



Revision of *Lophiotremataceae* (*Pleosporales*, *Dothideomycetes*): *Aquasubmersaceae*, *Cryptocoryneaceae*, and *Hermatomycetaceae* fam. nov.

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Key words

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Abstract The family *Lophiotremataceae* (*Pleosporales*, *Dothideomycetes*) is taxonomically revised on the basis of morphological observations and phylogenetic analyses of sequences of nuclear rDNA SSU, ITS, and LSU regions and *tef1* and *rpb2* genes. A total of 208 sequences were generated from species of *Lophiotremataceae* and its relatives. According to phylogenetic analyses, *Lophiotremataceae* encompasses the genus *Lophiotrema* and five new genera: *Atrocalyx*, *Crassimassarina*, *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus*. These genera are characterised by ascomata with or without a slit-like ostiole and pycnidial conidiomata. Three new families, *Aquasubmersaceae*, *Cryptocoryneaceae*, and *Hermatomycetaceae*, are proposed. Two genera previously recognised as members of *Lophiotremataceae*, namely, *Aquasubmersa* having ascomata with a papillate ostiolar neck and pycnidial conidiomata and *Hermatomyces* possessing sporodochial conidiomata and dimorphic (lenticular and cylindrical) conidia, are included in *Aquasubmersaceae* and *Hermatomycetaceae*, respectively. *Cryptocoryneum*, characterised by the presence of stromatic sporodochia, cheiroid conidia, and conidial arms developed downward from the cap cells, is placed in *Cryptocoryneaceae*. Two new genera, *Antealophiotrema* and *Pseudolophiotrema*, are established, but their familial placements remain unresolved. *Antealophiotrema* bears ascocata morphologically similar to those of *Lophiotrema*, but is differentiated from the latter by having ascocata with a well-developed peridium and a monodictys-like asexual morph. *Pseudolophiotrema* is also similar to *Lophiotrema*, but can be distinguished by ascocata with a thin peridium. A total of three new families, seven new genera, eight new species, and two new combinations are described and illustrated.

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INTRODUCTION

Lophiotremataceae was originally established as a monotypic family comprising the type genus *Lophiotrema* (Hirayama & Tanaka 2011). This genus is relatively common, known from throughout the world, and new species are continually being discovered (Mathiassen 1993, Tanaka & Harada 2003, Zhang et al. 2009, Liu et al. 2015, Hyde et al. 2016). Species in *Lophiotrema* are characterised by immersed ascocata with a crest-like ostiolar neck and cylindrical asci. Although the taxonomic validity of *Lophiotremataceae* has been confirmed by molecular studies, only a few of its members have been confirmed based on DNA studies (Hyde et al. 2013).

Several major taxonomic and phylogenetic investigations have been carried out to discover missing lineages in *Ascomycota* (Zhang et al. 2012, Ariyawansa et al. 2015, Liu et al. 2015, Doilom et al. 2016, Hashimoto et al. 2016, Hyde et al. 2016). In these studies, three interesting genera, *Aquasubmersa*, *Cryptocoryneum*, and *Hermatomyces*, were analysed phylogenetically suggesting a close relationship to *Lophiotremataceae*. *Aquasubmersa* was initially described as a coelomycetous genus (Zhang et al. 2012). Later, a second species of this

genus, *A. japonicum* with sexual and asexual morphs, was reported (Ariyawansa et al. 2015). The asexual genus *Hermatomyces* is characterised by having sporodochial conidiomata and dimorphic, i.e., lenticular and cylindrical, conidia (Chang 1995). Phylogenetic analyses using sequences of nuclear rDNA small subunit (18S; SSU), internal transcribed spacer (ITS) and large subunit (28S; LSU) regions and translation elongation factor 1- α (*tef1*) and DNA-directed RNA polymerase II second largest subunit (*rpb2*) genes have suggested that *Aquasubmersa* and *Hermatomyces* are closely related to *Lophiotrema* (Doilom et al. 2016, Tibpromma et al. 2016). On the basis of their phylogenetic studies, Doilom et al. (2016) and Tibpromma et al. (2016) proposed that *Aquasubmersa* and *Hermatomyces* are additional members of *Lophiotremataceae*. Another genus, *Cryptocoryneum*, is characterised by having stromatic sporodochia and cheiroid conidia (Schoknecht & Crane 1977). Recently, Hashimoto et al. (2016) assessed the taxonomic placement of *Cryptocoryneum* species on the basis of morphological observations and the results of a molecular phylogenetic analysis. Although their BLAST search of NCBI's GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov/genbank/>) suggested that the genus is also related to *Lophiotremataceae* (Hashimoto et al. 2016), they could not resolve the familial position of *Cryptocoryneum*. Many genera related to the family *Lophiotremataceae* still need to be re-evaluated.

During our on-going studies of ascomycetous fungi in Japan (Tanaka et al. 2010, 2011, 2015, Hashimoto et al. 2015a, b, 2016), we have collected 57 strains morphologically or phylogenetically related to *Lophiotremataceae*. The main objectives of the present study were to establish a taxonomic framework within *Lophiotremataceae* s.l. and to evaluate the circumscription

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Table 1 Specimens, isolates and new sequence accessions used in this study.

Species	Family	Original no.	Specimen no. ^a	Strain no.	GenBank Accession no. ^b					
					SSU	LSU	tef1	rpb2	ITS	
<i>Antealophiotarema brunneosporum</i>		CBS 123095	CBS H-20222 ^H	LC194298	LC194340	LC194382	LC194419	LC194474		
<i>Aquasubmersa japonica</i>		MAFF 245218	HHUF 30468 ^H	LC061581 ¹	LC061586 ¹	LC194383	LC194420	LC061591 ¹		
		MAFF 245219	HHUF 30469 ^H	LC061582 ¹	LC194384	LC194421	LC194422	LC061592 ¹		
		MAFF 245220	HHUF 30470 ^H	LC061583 ¹	LC061588 ¹	LC194385	LC194422	LC061593 ¹		
		MAFF 245613 = NBRC 112316	CBS 122364	LC194299	LC194341	LC194386	LC194423	LC194476		
		MAFF 245617	CBS H-20221 ^H	LC194300	LC194342	LC194387	LC194424	LC194476		
		HHUF 30512 ^H	HHUF 30512 ^H	LC194301	LC194343	LC194388	LC194425	LC194477		
		HHUF 29084 ^H	HHUF 29084 ^H	LC194302	LC194344	LC194389	LC194426	LC194478		
		MAFF 245614 = NBRC 112317	HHUF 30507 ^H	LC194303	LC194345	LC194390	LC194427	LC194479		
		MAFF 245616	HHUF 30510 ^H	LC194304	LC194346	LC194391	LC194428	LC194480		
		MAFF 245615 = NBRC 112318	HHUF 30509 ^H	LC194305	LC194347	LC194392	LC194429	LC194481		
		MAFF 245365 = NBRC 111758	HHUF 30477 ^H	LC194306	LC194348	LC096136 ²	LC096143 ²	LC096154 ²		
		MAFF 245366 = NBRC 111759	HHUF 30478 ^H	LC194307	LC194349	LC096137 ²	LC194431	LC096155 ²		
		CBS 123059	UPS F-6329989	LC194308	LC194350	LC096138 ²	LC194432	LC096156 ²		
		CBS 122829	–	LC194309	LC194351	LC096139 ²	LC096143 ²	LC096157 ²		
		CBS 122633	–	LC194310	LC194352	LC096140 ²	LC194434	LC096158 ²		
		MAFF 245367 = NBRC 111760	HHUF 30479 ^H	LC194311	LC194353	LC096141 ²	LC096145 ²	LC096159 ²		
		MAFF 245368	HHUF 30480 ^H	LC194312	LC194354	LC096142 ²	LC194436	LC096160 ²		
		MAFF 245369	HHUF 30481 ^H	LC194313	LC194355	LC096143 ²	LC194437	LC096161 ²		
		MAFF 245370 = NBRC 111761	HHUF 30482 ^H	LC194314	LC194356	LC096144 ²	LC194438	LC096162 ²		
		MAFF 245371	HHUF 30483 ^H	LC194315	LC194357	LC096145 ²	LC194439	LC096163 ²		
		MAFF 245372	HHUF 30484 ^H	LC194316	LC194358	LC096146 ²	LC194440	LC096164 ²		
		MAFF 245373	HHUF 30485 ^H	LC194317	LC194359	LC096147 ²	LC194441	LC096165 ²		
		MAFF 245374 = NBRC 111762	HHUF 30486 ^H	LC194318	LC194360	LC096148 ²	LC194442	LC096166 ²		
		MAFF 245375	HHUF 30487 ^H	LC194319	LC194361	LC096149 ²	LC194443	LC096167 ²		
		MAFF 245376	HHUF 30488 ^H	LC194320	LC194362	LC096150 ²	LC194444	LC096168 ²		
		MAFF 245377 = NBRC 111763	HHUF 30489 ^H	LC194321	LC194363	LC096151 ²	LC194445	LC096169 ²		
		CBS 113641	HHUF 30490 ^H	LC194322	LC194364	LC096152 ²	LC194446	LC096170 ²		
		–	–	LC194323	LC194365	LC096153 ²	LC194447	LC096171 ²		
		C. japonicum	KT 2563	HHUF 30505 ^H	LC194324	LC194366	LC194393	LC194448	LC194482	
		KT 361	HHUF 30518 ^H	LC194325	LC194367	LC194394	LC194449	LC194483		
		KT 3413	HHUF 30480 ^H	LC194326	LC194368	LC194395	LC194450	LC194484		
		yoné 36	HHUF 30484 ^H	LC194327	LC194369	LC194396	LC194451	LC194485		
		yoné 157	HHUF 30485 ^H	LC194328	LC194370	LC194397	LC194452	LC194486		
		KT 2913	HHUF 30486 ^H	LC194329	LC194371	LC194398	LC194453	LC194487		
		KT 3487	HHUF 30487 ^H	LC194330	LC194372	LC194399	LC194454	LC194488		
		KT 3071	HHUF 30488 ^H	LC194331	LC194373	LC194400	LC194455	LC194489		
		KT 3241	HHUF 30489 ^H	LC194332	LC194374	LC194401	LC194456	LC194490		
		–	HHUF 30490 ^H	LC194332	LC194375	LC194402	LC194457	LC194491		
		C. longicondensatum	–	LC194332	LC194376	LC194403	LC194458	LC001709 ³		
		C. paracondensatum	–	LC194332	LC194377	LC194404	LC194459	LC194493		
		C. pseudoristonei	–	LC194332	LC194378	LC194405	LC194460	AB539109 ⁶		
		Cryptocoryneum sp.	–	LC194332	LC194379	LC194406	LC194461	AB539109 ⁶		
		Galeaticarpia aomoriensis	–	LC194332	LC194380	LC194407	LC194462	AB524786 ⁵		
		Hematomycetaceae	–	LC194332	LC194381	LC194408	LC194463	AB524786 ⁵		
		Hematomycetaceae	–	LC194332	LC194382	LC194409	LC194464	LC194464		
		Hematomycetaceae	–	LC194332	LC194383	LC194410	LC194465	LC194465		
		Hematomycetaceae	–	LC194332	LC194384	LC194411	LC194466	LCB39110 ⁶		
		Hematomycetaceae	–	LC194332	LC194385	LC194412	LC194467	LCB39110 ⁶		
		Hematomycetaceae	–	LC194332	LC194386	LC194413	LC194468	LCB39110 ⁶		
		Hematomycetaceae	–	LC194332	LC194387	LC194414	LC194469	LCB39110 ⁶		
		L. nucula	KT 1424-1	HHUF 30079 ^H	LC194332	LC194388	LC194415	LC194470	LC194502	
		L. eburnoides	KT 2748	HHUF 30506 ^H	LC194332	LC194389	LC194416	LC194471	LC194503	
		L. Fallopiae	KT 656	HHUF 27547	LC194332	LC194390	LC194417	LC194472	LC194504	
		L. neoarundinaria	KT 856	HHUF 30526	LC194332	LC194391	LC194418	LC194473	LC194505	
		Lophiotarema boreale	KT 1034	HHUF 30015	LC194332	LC194392	LC194419	LC194477	LC194506	
		Lophiotarema	KT 2200	HHUF 30014	LC194332	LC194393	LC194420	LC194478	LC194507	
		Lophiotarema	KT 17	HHUF 30511	LC194332	LC194394	LC194421	LC194479	LC194508	
		Lophiotarema	KT 588	HHUF 27368	LC194332	LC194395	LC194422	LC194480	LC194509	
		Lophiotarema	KT 713	HHUF 27328	LC194332	LC194396	LC194423	LC194471	LC194510	
		Lophiotarema	KT 756	HHUF 27330	LC194332	LC194397	LC194424	LC194472	LC194511	
		Lophiotarema	–	–	LC194332	LC194398	LC194425	LC194473	LC194512	
		Lophiotarema	–	–	LC194332	LC194399	LC194426	LC194474	LC194513	
		Lophiotarema	–	–	LC194332	LC194399	LC194427	LC194475	LC194514	
		Lophiotarema	–	–	LC194332	LC194399	LC194428	LC194476	LC194515	
		Lophiotarema	–	–	LC194332	LC194399	LC194429	LC194477	LC194516	
		Lophiotarema	–	–	LC194332	LC194399	LC194430	LC194478	LC194517	
		Lophiotarema	–	–	LC194332	LC194399	LC194431	LC194479	LC194518	
		Lophiotarema	–	–	LC194332	LC194399	LC194432	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194433	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194434	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194435	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194436	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194437	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194438	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194439	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194440	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194441	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194442	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194443	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194444	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194445	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194446	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194447	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194448	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194449	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194450	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194451	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194452	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194453	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194454	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194455	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194456	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194457	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194458	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194459	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194460	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194461	LC194479	LC194519	
		Lophiotarema	–	–	LC194332	LC194399	LC194462	LC194479	LC194519	</td

Table 2 Specimens, isolates and GenBank accession numbers of species used in the phylogenetic study.

Species	Family	Specimen no. ^a	Strain no.	GenBank Accession no.				
				SSU	LSU	terf1	rpb2	ITS
<i>Anniculicola immersa</i>	<i>Anniculicolaceae</i>	CBS H-20226 ^H	CBS 123083	GU456295	FJ795498	GU456273	GU456358	—
<i>A. parva</i>	<i>Anniculicolaceae</i>	CBS H-20227 ^H	CBS 123092	GU296134	GU301797	GU349065	—	—
<i>Anteagloniaceum abbreviatum</i>	<i>Anteagloniaceae</i>	ANM 925.1	—	—	GQ221877	GQ221924	—	—
<i>A. globosum</i>	<i>Anteagloniaceae</i>	ANM 925.2 ^H	—	—	GQ221879	GQ221925	—	—
<i>A. parvulum</i>	<i>Anteagloniaceae</i>	SMH 5283 ^P	—	—	GQ221911	GQ221919	—	—
<i>A. parvulum</i>	<i>Anteagloniaceae</i>	MFLUCC 14-0815	KU922912	KU922911	KU922919	—	—	—
<i>Anteagloniaceae</i>	<i>Anteagloniaceae</i>	MFLU 16-0472	MFLUCC 14-0817	KU922914	KU922913	—	—	—
<i>Anteagloniaceae</i>	<i>Anteagloniaceae</i>	MFLU 16-0474	MFLUCC 14-0821	KU922916	KU922915	KU922921	—	—
<i>Anteagloniaceae</i>	<i>Anteagloniaceae</i>	MFLU 16-0470	MFLUCC 14-0823	KU922918	KU922917	KU922922	—	—
<i>Anteagloniaceae</i>	<i>Anteagloniaceae</i>	MFLU 16-0471 ^H	MFLUCC 14-0816	KU922910	KU922909	KU922920	—	—
<i>A. thailandicum</i>	<i>Aquasubmersaceae</i>	MFLU 111001 ^H	MFLUCC 11-0401 = IFRDCC 2572	JX276956	JX276955	—	—	JX276954
<i>Aquasubmersa miricensis</i>	<i>Byssolophis spherooides</i>	—	IFRDCC 2053	GU456296	GU456318	GU456263	GU456348	—
<i>Byssolophis spherooides</i>	<i>Hermatomyctaceae</i>	MFLU 15-3437 ^H	MFLUCC 14-1140	KU712465	KU764695	KU872757	KU712486	KU144917
<i>Hermaptomyctaceae</i>	<i>Hermaptomyctaceae</i>	MFLU 15-3438 ^H	MFLUCC 14-1141	KU712466	KU764696	KU872758	—	KU144918
<i>Hermaptomyctaceae</i>	<i>Hermaptomyctaceae</i>	MFLU 15-3439 ^P	MFLUCC 14-1142	KU712467	KU764697	—	KU712487	KU144919
<i>H. thailandica</i>	<i>Hermaptomyctaceae</i>	MFLU 15-3440 ^H	MFLUCC 14-1143	KU712468	KU764692	KU872754	KU712488	KU144920
<i>Hermaptomyctaceae</i>	<i>Hermaptomyctaceae</i>	MFLU 15-3441 ^P	MFLUCC 14-1144	KU712469	KU764693	KU872755	KU712489	KU144921
<i>Hermaptomyctaceae</i>	<i>Hermaptomyctaceae</i>	MFLU 15-3442 ^P	MFLUCC 14-1145	KU712470	KU764694	KU872756	KU712490	KU144922
<i>Lepidosphaeria nicotiae</i>	<i>Festudinaceae</i>	CBS 101341	—	DQ678067	DQ677910	DQ677963	—	—
<i>Lophiostoma arundinis</i>	<i>Lophiostomataceae</i>	—	—	DQ782383	DQ782384	DQ782386	AJ496633	—
<i>Lophiotrema bambusae</i>	<i>Lophiotremataceae</i>	MFLU 11-0150	MFLUCC 10-0558	KX672159	KX672154	KX672162	KX672161	KX672149
<i>L. crenatum</i>	<i>Lophiostomataceae</i>	—	CBS 621.86	DQ678017	DQ677912	DQ677965	—	—
<i>Polylophosphaeria fusca</i>	<i>Tetraplosphaeriaceae</i>	MFLU 11-0155 ^H	MFLUCC 11-0205	AB524463	AB524604	—	—	AB524789
<i>Pseudoastrophaerella bambusae</i>	<i>Pseudoastrophaerellaceae</i>	MFLU 11-0207 ^H	MFLUCC 11-0171	KT955455	KT955475	KT955437	KT955414	—
<i>P. longicolla</i>	<i>Pseudoastrophaerellaceae</i>	MFLU 11-0145 ^H	MFLUCC 10-0553	—	KT955476	KT955438	KT955420	—
<i>P. thailandensis</i>	<i>Pseudoastrophaerellaceae</i>	HHUF 29399 ^H	JCM 13175 = MAFF 239685	KT955456	KT955477	KT955439	KT955411	—
<i>Pseudotetraplospora curvappendiculata</i>	<i>Tetraplospora curvappendiculata</i>	HHUF 28582 ^H	JCM 12852 = MAFF 239495	AB524467	AB524608	—	—	AB524792
<i>Quadrirura septentrionalis</i>	<i>Tetraplospora curvappendiculata</i>	HHUF 28781 ^P	CBS 125429	AB524474	AB524615	—	—	AB524799
<i>Tetraplospora sasicola</i>	<i>Tetraplospora curvappendiculata</i>	HHUF 27566 ^H	JCM 13167 = MAFF 239677	AB524490	AB524631	—	—	AB524807
<i>Tripliosphaeria maxima</i>	<i>Tetraplospora curvappendiculata</i>	HHUF 29390 ^H	AB524496	AB524637	—	—	—	AB524812
<i>Ulospora bilgramii</i>	<i>Tetraplospora curvappendiculata</i>	—	DQ678025	DQ677976	DQ677921	DQ677974	—	—
<i>Vermulina enalia</i>	<i>Tetraplospora curvappendiculata</i>	—	GU479803	GU479803	GU479864	GU479836	—	—

^a H = holotype, P = paratype.

of this family based on morphological observations and phylogenetic analyses of SSU, ITS, and LSU nuclear rDNA and *tef1* and *rpb2* gene sequences.

MATERIALS AND METHODS

Isolation and morphological observation

All fungal structures were observed in preparations mounted in distilled water. Morphological characters were observed by differential interference and phase contrast microscopy (Olympus BX53), with images captured with an Olympus digital camera (DP21). A total of 57 single-spore isolates were used for morphological observations and phylogenetic analyses (Table 1). Colony characteristics of cultures grown on potato dextrose agar (PDA; Difco) were observed after 3 wk cultivation at 20 °C in the dark. Colours were noted as described by Rayner (1970). To induce sexual or asexual fructification in culture, 5-mm squares of mycelial agar were placed on water agar including sterilised natural substrate, such as rice straws and pine needles, and the plates were incubated at 20 °C for 2 wk in the dark. When the substrate was colonised, the plates were incubated at 20 °C under blacklight blue illumination for 2 mo to observe sporulation. Cultures were deposited in the Japan Collection of Microorganisms (JCM), the NITE Biological Resource Centre (NBRC), and the GeneBank Project, NARO, Japan (MAFF). Specimens were deposited in the fungus herbarium of Hirosaki University (HHUF).

Phylogenetic analyses

DNA extraction was carried out with an ISOPLANT II kit (Nippon Gene, Japan) in accordance with the manufacturer's protocol. Partial SSU, complete ITS, and partial LSU nuclear rDNA regions and partial *tef1* and partial *rpb2* genes were amplified by PCR with the primer pairs NS1/NS4, ITS1/ITS4 (White et al. 1990), LR0R/LR7 (Rehner & Samuels 1994, Vilgalys & Hester 1990), EF1-983F/EF1-2218R (Rehner & Buckley 2005), and fRPB2-5F/fRPB2-7cR (Liu et al. 1999), respectively. Amplifications were performed in 25 µL volumes consisting of 2 µL DNA extract, 2.5 µL of 10x TEMPase Buffer I, 10 mM dNTP mix, 1 µL of each 20-pM primer, 25 mM MgCl₂, 14.5 µL MilliQ water, and 0.5 µL TEMPase Hot Start DNA polymerase (Ampliqon, Denmark). PCRs were carried out on a PC 320 thermocycler (ASTEC, Japan) as follows: 95 °C for 15 min, followed by 35 cycles of 1 min at 94 °C, 1 min at the designated annealing temperature (42.2 °C for SSU, 61.5 °C for ITS, 46 °C for LSU, 60 °C for *tef1*, and 58 °C for *rpb2*), and 1 min at 72 °C, with a final denaturation step of 7 min at 72 °C. The PCR products were sequenced directly at SolGent (South Korea).

Newly generated sequences were deposited in GenBank (Table 1). Sequences of 32 taxa from *Amniculicolaceae*, *Anteagloniaceae*, *Aquasubmersaceae*, *Hermatomycetaceae*, *Lophiotremataceae*, *Pseudoastrosphaeriellaceae*, *Testudinaceae*, and *Tetraplosphaeriaceae* were also phylogenetically analysed (Table 2). *Lophiostoma crenatum* and *L. arundinis* (*Lophiotremataceae*) were used as outgroups. All sequences were aligned using the MUSCLE algorithm as implemented in MEGA v. 5 (Tamura et al. 2011). Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian methods. The optimum substitution models for each dataset were estimated by Kakusan4 (Tanabe 2011) based on the Akaike information Criterion (AIC; Akaike 1974) for the ML analysis and the Bayesian information Criterion (BIC; Schwarz 1978) for the Bayesian analysis. The ML analysis was performed using TreeFinder Mar 2011 (Jobb 2011) based on the models selected with the AICc4 parameter (proportional model among genes and proportional model among codons), namely, TN93ef+G for SSU, TN93+G for LSU, J2ef+G for ITS, F81+G for the *tef1* first codon position,

TIMef+G for the *tef1* second codon position, J2+G for the *tef1* third codon position, J2+G for the *rpb2* first codon position, JC69+G for the *rpb2* second codon position, and TN93ef+G for the *rpb2* third codon position. Bootstrap proportions (BPs) were obtained by 1 000 bootstrap replications. Bayesian analysis was performed with MrBayes v. 3.2.2 (Ronquist et al. 2012) with substitution models for different regions selected with the BIC4 parameter (i.e., proportional model among loci and among codons): K80+G for SSU, SYM+G for LSU, SYM+G for ITS, F81+G for the *tef1* first codon position, GTR+G for the *tef1* second codon position, GTR+G for the *tef1* third codon position, GTR+G for the *rpb2* first codon position, HKY85+G for the *rpb2* second codon position, and SYM+G for the *rpb2* third codon position. Two simultaneous, independent Metropolis-coupled Markov chain Monte Carlo (MCMC) runs were performed for 2 M generations with trees sampled every 1 000 generations. Convergence of the MCMC procedure was assessed from the average standard deviation of split frequencies (< 0.01) and effective sample size scores (all > 100) using MrBayes and Tracer v. 1.6 (Rambaut et al. 2014), respectively. The first 25 % of trees were discarded as burn-in, and the remaining trees were used to calculate 50 % majority rule trees and to determine posterior probabilities (PPs) for individual branches. The alignment was submitted to TreeBASE under study number S19310.

RESULTS

The ML and Bayesian phylogenetic analyses were conducted using an aligned sequence dataset comprising 970 nucleotide positions from SSU, 1 275 from LSU, 340 from ITS, 909 from *tef1*, and 1 023 from *rpb2*. The alignment contained a total of 89 taxa, which consisted of 84 taxa (94.4 %) in SSU, 89 (100 %) in LSU, 71 (79.8 %) in ITS, 81 (91 %) in *tef1*, 73 (82 %) in *rpb2* (Table 1, 2). No significant conflict was observed among individual gene phylogenies, allowing the five genes to be combined into a single dataset. This combined dataset provided higher confidence values for the familial level than did those of the individual gene trees (data not shown). ITS1 was excluded from the analyses because it contained too many ambiguously aligned regions. Of the 4 517 characters included in the alignment, 1 307 were variable and 3 189 were conserved. The ML tree with the highest log likelihood (-31261.0019) is shown in Fig. 1. The Bayesian likelihood score was -31334.0529. The topology recovered by the Bayesian analysis was almost identical to that of the ML tree except for the position of *Anteagloniaceae*.

In the phylogenetic tree shown in Fig. 1, *Lophiotremataceae* forms a moderately supported clade (77 % ML BP/1.00 Bayesian PP) (Fig. 1). The clade corresponding to *Lophiotrema* s.str. includes *L. eburnoides*, *L. fallopiae*, *L. neoarundinaria*, *L. neo-hysterioides*, *L. nucula*, and *L. vagabundum*. The results of the phylogenetic analyses suggest that four species previously placed in *Lophiotrema*, i.e., *L. boreale*, *L. brunneosporum*, *L. lignicola*, and *L. bambusae* (Mathiassen 1993, Zhang et al. 2009, Hyde et al. 2016), should be excluded from the genus. The first two species, *L. boreale* and *L. brunneosporum*, lie outside of *Lophiotremataceae*, but their familial placements are unresolved (Fig. 1). On the basis of these results, a new genus, *Antealophiotrema*, is established for *L. brunneosporum*. *Lophiotrema lignicola* is transferred to the new genus *Atrocalyx*, and a new combination, *Atrocalyx lignicola*, is proposed. The generic placement of *L. bambusae* and *L. boreale* remains unresolved because we were unable to examine any material of these species.

Four families, including the three new families, *Aquasubmersaceae*, *Cryptocoryneaceae*, and *Hermatomycetaceae*, 10 genera including seven new genera, nine species including seven new species, and two new combinations are described below.

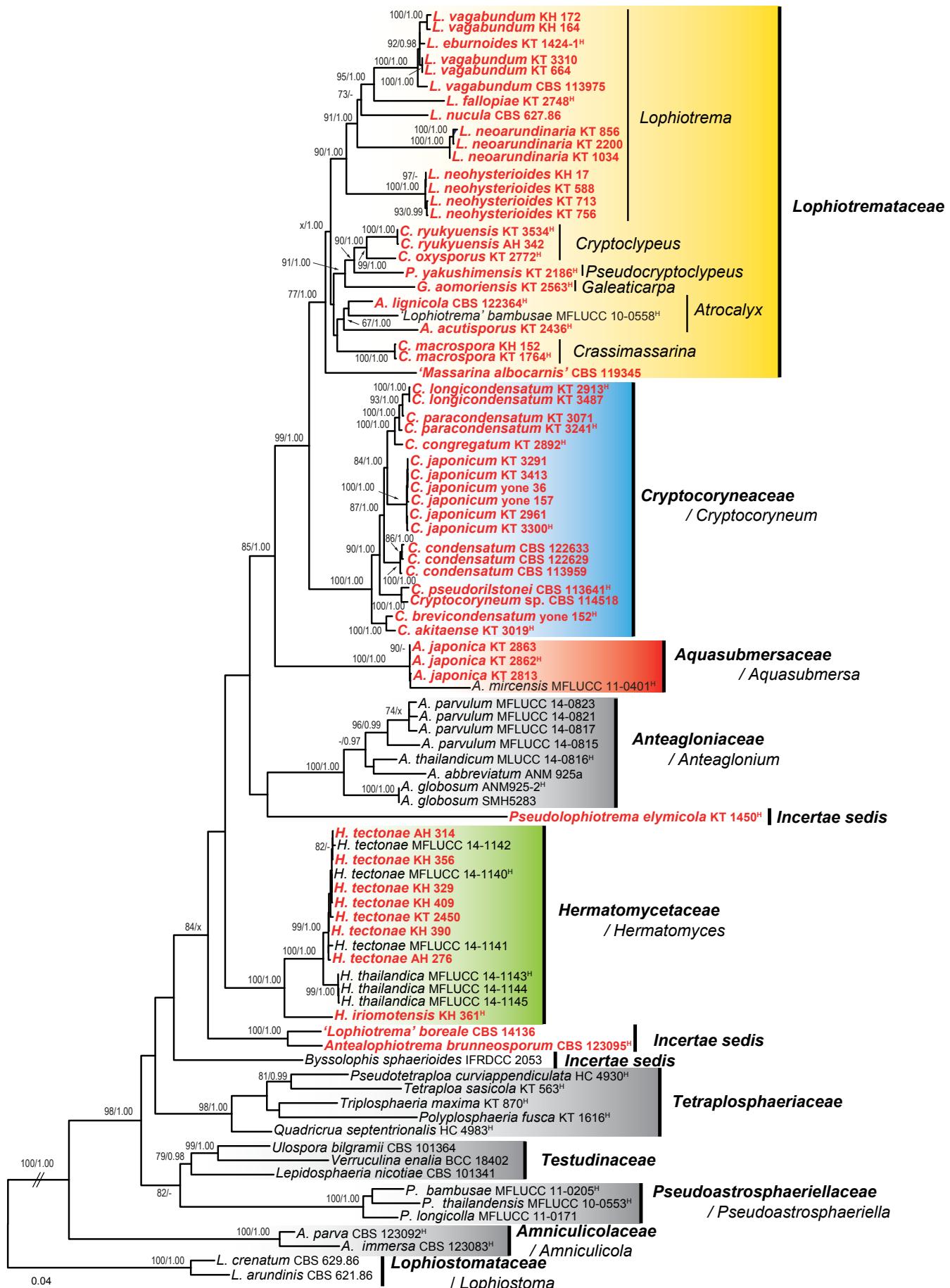


Fig. 1 Maximum-likelihood (ML) tree of *Aquasubmersaceae*, *Cryptocoryneaceae*, *Hermatomycetaceae*, and *Lophiotremataceae* based on SSU-ITS-LSU-tef1-rpb2 sequences. ML bootstrap proportion (BP) > 60 % and Bayesian posterior probabilities (PP) > 0.95 are presented at the nodes as ML BP/Bayesian PP. A hyphen ('-') indicates values < 60 % BP or 0.95 PP, and a node not present in the Bayesian analysis is shown with 'x'. Ex-holotype strains are indicated with a superscript H. The newly obtained sequences are shown in bold and red. The scale bar represents nucleotide substitutions per site.

Aquasubmersaceae A. Hashim. & Kaz. Tanaka, fam. nov. —
MycoBank MB819235

Type genus. *Aquasubmersa* K.D. Hyde & Huang Zhang, Cryptog. Mycol. 33: 340. 2012.

Saprobic on woody plants.

Sexual morph: Ascomata scattered to grouped, subglobose, semi-immersed, with a papillate ostiolar neck. Peridium composed of flattened, thin-walled, polygonal cells. Pseudoparaphyses septate, branched. Ascii bitunicate, cylindrical, with a short pedicel, 8-spored. Ascospores hyaline, broadly fusiform with rounded ends, septate.

Asexual morph: Conidiomata pycnidial, globose to subglobose, scattered, semi-immersed, ostiolate. Conidiophores absent. Conidiogenous cells holoblastic, lageniform, hyaline, smooth. Conidia hyaline, ellipsoidal, smooth.

Notes — Previous phylogenetic studies using SSU and LSU rDNA sequences placed *Aquasubmersa* in *Pleosporales* (Zhang et al. 2012, Ariyawansa et al. 2015). This genus was treated as a member of *Lophiotremataceae* in a recent study (Doilom et al. 2016). The results of our phylogenetic analyses based on SSU-ITS-LSU-tef1-rpb2 sequences strongly support (99 % ML BP/1.00 Bayesian PP) this genus as sister to a clade comprising *Lophiotremataceae* and *Cryptocoryneaceae* (Fig. 1). To accommodate the genus *Aquasubmersa*, we introduce a new family, *Aquasubmersaceae*. Species in *Aquasubmersaceae* share several common features, such as ascomata having a papillate ostiolar neck and pycnidial conidiomata. *Lophiotremataceae* differs from *Aquasubmersaceae* in having ascomata with a compressed, slit-like ostiole. *Cryptocoryneaceae* and *Hermatomycetaceae*, whose sexual morphs are still undetermined, can be easily distinguished from *Aquasubmersaceae*, which has ellipsoidal conidia, because two families have sporodochial conidiomata and cheiroid or lenticular conidia, respectively.

Aquasubmersa K.D. Hyde & Huang Zhang, Cryptog. Mycol. 33: 340. 2012

Type species. *Aquasubmersa mircensis* Huang Zhang & K.D. Hyde, Cryptog. Mycol. 33: 340. 2012.

Notes — The type species of this genus, *A. mircensis*, was reported only as an asexual morph (Zhang et al. 2012). Ariyawansa et al. (2015) subsequently introduced the second species, *A. japonica*, with sexual and asexual morphs. Both species occurred on submerged woody plants.

Cryptocoryneaceae A. Hashim. & Kaz. Tanaka, fam. nov. —
MycoBank MB819237

Type genus. *Cryptocoryneum* Fuckel, Fungi Rhenani Exsicc. XV–XVI: 25. 1865.

Saprobic on various plants.

Sexual morph: Undetermined.

Asexual morph: Conidiomata sporodochial, pulvinate, often confluent, dark brown to black. Conidiophores arising from stromatic cells, simple, septate, hyaline to pale brown. Conidiogenous cells monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown. Conidia solitary, acrogenous, branched, cheiroid, with dark brown to black cap cells firmly united together, multi-armed; basal cells brown, cuneiform, smooth, thin-walled; arms cylindrical, pale brown, branched at base, smooth, multi-septate.

Notes — *Cryptocoryneum* is characterised by having stromatic sporodochia, cheiroid conidia, and conidial arms that are developed downward from the cap cells (Schoknecht & Crane 1977). This genus was recently taxonomically and phylogeneti-

cally reassessed by Hashimoto et al. (2016). A BLAST search with ITS sequences of species in this genus suggested a close relationship with *Lophiotremataceae*, but their familial position remains unresolved (Hashimoto et al. 2016). According to our results, *Cryptocoryneum* forms a lineage that is phylogenetically distinct from *Lophiotremataceae* (Fig. 1). Although the sexual morph of *Cryptocoryneum* has not been determined, asexual morphs of this genus are phenotypically different from the coelomycetous asexual morph of *Lophiotremataceae* (Leuchtmann 1985, this study). We therefore introduce the new family *Cryptocoryneaceae* to accommodate the genus *Cryptocoryneum*.

Cryptocoryneum Fuckel, Fungi Rhenani Exsicc. XV–XVI: 25. 1865

Type species. *Cryptocoryneum fasciculatum* Fuckel, Fungi Rhenani Exsicc. XV–XVI: 25. Fung. Rhen. 1527. 1865. (= *Cryptocoryneum hysteroides* (Corda) Peyronel, Nuovo Giorn. Bot. Ital. 25: 449. 1918.)

Notes — For further information on this genus, see Hashimoto et al. (2016).

Hermatomycetaceae Locq. ex A. Hashim. & Kaz. Tanaka, fam. nov. — MycoBank MB819238

Hermatomycetaceae Locq., Mycologie générale et structural: 202. 1984. nom. inval. (Art. 36.1, Melbourne Code).

Type genus. *Hermatomyces* Speg., Anales Mus. Nac. Buenos Aires, ser. 3, 13: 445. 1911.

Saprobic on various plants.

Sexual morph: Undetermined.

Asexual morph: Conidiomata sporodochial, pulvinate, dark brown to black. Conidiophores mononematous, pale brown. Conidiogenous cells monoblastic, integrated, terminal, cylindrical. Conidia dimorphic; lenticular conidia ellipsoidal, muriform; cylindrical trans-septate, hyaline to brown.

Notes — The family *Hermatomycetaceae* sensu Locq. was informally proposed as a provisional name (Locquin 1984). *Hermatomycetaceae* is established here to accommodate the single genus *Hermatomyces*. Two of the most striking features of *Hermatomyces* are the sporodochial conidiomata and the dimorphic conidia, the latter existing in lenticular and cylindrical forms (Chang 1995). As a sexual morph of this genus has not been reported and a phylogenetic study has not been performed, this genus has long been treated as ‘*incertae sedis*’ within *Ascomycota* (Wijayawardene et al. 2012). Doilom et al. (2016) and Tibpromma et al. (2016) have suggested that this genus belongs to *Lophiotremataceae* on the basis of their phylogenetic analyses using SSU, LSU, *tef1*, and *rpb2*. Our analyses using additional species of *Lophiotremataceae* and its related taxa revealed that *Hermatomyces* is distantly related to *Lophiotremataceae* (Fig. 1).

Hermatomyces Speg., Anales Mus. Nac. Buenos Aires, ser. 3, 13: 445. 1911

Type species. *Hermatomyces tucumanensis* Speg., Anales Mus. Nac. Buenos Aires, ser. 3, 13: 446. 1911.

Saprobic on various plants.

Sexual morph: Undetermined.

Asexual morph: Conidiomata sporodochial, pulvinate, often confluent, dark brown to black. Conidiophores mononematous, septate, pale brown. Conidiogenous cells monoblastic, integrated, terminal, cylindrical. Conidia dimorphic; lenticular conidia ellipsoidal, muriform, dark brown to black at central cells, pale brown at peripheral cells; cylindrical conidia composed of black peripheral cells and dark brown upper cells, hyaline at lower cells.

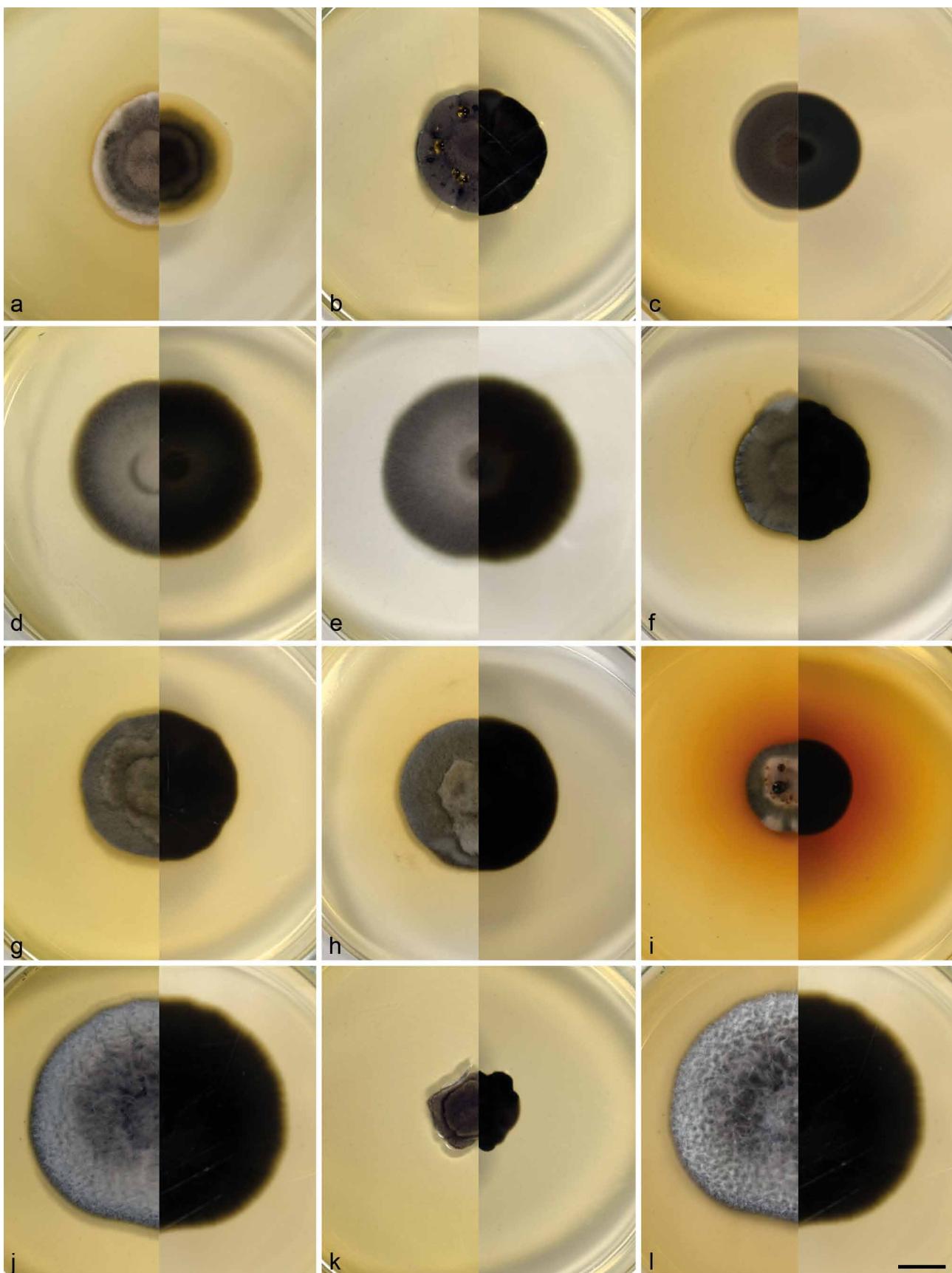


Fig. 2 Colony characters of lophiotremataceous species used in this study on PDA within 3 wk at 20 °C in the dark (left: upper, right: reverse). a. *Hermato- myces iriomotensis* (MAFF 245730 = NBRC 112471, ex-holotype culture); b. *Atrocalyx acutisporus* (MAFF 245613 = NBRC 112316, ex-holotype culture); c. *A. lignicola* (CBS 122364, ex-holotype culture); d. *Crassimassarina macrospora* (JCM 13096 = MAFF 239606, ex-holotype culture); e. *C. macrospora* (MAFF 245617, ex-paratype culture); f. *Cryptoclypeus oxysporus* (MAFF 245614 = NBRC 112317, ex-holotype culture); g. *C. ryukyuensis* (MAFF 245615 = NBRC 112318, ex-holotype culture); h. *C. ryukyuensis* (MAFF 245616, ex-paratype culture); i. *Galeaticarpa aomoriensis* (MAFF 245618 = NBRC 112319, ex-holotype culture); j. *Pseudocryptoclypeus yakushimensis* (MAFF 245622 = NBRC 112320, ex-holotype culture); k. *Antealophiotrema brunneosporum* (CBS 123095, ex-holotype culture); l. *Pseudolophiotrema elymicola* (JCM 13090 = MAFF 239600, ex-holotype culture). — Scale bar = 1 cm.

Notes — This genus was established by Spegazzini (1911) to accommodate *H. tucumanensis*. Since its establishment, 10 additional taxa have been described worldwide (Spegazzini 1911, Hughes 1953, Rao & De Hoog 1986, Castañeda-Ruiz & Heredia 2000, Leão-Ferreira et al. 2013, Prasher & Prasher 2014, Doilom et al. 2016, Tibpromma et al. 2016). Most species of this genus occur on angiosperms and monocots, with a few rarely reported from ferns (Castañeda-Ruiz & Heredia 2000) or gymnosperms (Mel'nik 2000). The most widespread species in the genus, *H. sphaericus*, has been reported from Africa, Eurasia, and Central and South America (Spegazzini 1911, Hughes 1953, Chang 1995, Matsushima 1993, Mel'nik 2000, Barbosa & Gusmão 2011).

***Hermatomyces iriomotensis* A. Hashim. & Kaz. Tanaka, sp. nov.** — MycoBank MB819239; Fig. 2a, 3

Etymology. Referring to the collection site.

Saprobic on woody plants.

Sexual morph: Undetermined.

Asexual morph: Conidiomata sporodochial, pulvinate, often confluent, dark brown to black. Conidiophores mononematous, short, pale brown, smooth, 2.5–3 µm wide, 17.5–22 µm high, sometimes reduced to conidiogenous cells. Conidiogenous cells monoblastic, terminal, integrated. Conidia dimorphic, lenticular and cylindrical. Lenticular conidia solitary, elliptical to almost round in one plane, smooth, muriform, with pale peripheral cells surrounding central dark brown to black cells, 30–36 × 20–27 µm (av. = 33.6 × 23.5 µm, n = 50), 18–24 µm thick (av. = 21.9 µm, n = 30) in lateral view. Cylindrical conidia straight to curved, with one to two columns, 3–7-septate, hyaline, sometimes pale brown at apical cell, constricted at the septa, 20.5–33 × 7–12.5 µm (av. = 28.7 × 9.6 µm, n = 20).

Culture characteristics — Colonies on PDA attaining 24–28 mm diam within 21 d at 20 °C in the dark, floccose, centrally

raised, straw (46: Rayner 1970) to grey olivaceous (107); reverse smoke grey (105) to grey olivaceous (107) (Fig. 2a); no sporulation observed.

Specimen examined. JAPAN, Okinawa, Isl. Iriomote, near Tropical botanic garden, on dead twigs of woody plant, 13 July 2011, K. Hirayama & K. Tanaka, KH 361 (HHUF 30518 holotype designated here, ex-holotype living culture MAFF 245730 = NBRC 112471).

Notes — Lenticular conidia of this species resemble those of *H. uniseriatum*, but *H. iriomotensis* can be distinguished from the latter species by the presence of 3–7-septate cylindrical conidia arranged in 1–2 columns (vs 2–3-septate and one row in the latter; Leão-Ferreira et al. 2013). *Hermatomyces iriomotensis* differs from *H. krabiensis*, which has cylindrical conidia with swollen lower cells (Tibpromma et al. 2016). Additionally, ITS sequences between these two taxa differed at seven positions.

***Lophiotremataceae* K. Hiray. & Kaz. Tanaka, Mycoscience 52: 405. 2011**

Type genus. *Lophiotrema* Sacc., Michelia 1 (no. 3): 338. 1878.

Saprobic on various plants.

Sexual morph: Ascomata immersed, erumpent at the apex, subglobose. Ostiolar neck crest-like or rarely papillate, mostly elongated and laterally compressed. Peridium composed of rectangular to globose cells. Pseudoparaphyses septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical, with a short stipe, 8-spored. Ascospores fusiform to broadly fusiform, hyaline, smooth.

Asexual morph: Conidiomata pycnidial, globose to subglobose, scattered, semi-immersed, ostiolate. Peridium composed of subglobose to angular, brown cells. Conidiophores absent. Conidiogenous cells holoblastic or phialidic, cylindrical to ampulliform, hyaline. Conidia ellipsoidal to cylindrical with rounded ends, hyaline, smooth, aseptate or multi-septate.

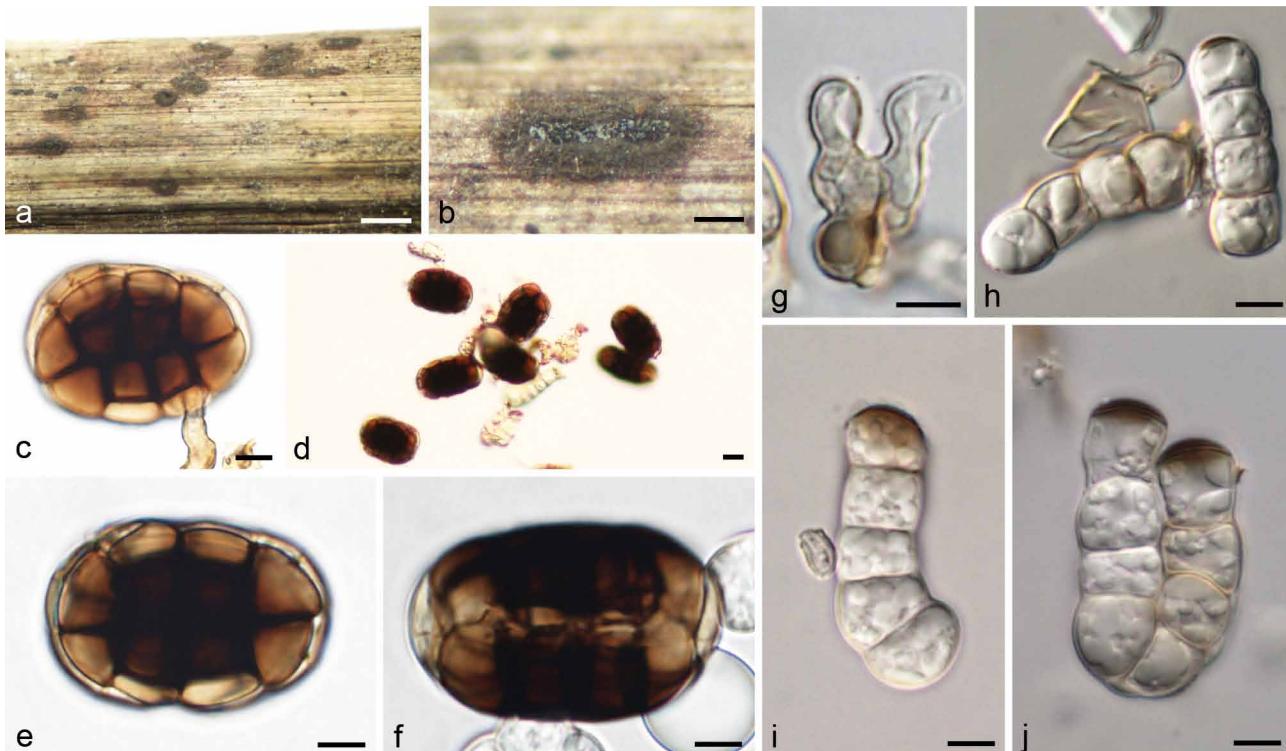


Fig. 3 *Hermatomyces iriomotensis*. a–b. Conidiomata on substrate; c. conidiogenous cell and immature lenticular conidium; d–f. lenticular conidia (f. lateral view); g. conidiogenous cells and immature cylindrical conidium; h–j. cylindrical conidia (all: HHUF 30518, holotype). — Scale bars: a = 1 mm; b = 200 µm; c, e–j = 5 µm; d = 10 µm.

Notes — As originally circumscribed *Lophiotremataceae* was a monotypic family comprising the genus *Lophiotrema* (Zhang et al. 2009, Hirayama & Tanaka 2011, Hyde et al. 2013). A somewhat broader familial concept for *Lophiotremataceae* was adopted by Doilom et al. (2016) and Tibpromma et al. (2016), who considered the family to comprise *Aquasubmersa*, *Hermatomyces*, and *Lophiotrema* on the basis of phylogenetic studies. However, the results of our morphological examination and phylogenetic analyses using SSU, ITS, LSU, *tef1*, and *rpb2* sequences suggest that this family encompasses *Lophiotrema* and five new genera.

Because *Aquasubmersa* and *Hermatomyces* were placed outside of *Lophiotremataceae* in our phylogenetic tree (Fig. 1), we treat these genera as belonging to the families *Aquasubmersaceae* and *Hermatomycetaceae*, respectively. One species of *Lophiotrema* (*L. lignicola*) grouped with *Atrocalyx*, a new genus in *Lophiotremataceae*, while two species (*Lophiotrema boreale* and *L. brunneosporum*) were placed outside of *Lophiotremataceae* entirely.

Marincowitz et al. (2008) have suggested that '*Massarina albocarnis*' (CBS 119345) has a phylogenetic affinity with *Lophiotrema* based on BLAST results involving ITS and LSU sequences. However, Beier et al. (2015), who observed the holotype specimen of *M. albocarnis*, has indicated that this species belongs to *Diaporthe* (*Sordariomycetes*). The isolate CBS 119345 may thus be misidentified. Unfortunately, we were unable to examine any morphological features of CBS 119345 because it did not sporulate in culture.

Lophiotrema Sacc., *Michelia* 1 (no. 3): 338. 1878

Type species. *Lophiotrema nucula* (Fr.) Sacc., *Michelia* 1 (no. 3): 338. 1878.

Notes — For further information on this genus, see Holm & Holm (1988), Tanaka & Harada (2003), Zhang et al. (2009), and Hirayama & Tanaka (2011). In the present study, we found that *Lophiotrema* s.str. should be limited to species having ascocarps with a slit-like ostiole and an ascocarpal wall of uniform thickness, ascospores with a short stipe, and pycnidial asexual morphs. Although *L. brunneosporum* with a monodictys-like asexual morph was sister to '*Lophiotrema*' *boreale* in our phylogenetic tree (Fig. 1), these two taxa were distinct from *Lophiotremataceae* s.str. (Fig. 1). Consequently, they should be treated as distinct lineages in *Dothideomycetes*. We were unable to morphologically examine *L. boreale* from an isolate of this species (CBS 114422) or the original specimen used for isolation. Further examination is required to clarify the taxonomic placement of this species.

Atrocalyx A. Hashim. & Kaz. Tanaka, gen. nov. — MycoBank MB819240

Etymology. From the Latin *atro-*, meaning black, and *calyx*, meaning cap.

Type species. *Atrocalyx acutisporus* A. Hashim. & Kaz. Tanaka.

Saprobic on woody plants.

Sexual morph: Ascocarps solitary to grouped, semi-immersed to immersed. Ostiolar neck crest-like, elongated and laterally compressed, surrounded by dark brown hyphae. Peridium composed of 2 zones at side. Pseudoparaphyses septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores broadly fusiform, hyaline, 1-septate, smooth.

Asexual morph: Conidiomata pycnidial, globose to subglobose, superficial, black, ostiolate. Peridium composed of elongated, brown cells. Conidiophores absent. Conidiogenous cells holoblastic, ampliform to cylindrical, hyaline. Conidia ellipsoidal, hyaline, smooth, aseptate.

Notes — The new genus *Atrocalyx* is established to accommodate *A. lignicola* (formerly *L. lignicola*) and a new species, *A. acutisporus*. These two species are characterised by a crest-like, elongated and laterally compressed ostiolar neck (Fig. 4b, 5b) surrounded by a well-developed peridium (up to 62.5 µm) (Fig. 4c, 5c). '*Lophiotrema*' *bambusae*, which was recently introduced as a species in *Lophiotrema* (Hyde et al. 2016), was nested within a moderately supported clade (67 % ML BP/1.00 Bayesian PP) along with these two species in our phylogenetic tree (Fig. 1). Because we could not observe any material of '*Lophiotrema*' *bambusae*, the generic placement of this species is pending.

The genus is morphologically similar to *Lophiotrema*, but can be distinguished from the latter by its well-developed peridium around the ostiolar neck and base (vs a poorly developed peridium up to 25 µm thick; Holm & Holm 1988).

Atrocalyx acutisporus A. Hashim. & Kaz. Tanaka, sp. nov. — MycoBank MB819241; Fig. 2b, 4

Etymology. Referring to the ascospores with acute ends.

Saprobic on dead twigs of woody plants.

Sexual morph: Ascocarps ellipsoidal, solitary to 2–4-grouped, immersed, 110–140 µm high, 190–210 µm diam. Ostiolar neck crest-like, elongated and laterally compressed, surrounded by dark brown hyphae. Peridium 20–30 µm thick at side, composed of 2 zones; outer zone 11–17 µm thick, composed of elongated, thin-walled, 8.5–12.5 × 2.5–3 µm, brown cells; inner zone 15–17 µm thick, composed of globose to rectangular, 7.5–9 × 4–5 µm, hyaline cells; near the ostiole 37.5–45 µm thick, composed of globose, brown to black cells; 19–37.5 µm thick at the base, composed of globose to rectangular, 1.8–2.5 µm diam cells. Pseudoparaphyses numerous, 1–1.5 µm wide, septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical, (66.5–)75–89.5 × 8–11 µm (av. = 80.8 × 9.2 µm, n = 10), with a short stipe (4–7.5 µm long, av. = 5.5 µm, n = 10), apically rounded with an ocular chamber, 8-spored. Ascospores broadly fusiform with acute ends, 13.5–18(–20) × 3–4(–5.5) µm (av. = 15.9 × 3.7 µm, n = 50), l/w 3.7–5.1 (av. = 4.4, n = 50), hyaline, with a septum nearly median (0.44–0.59, av. = 0.51, n = 50), slightly constricted at the septum, smooth, with an entire gelatinous sheath up to 2 µm thick.

Asexual morph: Conidiomata pycnidial, globose to subglobose, up to 145 µm high, 60–130 µm diam, 2–3-grouped, superficial, black, with a papillate ostiolar neck. Peridium 7.5–10 µm thick, composed of 3–4 layers of 7.5–13 × 1.5–3 µm, elongated, brown cells. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, 7.5–12 × 1.5–3 µm, ampliform to cylindrical, hyaline, smooth. Conidia ellipsoidal, 3–4 × 1.9–2 µm (av. = 3.5 × 2.0 µm, n = 50), l/w 1.5–2.1 (av. = 1.8, n = 50), hyaline, smooth, aseptate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 24–25 mm diam within 21 d at 20 °C in the dark, floccose, radiately, smoke grey (105); reverse olivaceous grey to olivaceous black (108) (Fig. 2b); asexual morph formed.

Specimen examined. JAPAN, Okinawa, Isl. Iriomote, Sono trail, on dead twigs of woody plant, 29 Sept. 2007, K. Tanaka & H. Yonezawa, KT 2436 (HHUF 30504 holotype designated here, ex-holotype living culture MAFF 245613 = NBRC 112316).

Notes — *Atrocalyx acutisporus* superficially resembles *A. lignicola*, but can be distinguished from the latter by its smaller ascospores (13.5–18(–20) × 3–4(–5.5) µm vs 20–26 × 4–5.5(–6) µm, respectively). ITS sequence differences between these two species were found at 16 of 529 nucleotide positions, with two gaps.

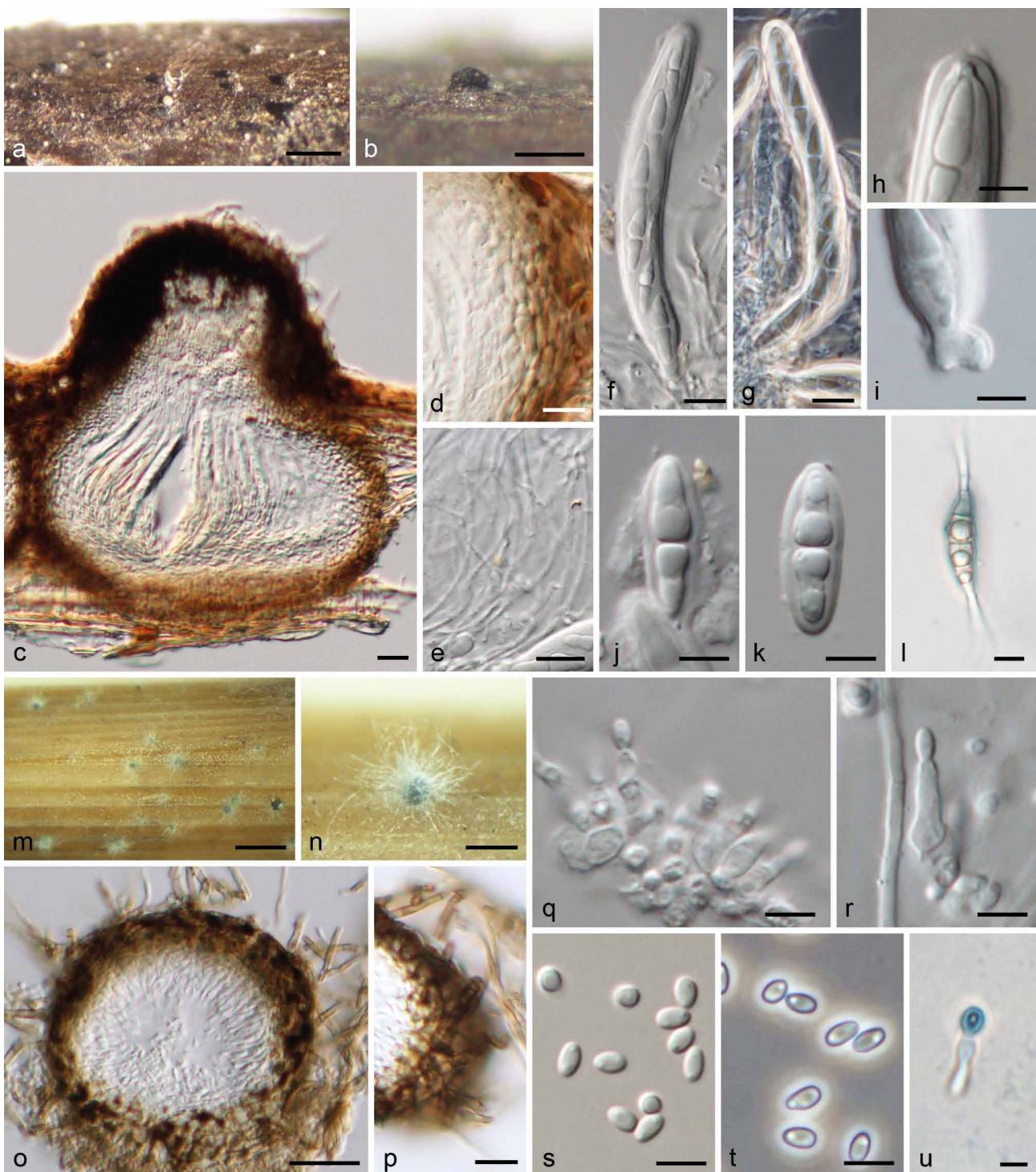


Fig. 4 *Atrocalyx acutisporus*. a–b. Appearance of ascocarps on substrate; c. ascocarp in longitudinal section; d. peridium of ascocarp; e. pseudoparaphyses; f–g. asci; h. ascus apex; i. ascus stipe; j–k. ascospores; l. germinating ascospore; m–n. conidiomata in culture; o. conidioma in longitudinal section; p. peridium of conidioma; q–r. conidiogenous cells; s–t. conidia; u. germinating conidium (a–l: HHUF 30504, holotype; m–u: MAFF 245613, ex-holotype culture). — Scale bars: a = 500 µm; b = 200 µm; c, o = 20 µm; d–g, p = 10 µm; h–l, q–u = 5 µm; m = 1 mm; n = 250 µm.

***Atrocalyx lignicola* (Ying Zhang, J. Fourn. & K.D. Hyde)**

A. Hashim. & Kaz. Tanaka, comb. nov. — MycoBank MB819242; Fig. 2c, 5

Basionym. *Lophiotrema lignicola* Ying Zhang, J. Fourn. & K.D. Hyde, Fung. Diversity 38: 238. 2009.

Saprobic on dead twigs of *Populus* sp.

Sexual morph: Ascocarps ellipsoidal, 330–380 µm high, 350–600 µm long, 230–400 µm wide, solitary to 4–5-grouped, semi-immersed. Ostiolar neck crest-like, elongated and laterally compressed, surrounded by dark brown hyphae. Peridium 27.5–42 µm thick at side, composed of 2 zones; outer zone 15–20 µm thick, composed of elongated, thin-walled, 10–12.5 ×

3.5–5 µm, brown cells; inner zone 15–22 µm thick, composed of globose to rectangular, 5–6.5 µm diam, hyaline cells; near the ostiole 57–62.5 µm thick, composed of dark brown cells; at base 35–46 µm thick, composed of globose to rectangular, 2.5–4(–6) µm diam, brown to black cells. Pseudoparaphyses numerous, 1–1.5 µm wide, septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 100–146 × 12.5–17 µm (av. = 125.2 × 13.4 µm, n = 11), with a short stipe (4–18 µm long, av. = 8.4 µm, n = 11), apically rounded with an ocular chamber, 8-spored. Ascospores broadly fusiform with rounded ends, 20–26 × 6.5–9.5 µm (av. = 22.3 × 7.4 µm, n = 50), hyaline, with a septum nearly median (0.45–0.54, av. = 0.50, n = 50), slightly constricted at the septum, smooth, with an

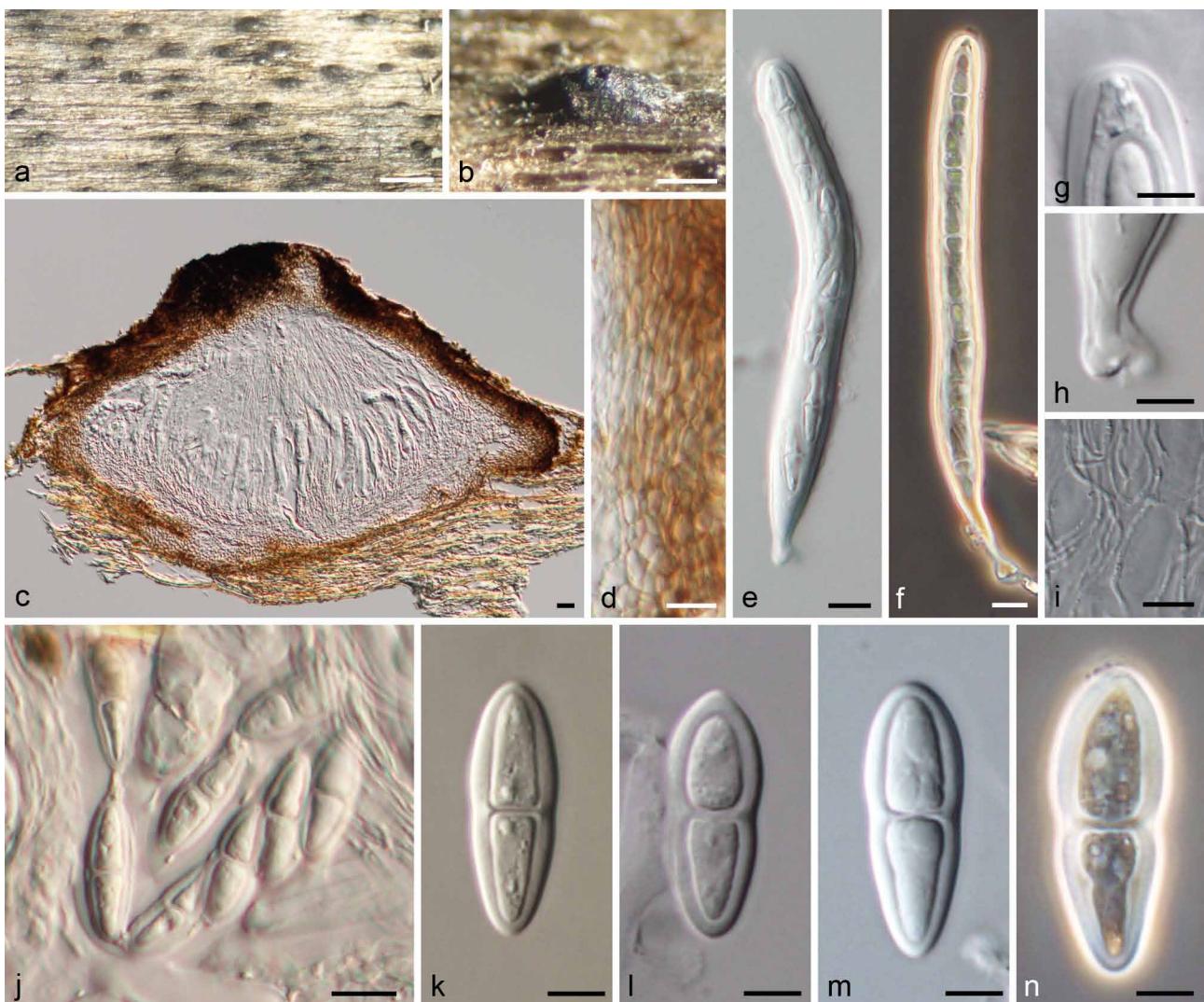


Fig. 5 *Atrocalyx lignicola*. a–b. Appearance of ascocarps on substrate; c. ascocarp in longitudinal section; d. peridium of ascocarp; e–f. ascospores; g. ascus apex; h. ascus stipe; i. pseudoparaphyses; j–n. ascospores (all: CBS H-20221, holotype). — Scale bars: a = 1 mm; b = 250 µm; c = 20 µm; d–f, i = 10 µm; g, h, j–n = 5 µm.

entire gelatinous sheath up to 2 µm thick. Senescent ascospores 3-septate, yellowish.

Asexual morph: Undetermined.

Culture characteristics — Colonies on PDA attaining 23–26 mm diam within 21 d at 20 °C in the dark, velvety, plane, smoke grey (105); reverse olivaceous black (108) (Fig. 2c); no sporulation observed.

Specimen examined. BELGIUM, Hainaut, Orval, ruisseau de Williers, on decorticated trunk of *Populus* sp., 29 Sept. 2006, J. Fournier (CBS H-20221 holotype, ex-holotype living culture CBS 122364).

Notes — *Atrocalyx lignicola* was first described as a species of *Lophiotrema* on the basis of its 1-septate, hyaline, strongly constricted ascospores (Zhang et al. 2009). According to our morphological observations of the holotype specimen, however, this species is not typical for the genus *Lophiotrema*; in particular, *A. lignicola* possesses ascocarps with well-developed peridium (Fig. 5c).

***Crassimassarina* A. Hashim. & Kaz. Tanaka, gen. nov.** — MycoBank MB819243

Etymology. After its morphological similarity to *Massarina*, but with well-developed ascocarpal wall.

Type species. *Crassimassarina macrospora* A. Hashim. & Kaz. Tanaka.

Saprobic on dead twigs of woody plants.

Sexual morph: *Ascomata* solitary to grouped, immersed to erumpent, subglobose. *Ostiolar neck* papillate, without slit-like ostiole, composed of carbonaceous, thick-walled, black cells. *Peridium* composed of rectangular, thin-walled cells. *Pseudoparaphyses* trabeculate, septate, branched and anastomosed. *Ascospores* broadly fusiform with rounded ends, straight, 1-septate, hyaline, smooth.

Asexual morph: *Conidiomata* pycnidial, globose to subglobose, scattered, semi-immersed, solitary, black, ostiolate. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, cylindrical, hyaline. *Conidia* cylindrical with rounded ends, hyaline, smooth, multi-septate.

***Crassimassarina macrospora* A. Hashim. & Kaz. Tanaka, sp. nov.** — MycoBank MB819244; Fig. 2d–e, 6

Etymology. Referring to the large ascospores.

Saprobic on dead twigs of *Cornus controversa*.

Sexual morph: *Ascomata* solitary to 4–5-grouped, immersed, erumpent at the apex, subglobose in section, 450–620 µm high, 380–700 µm diam. *Ostiolar neck* papillate, without slit-like ostiole, composed of carbonaceous, thick-walled, black cells. *Peridium* 25–60 µm thick, composed of 6–8 layers of rectangular, thin-walled, 5–12.5 µm diam cells, surrounded by brown

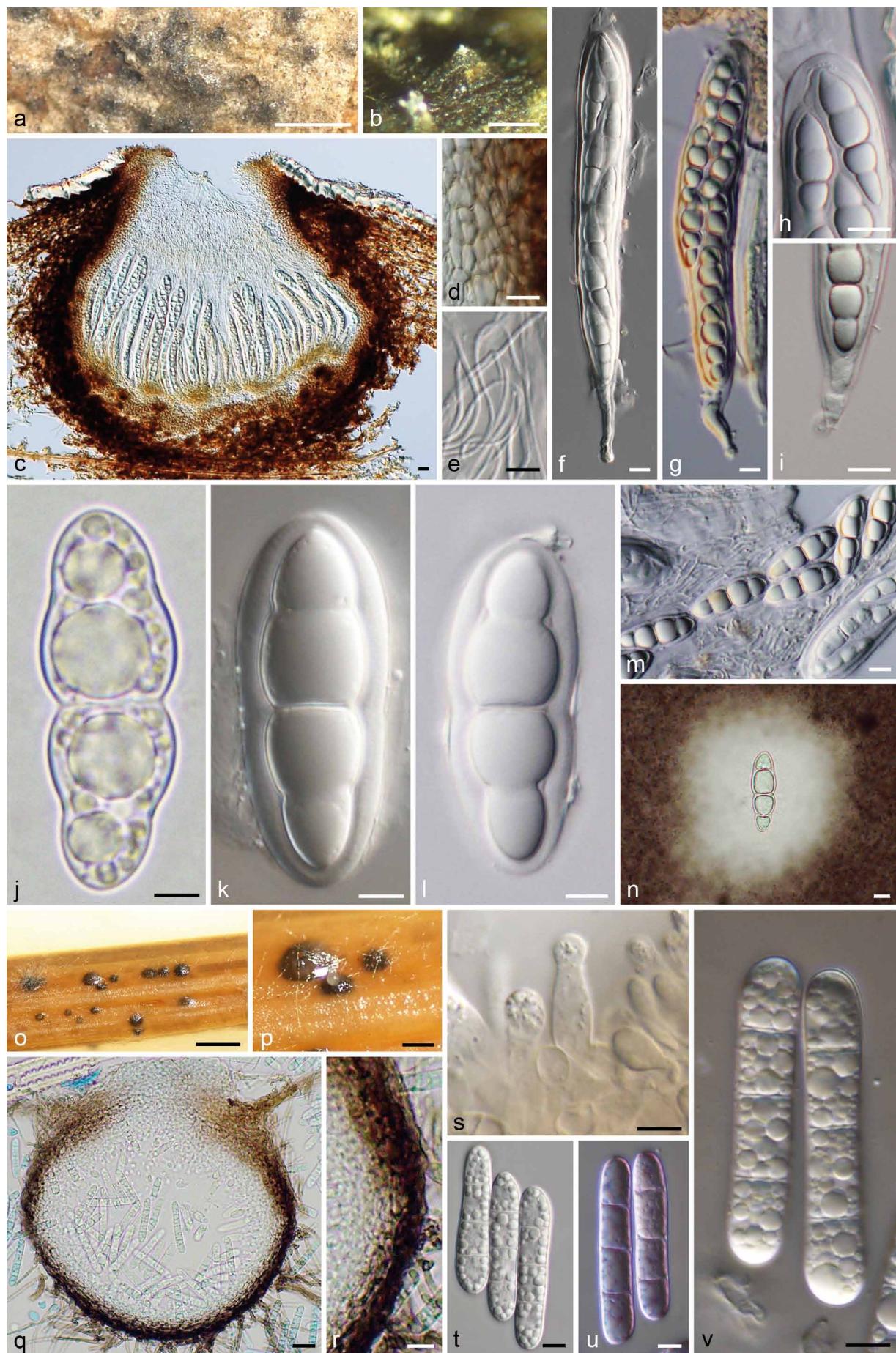


Fig. 6 *Crassimassarina macrospora*. a–b. Appearance of ascocarps on substrate; c. ascocarp in longitudinal section; d. peridium of ascocarp; e. pseudoparaphyses; f–g. ascii; h. ascus apex; i. ascus stipe; j–m. ascospores; n. ascospore with a gelatinous sheath (in India ink); o–p. conidiomata in culture; q. conidioma in longitudinal section; r. peridium of conidioma; s. conidigenous cells; t–v. conidia (u in Trypan Blue) (a, c–f, j–k, n: HHUF 29084, holotype; b, g–i, l–m: HHUF 30512, paratype; o–s, u–v: JCM 13096 = MAFF 239606, ex-holotype culture; t: MAFF 245617, ex-paratype culture). — Scale bars: a, o = 1 mm; b, p = 250 µm; c, q = 20 µm; d–g, m–n, r = 10 µm; h–l, s–v = 5 µm.

hyphae (2–3 µm thick). *Pseudoparaphyses* numerous, trabeculate, 1–1.5 µm wide, septate, branched and anastomosed. *Asci* numerous, bitunicate, fissitunicate, cylindrical, 165–200 × 25–33 µm (av. = 183.5 × 27.9 µm, n = 7), with a short stipe (7.5–26 µm long, av. = 16.8 µm, n = 7), apically rounded with an ocular chamber, 8-spored. *Ascospores* broadly fusiform with rounded ends, straight, (29.5–)33–42 × 9–15 µm (av. = 37.8 × 13.0 µm, n = 55), l/w 2.1–4.0 (av. = 2.9, n = 55), 1(–3)-septate, with a submedian primary septum (0.51–0.58(–0.71), av. = 0.53, n = 55), strongly constricted at the septum and midpoints of each cell, hyaline, smooth, guttulate when young, with an entire gelatinous sheath (1–3 µm wide at sides).

Asexual morph: *Conidiomata* pycnidial, globose to subglobose, up to 240 µm high, 190–250 µm diam, scattered, semi-immersed, solitary, black, with a papillate ostiolar neck. *Peridium* 15–20 µm thick, composed of 4–6 layers of 4–7 µm diam, subglobose to rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, 6–14 × 3–5 µm, cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, (32–)36–55(–58) × 6–8 µm (av. = 44.1 × 7.2 µm, n = 50), l/w 4.7–8.1 (av. = 6.2, n = 50), hyaline, smooth, 3(–7)-septate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 36–40 mm diam within 21 d, velvety, plane, smoke grey (105), grey olivaceous (107) at margin; reverse olivaceous black (108) (Fig. 2d–e); asexual morph formed.

Specimens examined. JAPAN, Ibaraki, Tsukuba, Amakubo, Tsukuba botanical garden, on dead twigs of *Cornus controversa*, 20 Nov. 2004, Y. Ooki, KT 1764 (HHUF 29084 holotype designated here, ex-holotype living culture JCM 13096 = MAFF 239606); ibid., KT 1765 (HHUF 29085 paratype); Aomori, Minamitsugaru, Owani, on dead twigs of woody plant, 28 June 2008, K. Hirayama & K. Tanaka, KH 152 (HHUF 30512 paratype, ex-paratype living culture MAFF 245617).

Notes — This genus is morphologically similar to *Massarina* and genera in *Pleomassariaceae* s.l. (Barr 1982, Tanaka et al. 2005, 2015) in having large, immersed ascomata with a short papillate ostiolar neck and relatively large ascospores. However, *Crassimassarina* is different from *Massarina* in having a well-developed ascosomal wall; it differs from pleomassariaceous genera in having trabeculate pseudoparaphyses and hyaline ascospores. The asexual morph of *Crassimassarina* resembles that of *Stagonospora* in regards to its pycnidial conidiomata, conidiophores with reduced conidiogenous cells, and multi-septate cylindrical conidia; however, the latter genus differs from *Crassimassarina* in having phialidic conidiogenous cells (Quaedvlieg et al. 2013, Tanaka et al. 2015). *Massarina* and *Stagonospora* (*Pleosporales*, *Massarinaceae*) are phylogenetically distinct lineages from *Lophiotremataceae* containing *Crassimassarina*.

Crassimassarina can be distinguished from other genera in *Lophiotremataceae* by its ascomata lacking a slit-like ostiole, an ascosomal peridium composed of carbonaceous cells (Fig. 6c), and multi-septate, large conidia (Fig. 6t–v).

ITS sequences of the two examined isolates of *C. macrospora* differed at only two positions, with two gaps. Morphological features and culture characteristics of these isolates were completely identical (Fig. 2d–e, 6k–l).

Cryptoclypeus A. Hashim. & Kaz. Tanaka, gen. nov. — MycoBank MB819245

Etymology. Referring to the ascomata covered by a less-developed clypeus.

Type species. *Cryptoclypeus ryukyuensis* A. Hashim. & Kaz. Tanaka.

Saprobic on dead twigs of bamboo.

Sexual morph: *Ascomata* solitary to grouped, immersed. *Ostiolar neck* crest-like, elongated, laterally compressed, with a

slit-like ostiole and less-developed clypeus. *Peridium* composed of rectangular, thin-walled, pale brown cells. *Pseudoparaphyses* septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* fusiform, multi-septate, hyaline, smooth.

Asexual morph: *Conidiomata* pycnidial, globose to subglobose, grouped, immersed, ostiolate. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* absent. *Conidiogenous cells* phialidic, ampliform to cylindrical. *Conidia* cylindrical with slightly angular ends, hyaline, smooth, 1-septate.

Notes — Two species of *Cryptoclypeus* share common characteristics, e.g., ascomata with a less-developed clypeus (up to 450 µm wide), and a peridium composed of rectangular cells (Fig. 7c–d, 8c–d). In the phylogenetic tree, they formed a well-supported clade in *Lophiotremataceae* (99 % ML BP/1.00 Bayesian PP) (Fig. 1).

The sexual morph of *Cryptoclypeus* is similar to that of *Tetraploa* (*Tetraplosphaeriaceae*), which also has ascomata with a clypeus and narrowly fusiform ascospores (Tanaka et al. 2009). *Cryptoclypeus*, however, can be distinguished from the latter genus by having a well-developed neck with a slit-like ostiole. The asexual morph of *Cryptoclypeus* superficially resembles that of *Bambusicola* (*Bambusicolaceae*), but is easily distinguishable from the latter by having phialidic conidiogenous cells rather than annellidic ones (Dai et al. 2012).

Cryptoclypeus oxysporus A. Hashim. & Kaz. Tanaka, sp. nov.

— MycoBank MB819246; Fig. 2f, 7

Etymology. Referring to the sharp ascospores.

Saprobic on dead culm of *Sasa* sp.

Sexual morph: *Ascomata* ellipsoidal, solitary, immersed, 245–310 µm high, 180–240 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, with less-developed clypeus (230–320 µm wide). *Peridium* uniform, 15–17.5 µm thick, composed of rectangular, thin-walled, 6.5–14 × 2.5–4.5 µm, pale brown cells. *Pseudoparaphyses* numerous, 0.5–1 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 71–100 × 6–9 µm (av. = 85.8 × 7.7 µm, n = 10), with a short stipe (5.5–11 µm long, av. = 7.8 µm, n = 10), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with acute ends, straight, 19–27 × 3–4.5 µm (av. = 23.3 × 3.2 µm, n = 50), l/w 5.5–8.9 (av. = 7.2, n = 50), 3-septate, slightly constricted at the primary septum nearly median (0.46–0.55, av. = 0.50, n = 50), hyaline, smooth.

Asexual morph: Undetermined.

Culture characteristics — Colonies on PDA attaining 24–28 mm diam within 21 d at 20 °C in the dark, velvety, radiately, centrally raised, smoke grey (105) to grey olivaceous (107); reverse olivaceous black (108) (Fig. 2f); no sporulation observed.

Specimen examined. JAPAN, Iwate, Hanamaki, near Dai spa, on dead culm of *Sasa* sp., 25 June 2011, K. Tanaka, KT 2772 (HHUF 30507 holotype designated here, ex-holotype living culture MAFF 245614 = NBRC 112317).

Notes — This species can be distinguished from *C. ryukyuensis* by its slightly larger ascospores with acute ends (Fig. 7k–n) (19–27 × 3–4.5 µm vs 15–24 × 3–4.5 µm, with rounded ends in the latter species; Fig. 8k–m). ITS sequences between these two species differed at 17–18 positions, with eight gaps.

Cryptoclypeus ryukyuensis A. Hashim. & Kaz. Tanaka, sp. nov. — MycoBank MB819247; Fig. 2g–h, 8

Etymology. Referring to the collection site.

Saprobic on dead twigs of *Pleioblastus linearis*.

Sexual morph: *Ascomata* ellipsoidal, solitary to 3–5-grouped, immersed, 220–240 µm high, 330–380 µm diam. *Ostiolar neck*

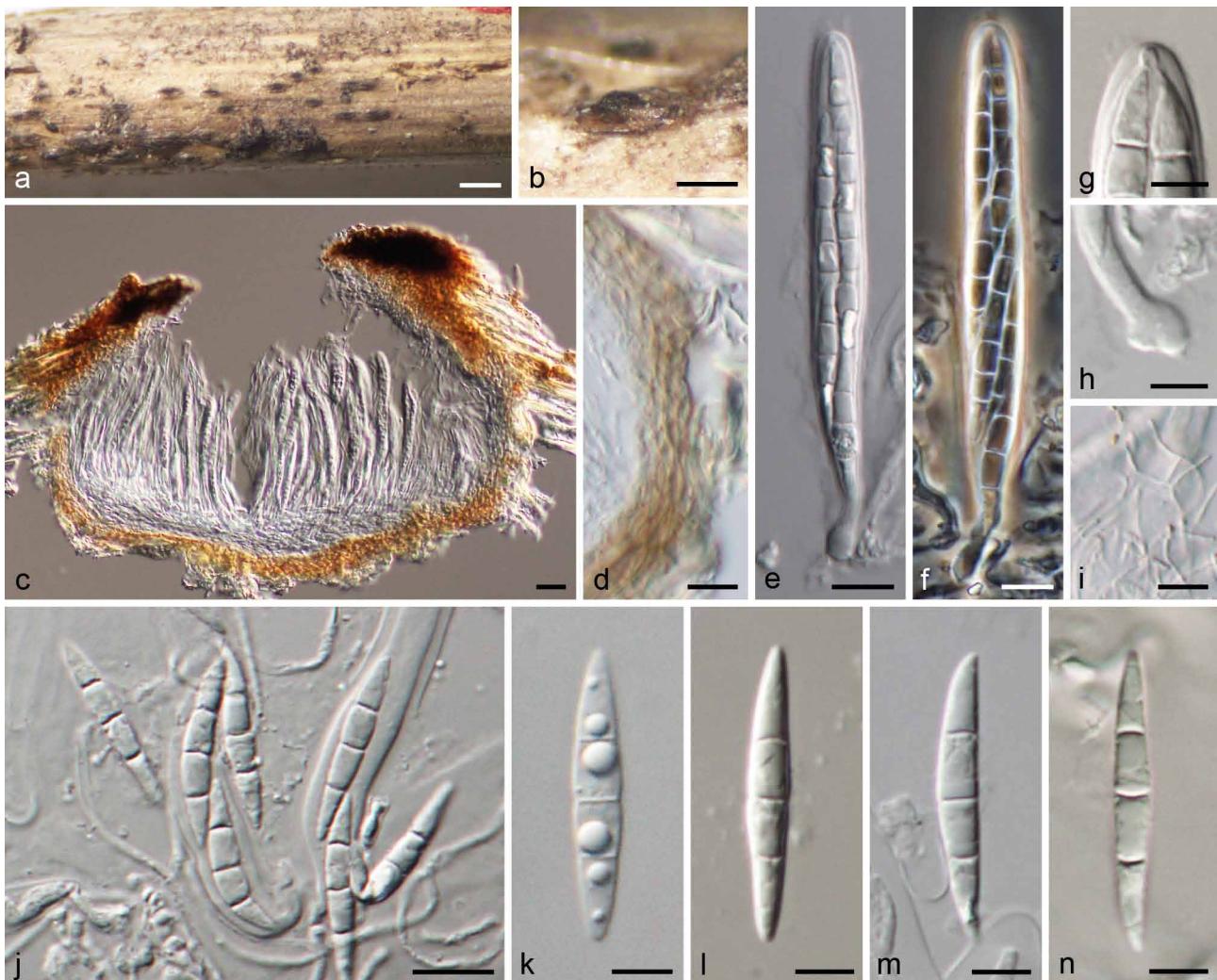


Fig. 7 *Cryptoclypeus oxysporus*. a–b. Appearance of ascomata on substrate; c. ascoma in longitudinal section; d. peridium of ascoma; e–f. ascii; g. ascus apex; h. ascus stipe; i. pseudoparaphyses; j–n. ascospores (all: HHUF 30507, holotype). — Scale bars: a = 1 mm; b = 250 µm; c = 20 µm; d–f, i = 10 µm; g–h, j–n = 5 µm.

crest-like, elongated, laterally compressed, with less-developed black clypeus (380–450 µm wide). Peridium uniform, 22.5–25 µm thick at side, composed of rectangular, thin-walled, 10–22.5 × 5–6.5 µm, pale brown cells. Pseudoparaphyses numerous, 1–1.5 µm wide, septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical, 72.5–100.5 × 6–10 µm (av. = 87.0 × 7.8 µm, n = 20), with a short stipe (4–9 µm long, av. = 6.5 µm, n = 20), apically rounded with an ocular chamber, 8-spored. Ascospores fusiform with rounded ends, straight, 15–24 × 3–4.5 µm (av. = 19.7 × 3.8 µm, n = 50), l/w (3.8–)4.3–6.6 (av. = 5.2, n = 50), 3-septate, slightly constricted at the primary septum nearly median (0.45–0.53, av. = 0.50, n = 50), hyaline, smooth, guttulate when young.

Asexual morph: Conidiomata pycnidial, globose to subglobose, up to 230 µm high, 230–340 µm diam, 3–5-grouped, immersed. Ostiolar neck up to 50 µm high, carbonaceous, papillate. Peridium 17.5–27.5 µm thick, composed of 7–10 layers of 4–8 × 2.5–6.5 µm, subglobose to rectangular, brown cells. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, 7–12 × 2–3 µm, ampliform to cylindrical, hyaline, smooth. Conidia cylindrical with slightly angular ends, 11.5–15 × 2–2.5 µm (av. = 12.9 × 2.1 µm, n = 50), l/w 5.1–7.0(–8.3) (av. = 6.3, n = 50), hyaline, smooth, 1-septate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 28–30 mm diam within 21 d at 20 °C in the dark, velvety, radiately, centrally raised, smoke grey (105) to grey olivaceous (107); reverse greenish black (124) (Fig. 2g–h); no sporulation observed.

Specimens examined. JAPAN, Okinawa, Kunigami-son, Okuma, Mt Yonaha, on dead culm of *Pleioblastus linearis*, 18 May 2015, A. Hashimoto et al., AH 342 (HHUF 30510 paratype, ex-paratype living culture MAFF 245616); Yona, Mt Fuenchiji, on dead culm of *Pleioblastus linearis*, 19 May 2015, K. Tanaka et al., KT 3534 (HHUF 30509 holotype designated here, ex-holotype living culture MAFF 245615 = NBRC 112318).

Notes — Ex-holotype and ex-paratype isolates of *C. ryukyuensis* are derived from sexual and asexual morphs, respectively. Unfortunately, a connection between sexual and asexual forms of this species could not be confirmed in culture. Nonetheless, *rpb2* sequences were completely identical between the two strains, while *ITS* sequences differed at only one position and *tef1* sequences differed at two, neither of which caused an amino acid substitution. Culture characteristics were also identical (Fig. 2g–h). We therefore regard these isolates as conspecific.

Galeaticarpa A. Hashim. & Kaz. Tanaka, *gen. nov.* — MycoBank MB819248

Etymology. From the Latin *galea*, meaning helmet, and *carpa*, meaning fruiting body.

Type species. *Galeaticarpa aomoriensis* A. Hashim. & Kaz. Tanaka.

Saprobic on woody plants.

Sexual morph: Ascomata solitary to grouped, immersed to erumpent, subglobose. Ostiolar neck elongated, laterally compressed, surrounded by well-developed clypeus. Peridium

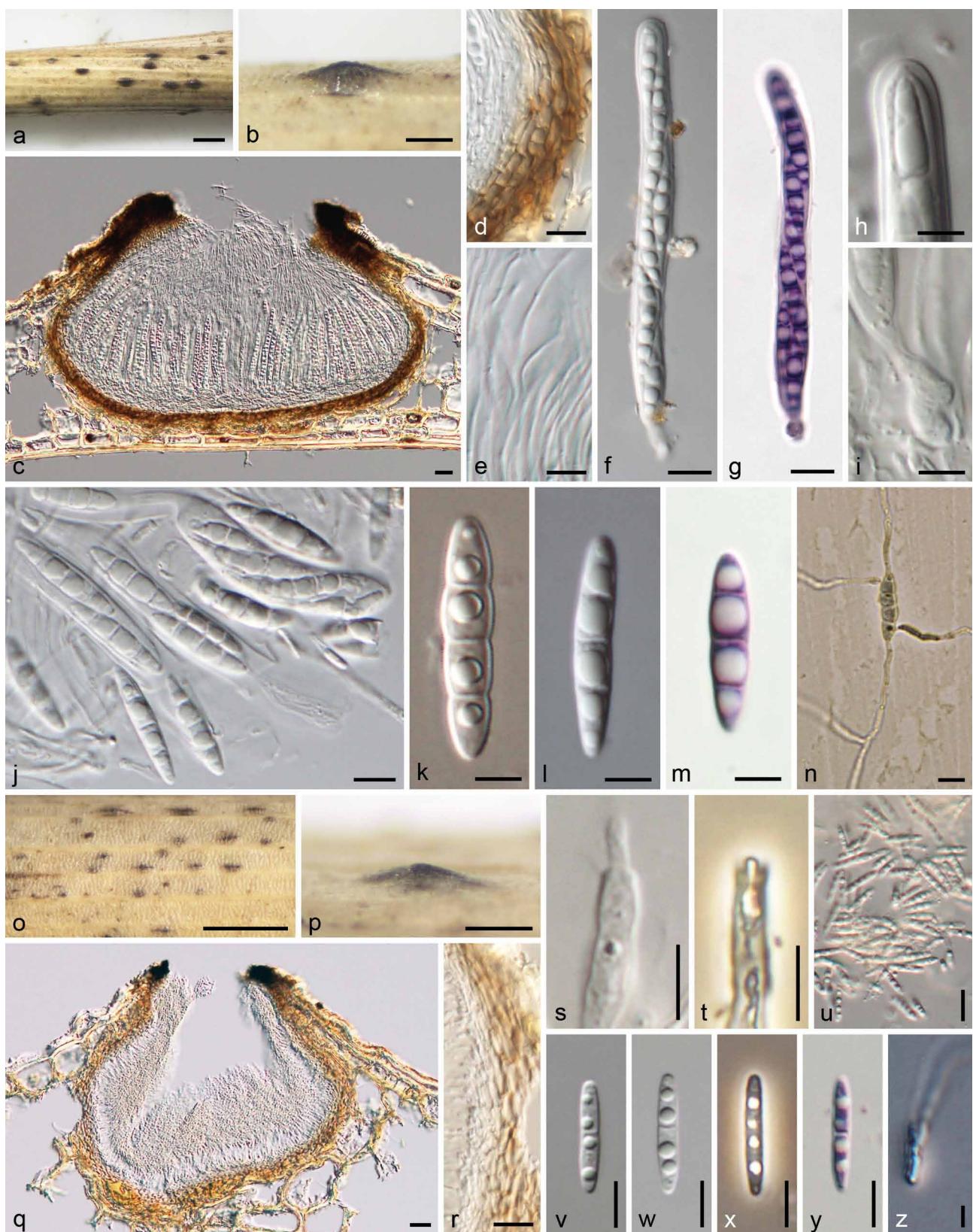


Fig. 8 *Cryptoclypeus ryukyuensis*. a–b. Appearance of ascocarps on substrate; c. ascocarp in longitudinal section; d. peridium of ascocarp; e. pseudoparaphyses; f–g. asci (g in Trypan Blue); h. ascus apex; i. ascus stipe; j–m. ascospores (m in Trypan Blue); n. germinating ascospore; o–p. conidiomata on substrate; q. conidioma in longitudinal section; r. peridium of conidioma; s–t. conidiogenous cells; u–y. conidia (y in Trypan Blue); z. germinating conidium (a–n: HHUF 30509, holotype; o–z: HHUF 30510, paratype). — Scale bars: a, o = 1 mm; b, p = 250 µm; c, q = 20 µm; d–g, j, n, r, u = 10 µm; h–i, k–m, s–t, v–z = 5 µm.

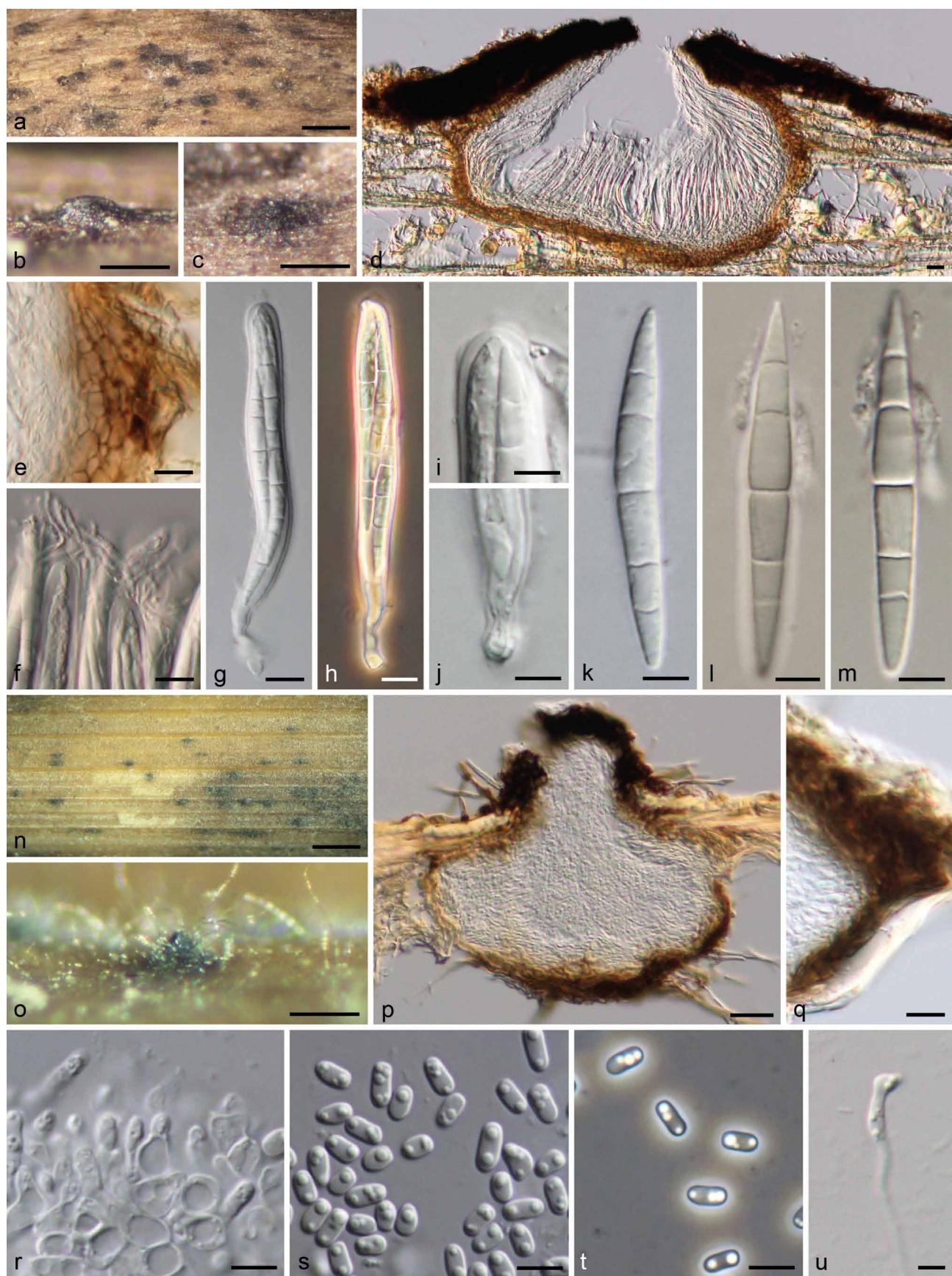


Fig. 9 *Galeaticarpa aomoriensis*. a–c. Appearance of ascomata on substrate; d. ascoma in longitudinal section; e. peridium of ascoma; f. pseudoparaphyses; g–h. asci; i. ascus apex; j. ascus stipe; k–m. ascospores; n–o. conidiomata in culture; p. conidioma in longitudinal section; q. peridium of conidioma; r. conidiogenous cells; s–t. conidia; u. germinating conidium (a–m: HHUF 30505, holotype; n–u: MAFF 245618 = NBRC 112319, ex-holotype culture). — Scale bars: a, n = 1 mm; b–c, o = 250 µm; d, p = 20 µm; e–h, q = 10 µm; i–m, r–u = 5 µm.

composed of rectangular, brown cells. *Pseudoparaphyses* septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores fusiform, multi-septate, hyaline, smooth.

Asexual morph: Conidiomata pycnidial, globose to subglobose, grouped, immersed. Ostiolar neck carbonaceous, papillate. Peridium composed of rectangular, pale brown to brown cells. Conidiophores absent. Conidiogenous cells holoblastic, cylindrical, hyaline, smooth. Conidia ellipsoidal with rounded ends, hyaline, smooth, aseptate.

***Galeaticarpa aomoriensis* A. Hashim. & Kaz. Tanaka, sp. nov.** — MycoBank MB819249; Fig. 2i, 9

Etymology. Referring to the collection site.

Saprobic on dead twigs of woody plants.

Sexual morph: Ascomata ellipsoidal, solitary to 4–5-grouped, immersed to erumpent, subglobose, 265–285 µm high, 370–400 µm diam. Ostiolar neck crest-like, elongated, laterally compressed, surrounded by well-developed clypeus (620–750 µm wide). Peridium uniform, 15–26 µm thick, composed of 3–5 layers of rectangular, thin-walled, 7.5 × 3–6.5 µm, brown cells. *Pseudoparaphyses* numerous, 1–1.5 µm wide, septate, branched and anastomosed. Asci numerous, bitunicate, fissitunicate, cylindrical, 78–102 × 8.5–11 µm (av. = 93.5 × 9.7 µm, n = 11), with a short stipe (4–11 µm long, av. = 7.2 µm, n = 11), apically rounded with an ocular chamber, 8-spored. Ascospores fusiform with acute ends, straight, 31–47 × 4–5.5 µm (av. = 40.6 × 4.7 µm, n = 50), l/w (5.8–)7.2–10.5 (av. = 8.7, n = 50), 5-septate, with a primary septum nearly median (0.46–0.57, av. = 0.51, n = 50), slightly constricted at the primary septum, hyaline, smooth.

Asexual morph: Conidiomata pycnidial, flask-shaped, up to 135 µm high in section, 210–250(–420) µm diam, 3–5-grouped, semi-immersed. Ostiolar neck 32–50 µm high, carbonaceous, papillate. Peridium 10–12.5 µm wide, composed of 2–3 layers of 10–12 × 3–4 µm, rectangular, pale brown to brown cells. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, 5.5–11 × 2.5–3 µm, cylindrical, hyaline, smooth. Conidia ellipsoidal with rounded ends, 3.5–6 × 1.8–2.2 µm (av. = 4.5 × 2.0 µm, n = 50), l/w 1.8–3.0 (av. = 2.2, n = 50), hyaline, smooth, aseptate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 17–19 mm diam within 21 d at 20 °C in the dark, velvety, plane, smoke grey (105); reverse chestnut (40), brown vinaceous (84) pigment produced (Fig. 2i); asexual morph formed.

Specimen examined. JAPAN, Aomori, Nishimeya, Shirakami, Ooshirosawa stream, on dead twigs of dead woody plant, 30 Aug. 2008, K. Tanaka et al., KT 2563 (HHUF 30505 holotype designated here, ex-holotype living culture MAFF 245618 = NBRC 112319).

Notes — A new monotypic genus, *Galeaticarpa*, is proposed here for species having ascomata with a clypeus, cylindrical asci with a short stipe, and fusiform, multi-septate, hyaline ascospores. These morphological characters are similar to those of *Astrophaeriella*, but *Galeaticarpa* can be distinguished from *Astrophaeriella* by its crest-like ostiolar neck and uniformly developed peridium (vs a poorly developed peridium at the base in *Astrophaeriella*; Chen & Hsieh 2004, Phookamsak et al. 2015). These two genera are distantly related and belong to the families Lophiotremataceae and Astrophaeriellaceae, respectively.

Cryptoclypeus, *Galeaticarpa*, and *Pseudocryptoclypeus* are morphologically similar to one another in having ascomata with a clypeus, but *Galeaticarpa* can be distinguished from these other genera by its most striking features — a well-developed clypeus (up to 750 µm wide) (Fig. 9a–d) and flask-shaped

conidiomata (Fig. 9p). In addition to phenotypic differences, *Cryptoclypeus* and *Pseudocryptoclypeus* tend to occur exclusively on bamboos, while *Galeaticarpa* occurs on woody plant hosts. Several bambusicolous fungi have been reported to be phylogenetically unrelated to genera and species on other host plants, even though they have morphological similarities with those groups (Tanaka et al. 2009, Hashimoto et al. 2015b). Further discovery of new lineages related to these genera is needed to clarify the relationship between their evolution and host preference.

***Pseudocryptoclypeus* A. Hashim. & Kaz. Tanaka, gen. nov.**

— MycoBank MB819250

Etymology. After its morphological similarity to *Cryptoclypeus*.

Type species. *Pseudocryptoclypeus yakushimensis* A. Hashim. & Kaz. Tanaka.

Saprobic on bamboo.

Sexual morph: Ascomata scattered to grouped, immersed. Ostiolar neck crest-like, elongated, laterally compressed, with less-developed clypeus. Peridium composed of compressed, rectangular, thin-walled, brown cells. *Pseudoparaphyses* septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores fusiform with acute ends, multi-septate, hyaline, smooth.

Asexual morph: Conidiomata pycnidial, globose to subglobose, grouped, immersed, ostiolate. Peridium composed of rectangular, pale brown cells. Conidiophores absent. Conidiogenous cells holoblastic, ampliform to cylindrical, hyaline. Conidia cylindrical with rounded ends, hyaline, smooth, 1-septate.

***Pseudocryptoclypeus yakushimensis* A. Hashim. & Kaz.**

Tanaka, sp. nov. — MycoBank MB819251; Fig. 2j, 10

Etymology. Referring to the collection site.

Saprobic on dead culms of bamboo.

Sexual morph: Ascomata ellipsoidal, 4–5-grouped, immersed, 325–380 µm high, 250–260 µm diam. Ostiolar neck crest-like, elongated, laterally compressed, with less-developed clypeus (200–315 µm wide). Peridium 17.5–25 µm thick of 2 zones at side; outer zone 10–17.5 µm thick of compressed, thin-walled, 12–17 × 1.5–4.2 µm, brown cells; inner zone of 7.5–10 µm thick, rectangular, 5–9 × 4–6 µm, pale brown cells. *Pseudoparaphyses* 0.5–1 µm wide, septate, branched, anastomosed. Asci bitunicate, fissitunicate, cylindrical, 92.5–127 × 7.5–10 µm (av. = 104.8 × 8.2 µm, n = 15), with a short stipe (5–15 µm long, av. = 8.2 µm, n = 15), apically rounded with an ocular chamber, 8-spored. Ascospores fusiform with acute ends, straight, 20–32.5 × 3–5 µm (av. = 25.4 × 4.0 µm, n = 50), l/w 4.7–8.1 (av. = 6.4, n = 50), 5-septate, with a primary septum nearly median (0.44–0.57, av. = 0.50, n = 50), slightly constricted at the primary septum, hyaline, smooth, with gelatinous pad at each end.

Asexual morph: Conidiomata pycnidial, globose to subglobose, up to 375 µm high, 400–480 µm diam, 5–6-grouped, immersed. Ostiolar neck carbonaceous, papillate, 47.5–77.5 µm high. Peridium 10–15 µm thick, composed of 3–4 layers of 7.5–10 × 0.8–1.0 µm, rectangular, pale brown cells. Conidiophores absent. Conidiogenous cells holoblastic, 12–16 × 2.5–3 µm, ampliform to cylindrical, hyaline, smooth. Conidia cylindrical with rounded ends, 9–14 × 2.5–3.5 µm (av. = 12.0 × 3.0 µm, n = 50), l/w 3.3–4.8 (av. = 4.0, n = 50), hyaline, smooth, 1-septate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 32–52 mm diam within 21 d at 20 °C in the dark, floccose, plane, smoke grey (105); reverse grey olivaceous (107) to olivaceous black (108) (Fig. 2j); asexual morph formed.

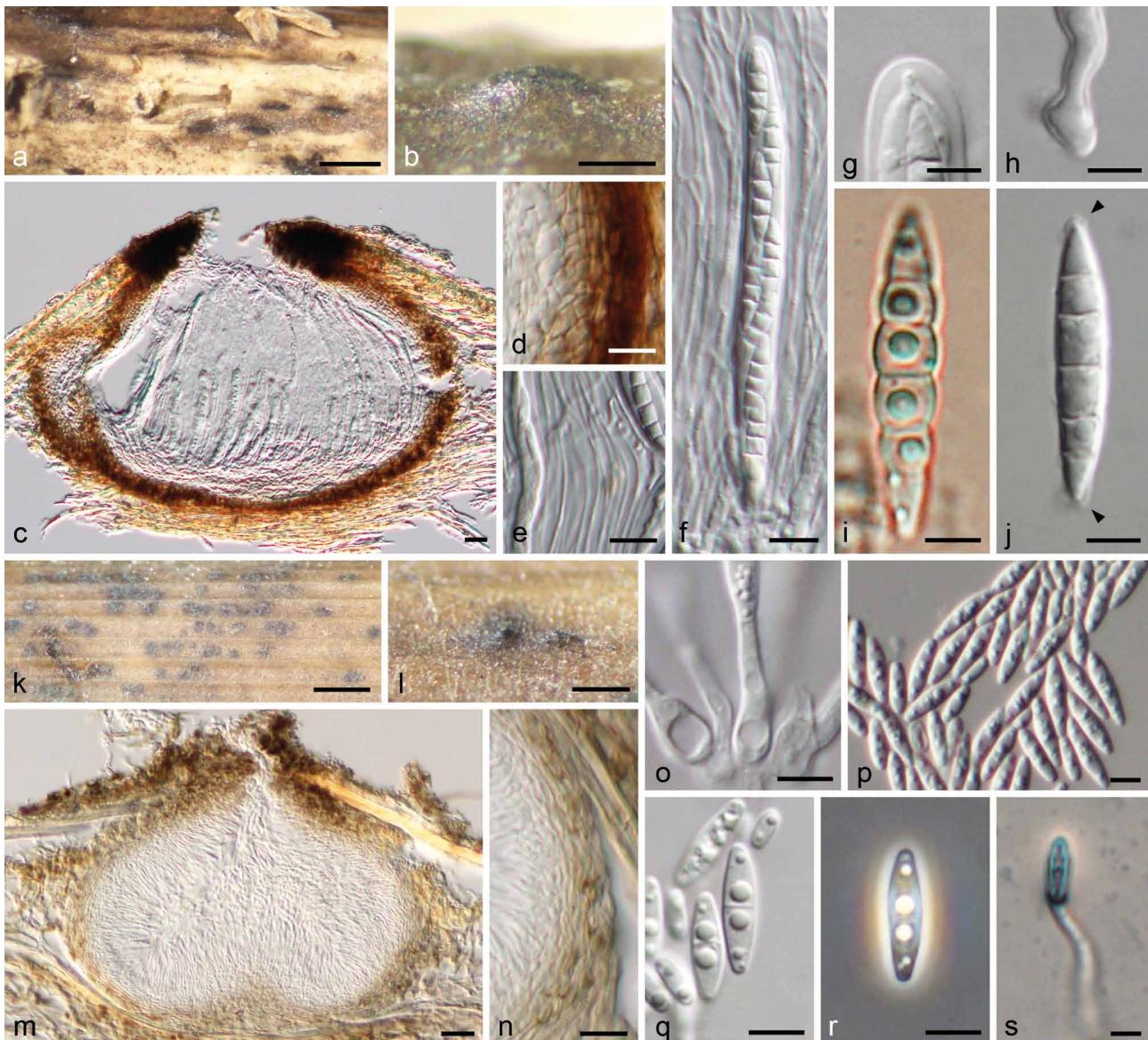


Fig. 10 *Pseudocryptoclypeus yakushimensis*. a–b. Appearance of ascocarps on substrate; c. ascocarp in longitudinal section; d. peridium of ascocarp; e. pseudoparaphyses; f. ascus; g. ascus apex; h. ascus stipe; i–j. ascospores (j arrowheads indicate gelatinous pad); k–l. conidiomata in culture; m. conidioma in longitudinal section; n. peridium of conidioma; o. conidiogenous cells; p–r. conidia; s. germinating conidium (a–j: HHUF 30503, holotype; k–s: MAFF 245622 = NBRC 112320, ex-holotype culture). — Scale bars: a, k = 1 mm; b = 200 µm; c, m = 20 µm; d–f, n = 10 µm; g–j, o–s = 5 µm; l = 250 µm.

Specimen examined. JAPAN, Kagoshima, Isl. Yakushima, Nagata, on dead culms of bamboo, 16 Mar. 2007, K. Tanaka & H. Yonezawa, KT 2186 (HHUF 30503 holotype designated here, ex-holotype living culture MAFF 245622 = NBRC 112320).

Notes — *Pseudocryptoclypeus* is similar to *Cryptoclypeus* in having a less-developed clypeus in ascocarps, multi-septate ascospores, pycnidial conidiomata, and 1-septate, hyaline conidia. *Pseudocryptoclypeus* has an ascocarpal peridium composed of 2 zones and holoblastic conidiogenous cells (Fig. 10d, o) rather than the ascocarpal wall with 1 zone and phialidic conidiogenous cells of *Cryptoclypeus* (Fig. 8d, s–t). Although these two genera constituted a highly supported clade (90 % ML BP/1.00 Bayesian PP) in our phylogenetic tree (Fig. 1), they had 43–45 base differences with 61–63 gaps in their ITS regions.

INCERTAE SEDIS

Antealophiotrema A. Hashim. & Kaz. Tanaka, gen. nov. — MycoBank MB819253; Fig. 2k, 11

Etymology. Formerly belonging to *Lophiotrema*.

Type species. *Antealophiotrema brunneosporum* (Ying Zhang, J. Fourn. & K.D. Hyde) A. Hashim. & Kaz. Tanaka.

Saprobic on woody plants.

Sexual morph: Ascomata subglobose to depressed ellipsoidal. Ostiolar neck crest-like, elongated, laterally compressed. Peridium composed of globose to rectangular, hyaline to brown cells, with brown hyphae at side. Pseudoparaphyses septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical to clavate, 4–8-spored. Ascospores narrowly fusiform, 1-septate, brown, smooth.

Asexual morph: Mycelium superficial, brown. Conidiophores absent. Conidiogenous cells holoblastic, integrated, terminal, brown, truncate, solitary, acrogenous. Conidia globose to ovoid, black, muriform.

Antealophiotrema brunneosporum (Ying Zhang, J. Fourn. & K.D. Hyde) A. Hashim. & Kaz. Tanaka, comb. nov. — MycoBank MB819253; Fig. 2k, 11

Basionym. *Lophiotrema brunneosporum* Ying Zhang, J. Fourn. & K.D. Hyde, Fung. Diversity 38: 240. 2009.

Saprobic on decorticated wood of *Salix* sp.

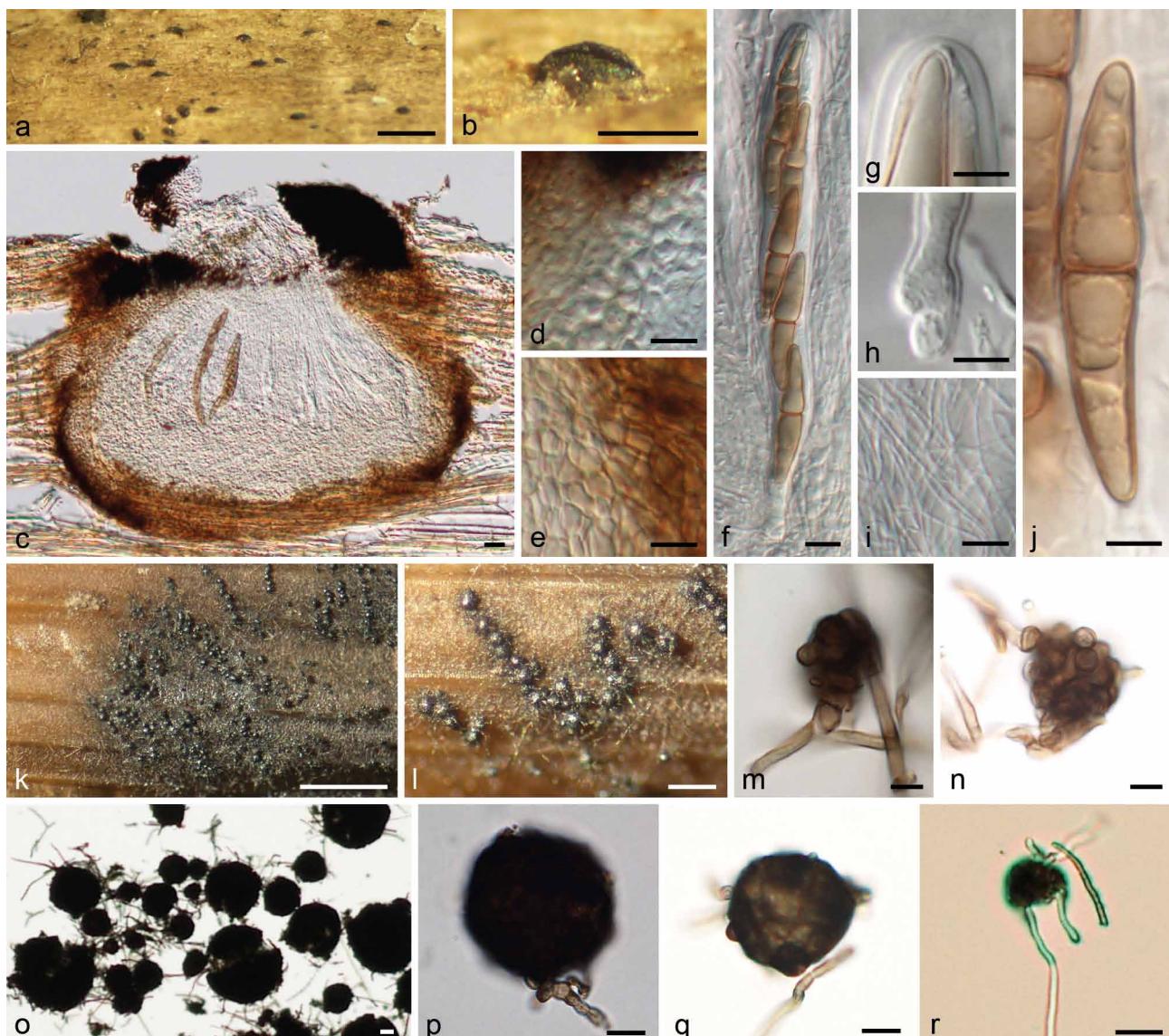


Fig. 11 *Antealophiotrema brunneosporum*. a–b. Appearance of ascocarps on substrate; c. ascocarp in longitudinal section; d. peridium of ascocarp near ostiole; e. peridium of ascocarp at side; f. ascus; g. ascus apex; h. ascus stipe; i. pseudoparaphyses; j. ascospore; k–l. conidia in culture; m–n. conidiogenous cells and immature conidia; o–q. conidia; r. germinating conidium (a–j: CBS H-20222, holotype; k–r: CBS 123095, ex-holotype culture). — Scale bars: a, k = 1 mm; b, l = 250 µm; c, o, r = 20 µm; d–f, i, p–q = 10 µm; g–h, j, m–n = 5 µm.

Sexual morph: Ascomata subglobose to depressed ellipsoidal, up to 380 µm high, 460–530 µm diam. Ostiolar neck crest-like, elongated, laterally compressed. Peridium ununiform, 42.5–62.5 µm thick, composed of 2 zones; outer zone 22.5–25 µm thick, composed of moderately thick-walled, rectangular, (5–)7–9 × 5–7.5 µm, brown cells, with brown hyphae; inner zone 20–32.5 µm thick of thin-walled, rectangular, 7–9 × 4–7.5 µm, hyaline to pale brown cells; at base 27.5–35 µm thick, of globose to rectangular, 3.5–7 × 3.5–4 µm, pale brown cells. Pseudoparaphyses numerous, 0.8–1.5 µm wide, septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical to clavate, 119–148 × 13–15 µm (av. = 138.2 × 14.3 µm, n = 8), with a short stipe (8.5–16 µm long, av. = 10.9 µm, n = 8), apically rounded with an ocular chamber, with biserrate 4–8 ascospores. Ascospores narrowly fusiform with slightly rounded ends, 34.5–48 × 6.5–10 µm (av. = 39.8 × 8.0 µm, n = 32), I/w 4.1–6.1 (av. = 5.0, n = 32), with a septum nearly median (0.44–0.54, av. = 0.49, n = 50), strongly constricted at the septum, brown, smooth.

Asexual morph: Mycelium superficial, brown. Conidiophores absent. Conidiogenous cells holoblastic, integrated, terminal,

brown, truncate, solitary, acrogenous. Conidia globose to obovoid, black, 27.5–85 µm diam (av. = 52.0 µm, n = 57), muriform.

Culture characteristics — Colonies on PDA attaining 12–17 mm diam within 21 d at 20 °C in the dark, floccose, centrally raised, grey olivaceous (107); reverse olivaceous black (108) (Fig. 2k); asexual morph formed.

Specimen examined. FRANCE, Ariège, Rimont, Las Muros, on decorticated wood of *Salix* sp., 24 Sept. 2006, J. Fournier & K.D. Hyde (CBS H-20222 holotype, ex-holotype culture CBS 123095).

Notes — The transfer of *L. brunneosporum* to *Antealophiotrema* is based on its morphological features, as it differs from *Lophiotrema* s.str. in having a well-developed peridium (up to 62.5 µm thick, Fig. 11c) as well as a monodictys-like asexual morph (Fig. 11k–r). In our phylogenetic tree, this species and '*Lophiotrema*' *boreale* (CBS 114422) formed a fully supported clade (100% ML BP/1.00 Bayesian PP) outside of *Lophiotremataceae* and are recognised as a lineage distinct from *Lophiotrema* s.str. (Fig. 1). We treat *Antealophiotrema* as 'incertae sedis' in *Pleosporales* at present; additional taxa related to this monotypic genus will be required to resolve its familial placement.

***Pseudolophiotrema* A. Hashim. & Kaz. Tanaka, gen. nov. —**

Mycobank MB819254

Etymology. After its morphological similarity to *Lophiotrema*.Type species. *Pseudolophiotrema elymicola* A. Hashim. & Kaz. Tanaka.

Saprobic on herbaceous plants.

Sexual morph: Ascomata grouped, immersed, globose. Ostiolar neck crest-like, elongated, laterally compressed. Peridium composed of compressed, thin-walled, pale brown cells. Pseudoparaphyses numerous, septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores fusiform, 1-septate, hyaline, smooth.

Asexual morph: Undetermined.***Pseudolophiotrema elymicola* A. Hashim. & Kaz. Tanaka, sp. nov. —** MycoBank MB819255; Fig. 2I, 12

Etymology. Referring to the generic name of the host plant.

Saprobic on dead leaves of *Leymus mollis*.

Sexual morph: Ascomata grouped, immersed, globose, 200–300 µm high, 190–340 µm diam. Ostiolar neck crest-like, elongated, laterally compressed. Peridium uniform, 10–12.5 µm thick composed of compressed, thin-walled, 5–12 × 2–3.5 µm, pale brown cells. Pseudoparaphyses numerous, 1.5–2 µm wide, septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical, 82–108 × 10–17 µm (av. = 92.4 × 13.9 µm, n = 11), with a short stipe (5–8 µm long, av. = 6.3 µm, n = 10), apically rounded with an ocular chamber, 8-spored. Ascospores fusiform with acute ends, straight, 20–28 × 4.5–7 µm (av. = 22.7 × 5.5 µm, n = 50), l/w 3.2–5.1 (av. = 4.2, n = 50), with a septum nearly median (0.47–0.56, av. = 0.51, n = 50), strongly constricted at the septum and midpoint of each cell, hyaline, smooth, with a gelatinous sheath.

Asexual morph: Undetermined.

Culture characteristics — Colonies on PDA attaining 18–19 mm diam within 21 d at 20 °C in the dark, velvety, plane, radiate-

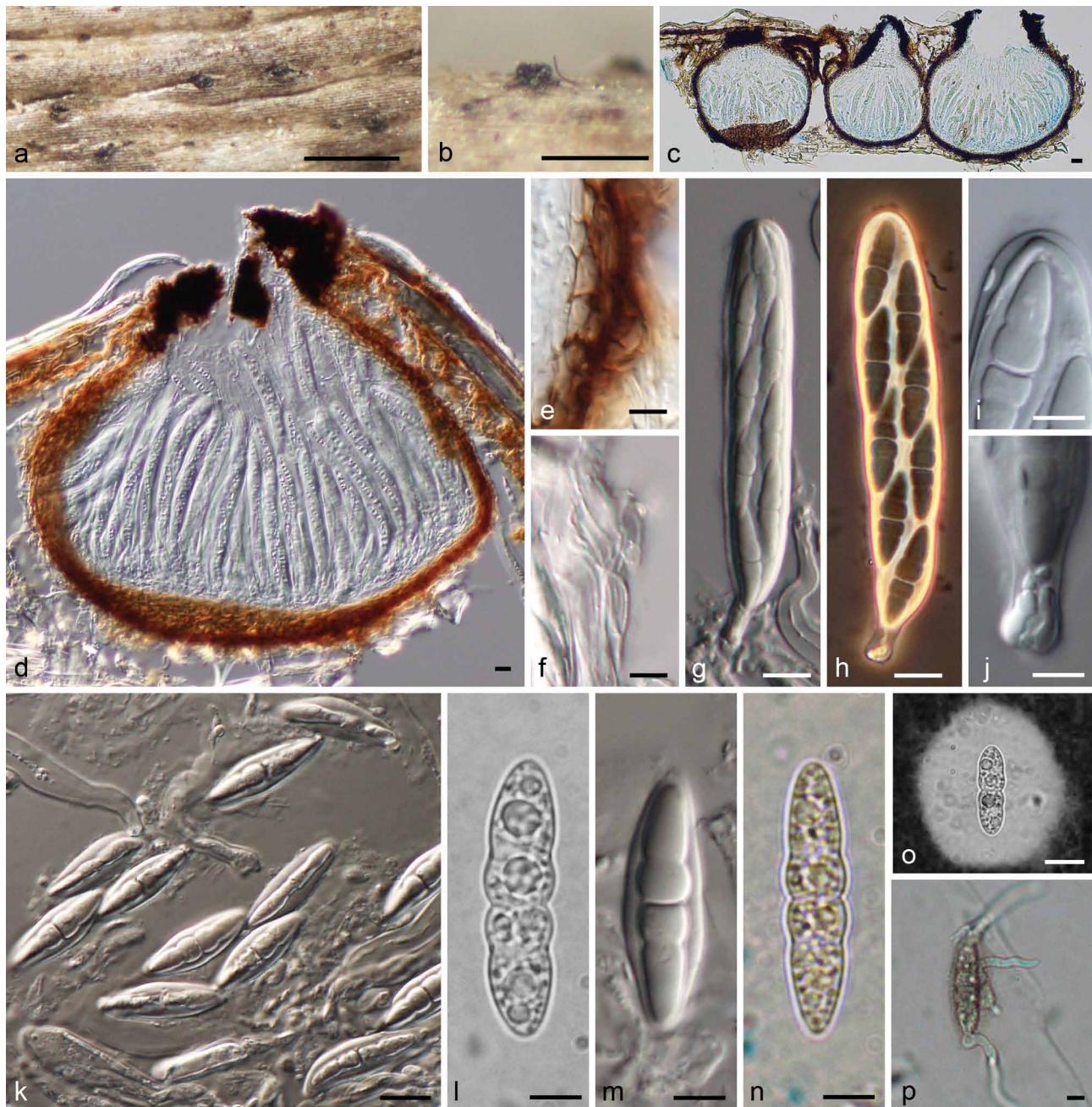


Fig. 12 *Pseudolophiotrema elymicola*. a–b. Appearance of ascocarps on substrate; c–d. ascocarp in longitudinal section; e. peridium of ascocarps; f. pseudoparaphyses; g–h. ascospores; i. ascus apex; j. ascus stipe; k–n. ascospores; o. ascospore with a gelatinous sheath (in India ink); p. germinating ascospore (a–m, o–p: HHUF 28984, holotype; n: JCM 13090 = MAFF 239600, ex-holotype culture). — Scale bars: a = 500 µm; b = 250 µm; c–d = 20 µm; e–h, k, o–p = 10 µm; i–j, l–n = 5 µm.

ly, smoke grey (105); reverse grey olivaceous (107) (Fig. 2l); sexual morph formed.

Specimen examined. JAPAN, Hokkaido, Yufutsu, on dead leaves of *Leymus mollis*, 1 Sept. 2003, Y. Harada, KT 1450 (HHUF 28984 holotype designated here, ex-holotype culture JCM 13090 = MAFF 239600).

Notes — *Pseudolophiotrema* is quite similar to *Lophiotrema* in having a crest-like ostiolar neck, a peridium of uniform thickness, cylindrical ascii with a short stipe, and 1-septate hyaline ascospores with a gelatinous sheath. However, it can be separated from the latter genus by its thinner ascomatal wall (10–12.5 µm thick; Fig. 12d–e) composed of compressed cells vs the 20–30 µm thick wall made up of rectangular cells in *Lophiotrema* (Holm & Holm 1988).

In our phylogenetic tree, the genus *Pseudolophiotrema* was placed completely outside of Lophiotremataceae, and was also separate from the families Cryptocoryneaceae and Aquasubmersaceae (Fig. 1). The familial placement of *Pseudolophiotrema* remains unclear. Additional taxa belonging to this genus are needed to fully understand its taxonomic affiliations within Pleosporales.

DISCUSSION

Lophiotremataceae was established by Hirayama & Tanaka (2011) to accommodate *Lophiotrema*. Subsequently, *Aquasubmersa* and *Hermatomyces* were recognised as additional members of this family on the basis of molecular phylogenetic analyses (Ariyawansa et al. 2015, Doilom et al. 2016, Hyde et al. 2016). A phylogenetic relationship between *Cryptocoryneum* and Lophiotremataceae was also suggested by a BLAST search of ITS sequences of species in that genus (Hashimoto et al. 2016). Although tree topologies generated in previous molecular studies have suggested that the above-mentioned genera might be members of Lophiotremataceae, statistical support for a Lophiotremataceae s.lat. clade has been relatively weak (Ariyawansa et al. 2015, Doilom et al. 2016, Hashimoto et al. 2016, Hyde et al. 2016). Our results do not support the monophyly of Lophiotremataceae s.lat. as recognised in several previous studies (Fig. 1). Instead, the various asexual morphs found in *Aquasubmersa*, *Cryptocoryneum*, and *Hermatomyces* belong to separate families whose monophyly is strongly supported (Fig. 1). We thus consider Lophiotremataceae to be restricted to *Lophiotrema* and five new genera (*Atrocalyx*, *Crassimassarina*, *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus*), all of which have ascomata with or without slit-like ostioles and pycnidial conidiomata. We also conclude that *Aquasubmersa* (a freshwater lineage having papillate ascomatal ostioles and pycnidial conidiomata with 1-celled conidia), *Cryptocoryneum* (having sporodochial conidiomata with cheiroid conidia), and *Hermatomyces* (having sporodochial conidiomata with lenticular and/or cylindrical conidia), which have provisionally been regarded as genera of Lophiotremataceae, should be placed in their own families given their different asexual morphs.

Except for *Crassimassarina*, genera accepted in the present study as members of Lophiotremataceae (i.e., *Lophiotrema* s.str. and the newly introduced genera *Atrocalyx*, *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus*) have features that are generally consistent with traditional generic concepts of *Lophiotrema* s.lat. (Holm & Holm 1988, Tanaka & Harada 2003, Zhang et al. 2009, Hirayama & Tanaka 2011). In contrast, *Crassimassarina* has some characteristics atypical of Lophiotremataceae: large, immersed ascomata with a papillate ostiolar neck, resembling those of genera in Pleomassariaceae s.lat. (Barr 1982, Tanaka et al. 2005), as well as pycnidial conidiomata with multi-septate cylindrical conidia resembling

those of *Stagonospora* (Quaedvlieg et al. 2013, Tanaka et al. 2015). The phylogenetic placement of this morphologically distinct lineage in Lophiotremataceae led us to conduct detailed observations of the above lophiotremataceous genera. These observations revealed morphological variations among ascocata with slit-like ostioles in Lophiotremataceae, such as ascocata with a distinct clypeus around the ostiolar neck (in *Cryptoclypeus*, *Galeaticarpa* and *Pseudocryptoclypeus*, Fig. 7c, 8c, 9d, 10c), ascocata with a well-developed peridium (*Atrocalyx*, Fig. 4c, 5c), and ascocata with a less-developed peridium of a uniform thickness up to 25 µm (*Lophiotrema*; Holm & Holm 1988). These morphologically distinguishable groups constitute phylogenetically distinct lineages that also correspond to their differing asexual morphs (Fig. 4o, 6q, 8q, 9p, 10m). For example, the sexual morphs of *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus* have similar morphological features, but these genera can be separated from one another according to the morphology of their conidiomata or conidiogenous cells (see Notes on each genus). Consequently, we treat these five genera as separate from *Lophiotrema* s.str. on the basis of holomorph morphology and phylogenetic relationships. *Antealophiotrema brunneosporum*, originally misidentified as a species in *Lophiotrema* because of its morphological resemblance to that genus (Zhang et al. 2009), is only distantly related to Lophiotremataceae. This species is clearly different from *Lophiotrema* in regard to the anatomical characteristics of its peridium and its monodictys-like asexual morph. The result of our present study strongly confirms that the presence of a slit-like ostiole is an unreliable character for delimitation of generic relationships.

Traditionally, the slit-like ostiole of the ascocata has been especially emphasised as a useful character for familial circumscription in ascomycetes (Chesters & Bell 1970). In earlier studies, *Lophiotrema* was placed in Lophiotremataceae according to this criterion (Chesters & Bell 1970, Leuchtmann 1985, Holm & Holm 1988, Barr 1992). Zhang et al. (2009) suggested that *Lophiotrema* is phylogenetically distinct from Lophiotremataceae but could not find any morphological differences between their sexual morphs. Hirayama & Tanaka (2011) re-evaluated the phylogenetic significance of several morphological features used for characterisation of each genus in previous studies. After determining that ascus shape and length of ascus stipe are reliable taxonomic indicators to delineate these two genera, they established Lophiotremataceae to accommodate *Lophiotrema* (Hirayama & Tanaka 2011). Our results, however, strongly confirm that several lophiotrema-like species, i.e., *Antealophiotrema brunneosporum*, '*Lophiotrema*' boreale, and *Pseudolophiotrema elymicola*, deviate from Lophiotremataceae. These species were originally misidentified or provisionally identified as species in *Lophiotrema* on the basis of their morphological resemblance to the genus (Mathiassen 1989, 1993, Zhang et al. 2009), but were found to be phylogenetically separate from *Lophiotrema* s.str. in this study. Previous morphological circumscriptions of Lophiotremataceae and *Lophiotrema* (Holm & Holm 1988, Mathiassen 1989, 1993, Tanaka & Harada 2003, Zhang et al. 2009, Hirayama & Tanaka 2011, Ariyawansa et al. 2015, Doilom et al. 2016, Hyde et al. 2016) were obviously fairly broad concepts that did not reflect their phylogenetic relationships. We additionally discovered several morphological variants of ascocata having slit-like ostioles (e.g. *Atrocalyx*, *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus*), revealed the phylogenetic position of a genus without slit-like ostioles in Lophiotremataceae (i.e., *Crassimassarina*), and observed asexual morphs of genera belonging to Lophiotremataceae. More precise morphological examination of both sexual and asexual morphs in this family will be needed to define familial concepts of Lophiotremataceae and its relatives.

The phylogenetic significance of slit-like ostioles of ascocarps should also be re-evaluated because this phenotypic character is now known in several families, such as *Aigialaceae* (Suetrong et al. 2009), *Ligninsphaeriaceae* (Zhang et al. 2016), and *Lophiostomataceae* (Thambugala et al. 2015). The slit-like ostiole should be regarded as a character that has evolved multiple times independently within *Dothideomycetes*, similar to the parallel evolution of hysterothelial ascocarps in *Anteagloniaceae* (Mugambi & Huhndorf 2009), *Gloniaceae* (Boehm et al. 2009), *Hysteriaceae* (Boehm et al. 2009), *Lophiostomataceae* (Thambugala et al. 2015), and *Mytilinidiaceae* (Boehm et al. 2009).

Recent molecular studies on *Dothideomycetes* have revealed hidden lineages and prompted revision of several families in this class (Crous et al. 2015, Guatimosim et al. 2015, Knapp et al. 2015, Tanaka et al. 2015, Jaklitsch & Voglmayr 2016, Van Nieuwenhuijzen et al. 2016). Although multiple molecular systematic studies, mainly of pathogenic fungi of woody plants (Phillips et al. 2013, Slippers et al. 2013, Alves et al. 2014, Fan et al. 2015, Trakunyingcharoen et al. 2015), have generated a robust phylogeny for *Botryosphaerales*, many additional new lineages in this group, including endophytes and saprophytes, have been discovered from various niches (Thambugala et al. 2014, Crous et al. 2015, 2016, Wyka & Broders 2016, Osorio et al. 2017, Yang et al. 2017). Drawing on many recent collections, our study has revealed previously unrecognised diversity within *Lophiotremataceae* and its relatives and the potential taxonomic importance of the asexual morphs in defining families. To build a comprehensive taxonomic framework, further morphological surveys based on additional collections together with more molecular data are needed.

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