

# Taxonomic revision of *Lophiostoma* and *Lophiotrema* based on reevaluation of morphological characters and molecular analyses

Kazuyuki Hirayama · Kazuaki Tanaka

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**Abstract** *Lophiostoma* and *Lophiotrema* share several morphological and ecological features. They have been regarded as closely related genera within the family Lophiostomataceae, but their morphological circumscriptions have been uncertain. To clarify the generic definitions of *Lophiostoma* and *Lophiotrema*, we conducted phylogenetic analyses of 29 isolates of these genera based on the SSU and LSU nrDNA sequences, and also reevaluated several key characters previously used for their generic characterization. Our results clearly confirmed that *Lophiostoma* and *Lophiotrema* are distinct genera belonging to different families; the ascus shape, including length of the ascus stipe, is a reliable taxonomic indicator to allow discrimination between the genera. In *Lophiostoma* species, asci are clavate with relatively long stipes [mostly (10–) 15–30 µm in length], whereas in *Lophiotrema* the asci are cylindrical with short stipes (up to 15 µm long). A new family, Lophiotremataceae, is proposed to accommodate species in the *Lophiotrema* clade that was distantly placed from the Lophiostomataceae within the Pleosporales. *Lophiostoma quadrisporum*, collected from twigs of *Liriodendron tulipifera*, is described as a new species with distinctive 4-spored asci. *Lophiotrema vitigenum*, which has clavate asci with long stipes, is transferred to *Lophiostoma*.

**Keywords** Ascomycota · Dothideomycetes · Lophiotremataceae · Pleosporales · Systematics

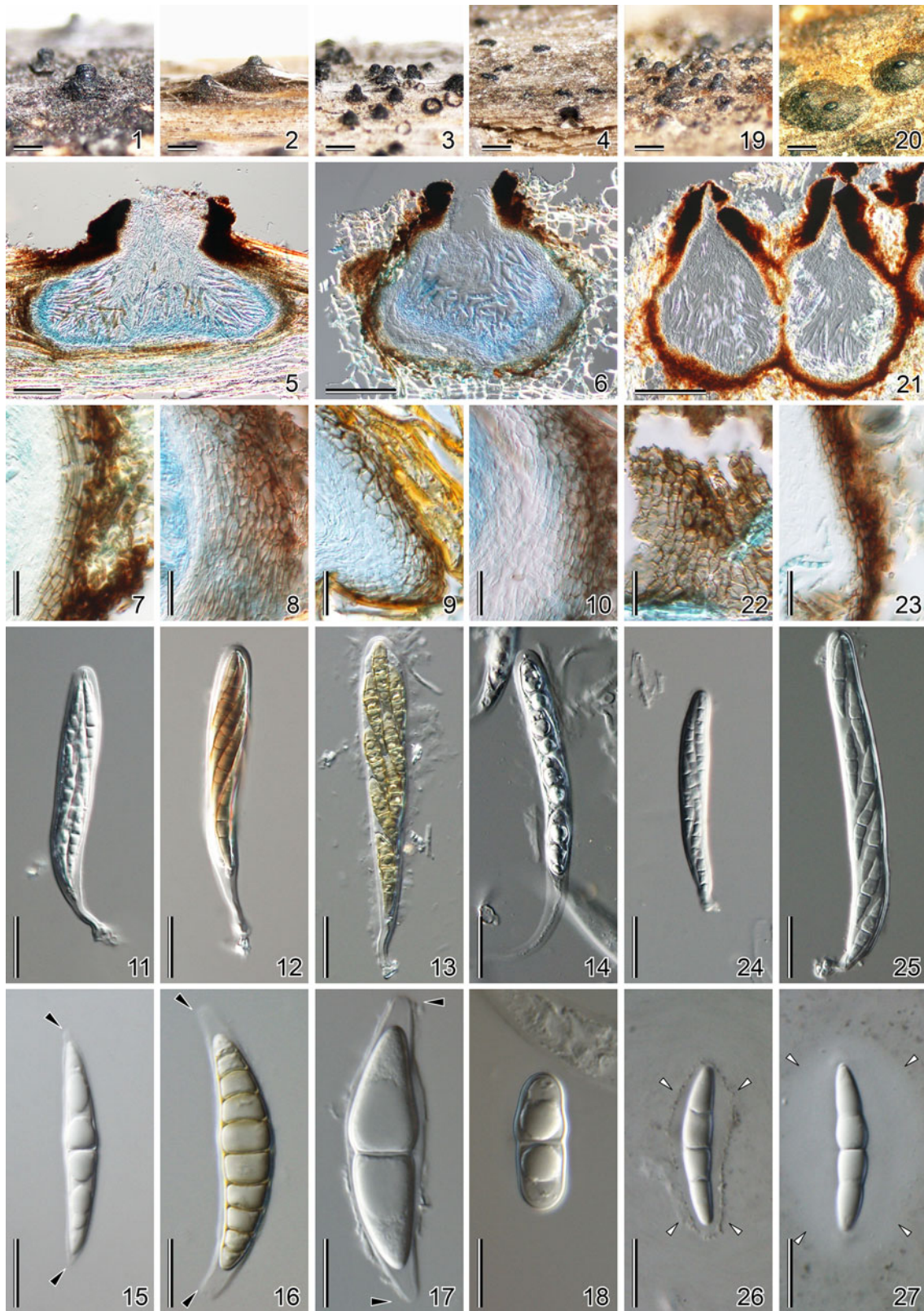
K. Hirayama · K. Tanaka (✉)  
Faculty of Agriculture and Life Science, Hirosaki University,  
3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan  
e-mail: k-tanaka@cc.hirosaki-u.ac.jp

K. Hirayama  
The United Graduate School of Agricultural Sciences,  
Iwate University, 18-8 Ueda 3 chome, Morioka 020-8550, Japan

## Introduction

*Lophiostoma* Ces. & De Not. and *Lophiotrema* Sacc. are bitunicate ascomycetes in the Pleosporales, Dothideomycetes. Most species within these genera occur mainly on the twigs or bark of various woody plants (Holm and Holm 1988; Tanaka et al. 2010a). Some species, however, are frequently found on the culms of herbaceous plants, such as reeds (Tanaka and Harada 2003a), palms (Hyde et al. 2000), and bamboos (Cai et al. 2003). These species are considered as saprobes on the foregoing substrates in terrestrial (Holm and Holm 1988), freshwater (Hyde and Aptroot 1998), and marine environments (Hyde et al. 1992). The two genera share several morphological features (Figs. 1–27), such as carbonaceous ascomata with a laterally compressed apex (termed as a crest-like beak with a slit-like ostiole; Figs. 1–4, 19, 20), fissitunicate asci (Figs. 11–14, 24, 25), and hyaline to dark brown, one- to multiseptate ascospores (Figs. 15–18, 26, 27) (Holm and Holm 1988). These two genera have, therefore, been regarded as closely related genera within the Lophiostomataceae (Barr 1992).

*Lophiostoma* and *Lophiotrema* have been distinguished based on Saccardo's sporological principles: "phaeophragmiae" in *Lophiostoma* and "hyalophragmiae" in *Lophiotrema* (Saccardo 1878). Chesters and Bell (1970a), however, synonymized *Lophiotrema* under *Lophiostoma* because they considered that neither ascospore color nor number of transverse septa could be used for generic delimitation. Although this opinion was accepted by Leuchtman (1985), these were reestablished as separate genera by Holm and Holm (1988), based primarily on peridial structure of ascomata and ascus shape. Namely, *Lophiostoma* has an ascomatal wall that is broader laterally at the base (~50 µm) and composed of parallel, long,



◀ **Figs. 1–27** Morphological features of representative species of *Lophiostoma* (1–18) and *Lophiotrema* (19–27). **1–4, 19, 20** Ascomata erumpent or immersed on host surface. **5, 6, 21** Longitudinal sections through ascomata. **7–10, 22, 23** Sections through ascomatal walls, composed of parallel rows of rectangular cells (**7, 8**), rectangular to prismatic cells (**9, 10, 22**) and prismatic cells (**23**). **11–14** Asci clavate with a long stipe. **15** Ascospore hyaline, 1-septate, with terminal short appendages (*arrowheads*). **16** Ascospore pigmented, 9-septate, with terminal long appendages (*arrowheads*). **17** Ascospore hyaline, 1-septate, with terminal long appendages (*arrowheads*). **18** Ascospore hyaline, 1-septate, without appendage or sheath. **24, 25** Asci cylindrical with a short stipe. **26** Ascospore hyaline, 3-septate, with an entire sheath (*arrowheads*). **27** Ascospore hyaline, 1-septate, with an entire sheath (*arrowheads*). **1–18** *Lophiostoma* species: **1, 7, 11, 15** from *L. macrostomum* (**1, 15** = HHUF 27290; **7, 11** = HHUF 27293); **2, 8, 12** from *L. arundinis* (**2** = HHUF 27305, **8** = HHUF 27413, **12** = HHUF 27304); **3** from *L. fuckelii* (HHUF 27325); **4, 6, 10, 14, 18** from *L. quadrisporum* (HHUF 27321); **5** from *L. caulium* “var. *d*” (HHUF 27310); **9** from *L. sagittiforme* (HHUF 29754); **13** from *L. caudatum* (HHUF 27319); **16** from *L. caulium* “var. *f*” (HHUF 27313); **17** from *L. vitigenum* (HHUF 26930). **19–27** *Lophiotrema* species: **19, 21, 23, 24, 26** from *L. neohysterioides* (**19, 24, 26** = HHUF 27328; **21, 23** = HHUF 27331); **20, 22** from *L. neoarundinaria* (HHUF 27547); **25, 27** from *L. vagabundum* (HHUF 27323). **Bars 1–4, 19, 20** 200  $\mu\text{m}$ ; **5, 6, 21** 100  $\mu\text{m}$ ; **7–10, 22, 23** 20  $\mu\text{m}$ ; **11–18, 24–27** 10  $\mu\text{m}$

prismatic cells, and it has clavate asci. In contrast, *Lophiotrema* has an ascomatal wall of entirely equal thickness ( $\sim 25 \mu\text{m}$ ) composed of textura angularis to globosa, and it has cylindrical asci. These generic circumscriptions have been followed by later authors (Barr 1992; Mathiassen 1993; Yuan and Zhao 1994; Tanaka and Harada 2003a,b; Tanaka and Hosoya 2008; Eriksson 2009).

Taxonomic revision of *Lophiostoma* and *Lophiotrema* has been carried out on morphological grounds (e.g., Lehmann 1886; Berlese 1894; Chesters and Bell 1970a). Recent molecular analyses have revealed phylogenetic relationships and species validities of the lophiostomatoid fungi in the Pleosporales (Schoch et al. 2006, 2009; Tanaka and Hosoya 2008; Mugambi and Huhndorf 2009a). Several unrelated species without any compressed crest-like beak on their ascomata, previously described as *Lophiostoma*, have recently been excluded from the genus. For example, *L. breviappendiculatum* Kaz. Tanaka et al. (Tanaka et al. 2005) and *L. ingoldianum* (Shearer & K.D. Hyde) Aptroot & K.D. Hyde (Shearer and Hyde 1997; Hyde et al. 2002), occurring in freshwater habitats, were transferred to *Lindgomyces* K. Hiray. et al. based on analyses of the small and large subunit nuclear ribosomal DNA (SSU and LSU nrDNA) and morphological reevaluation (Shearer et al. 2009; Hirayama et al. 2010). *Lophiostoma mangrovei* Kohlm. & Vittal, found on marine mangroves (Kohlmeyer and Vittal 1986), was treated as a species in *Rimora* Kohlm. et al. based on analyses of four genes (Suetrong et al. 2009). In general, typical species in accordance with the generic concept of *Lophiostoma* (Holm and Holm 1988) appear to represent a natural group derived from a

single ancestor. On the other hand, taxonomic circumscription of the genus *Lophiotrema* would be problematic. Zhang et al. (2009b) clearly indicated that *Lophiostoma* and *Lophiotrema* are phylogenetically distinct genera based on molecular study. However, they considered that morphological criteria, particularly the peridial structure of ascomata formerly used to separate these lophiostomatoid genera, are unable to provide differentiation between *Lophiostoma* and *Lophiotrema* because the ascomata within these genera are almost identical (Zhang et al. 2009b). Then, they described two new species of *Lophiotrema* based on their close phylogenetic relationship to the type of *Lophiotrema* (*L. nucula* Rehm) (Zhang et al. 2009b), but morphological differentiation between *Lophiostoma* and *Lophiotrema* has consequently remained unclear.

The phylogenetic study of lophiostomatoid genera conducted by Zhang et al. (2009b) further revealed that *Lophiotrema* is not a member of Lophiostomataceae; this observation contrasted with the traditional classification of the genus (Saccardo 1883; Clements and Shear 1931; Barr 1992). Subsequently, on the basis of further molecular analyses using five DNA regions [SSU and LSU nrDNA, the translation elongation factor-1 alpha (*TEF1*), and the largest and second largest subunits of RNA polymerase II (*RPB1* and *RPB2*)], Zhang et al. (2009a) found that two species previously placed in *Lophiostoma* should be transferred to *Lophiotrema*, and that the monophyletic clade of *Lophiotrema* is related to the Testudinaceae rather than the Lophiostomataceae. Familial placement of *Lophiotrema*, however, remained uncertain, mostly because of the lack of a morphological circumscription of the genus.

In this study, we carried out phylogenetic analyses of *Lophiostoma* and *Lophiotrema* based on SSU and LSU nrDNA sequences, using 29 isolates from these genera. The taxonomic significance of several key characters previously used for the morphological delimitation of these genera was reevaluated. Our purpose was to clarify the morphological circumscriptions of *Lophiostoma* and *Lophiotrema* and to reveal the familial placement of *Lophiotrema*.

## Materials and methods

### Morphological studies and fungal isolates

Specimens of *Lophiostoma* and *Lophiotrema* from the herbarium of Hirosaki University (HHUF) (Table 1) were used for microscopic observation following the method described by Hirayama et al. (2010). Special attention was given to key characters used in the delimitation of

**Table 1** Specimens, isolates, and GenBank accession numbers of *Lophiostoma* and *Lophiotrema* used in this study

Species name	Original no.	Specimen no.	Culture collection no.	Collection site	Substrate	GenBank no.
<i>Lophiostoma arundinis</i>	KT 606	HHUF 27304	JCM 13550	Aomori, JPN	<i>Phragmites australis</i>	<b>AB618679</b>
	KT 651	HHUF 27305	JCM 13551/MAFF 239449	Aomori, JPN	<i>Phragmites australis</i>	<b>AB618680</b>
	KT 668	HHUF 27413	–	Aomori, JPN	<i>Phragmites australis</i>	–
<i>Lophiostoma caudatum</i>	KT 530	HHUF 27319	MAFF 239453	Aomori, JPN	<i>Dactylis glomerata</i>	<b>AB619000</b>
	KT 603	HHUF 27306	MAFF 239450	Aomori, JPN	Herbaceous plant	<b>AB619001</b>
<i>Lophiostoma caulium</i> “var. a”	KT 633	HHUF 27307	JCM 17669	Aomori, JPN	Herbaceous plant	<b>AB619002</b>
<i>Lophiostoma caulium</i> “var. d”	KT 604	HHUF 27309	JCM 17668	Aomori, JPN	Herbaceous plant	<b>AB619003</b>
	KT 777	HHUF 27310	MAFF 239451	Aomori, JPN	Herbaceous plant	<b>AB619004</b>
<i>Lophiostoma caulium</i> “var. f”	KT 573	HHUF 27313	MAFF 239452	Aomori, JPN	Herbaceous plant	<b>AB619005</b>
	KT 686-1	HHUF 27315	JCM 17670	Aomori, JPN	Woody plant	<b>AB619006</b>
<i>Lophiostoma fackelii</i>	KT 794	HHUF 27311	JCM 17671	Aomori, JPN	<i>Dactylis glomerata</i>	<b>AB619007</b>
	KH 161	HHUF 30076	JCM 17672	Hokkaido, JPN	<i>Vitis coignetiae</i>	<b>AB619008</b>
<i>Lophiostoma macrostomum</i>	KT 634	HHUF 27325	MAFF 239458	Aomori, JPN	Unknown plant	<b>AB619009</b>
	KT 508	HHUF 27288	JCM 13544	Aomori, JPN	<i>Morus bombycis</i>	<b>AB619010</b>
<i>Lophiostoma quadrisporum</i>	KT 635	HHUF 27290	JCM 13545	Aomori, JPN	Herbaceous plant	AB433273
	KT 709	HHUF 27293	JCM 13546/MAFF 239447	Aomori, JPN	Unknown plant	AB433274
<i>Lophiostoma sagittiforme</i>	KT 843	HHUF 27321	MAFF 239455	Iwate, JPN	<i>Liriodendron tulipifera</i>	<b>AB619011</b>
	KT 1934	HHUF 29754	JCM 15100	Kagoshima, JPN	<i>Machilus japonica</i>	AB369267
<i>Lophiostoma semiliberum</i>	KT 622	HHUF 27299	JCM 13548	Aomori, JPN	Herbaceous plant	<b>AB619012</b>
	KT 652	HHUF 27298	JCM 13547	Aomori, JPN	<i>Phragmites australis</i>	<b>AB619013</b>
<i>Lophiostoma vitigenum</i>	KT 828	HHUF 27300	JCM 13549/MAFF 239448	Aomori, JPN	Herbaceous plant	<b>AB619014</b>
	HH 26930	HHUF 26930	JCM 13534/MAFF 239459	Aomori, JPN	<i>Vitis coignetiae</i>	<b>AB619015</b>
<i>Lophiostoma winteri</i>	HH 26931	HHUF 26931	JCM 17676	Aomori, JPN	<i>Vitis coignetiae</i>	<b>AB619016</b>
	KT 740	HHUF 27317	JCM 17648	Hokkaido, JPN	Unknown plant	<b>AB619017</b>
<i>Lophiotrema neoarundinaria</i>	KT 764	HHUF 27316	MAFF 239454	Hokkaido, JPN	<i>Polygonum</i> sp.	<b>AB619018</b>
	KT 856	HHUF 27547	MAFF 239461	Nagano, JPN	<i>Phyllostachys bambusoides</i>	AB524455
<i>Lophiotrema neohysterioides</i>	KT 1034	HHUF 30015	NBRC 106239	Tochigi, JPN	<i>Phyllostachys bambusoides</i>	AB524457
	KT 2200	HHUF 30014	NBRC 106238	Kagoshima, JPN	<i>Phyllostachys bambusoides</i>	AB524456
<i>Lophiotrema nucula</i>	KT 686-2	HHUF 27331	–	Aomori, JPN	Woody plant	–
	KT 713	HHUF 27328	JCM 17673	Aomori, JPN	<i>Robinia pseudoacacia</i>	<b>AB618701</b>
<i>Lophiotrema vagabundum</i>	KT 756	HHUF 27330	MAFF 239457	Hokkaido, JPN	Woody plant	<b>AB618702</b>
	–	–	CBS 113826/JCM 14132	Upland, SWE	<i>Fraxinus excelsior</i>	<b>AB619021</b>
<i>Lophiotrema vagabundum</i>	KH 164	HHUF 30077	JCM 17674	Hokkaido, JPN	<i>Vitis coignetiae</i>	<b>AB619022</b>
	KH 172	HHUF 30078	JCM 17675	Hokkaido, JPN	<i>Vitis coignetiae</i>	<b>AB619023</b>
<i>Lophiotrema angustifolium</i>	KT 664	HHUF 27323	MAFF 239456	Aomori, JPN	Woody plant	<b>AB618706</b>
	–	–	CBS 113975/JCM 14138	Upland, SWE	<i>Epilobium angustifolium</i>	<b>AB618707</b>

The sequences determined in this study are in bold  
 JPN Japan, SWE Sweden

*Lophiostoma* and *Lophiotrema*, including size and peridial structure of ascomata, ascus shape, and ascospore morphology. Fungal cultures used were deposited in the Japan Collection of Microorganisms (JCM), the National Institute of Agrobiological Sciences (MAFF), and the NITE Biological Resource Center (NBRC) (Table 1).

### Phylogenetic analyses

DNA was extracted from a total of 29 isolates, including two strains obtained from the Centraalbureau voor Schimmelcultures (CBS) (see Table 1). Approximately 1,300 nucleotides at the 5'-end of the partial SSU and LSU nrDNA were amplified by polymerase chain reaction (PCR) using the primer pairs NS1–NS4 for the SSU (White et al. 1990) and LROR–LR7 for the LSU (Rehner and Samuels 1994). Methods of DNA extraction and PCR amplification have been described by Hirayama et al. (2010).

The SSU and LSU sequences of *Lophiostoma* and *Lophiotrema* species were aligned alongside those of related species from GenBank (Table 2). Sequences of *Dothidea insculpta* Wallr., an outgroup taxon, were used to root trees. Preliminary multiple alignment of sequences was conducted using MEGA 4 (Tamura et al. 2007). Gaps and ambiguous regions were excluded from analyses. The aligned dataset was subjected to three phylogenetic analyses: maximum parsimony (MP) using a close-neighbor-interchange heuristic search with an initial tree obtained by random addition sequence (100 replicates), neighbor-joining (NJ) analysis based on the Kimura two-parameter model, and Bayesian analyses using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). The final alignment was deposited in TreeBASE (<http://www.treebase.org>).

Bootstrap values (BV) for MP and NJ analyses were computed from 1,000 replicates. MrModeltest version 2.3 (Nylander 2004), in conjunction with PAUP version 4.0b10 (Swofford 2003), were used to select substitution models for Bayesian analyses. On the basis of the Akaike information criterion, a general time-reversible, invariant, g-distributed (GTR + I + G) model was applied. Two runs with ten chains of Markov Chain Monte Carlo (MCMC) iterations were performed for 5 million generations, keeping 1 tree every 100 generations. Runs were deemed to have converged if the mean standard deviation of split frequencies became less than 0.01. The first 0.8 million generations of the dataset were discarded as burn-in, and the remaining 42,000 trees were used to calculate 50% majority rule trees and to determine Bayesian posterior probabilities (BPP) for individual branches.

## Results

### Analyses of combined SSU and LSU nrDNA sequences

A combined alignment of the SSU (884 bp) and LSU (723 bp) regions consisting of 74 strains was generated. SSU region insertions found in *Delitschia didyma* Auersw. (512–808, 1247–1591) and *Neottiosporina paspali* (G.F. Atk.) B. Sutton & Alcorn (487–841) were excluded from the alignment. Of 1,607 characters, 425 (26.4%) were variable, and of these 306 (19.0%) were parsimony informative. A MP analysis of the dataset resulted in 51 equally parsimonious trees with a length of 1,212 steps (consistency index = 0.461, retention index = 0.797). The trees obtained from NJ and Bayesian analyses were topologically similar to the MP tree. One of the 51 MP trees is shown in Fig. 28.

*Lophiostoma* and *Lophiotrema* formed distinct monophyletic clades (Fig. 28). All *Lophiostoma* species and *Lophiotrema vitigenum* Kaz. Tanaka & Y. Harada (HH 26930 and 26931) grouped with *Lophiostoma macrostomum* (Tode) Ces. & De Not. (the type species of the genus *Lophiostoma*) in a strongly supported clade (99% BV, 1.00 BPP). A new species, *Lophiostoma quadrisporum*, and *L. fuckelii* Sacc. were sister to all other taxa in the *Lophiostoma* clade. Species in *Lophiotrema*, including the type species of the genus (*L. nucula*), clustered in a well-supported lineage (91–97% BV, 1.00 BPP) in a basal position of a main pleosporalean clade composed of the Lophiostomataceae and several other families (Fig. 28).

## Taxonomy

Several characters, such as size and peridial structure of ascomata, shape and stipe length of asci, and ascospore morphology, were examined (Figs. 1–27) and are shown on our tree (Fig. 28). The shape and stipe length of asci appear to have diagnostic value for the separation of *Lophiostoma* and *Lophiotrema*. A new family, Lophiotremataceae, is proposed to accommodate the genus *Lophiotrema*, based on morphological and molecular evidence. One new species and one new combination within *Lophiostoma* are described below. Their detailed descriptions and illustrations are found in Tanaka and Harada (2003b).

**Lophiotremataceae** K. Hiray. & Kaz. Tanaka, fam. nov.

MycoBank no.: MB 561063

Ascomata subglobosa vel globosa. Rostrum compressum, cum ostiolo rimiformi. Pseudoparaphyses copiosae, septatae, ramificantes et anastomosantes. Asci fissitunicati, cylindrici, brevistipitati vel sessiles. Ascosporae fusiformes

**Table 2** Additional sequences obtained from Genbank

Species	Strain	GenBank accession no.	
		SSU	LSU
<b>Dothideomycetes</b>			
<i>Amniculicola immersa</i>	CBS 123083	GU456295	FJ795498
<i>Amniculicola parva</i>	CBS 123092	GU296134	GU301797
<i>Arthopyrenia salicis</i>	CBS 368.94	AY538333	AY538339
<i>Ascochyta fabae</i>	CBS 524.77	EU754034	EU754133
<i>Delitschia didyma</i>	UME 31411	AF242264	DQ384090
<i>Delitschia winteri</i>	CBS 225.62	DQ678026	DQ678077
<i>Didymella exigua</i>	CBS 183.55	EU754056	EU754155
<i>Helicascus nypae</i>	BCC 36751	GU479754	GU479788
<i>Lentithecium arundinaceum</i>	CBS 619.86	GU296157	DQ813509
<i>Lentithecium fluviatile</i>	CBS 122367	GU296158	FJ795451
<i>Leptosphaeria doliolum</i>	CBS 505.75	GU296159	GU301827
<i>Leptosphaeria maculans</i>	DAOM 2229267	DQ470993	DQ470946
<i>Lindgomyces cinctosporae</i>	Raja R56-1	AB522430	AB522431
<i>Lindgomyces ingoldianus</i>	ATCC 200398	AB521719	AB521736
<i>Lophiostoma compressum</i>	IFRD 2014	FJ795480	FJ795437
<i>Lophiostoma crenatum</i>	CBS 629.86	DQ678017	DQ678069
<i>Lophiostoma heterosporum</i>	CBS 644.86	AY016354	AY016369
<i>Lophiostoma scabridisporum</i> 1	BCC 22835	GQ925831	GQ925844
<i>Lophiostoma scabridisporum</i> 2	BCC 22836	GQ925832	GQ925845
<i>Lophiotrema lignicola</i>	CBS 122364	FJ795488	FJ795445
<i>Massaria inquinans</i>	M 19	HQ599444	HQ599402
<i>Massaria platanoidea</i>	M 7	HQ599457	HQ599420
<i>Massarina eburnea</i>	JCM 14422	AB521718	AB521735
<i>Montagnula opulenta</i>	CBS 168.34	AF164370	DQ678086
<i>Morosphaeria ramunculicola</i>	JK 5304B	GU479760	GU479794
<i>Neotestudina rosatii</i>	CBS 690.82	DQ384069	DQ384107
<i>Neottiosporina paspali</i>	CBS 331.37	EU754073	EU754172
<i>Phaeodothis winteri</i>	CBS 182.58	GU296183	GU301857
<i>Phaeosphaeria avenaria</i>	CBS 602.86	AY544725	AY544684
<i>Phaeosphaeria juncophila</i>	CBS 575.86	GU456307	GU456328
<i>Pleospora herbarum</i>	CBS 714.68	DQ767648	DQ678049
<i>Preussia terricola</i>	DAOM 230091	AY544726	AY544686
<i>Pseudotetraploa curviappendiculata</i>	JCM 12852	AB524467	AB524608
<i>Setosphaeria monoceras</i>	CBS 154.26	AY016352	AY016368
<i>Triplosphaeria maxima</i>	JCM 13172	AB524496	AB524637
<i>Ulospora bilgramii</i>	CBS 110020	DQ384071	DQ384108
<i>Westerdykella cylindrica</i>	CBS 454.72	AY016355	AY004343
<b>Outgroup</b>			
<i>Botryosphaeria dothidea</i>	CBS 115476	DQ677998	DQ678051
<i>Dothidea insculpta</i>	CBS 189.58	DQ247810	DQ247802
<i>Spencermartinsia viticola</i>	CBS 117009	DQ678036	DQ678087

SSU small subunit, LSU large subunit

vel cylindricae, uni-vel multiseptatae, hyalinae vel brunneae, cum vel sine tunicis gelatinosis.

Ascomata subglobose to globose, scattered to crowded. Beak compressed, with a slit-like ostiole. Ascumatal wall

composed of pale brown, small, thin-walled cells. Pseudoparaphyses filamentous, numerous, septate, branched, anastomosing. Asci fissitunicate, cylindrical, with a short stipe or sessile, rounded at the apex, with an apical

chamber. Ascospores fusiform to cylindrical, 1- to multi-septate, hyaline to brown, with or without an entire gelatinous sheath.

Typus genus: *Lophiotrema* Sacc.

***Lophiostoma quadrisporum*** K. Hiray. & Kaz. Tanaka, sp. nov. Figs. 4, 6, 10, 14, 18

Mycobank no.: MB 561064

Ascomata 300–360  $\mu\text{m}$  alta, 300–435  $\mu\text{m}$  diametro, subglobosa vel globosa. Rostrum 130–200  $\mu\text{m}$  latum, cristatum. Paries ascomatis 10–20  $\mu\text{m}$  crassus, ex cellulis prismaticis compositus. Pseudoparaphyses copiosae, 1–2  $\mu\text{m}$  latae. Asci (70–)80–110(–120)  $\times$  (8–)9–11.5  $\mu\text{m}$ , fissitunicati, clavati, cum longistipitibus, quadrispori. Ascospores 19–24.5  $\times$  6–9.5  $\mu\text{m}$ , ellipsoidei-fusiformes, uniseptatae, hyalinae.

Misapplied name: *Lophiotrema nucula* auct. non (Fr.) Sacc.; Kaz. Tanaka & Y. Harada, Mycoscience 44: 116, 2003.

Etymology: In reference to the 4-spored asci.

Specimen examined: Japan, Iwate, Morioka, Ueda, campus of Iwate Univ., on twigs of *Liriodendron tulipifera* L., 11 Jan. 2002, coll. Y. Harada, KT 843 (HHUF 27321, holotype designated here; ex-holotype isolate MAFF 239455).

***Lophiostoma vitigenum*** (Kaz. Tanaka & Y. Harada) K. Hiray. & Kaz. Tanaka, comb. nov. Fig. 17

Mycobank no.: MB 561065

$\equiv$  *Lophiotrema vitigenum* Kaz. Tanaka & Y. Harada, Mycoscience 44: 119, 2003 (basionym).

Specimens examined: Japan, Aomori, Hirosaki, Kudoji, on twigs of *Vitis coignetiae* Pulliat ex Planch., 27 Oct. 2001, coll. S. Hatakeyama (HHUF 26930 holotype of basionym; ex-holotype isolate JCM 13534 = MAFF 239459); ibid (HHUF 26931 isotype of basionym; ex-isotype isolate JCM 17676).

## Discussion

### Monophylies of *Lophiostoma* and *Lophiotrema*

All phylogenetic trees obtained in our study confirmed clearly that *Lophiostoma* and *Lophiotrema* are separate genera belonging to different families (see Fig. 28); this has been indicated in several previous papers (Schoch et al. 2009; Zhang et al. 2009a, b). Zhang et al. (2009b) suggested that species in *Lophiostoma* could be divided

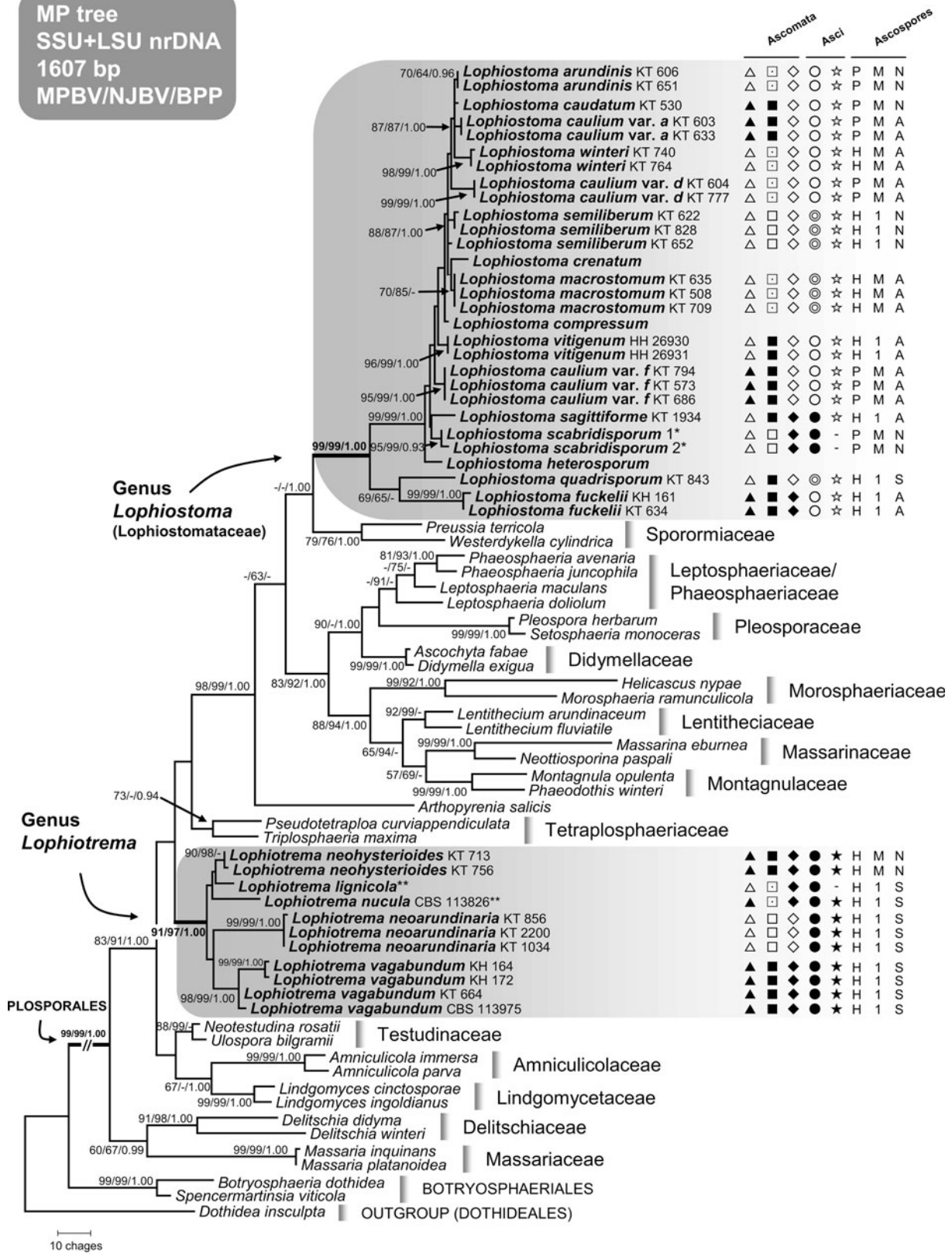
phylogenetically into two distinct lineages based on analyses of SSU + LSU nrDNA and *RPB2*. They described these as “*Lophiostoma* clade I,” including several melanommataceous genera (e.g., *Melanomma* Nitschke ex Fuckel and *Herpotrichia* Fuckel), and “*Lophiostoma* clade II,” including most *Lophiostoma* species. They introduced two species in *Lophiostoma* clade I as new species of the genus (*L. rugulosum* Yin. Zhang et al. and *L. glabrotunicatum* Yin. Zhang et al.), because a sequence of *L. macrostomum* (the type species of *Lophiostoma*) retrieved from GenBank (DQ384094; voucher Lundqvist 20504 in S) also nested within this clade (Zhang et al. 2009b). However, species in clade I, as well as *L. rugulosum* and *L. glabrotunicatum* (Zhang et al. 2009b), appear to be more closely related to the Melanommataceae recently redefined (Mugambi and Huhndorf 2009a) rather than lophiostomatoid fungi, based on morphological feature of ascomata without a laterally compressed crest-like beak or slit-like ostiole. Mugambi and Huhndorf (2009a) suggested that the GenBank sequences of *L. macrostomum* (DQ384094) may be based on a misidentification. Taxa in “*Lophiostoma* clade II” sensu Zhang et al. (2009b) are currently accepted as *Lophiostoma* sensu stricto by several authors (Mugambi and Huhndorf 2009a; Schoch et al. 2009; Suetrong et al. 2009; Tanaka et al. 2010b). In the other genus, *Lophiotrema*, all species used in our study formed a highly supported monophyly with the exception of *Lophiostoma vitigenum*, and this clade was distantly placed from the *Lophiostoma* within the Pleosporales (Fig. 28).

### Morphological circumscriptions of *Lophiostoma* and *Lophiotrema*

There has been some controversy about the circumscription of both *Lophiostoma* and *Lophiotrema*. The following morphological criteria have been used in the delimitation of both genera: (1) ascomatal size [large (200–700  $\mu\text{m}$ ) in *Lophiostoma* versus small (up to 200  $\mu\text{m}$ ) in *Lophiotrema*; Tang et al. 2003], (2) thickness of ascomatal peridium ( $\sim$ 50  $\mu\text{m}$  vs. 25  $\mu\text{m}$ ; Holm and Holm 1988), (3) peridial cell type (parallel, long, prismatic cells vs. textura angularis to globosa; Holm and Holm 1988), (4) ascus shape (clavate vs. cylindrical; Holm and Holm 1988), (5) ascospore color (pigmented vs. hyaline; Saccardo 1878), (6) ascospore septation (1- to several septate vs. 1-septate; Holm and Holm 1988), and (7) ascospore appendages (with or without appendages vs. with or without a gelatinous sheath; Holm and Holm 1988). In addition to these characters, we have noted length of ascus stipe for each species to help elucidate generic boundaries (see Fig. 28).

A classical understanding following a generic concept based on Saccardoan spore morphology such as color and septation is obviously uninformative (Fig. 28). Saccardo

MP tree  
SSU+LSU nrDNA  
1607 bp  
MPBV/NJBV/BPP





◀ **Fig. 28** One of the 51 most parsimonious (MP) trees based on a combined dataset of small subunit (SSU) (884 bp) and large subunit (LSU) (723 bp) rDNA. Most parsimonious (MP) and neighbor-joining (NJ) bootstrap values greater than 50% and Bayesian posterior probabilities above 0.90 are indicated at the nodes as MPBV/NJBV/BPP. Hyphen (“-”) indicates values lower than 50% (BV) or 0.90 (BPP). Tree length = 1,212, consistency index = 0.461, retention index = 0.797. The tree was rooted to *Dothidea insculpta* (Dothideales). Taxonomic criteria of *Lophiostoma* and *Lophiotrema* are noted after the species name as follows. Ascomatal size: *filled triangles*, up to 350  $\mu\text{m}$ ; *open triangles*, more than 350  $\mu\text{m}$ . Thickness of ascomatal peridium: *filled squares*, up to 25  $\mu\text{m}$ ; *open squares with a dot*, 25–50  $\mu\text{m}$ ; *open squares*, more than 50  $\mu\text{m}$ . Peridial cell type: *open diamonds*, parallel, long, prismatic cells; *filled diamonds*, small cells of textura angularis to globosa. Ascus shape: *open circles*, clavate; *double circles*, clavate to cylindrical; *filled circles*, cylindrical. Ascus stipe length: *filled five-pointed stars*, up to 15  $\mu\text{m}$ ; *open five-pointed stars*, more than 15  $\mu\text{m}$ . Ascospore color: *P* pigmented, *H* hyaline. Ascospore septation: *I* 1-septate, *M* multiseptate. Ascospore appendage: *A* appendage, *S* sheath, *N* none. Morphological data of the taxa with *asterisks* were obtained from Abdel-Wahab and Jones (2000) (\*) and from Holm and Holm (1988) and/or Zhang et al. (2009a) (\*\*)

(1878) erected *Lophiotrema* to include fungi that have ascomata with a crest-like beak similar to those of *Lophiostoma*, but with hyaline, multi-septate ascospores. Spore color was not considered of primary taxonomic importance in defining genera (Chesters and Bell 1970a), but spore septation was used as a criterion in the key to lophiostomatoid genera provided by Holm and Holm (1988). However, the presence of species with pigmented ascospores in the *Lophiotrema* clade, e.g., *L. rugulosum* and *L. glabrotunicatum* (Zhang et al. 2009b), and species with 3-septate ascospores, e.g., *L. neohysterioides* M.E. Barr (Fig. 28), rejects the diagnostic value of spore color and septation in the separation of these genera.

Another major generic concept, emphasizing differences in ascomatal peridium (thickness and cell structure), was proposed by Holm and Holm (1988); this distinction has been widely accepted by many authors (e.g., Barr 1992; Mathiassen 1993; Yuan and Zhao 1994; Tanaka and Harada 2003a,b; Tanaka and Hosoya 2008; Eriksson 2009). Zhang et al. (2009b), however, concluded, from morphological comparison of both genera based on type specimens, that peridial thickness and structure do not have taxonomic significance. This conclusion is generally confirmed by our analyses (Fig. 28).

The protruding appendages at the ends of ascospores were sometimes regarded as reliable features for the characterization of *Lophiostoma* species (Holm and Holm 1988; Tang et al. 2003). Appendages are certainly found only in species within the *Lophiostoma* clade, and not in the *Lophiotrema* clade (Fig. 28). It seems likely that the reason for many species with appendaged ascospores within the *Lophiostoma* clade relates to their habitats. Many are reported from aquatic environments, including,

for example, *L. armatisporum* (K.D. Hyde et al.) E.C.Y. Liew et al., *L. bipolare* (K.D. Hyde) E.C.Y. Liew et al., and *L. frondisubmersum* (K.D. Hyde) E.C.Y. Liew et al., and all these have bipolar appendages on their ascospores (Hyde et al. 1992; Hyde 1994, 1995). Although these aquatic species are not included in our analyses, they have previously been phylogenetically verified as *Lophiostoma* (Liew et al. 2002; Tanaka and Hosoya 2008). Several typical *Lophiostoma* species (e.g., *L. macrostomum* and *L. caulium* (Fr.) Ces. & De Not.) have been found frequently on reeds or herbaceous debris in riparian habitats (Tanaka and Harada 2003a); these also have ascospores with protruded appendages (Fig. 28). These extracellular structures are generally considered adaptations to aquatic or humid habitats, better enabling the ascospores to attach to substrates (Shearer 1993; Jones 2006; Vijaykrishna et al. 2006). The phylogenetic significance of spore appendages for generic separation is therefore considered doubtful, because these may evolve convergently among unrelated taxa in aquatic habitats (Hirayama et al. 2010).

Our results strongly confirm that ascus shape is a reliable taxonomic indicator to differentiate between *Lophiostoma* and *Lophiotrema*. This character has already been proposed for this purpose; *Lophiostoma* is usually characterized by clavate asci and *Lophiotrema* by cylindrical asci (Holm and Holm 1988). However, the border between “clavate” and “cylindrical” has sometimes been ambiguous or confusing. We have thus provided the additional character of ascus stipe length (from the base of the ascospore arranged at the lowest position to the base of the stipe) to help define ascus shape. In *Lophiostoma* species, the clavate asci were found to have a relatively long stipe [mostly (10–) 15–30  $\mu\text{m}$  in length], whereas in *Lophiotrema* the cylindrical asci have a sessile to short stipe (up to 15  $\mu\text{m}$ ). The stipe length of two species, *L. quadrisporum* and *L. vitigenum*, previously reported as *Lophiotrema* (Tanaka and Harada 2003b) but actually belonging to *Lophiostoma*, is also relatively long (15–33  $\mu\text{m}$  and 12–24  $\mu\text{m}$ , respectively). Although this character has never previously been used for the differentiation of lophiostomatoid genera, we suggest that it should be used and tested in further taxonomic revisions of these genera.

#### Familial placement of *Lophiotrema*

Because there is no appropriate family in current Dothideomycetes classification (Lumbsch and Huhndorf 2010), we have established a new family, Lophiotremataceae, on morphological and molecular grounds, to accommodate *Lophiotrema* species. Traditionally, *Lophiotrema* has been placed in the Lophiostomataceae along with *Lophiostoma* (Saccardo 1883; Clements and Shear 1931; Barr 1992; Lumbsch and Huhndorf 2007; Kirk et al. 2008). However,

its distant relationship with the Lophiostomataceae has been suggested (Schoch et al. 2009; Zhang et al. 2009a,b) and confirmed by our study, using sequences of more than ten strains of *Lophiotrema* (Fig. 28). Phylogenetically, the *Lophiotrema* clade is close to the families Testudinaceae (Tanaka et al. 2009; Zhang et al. 2009a,b) and Tetraplosphaeriaceae (Schoch et al. 2009) in the Pleosporales. Morphologically, however, species in the *Lophiotrema* clade are quite different from members of the Testudinaceae; the latter is characterized by cleistothecial ascomata and sculptured ascospores (von Arx 1971; Hawksworth 1979). Similarly, *Lophiotrema* species do not have the *Tetraploa*-like hyphomycetous anamorphs of taxa in the Tetraplosphaeriaceae (Tanaka et al. 2009). *Lophiostoma*-like fungi having ascomata with a long slit-like ostiole are known from other families, such as the Platystomaceae [*Ostropella* (Sacc.) Höhn. and *Xenolophium* Syd.; Mugambi and Huhndorf 2009a] and the Aigialaceae (*Rimora*; Suetrong et al. 2009). Molecular analyses of five genes (SSU, LSU nrDNA, *TEF1*, *RPB1*, and *RPB2*), however, do not support a close phylogenetic relationship between *Lophiotrema* and these families (Schoch et al. 2009). This finding indicates that fungi with lophiostomatoid ascomata may have evolved multiple times independently within the Dothideomycetes, as is the case for genera with hysterothecial ascomata with a slit-like ostiole, e.g., *Glonium* Muhl., *Hysterium* Pers., and *Hysterographium* Corda (Mugambi and Huhndorf 2009b). Familial circumscription placing much importance on the slit-like ostiolar opening (e.g., Lophiostomataceae sensu lato; Barr 1992) should not therefore be applied to the Lophiostomataceae, although this character may have taxonomic significance at generic level. Further molecular evidence and morphological evaluation of many species in related genera may be required to more clearly define the phenotypic circumscription of the Lophiostomataceae.

#### Notes on the species examined

*Lophiostoma caulium* has been considered to be a species complex of several related species (Chesters and Bell 1970a). Holm and Holm (1988) divided this “species” into five “varieties,” labeling them with the letters “var. *a*” to “var. *e*,” mainly on the basis of ascospore size and septation. Tanaka and Harada (2003a) followed these provisional decisions without formal taxonomic status and proposed a new taxon, “var. *f*,” for species with 9-septate ascospores. We analyzed three varieties (*L. caulium* “var. *a*, *d*, and *f*”) phylogenetically, and these are clearly shown to be three distinct species in our MP tree (see Fig. 28). Further taxonomic revision based on type specimens of the *L. caulium* complex and phylogenetic analyses using their epitype strains will be necessary to establish species names.

*Lophiostoma semiliberum* (Desm.) Ces. & De Not. has been interpreted to be an immature stage of *L. arundinis* (Pers.) Ces. & De Not. (Munk 1957; Eriksson 1967; Eriksson and Yue 1986) because of the similarities in their ascospore sizes and habitats. We have revealed that these are different taxa, as was suggested from their anamorphic (possibly spermatial) morphology in vitro (Tanaka and Harada 2003a).

*Lophiostoma vitigenum* and *L. fockelii* have been treated as *Lophiotrema* because of their rather small ascomata and peridia of equal thickness (Tanaka and Harada 2003b). As already mentioned, however, these characters do not have phylogenetic significance for generic separation. Because these species both have clavate asci with long stipes, they should be treated as *Lophiostoma* species. Placement of these taxa in *Lophiostoma* was verified by the molecular work of Mugambi and Huhndorf (2009a) and our own work.

*Lophiostoma quadrisporum*, described here as a new species, is based on a misidentified material of *Lophiotrema nucula* (HHUF 27321). Despite several discrepancies between the material and the description of *L. nucula* in terms of ascospore number and cultural characteristics, Tanaka and Harada (2003b) tentatively judged the specimen to be *L. nucula* on the basis of ascospore similarity. However, our reexamination of this specimen indicates that the fungus HHUF 27321 is neither congeneric nor conspecific with *L. nucula*. It is distinguished by ascospores that are somewhat wider than those of *L. nucula* [6–9.5 µm vs. (4–) 5–6.5 µm wide; Zhang et al. 2009b] and consistently 4-spored asci; *L. nucula* in contrast has 8-spored asci (Holm and Holm 1988). The clavate asci with long stipes (15–33 µm in length) of *L. nucula* sensu Tanaka and Harada (2003b) indicate its phylogenetic affinity with *Lophiostoma* rather than with *Lophiotrema*. This interpretation is evidently supported by our phylogenetic analyses (Fig. 28).

#### Further study

We have revealed that shape and stipe length of ascus are indicative in differentiating between *Lophiostoma* (Lophiostomataceae) and *Lophiotrema* (Lophiotremataceae). However, we would consider it equivocal to separate these at familial level based on this ascus character alone; more fundamental differences should define families. We considered that there were no differences in ascomatal wall anatomy between these genera; however, this opinion should be reexamined using a method of making precise ascomatal sections (Huhndorf 1991). Peridial anatomy is recognized as a good predictor of generic or familial relationship among some Ascomycota (Miller and Huhndorf 2005; Boehm et al. 2009). *Leptosphaeria* Ces. & De

Not. (Leptosphaeriaceae) and *Phaeosphaeria* I. Miyake (Phaeosphaeriaceae), for example, can be distinguished by their wall structure (scleroplectenchymatous in *Leptosphaeria* and pseudoparenchymatous in *Phaeosphaeria*) (Câmara et al. 2002). These wall traits can be observed only in well-made, thin ascomatal sections (3–4 µm thick) (Huhndorf 1992). Several species in *Lophiostoma* (e.g., *L. arundinis*, *L. fuckelii*, *L. macrostomum*, and *L. sagittiforme* Kaz. Tanaka & Hosoya) and *Lophiotrema* [e.g., *L. neoarundinaria* (Ellis & Everh.) Yin. Zhang et al., *L. neohysterioides*, and *L. vagabundum* (Sacc.) Sacc.] are known to produce ascomata in culture (Leuchtmann 1985; Tanaka and Harada 2003a,b,c; Tanaka and Hosoya 2008). An in vitro developmental study of the ascomata of these homothallic species based on the semi-thin sectioning, as was partly carried out for *L. fuckelii* (Chesters and Bell 1970b), may help to further resolve important morphological differences between *Lophiostoma* and *Lophiotrema*.

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