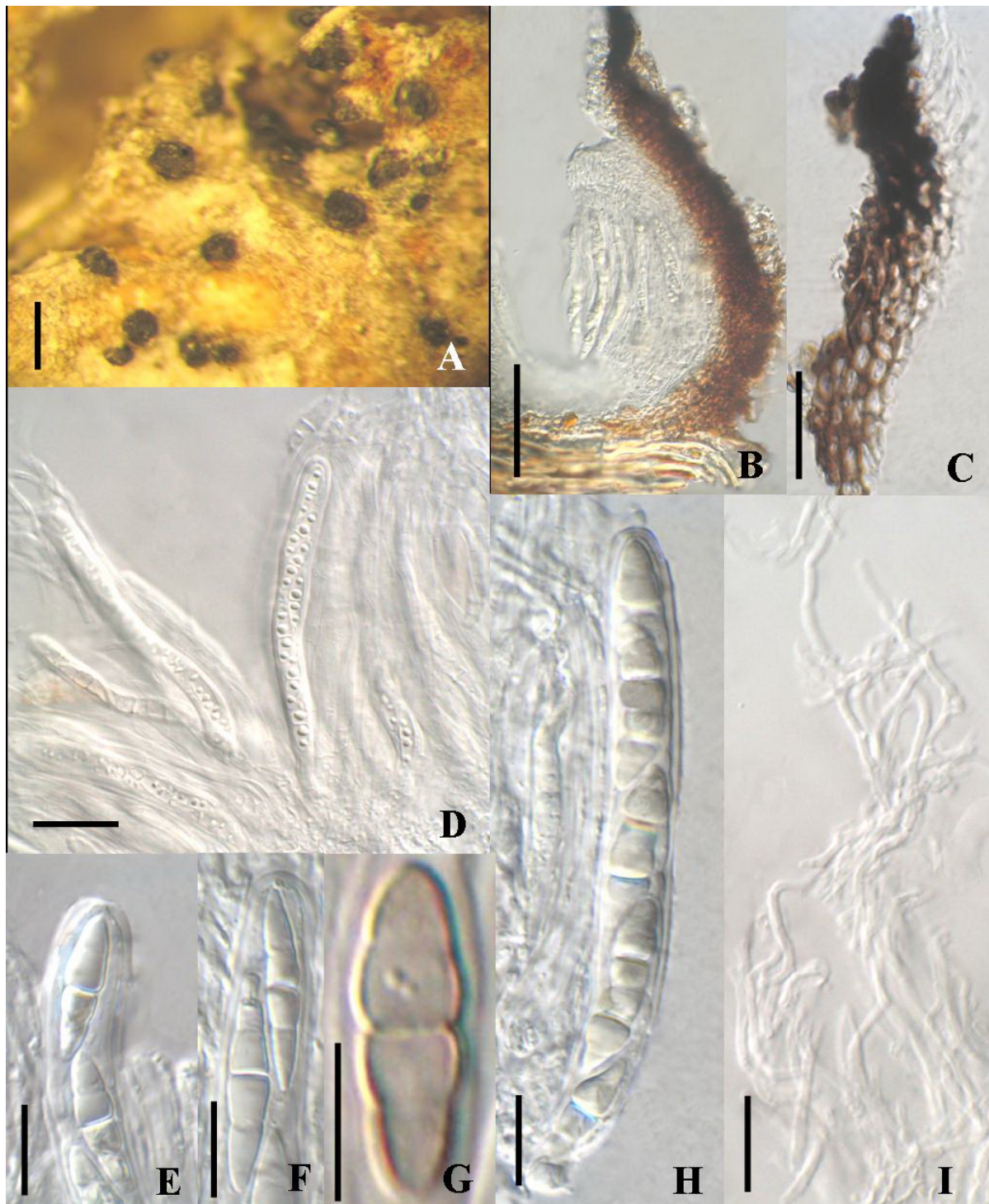


Fig. 4. *Lophiotrema nucula* (from Leptotype (UPS!)). **A.** Ascomata on the host surface. **B.** Section of an ascoma. **C.** Peridium structure near the apex. **D, H.** Asci in the pseudoparaphyses. **E, F.** Upper part of the asci, showing the small ocular chamber near the apex. **G.** Mature ascospores. **H.** Ascus in pseudoparaphyses. **I.** Pseudoparaphyses. **B-C** in water, **D-I** in lactic acid. Scale bars: **A** = 0.5 mm, **B** = 100 μ m, **C, D** = 30 μ m, **E-I** = 10 μ m.

senescent, 1-septate, 3-septate when old, deeply constricted at the median septum, the upper cell often broader than the lower one (Fig. 4G).

Specimen examined: Lectotype (UPS!) as *Sphaeria nucula* Fr. on decaying wood.

A relatively unambiguous definition of *Lophiotrema* was given by Holm and Holm (1988) based on a unique combination of ascomata, peridium, asci and ascospores characters. Ascomata are small to medium-sized and approximately pyriform with an often

reduced cylindrical neck which may even be absent. The peridium is approximately 20-30 μm of equal thickness, composed of an outer layer of *textura angularis* of uniformly pigmented cells, up to 12 μm , and an inner layer of very small hyaline cells, with somewhat thickened walls. Asci are cylindrical and spores are hyaline, at first 1-septate, later 3-septate, with distinct guttules, often with a mucilaginous sheath (Holm and Holm, 1988). Much emphasis was placed on ascospore characters by Holm and Holm (1988) when they described and distinguished the three *Lophiotrema* species: *L. boreale*, *L. nucula* and *L. vagabundum*. This generic concept was widely accepted by later workers (e.g. Barr, 1990; Yuan and Zhao, 1994; Kirk *et al.*, 2008). Emphasis was placed on the peridium and asci by Tanaka and Harada (2003b) to distinguish *Lophiotrema* from *Lophiostoma*. This peridium differences however, is not supported in the lectotype specimen we examined here, which has a flattened thin-walled base. While several of our recent collections from Europe fit Holm and Holm's description well, which differs from the lectotype specimen in: ascomata (immersed to slightly erumpent *vs.* erumpent to nearly superficial), length of asci (90-115 μm *vs.* 120-130 μm) and peridium (equal in thickness *vs.* with thinner base) (Figs 11B-D). Thus here we call these collections as *Lophiotrema nucula sensu* Holm. The DNA sequences of *L. nucula* used here are of Holm's collection. Thus further study is needed to clarify the specific status of *L. nucula sensu stricto*, and equal-thickness peridium could not serve as a diagnosing character of *Lophiotrema*. Presently, we temporarily treat *L. nucula sensu* Holm as *L. nucula sensu stricto* basing on their mostly comparable morphological characters.

Lophiotrema lignicola Yin. Zhang, J. Fourn. & K.D. Hyde, **sp. nov.** (Fig. 5)
MycoBank: 512792

Etymology: from the Latin "*lignicola*", in reference to the lignicolous substrate.

Ascomata 260-350 μm alta, 450-530 longa, semi-immersa, papillaris, gregarius. Asci 133-180 \times 15-17 μm , 8-sporei, latus-cylindrico, pedicellati, bitunicat. Ascosporeae 21-25 \times 6.5-8 μm , latus-fusiformes, hyalinae.

Ascomata 260-350 μm high \times 450-530 μm diam., gregarious, 2/3rds to semi-immersed

under a blackish pseudostroma, lenticular; papillate, apex often elongate and laterally compressed but not raised or forming a crest, with an elongated slit-like ostiole (Figs 5A, B). *Peridium* 20-30 μm thick at sides and base, 40-60 μm thick at apex, dark brown, pseudoparenchymatous, composed of two intergrading layers: outer layer of small cells 4-10 μm long, *textura angularis* to *prismatica*, moderately thick-walled at sides and base, very thick-walled at apex, with dark brown hyphal appendages 4-6 μm broad penetrating the surrounding wood and forming a pseudostroma 60-100 μm thick around the papilla; inner layer composed of less pigmented to hyaline cells *textura angularis*, very thin-walled (Figs 5C, D). *Hamathecium* of dense, long pseudoparaphyses, rarely septate, 1-1.5 μm broad, branching and anastomosing between and above asci, embedded in gelatinous matrix (Fig. 5E). *Asci* 133-180 $\mu\text{m} \times$ 15-17 μm ($\bar{x} = 160 \times 15.5$ μm , $n = 10$), 8-spored, bitunicate, fissitunicate, broad cylindrical, with a short, narrowed, twisted, pedicellate, with an ocular chamber and a minute apical ring visible in immature asci (Figs 5F, G). *Ascospores* 21-25 \times 6.5-8 μm ($\bar{x} = 23.5 \times 7.5$ μm , $n = 10$), uni to biseriata, broadly fusiform with broad to narrow rounded ends, hyaline, yellowish at maturity, 2-celled, cell wall up to 1.8 μm thick, slightly constricted at the median septum, the upper cell often slightly shorter and broader than the lower one, smooth, surrounded by an irregular hyaline gelatinous sheath 2-2.5 μm thick visible in 3% KOH but not seen in water or India ink (Figs 5H-J).

Culture characters (ex-holotype: CBS 122364)

On OA spreading, with sparse aerial mycelium and even, regular margins, reaching 20 mm diam.; surface olivaceous-grey. On PDA spreading with sparse to moderate aerial mycelium and regular margins, reaching 15 mm diam.; surface olivaceous-grey in middle, iron-grey in outer region and in reverse. On MEA erumpent, with moderate aerial mycelium and smooth, crenate margins, reaching 15 mm diam.; surface pale olivaceous-grey, reverse olivaceous-grey.

Specimen examined: BELGIUM, Hainaut, Orval, ruisseau de Williers, on decorticated trunk of *Populus* sp., 29 Sept. 2006, leg., det. J. Fournier [CBS H-20221, **holotype**].

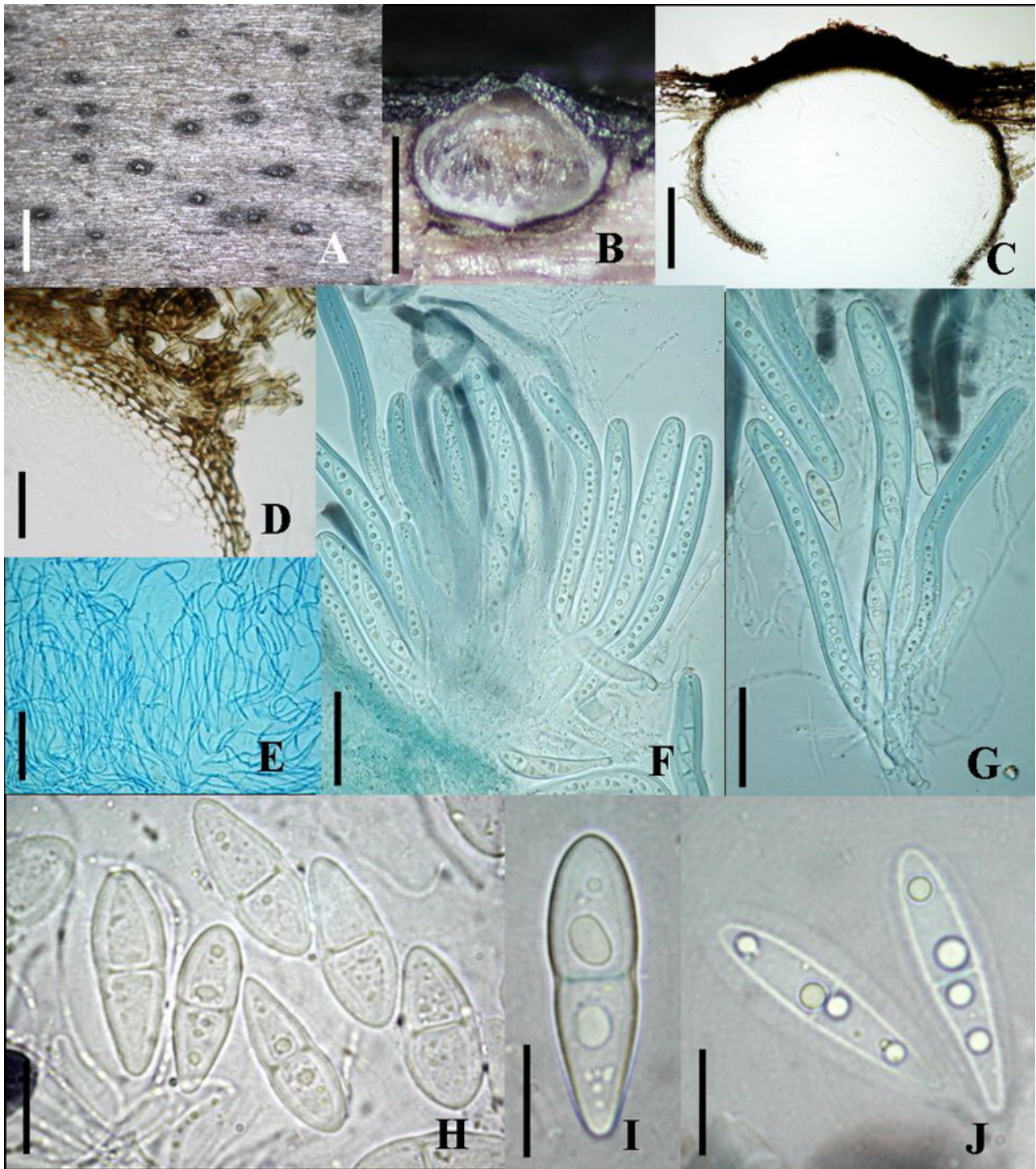


Fig. 5. A-J. *Lophiotrema lignicola* (from Holotype IFRD 2009). **A.** Ascomata on the host surface. **B.** Section of an ascoma in wood. **C.** Section of an ascoma. Note the peridium structure. **D.** Section of the partial peridium near apex. Note the peridium structure. **E.** Pseudoparaphyses. **F, G.** Asci in pseudoparaphyses. **H-J.** Ascospores. Note the sheath. **C, D, H** in water; **E** in cotton blue; **F, G** in chlorazol black. Scale bars: **A, B** = 0.5 μm , **C** = 100 μm , **D-G** = 50 μm , **H-J** = 10 μm .

Notes: The broadly cylindrical ascus with a short pedicel and hyaline one-septate ascospore surrounded by a broad mucilaginous sheath are similar to that found in the type species of the genus – *Lophiotrema nucula*, but the immersed ascomata of *L. lignicola* could readily distinguish the erumpent to nearly

superficial ascomata of *L. nucula*. Morphologically, *Lophiotrema lignicola* is also comparable with *Lophiostoma fuckelii sensu lato*, but the ascomata of *Lophiotrema lignicola* are much broader (260-350 \times 450-530 μm vs. (180-)310-340 \times (140-)215-260 μm), and the ascospores of the *L. lignicola* are also larger than those of

Lophiostoma fuckelii (21-25 × 6.5-8 µm vs. 15-20 × 4-5 µm) (Holm and Holm, 1988). The asci of the *Lophiotrema lignicola* are cylindrical while those of the *Lophiostoma fuckelii* are clavate (Holm and Holm, 1988). Another morphologically comparable species is *Lophiostoma glaciale*, but *L. glaciale* has clavate asci and a distinct crest on top of the ascomata.

Lophiotrema brunneosporum Yin, Zhang, J. Fourn. & K.D. Hyde, **sp. nov.** (Fig. 6)
MycoBank: 512793

Etymology: from the Latin “*brunneo-*” and “*sporum*”, in reference to the brown-coloured ascospore.

Ascomata 260-350 µm alta, 450-530 µm longa, immersa, solitarii. Asci 130-170 × 15-17(-26) µm, 4-8-sporei, clavati, bitunicat. Ascosporae 35-40 × 7.5-9 µm, contracte-fusiformes, bruneu.

Ascomata 260-350 µm high × 450-530 µm diam., papillate, immersed, subglobose to depressed ellipsoidal, papilla laterally flattened, appearing on host surface as narrowly ellipsoid blackish spots elongated with the grain of wood, with a central slit-like ostiole flush with the surface to most often slightly raised above surface, shiny black, forming a narrow crest 250-420 µm long, to 100 µm high (Figs 6A, B). Peridium 28-35 µm thick, dark brown, pseudoparenchymatous, composed of moderately thick-walled cells, cell walls 1-1.5 µm thick, lacking differentiated layers but heterogenous, composed of small isodiametric cells 2-4 µm diam. at base forming a *textura angularis* and larger cells 6-10 µm long varying from *textura angularis* to *textura prismatica* at sides and apex, with brown hyphal appendages originating from the outer cells; pseudostroma 50-65 µm thick, dark brown, composed of host cells and fungal cells (Fig. 6D). Hamathecium of dense, filamentous, 0.8-1.5 µm broad pseudoparaphyses, embedded in mucilage, rarely septate, anastomosing between and above the asci. Asci 130-170 × 15-17(-26) µm (\bar{x} = 155 × 16.5 µm, n = 10), 4-8-spored, bitunicate, fissitunicate, cylindro-clavate, pedicellate, with a large ocular chamber (Fig. 6C). Ascospores 35-40 × 7.5-9 µm (\bar{x} = 38 × 8 µm, n = 10), uni to biseriata, narrowly fusiform with narrow rounded ends, brown at maturity, 2-celled, deeply constricted at the median septum, the upper cell often shorter and broader than the lower one, smooth, containing refractive globules (Figs 6E-H).

Culture characters (ex-holotype: CBS 123095)

On OA erumpent with moderate aerial mycelium and even, crenate margins, reaching up to 10 mm diam.; olivaceous-grey on surface. On PDA erumpent, with sparse aerial mycelium, and even crenate margins, reaching 8 mm diam.; surface and reverse olivaceous-grey. On MEA erumpent, with moderate aerial mycelium, and even, crenate margins, reaching up to 8 mm diam.; surface pale olivaceous-grey; reverse iron-grey.

Specimens examined: FRANCE, Ariège, Rimont, Las Muros, on decorticated wood of *Salix* sp., 24 Sept. 2006, leg., det. J. Fournier & K.D. Hyde [CBS H-20222, **holotype**]; Peyrau, on rotten decorticated wood of *Acer campestre*, 23 Aug. 1997, leg., det. J. Fournier [IFRD2034].

Notes: The morphological characters such as compressed papilla, dense, filamentous pseudoparaphyses, and cylindro-clavate asci and fusiform one-septate ascospores fit *Lophiostomaceae* well. Phylogenetically, *Lophiotrema brunneosporum* forms a robust clade with *L. nucula*, and consequently we dispose *L. brunneosporum* in *Lophiotrema*.

The most outstanding character of this species is the reddish-brown ascospores, which is uncommon in *Lophiotrema*. Even lophiostomateous species with pigmented ascospores are usually multiseptate (≥ 3) (Holm and Holm, 1988). The reddish-brown 1-septate ascospores are comparable with those of *Trematosphaeria pertusa* and some species of *Botryosphaeria-ceae* (Phillips *et al.*, 2008; Zhang *et al.*, 2008), and thus ascospore colour has little polyphyletic significance.

Lophiostoma

Lophiostoma was formally established by Cesati and De Notaris (1863) and lectotypified by *L. macrostomum* (Holm, 1975 p. 481). *Lophiostoma* is morphologically a well studied genus (Chesters and Bell, 1970; Holm and Holm, 1988; Barr, 1990; Yuan and Zhao, 1994), and currently it comprises about 30 species (Tanaka and Harada, 2003a). The genus was characterized by Holm and Holm (1988) as having immersed to erumpent ascomata with a cylindrical or crest-like apex and full length slit-like ostiole; a peridium of unequal thickness, which was broader near the base (*Lophiostoma*-type); mostly clavate, bitunicate asci and 1- to several septate, hyaline to

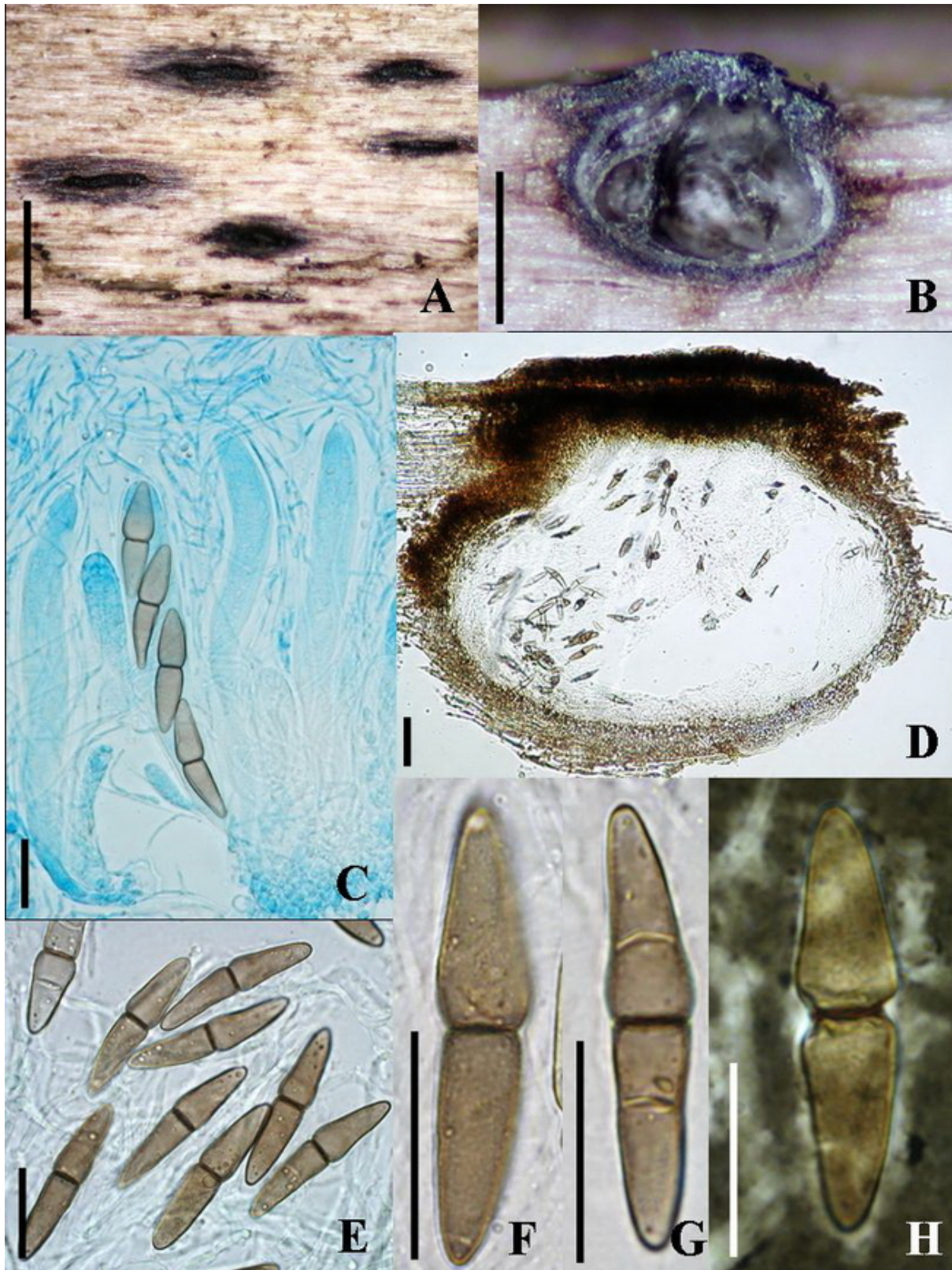


Fig. 6. *Lophiotrema brunneusporum* (from Holotype). **A.** Ascomata on the host surface. Note the compressed papilla. **B.** Section of an ascoma in wood. **C.** Four-spored mature and some immature asci in pseudoparaphyses. **D.** Section of the peridium. Note the peridium structure. **E-H.** Mature ascospores. **C** in Cotton blue, **D-G** in water, **H** in Indian ink. Scale bars: **A, B** = 0.5 mm, **C-H** = 20 μ m.

pigmented ascospores with terminal appendages or surrounded by a mucilaginous sheath. This definition was followed by Barr (1990) Yuan and Zhao (1994) and Hyde *et al.* (2002). Phylogenetic affinity with some *Massarina* species has been reported by Liew *et al.* (2002), but as yet no generic revision has been conducted.

Lophiostoma macrostomum (Tode) Ces. & De Not., Commentario della Società Crittogamologica Italiana 1: 219 (1863). (Fig. 7)

≡ *Sphaeria macrostoma* Tode, Fungi Mecklenburgenses Selecti 2: 12 (1791).

Other synonyms see Holm and Holm (1988).

Ascomata 400-600 µm high × 420-560 µm diam., densely scattered to gregarious, semi-immersed to erumpent, globose, subglobose, with a small to large flat crest-like apex which is variable in shape, up to 300 µm high and 480 µm broad (Figs 7A, B). *Peridium* 30-45 µm thick at the sides, thicker at the apex and thinner at the base, one-layered, composed of small lightly pigmented thin-walled cells of *textura prismatica*, cells *ca.* 6-9 × 3-4 µm diam., apex composed of pseudoparenchymatous cells (Fig. 7B). *Hamathecium* dense, very long, up to 3 µm near the base and less than 1.5 µm broad in the upper place, septate, embedded in mucilage, anastomosing and branching between and above the asci (Fig. 7F). *Asci* 110-145 × 10-15 µm (\bar{x} = 127.5 × 13 µm, *n* = 10), 8-spored, bitunicate, fissitunicate, clavate, pedicellate, with a small ocular chamber (J-) (Figs 7C-E). *Ascospores* 27-38(-43) × 5-7.5 µm (\bar{x} = 31.2 × 6.4 µm, *n* = 10), biseriate, fusiform, hyaline, usually one septate, with 3-5 septa and faintly brown when old, with (2-)3(-4) distinct oil drops in each cell, with short terminal appendages (Figs 7H-J), and ornamented with warts when spores are senescent (Fig. 7G).

Specimen examined: SWEDEN, Smaland, Femsjö par., Femsjö, on *Prunus*, leg. Elias Fries, det. Geir Mathiassen [UPS **Lectotype** (as *Sphaeria macrostoma* Fr.)].

The crest-like papilla has been regarded as a prominent morphological character of *Lophiostoma macrostomum* (Chesters and Bell, 1970; Holm and Holm, 1988). In the lectotype specimen (lectotypified by Holm, 1975), the raised area above the ascomata is up to 300 µm

high and 480 µm long and seen as a flattened or even Y-shaped crest (Fig 7). In *Lophiostoma curtum* and *Lophiotrema boreale* the raised area above the ascomata varies considerably in height or is even lacking (Holm and Holm, 1988). Thus the variable “crest-like raised area in *Lophiostomaceae*” was explained as an evolutionarily adaptation to the hard substrate within which the ascomata develop (Holm and Holm, 1988). The ascospores of *L. macrostomum* usually turn reddish-brown when mature, and minutely verrucose ornamentation was also found on the surface of the pigmented ascospores. Hyaline ascospores that became pigmented with aging are common in *Lophiostoma*, such as in *L. appendiculatum*, *L. massarioides*, *L. semiliberum*, *L. subcorticale* and *L. winteri* (Holm and Holm, 1988; Tanaka and Harada, 2003a). The phylogenic significance of this character should be observed carefully in the future but at present its phylogenetic significance is unclear as this also occurs in some *Lophiotrema* species.

Lophiostoma rugulosum Yin. Zhang, J. Fourn. & K.D. Hyde, **sp. nov.** (Fig. 8)
Mycobank: 512794

Etymology: from the Latin “*rugulosum*”, in reference to the “roughened” wall surface.

Ascomata 250-420 µm alta, 290-500 longa, subglobosa, superficialra, dispergere vel gregariculus, apex ostiolum latiusculus, nigra. *Asci* 130-155 × 12-15 µm, 8-spore, contracte-clavati vel fusiformes, bitunicat. *Ascosporeae* 24-31(-34) × 5-8 µm, fusiformes, hyalinae.

Ascomata 250-420 µm high × 290-500 µm diam., gregarious, scattered to rarely fused in small groups, slightly immersed, erumpent to nearly superficial, subglobose with flattened base broadly attached to the substrate, black, often bearing remnants of wood fibres; surface coarsely roughened and cracked to distinctly warted, apex non-papillate, typically opening through a wide, umbilicate pore 40-60 µm diam., usually surrounded by 4-6 radial ridges (Figs 8A, B). *Peridium* 50-65 µm thick, up to 80 µm thick at base angles, pseudoparenchymatous, leathery, comprising two cell types, outer cells 15-60 µm thick, dark brown, thicker at the apex, much thinner or absent at the base, comprising heavily melanized, thick-walled cells of *textura angularis*, inner cells 10-55 µm thick, of thin-walled hyaline cells of *textura angularis* to *textura prismatica* (Figs 8D, E). *Hamathecium* of tightly aggregated cellular

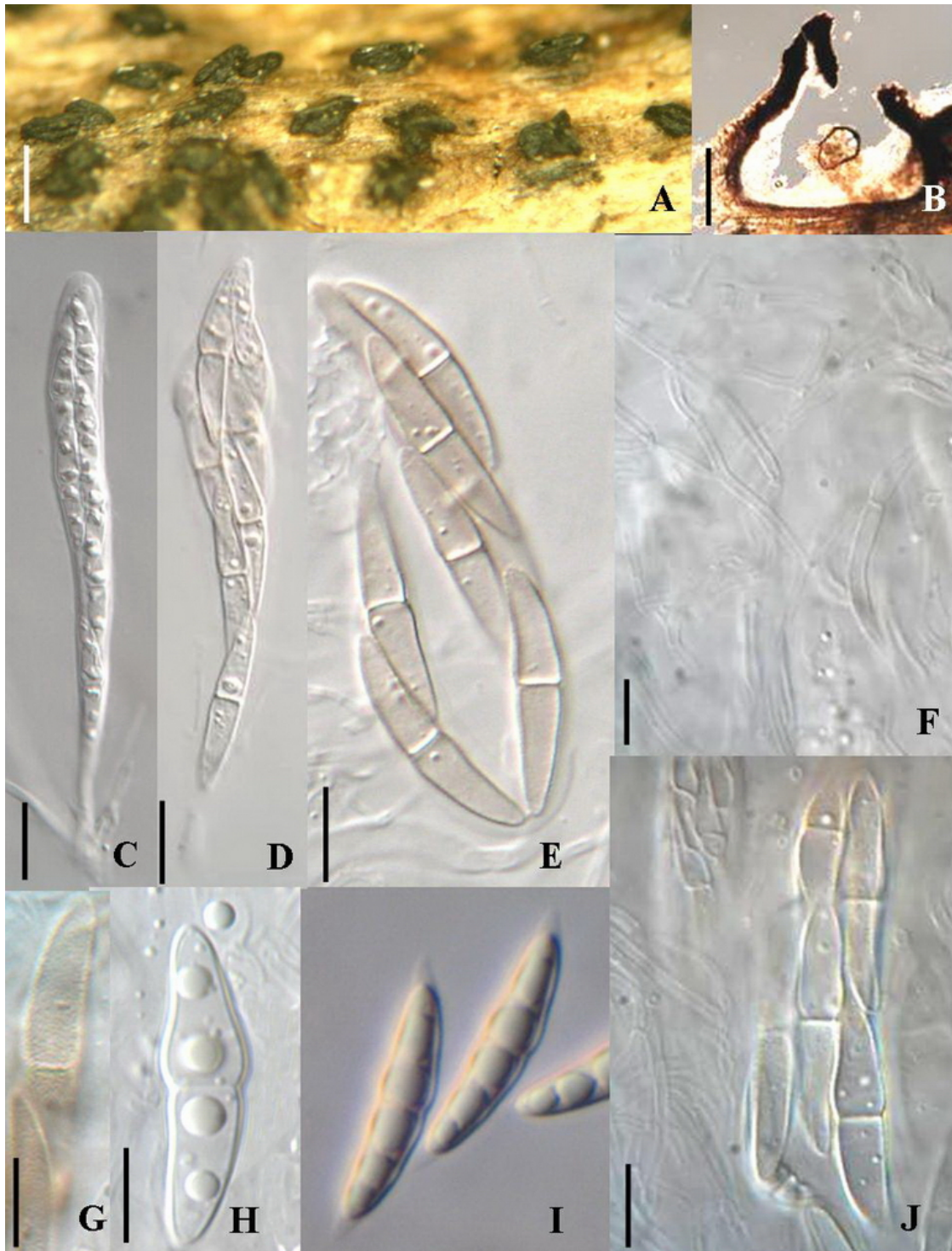


Fig. 7. *Lophiostoma macrostomum* (A-H, J from Leptotype (UPS!), I from IFRD 2005). A. Ascomata on the host surface. B. Section of the peridium. C-E. Ascus. F. Hamathecium. G-J. Ascospores. B-J in water. Scale bars: A = 500 μm , B = 200 μm , C-J = 10 μm .

pseudoparaphyses, 1.7-2.5 μm broad, embedded in mucilage, ramified and somewhat diverticulate, rarely anastomosing between and above the asci, apically ending into bunches of clavate cells 12-20 μm long \times 4-7 μm broad. *Asci* 130-155 \times 12-15 μm (\bar{x} = 150 \times 14 μm , n = 10), 8-spored, bitunicate, fissitunicate, cylindro-clavate, pedicellate, with a faint

ocular chamber (Fig. 8C). *Ascospores* 24-31(-34) \times 5-8 μm (\bar{x} = 29 \times 7 μm , n = 10), biseriolate, fusiform with broad to narrowly rounded to acute ends, with mucilaginous sheath to 3 μm thick, hyaline and one septate when young, turning yellowish-grey and verruculose while in the ascus, dark grey to sooty grey and become 1-3-septate when

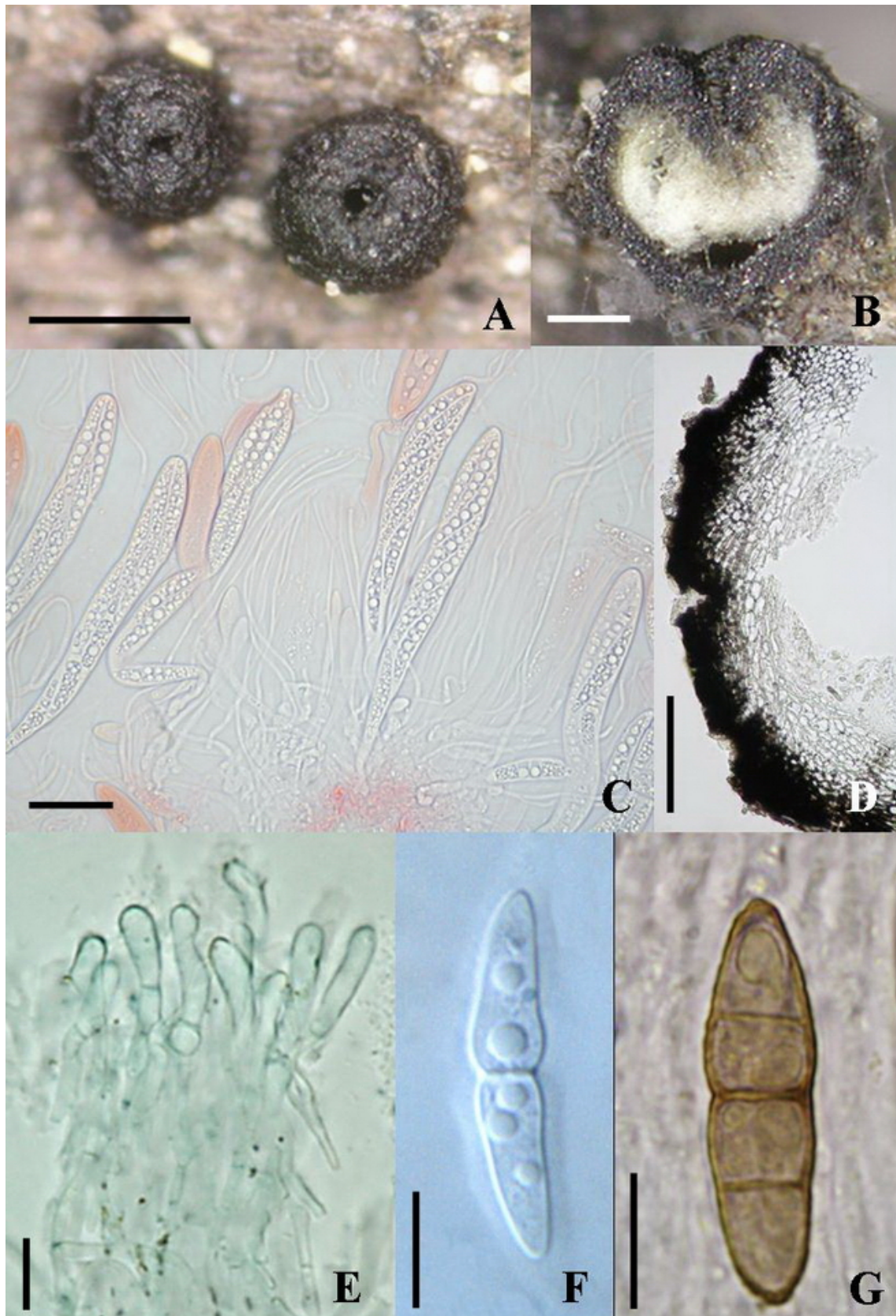


Fig. 8. *Lophiostoma rugulosum* (from Holotype IFRD 2011). **A.** Ascomata on the host surface. **B.** Section of an ascoma. **C.** Asci in the pseudoparaphyses. **D.** Peridium structure. **E.** Pseudoparaphyses. Note the swelling tips. **F, G.** Ascospore. **C** in Congo Red; **E** in chlorazol black; others in water. Scale bars: **A** = 500 μm , **B, D** = 100 μm , **C** = 20 μm , **E-G** = 10 μm .

senescent, slightly to deeply constricted at the septum, the upper cell often broader than the lower one (Figs 8F, G).

Culture characters (ex-type: CBS 123093)

On OA spreading with sparse aerial mycelium and even margins, reaching 10 mm diam.; surface olivaceous-grey with a prominent, wide margin of diffuse, bright yellow pigment. On PDA spreading, with sparse aerial mycelium and even crenate margins, reaching 17 mm diam.; surface and reverse iron-grey, smoke-grey at margin, slimy in middle. On OA hyphae smooth, hyaline to pale olivaceous, 3-4 μm wide, becoming swollen below septa, with cells up to 15 μm diam.; in aerial mycelium these cells aggregate at the ends of hyphae, forming densely packed round propagules up to 90 μm diam., consisting of somewhat curved, smooth, olivaceous cells up to 15 μm long and 7 μm wide; presumably these can be dislodged as conidia.

Specimens examined: FRANCE, Ariège, Rimont, Peyrau, 400m, on submerged wood of *Alnus glutinosa*, 31 Aug. 2006, leg., det. J. Fournier [CBS H-20223, **holotype**]; 31 Aug. 2000, leg., det. J. Fournier [IFRD2026]; 23 Sept. 2000, leg., det. J. Fournier [IFRD 2027]; on submerged wood of *Salix sp.*, 26 Jul. 2006, leg., det. J. Fournier [IFRD 2028]; on submerged wood of *Rhamnus cathartica*, 13 Aug. 2006, leg., det. J. Fournier [IFRD 2029]; Lescure, Bois du Pas du Baup, Le Volp, 500m, on partly submerged wood of *Quercus*, 26 Oct. 2006, leg., det. J. Fournier [IFRD2025].

Notes: The dense, filamentous hamathecium, cylindro-clavate asci, and hyaline, one-septate ascospores with mucilaginous sheath fit characters of *Lophiostoma* well. The peculiar wall structure and thickness, and the broad umbilicate ostioles surrounded by 4-6 radial ridges however, are the most distinctive features of this fungus, which is different from other morphologically comparable species and unusual for this genus. The presence of pseudoparaphyses with apically free ends differentiated into elongated swollen cells is likewise most unusual among *Pleosporales* and members of *Lophiostoma*. The ostiolar pore is widely open and empty on dry mature ascomata, but a whitish plug is present on fresh or rehydrated material. It can be assumed these swollen cells are involved in the formation of the apical plug. They lack pigmented deposits and are not aggregated into an epithecium, as it occurs in *Patellariaceae* which, moreover are

more cupulate to disc-shaped (Kutorga and Hawksworth, 1997; Zhang and Hyde, 2009). Compressed papilla is a highly variable character that the variation of both form and size can even be seen in the same specimen (Holm and Holm, 1988). Thus, based on phylogenetic result, here we assign *Lophiostoma rugulosum* in *Lophiostoma* despite its broad umbilicate ostioles.

In addition, this taxon appears fairly abundant in the two above mentioned brooks, regardless of any host preference, but was never encountered in other brooks or streams prospected in the same region, which indicates it may have narrow ecological requirements.

Lophiostoma glabrotunicatum Yin. Zhang, J. Fourn. & K.D. Hyde, **sp. nov.** (Fig. 9)
Mycobank: 512800

Etymology: from the Latin “*glabrotunicatum*”, in reference to the “smooth” wall surface.

Ascomata 260-380 μm alta, 360-420 longa, subglobosa, superficialia, dispergere, nigra. Asci 124-152 \times 11-13 μm , 8-spore, cylindrico vel sub-clavat, bitunicat. Ascosporeae 22-27 \times 6-7 μm , fusiformes, hyalinae.

Ascomata 260-380 μm high \times 360-420 μm diam., scattered or in small groups, superficial, with basal wall remaining immersed in host tissue not easily removed from the substrate, subglobose to depressed sphaerical, black, slightly roughened, brittle, often bearing remnants of wood fibres; apex hardly to weakly papillate, with a small ostiolar pore; underlying wood stained yellow (Figs 9A, B). **Peridium** 45-70 μm thick at sides and apex, up to 100 μm thick at base angles, much thinner at the base, leathery, dark brown, two-layered: outer layer 15-24 μm thick, *textura angularis* of dark brown thick-walled cells 5-10 μm diam., nearly opaque at apex, cell walls 2-3 μm thick, including a heavily pigmented outermost crust 5-10 μm thick with small protruding opaque cells, and inner layer 30-80 μm thick, *textura prismatica* to *textura angularis*, composed of hyaline thin-walled cells in lower part, and irregularly pigmented and moderately thick-walled cells toward the apex (Fig. 9C). **Hamathecium** of dense, long pseudoparaphyses, 2-2.5 μm broad, embedded in gelatinous matrix, septate, branching and anastomosing between and above the asci (Fig. 9G). **Asci** 124-152 \times 11-13 μm (\bar{x} = 145 \times 12 μm , n = 10), 8-spored, bitunicate, fissitunicate,

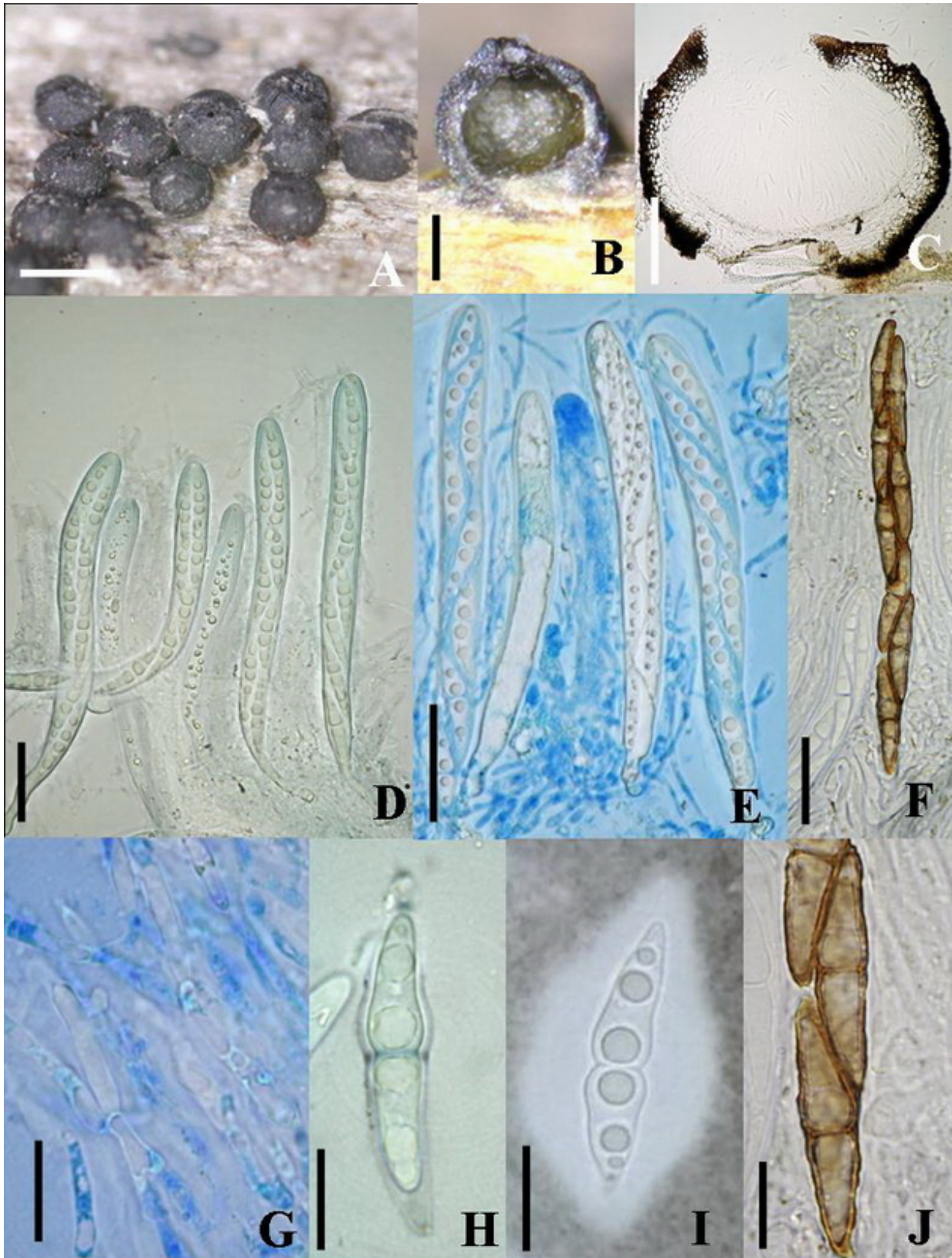


Fig. 9. *Lophiostoma glabrotunicatum* (from Holotype IFRD 2012). **A.** Superficial ascomata on the host surface. **B.** Section of an ascoma. **C.** Section of peridium. Note the two layered peridium structure. **D-F.** Asci with short pedicels in the pseudoparaphyses. Note the senescent ascus in **F.** **G.** Thick, septate and branching pseudoparaphyses. **H, I.** Ascospores in sheath. **J.** Senescent ascospores. **C, F, J** in water; **D, H** in chlorazol black; **E, G** in cotton blue; **I** in Indian ink. Scale bars: **A** = 500 μ m, **B, C** = 100 μ m, **D - F** = 20 μ m, **G - J** = 10 μ m.

clavate-cylindrical, with a short, narrowed, twisted, furcate pedicel which is 6-10 μm long, with a truncate ocular chamber and a minute ring visible on immature asci (Figs 9D-F). *Ascospores* 22-27 \times 6-7 μm (\bar{x} = 25 \times 6.5 μm , n = 10), obliquely uniseriate and partially overlapping or biseriate in places, fusiform with narrowly rounded ends, hyaline, two-celled, deeply constricted at the median septum, the upper cell often shorter and broader than the lower one, smooth, surrounded by an irregular hyaline gelatinous sheath 2.5-6 μm thick best seen in India ink. Senescent ascospores are brown and 2-3-septate, finely verrucose, a wide gelatinous sheath is present on immature ascospores, disappearing on old ascospores (Figs 9H-J).

Specimens examined: FRANCE, Ariège, Prat Communal, Loumet, on submerged wood of *Alnus glutinosa*, 1000m, 08 Sept. 2006, leg., det. J. Fournier [CBS H-20225, **holotype**, CBS H-20224, **isotype**]; Les Cabannes, Pierrefitte forest, on submerged wood of *Fagus sylvatica*, 1400 m, 15 Oct. 2007, leg., det. J. Fournier [IFRD2035]; Ustou, Cirque de Cagateille, on submerged wood of *Salix* sp., 1150 m, 11 Jun. 2007, leg., det. J. Fournier [IFRD2030].

Notes: All of the morphological characters such as dense, long pseudoparaphyses, clavate-cylindrical asci, hyaline, one-septate, fusiform ascospores with a broad sheath suggest that this taxon belongs to the *Lophiostomataceae*, and that *L. glabrotunicatum* is phylogenetically closely related with *Lophiostoma* (Fig. 1). The papilla of this species does not, however, agree with the concept of *Lophiostoma*. But the widely variable character and young evolutionary status of the crest-like apex of *L. macrostomum* might help to explain the variable apex morphology of this group (Fig. 1, Clade *Melanommataceae*) (Holm and Holm, 1988). Ascospores of *L. glabrotunicatum* are comparable with those of *L. aquaticum* and *Massarina submediana*, but the superficial, subglobose ascomata and the yellow staining of the underlying wood are not in agreement (Aptroot, 1998). *Lophiostoma glabrotunicatum* has been repeatedly recovered from the mountainous area of France exclusively between 1000-1400 m elevations. This might indicate its ecological preference for cold water in the high altitude mountainous area.

Massarina

Massarina was introduced by Saccardo (1883) for species of pyrenocarpous ascomycetes that had previously been placed in *Massaria* De Not., but typically had hyaline ascospores (Bose, 1961). The family *Massarinaceae* was described by Munk (1956) to accommodate *Massarina*. This family was not commonly used and *Massarina* was later placed within the *Lophiostomataceae* in the *Pleosporales* (Bose, 1961; Eriksson and Yue, 1986; Barr, 1987, 1990). Of the 160 epithets listed in his monograph, Aptroot accepted only 43 species (Aptroot, 1998). The concept of *Massarina* was widely accepted as having single or aggregated, immersed to erumpent, spherical to hemispherical, pseudothecioid ascomata; cellular pseudoparaphyses; bitunicate, cylindrical to clavate or obpyriform asci; and hyaline, 1-3(-7)-septate, fusiform to long ellipsoid ascospores that mostly have a mucilaginous sheath or appendages (Aptroot, 1998; Hyde and Aptroot, 1998; Tanaka and Harada, 2003c). Recent morphological, molecular and anamorphic results indicate, however, that *Massarina* is polyphyletic (Hyde, 1995; Kirk *et al.*, 2008; Liew *et al.*, 2002). We believe that *Massarina sensu stricto* should be confined to the generic type (*M. eburnea*) and very similar species (e.g. *M. cisti*).

Massarina eburnea (Tul. & C. Tul.) Sacc., Syll. Fung. 2: 153 (1883). (Fig. 10)

= *Massaria eburnea* Tul. & C. Tul., Selecta Fungorum Carpologia 2: 239 (1863), nom. nov. [non sensu Tulasne & Tulasne]

Other synonyms see Hyde (1995).

Ascomata to 250 μm high \times 500-700 μm diam., solitary or in small clusters, forming under raised dome-shaped areas, with blackened centres, with a central ostiole, immersed within the cortex of thin dead branches, ellipsoidal, rounded from above, clypeate, neck central, short and barely noticeable on host surface (Fig. 10A). Clypeus ca. 250 μm diam., 60 μm thick, brown, comprising compact brown-walled cells of *textura angularis* to *globulosa* beneath host epidermal cells (Fig. 10B). *Peridium* ca. 20 μm

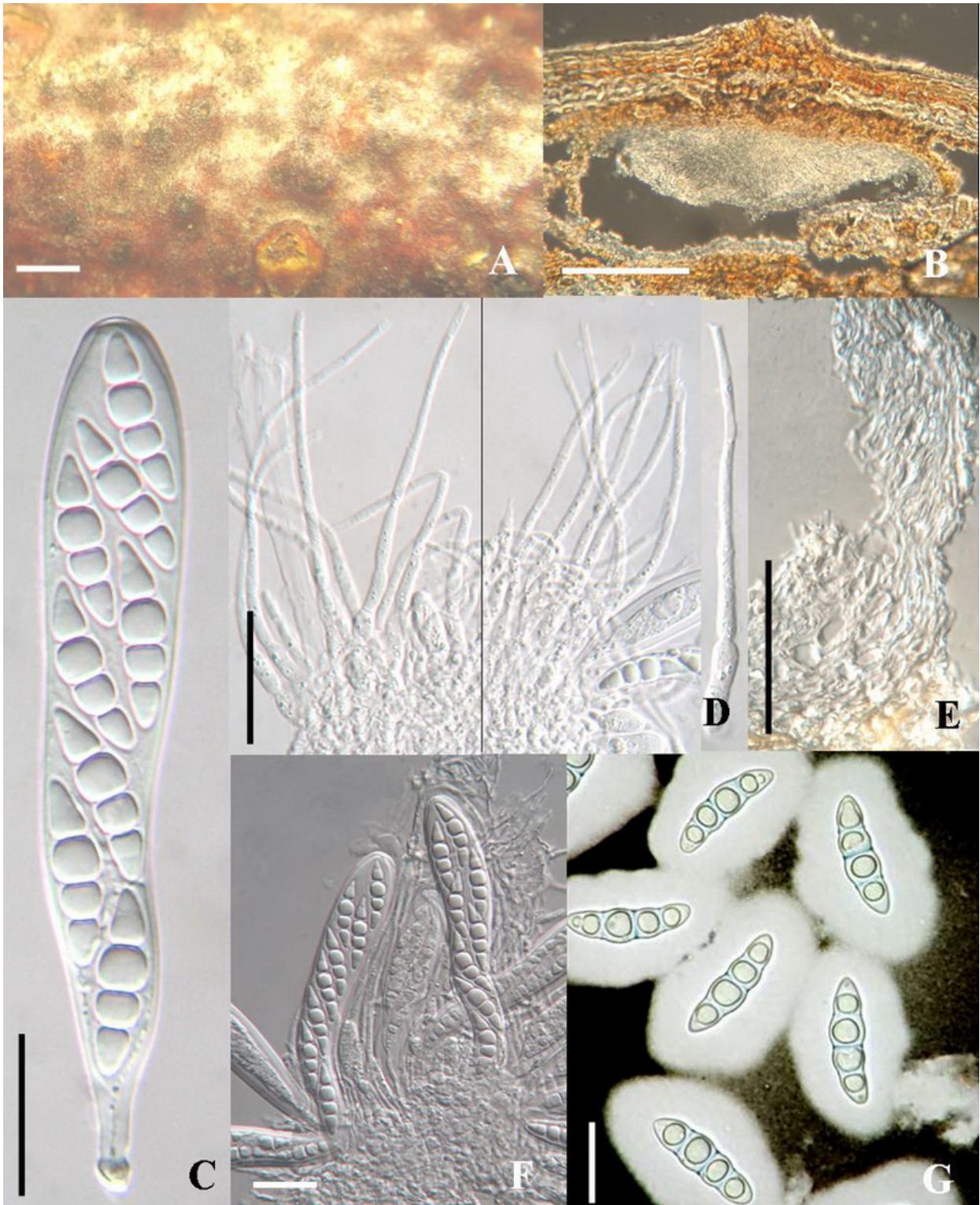


Fig. 10. *Massarina eburnea* (from IFRD 2006). **A.** Ascomata on the host surface. **B.** Section of an ascoma. **C.** Ascus with short pedicel. **D.** Cellular pseudoparaphyses. **E.** Section of the peridium comprising a few layers of compressed cells. **F.** Asci in pseudoparaphyses. **G.** Ascospores with three septa. **A-F** in water; **G** in Indian Ink. Scale bars: **A** = 500 μm, **B** = 100 μm, **C-G** = 20 μm.

thick comprising 3-5 layers of hyaline compressed cells, fusing at the outside with the host (Fig. 10E). *Hamathecium* tapering cellular pseudoparaphyses, ca. 2 μm broad, septate, embedded in a gelatinous matrix, without anastomosing (Fig. 10D). *Asci* 108-170 × 18-22 μm (\bar{x} = 144.5 × 18.8 μm, n = 10), 8-

spored, cylindric-clavate, pedicellate, bitunicate, fissitunicate, (1-)2-seriate, apically rounded, with an ocular chamber and faint ring (J-) (Figs 10C, F). *Ascospores* 30-38 × 8-12 μm (\bar{x} = 32.4 × 8.6 μm, n = 10), fusiform to ellipsoidal, 4-celled, constricted at the septa, hyaline, with acute rounded ends and

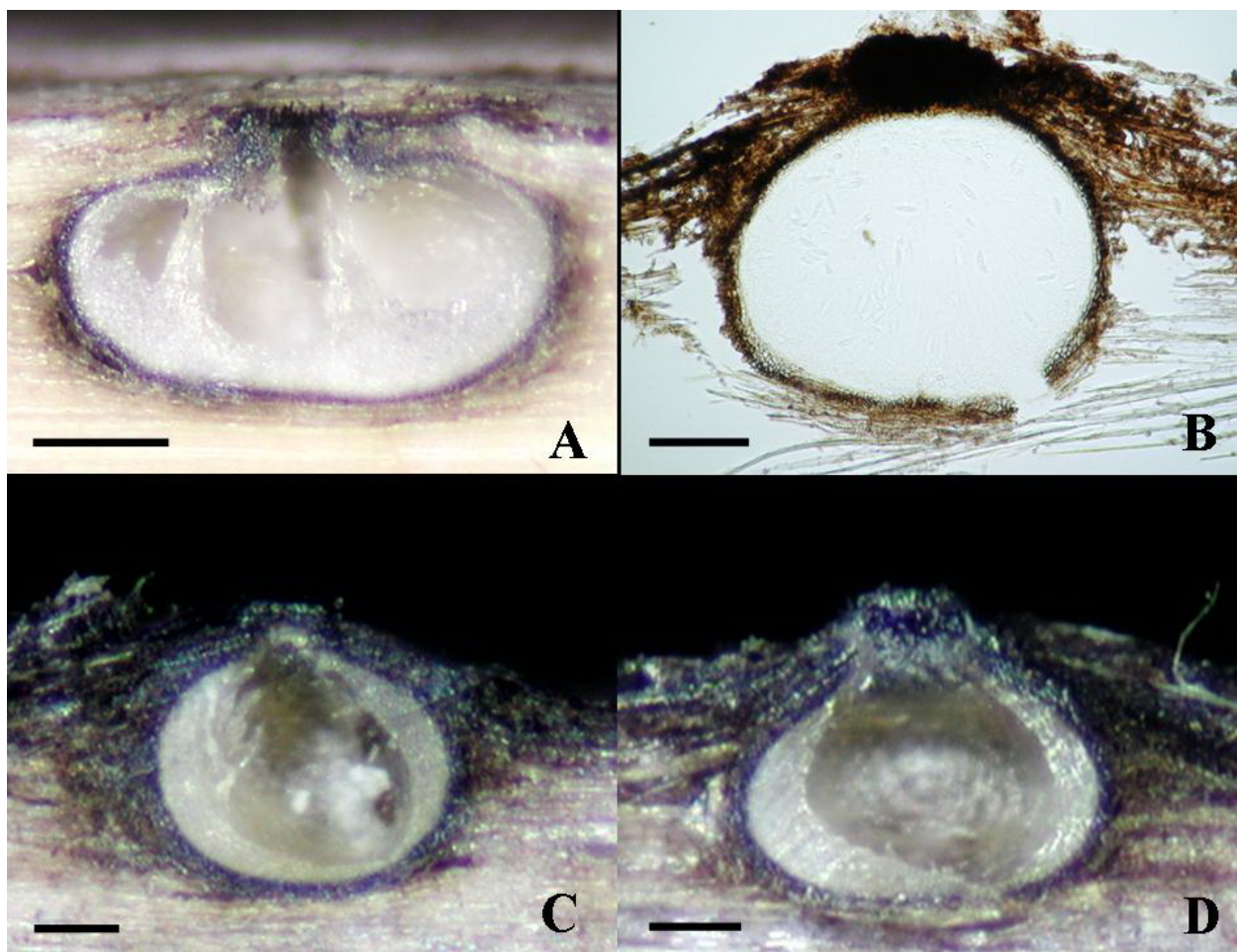


Fig. 11. **A.** *Keissleriella linearis*. Section of an ascoma, note the setae in ostiole. **B-D.** *Lophiotrema nucula* sensu Holm. **B.** Peridium section of an ascoma. Note the peridium in equal thickness. **C, D.** Habitate section of ascomata. Scale bars: **A** = 100 μ m; **B-D** = 50 μ m.

surrounded by (5-8 μ m) mucilaginous sheath (Fig. 10G).

Specimens examined: FRANCE, on twig of *Fagus* sp., Desmazières 1764. P (holotype of *Sphaeria pupula* var. *minor*). AUSTRIA, Silesia, Karlsbrunn, on dead twigs of *Fagus sylvatica* L., Aug. and Sept. 1890, Niessl., De Thümen, Mycotheca universalis no. 1951 sub *Massarina eburnea*, ETH. SAXONIA, Königsbrunn, on twigs of *Fagus sylvatica*, April 1882, W. Krieger, Rabenhorst & Winter, Fungi europaei no. 2767, ETH (lectotype). FRANCE, on a dead twig of *Fagus sylvatica*, Deux Sèvres, Villiers en Bois, Forêt de Chizé, Rimbaud, 14 Apr. 2008, leg. det. Paul Leroy [IFRD2006].

In the holotype of *Sphaeria pupula* var. *minor* (P) and lectotype of *Massarina eburnea* (ETH), ascospores are reported as “not constricted at the septa” (Hyde, 1995). However in one of our recent collections, constrictions at the ascospore septa were observed (Fig. 10), which was consistent with the description by Fallah and Shearer (2001). This might be because this character is not clear in the old (over 100 years) and dry herbarium specimens

or it may be variable between collections. Thus fresh epitypes are recommended to be assigned in order to get more reliable information about the morphology and phylogeny of a particular group of fungi (Hyde and Zhang, 2008; Zhang *et al.*, 2008).

Conclusions — one new genus, five new species and three new combinations are introduced here. Both *Massarina* and *Lophiostoma* are polyphyletic. *Lophiostoma* is phylogenetically divided into two groups: *Lophiostoma* I and *Lophiostoma* II. *Massarina* sens. lat. is mainly divided into two groups: *Massarina* and *Lentithecium*. Although the “*Lophiotrema*-like” peridium is refuted by checking the lectotype material, *Lophiotrema* still represents a separate genus from *Lophiostoma*. This study also indicated that ascospore and apical morphology are morphological characters that have phylogenetic significance, though they are not fully reliable for generic level classification.

Furthermore, a compressed apex has more phylogenetic significance than a crest-like apex. Several new species are described from France, a part of the world where these taxa have been relatively well studied. *Massarina* and *Lophiostoma* species are common in the tropics on submerged as well as terrestrial wood (see Pinruan *et al.*, 2007; Kodsueb *et al.*, 2008), however the correct identity of these taxa need confirmation using molecular techniques.

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