



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 ascomata morphologically similar to those of *Lophiotrema*, but is differentiated from the latter by having ascomata with a well-developed peridium and a monodictys-like asexual morph. *Pseudolophiotrema* is also similar to *Lophiotrema*, but can be distinguished by ascomata 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 ascomata 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.lat. 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.	SSU	LSU	GenBank Accession no. ^b	ITS
							<i>tef1</i>	<i>rpb2</i>
<i>Anteaelephiotrema brunneosporum</i>	incentae sedis	–	CBS H-20222 ^H	CBS 123095	LC194298	LC194340	LC194382	LC194419
<i>Aquasubmersa japonica</i>	Aquasubmersaceae	KT 2813	HHUF 30468 ^H	MAFF 245218	LC061581 ¹	LC061586 ¹	LC194383	LC194420
	Aquasubmersaceae	KT 2862	HHUF 30469 ^H	MAFF 245219	LC061582 ¹	LC061587 ¹	LC194384	LC194421
	Aquasubmersaceae	KT 2863	HHUF 30470 ^P	MAFF 245220	LC061583 ¹	LC061588 ¹	LC194385	LC194422
<i>Atrocalyx acutisporus</i>	Lophiotremataceae	KT 2436	HHUF 30504 ^H	MAFF 245613 = NBRC 112316	LC194299	LC194341	LC194386	LC194475
<i>A. lignicola</i>	Lophiotremataceae	–	CBS H-20221 ^H	CBS 122364	LC194300	LC194342	LC194387	LC194476
<i>Crassmassarina macrospora</i>	Lophiotremataceae	KH 152	HHUF 30512 ^P	MAFF 245617	LC194301	LC194343	LC194388	LC194425
	Lophiotremataceae	KT 1764	HHUF 29054 ^H	JCM 13096 = MAFF 239606	LC194302	LC194344	LC194389	LC194478
<i>Cryptoclypeus oxysporus</i>	Lophiotremataceae	KT 2772	HHUF 30507 ^H	MAFF 245614 = NBRC 112317	LC194303	LC194345	LC194390	LC194427
<i>C. ryukyensis</i>	Lophiotremataceae	AH 342	HHUF 30510 ^P	MAFF 245616	LC194304	LC194346	LC194391	LC194428
	Lophiotremataceae	KT 3534	HHUF 30509 ^H	MAFF 245619 = NBRC 112318	LC194305	LC194347	LC194392	LC194429
<i>Cryptocoryneum akitaense</i>	Cryptocoryneaceae	KT 3019	HHUF 30477 ^H	MAFF 245365 = NBRC 111758	LC194306	LC194348	LC096136 ²	LC194481
<i>C. brevicondensatum</i>	Cryptocoryneaceae	none 152	HHUF 30478 ^H	MAFF 245366 = NBRC 111759	LC194307	LC194349	LC096137 ²	LC096155 ²
<i>C. condensatum</i>	Cryptocoryneaceae	–	UPS F-632989	CBS 113959	LC194308	LC194350	LC096138 ²	LC194432
	Cryptocoryneaceae	–	–	CBS 122629	LC194309	LC194351	LC096139 ²	LC194433
	Cryptocoryneaceae	–	–	CBS 122633	LC194310	LC194352	LC096140 ²	LC194434
	Cryptocoryneaceae	KT 2892	HHUF 30479 ^H	MAFF 245367 = NBRC 111760	LC194311	LC194353	LC096141 ²	LC194435
	Cryptocoryneaceae	KT 2961	HHUF 30480 ^P	MAFF 245368	LC194312	LC194354	LC096142 ²	LC096160 ²
<i>C. japonicum</i>	Cryptocoryneaceae	KT 3291	HHUF 30481 ^P	MAFF 245369	LC194313	LC194355	LC096143 ²	LC096161 ²
	Cryptocoryneaceae	KT 3300	HHUF 30482 ^H	MAFF 245370 = NBRC 111761	LC194314	LC194356	LC096144 ²	LC194438
	Cryptocoryneaceae	KT 3413	HHUF 30483 ^P	MAFF 245371	LC194315	LC194357	LC096145 ²	LC096163 ²
	Cryptocoryneaceae	none 36	HHUF 30484 ^P	MAFF 245372	LC194316	LC194358	LC096146 ²	LC096164 ²
	Cryptocoryneaceae	none 157	HHUF 30485 ^P	MAFF 245373	LC194317	LC194359	LC096147 ²	LC194441
<i>C. longicondensatum</i>	Cryptocoryneaceae	KT 2913	HHUF 30486 ^H	MAFF 245374 = NBRC 111762	LC194318	LC194360	LC096148 ²	LC194442
	Cryptocoryneaceae	KT 3487	HHUF 30487 ^P	MAFF 245375	LC194319	LC194361	LC096149 ²	LC096162 ²
<i>C. paracocondensatum</i>	Cryptocoryneaceae	KT 3071	HHUF 30488 ^P	MAFF 245376	LC194320	LC194362	LC096150 ²	LC096168 ²
	Cryptocoryneaceae	KT 3241	HHUF 30489 ^H	MAFF 245377 = NBRC 111763	LC194321	LC194363	LC096151 ²	LC096169 ²
<i>C. pseudofilistonei</i>	Cryptocoryneaceae	–	HHUF 30490 ^H	CBS 113641	LC194322	LC194364	LC096152 ²	LC194446
<i>Cryptocoryneum</i> sp.	Cryptocoryneaceae	–	–	CBS 114518	LC194323	LC194365	LC096153 ²	LC194447
<i>Galeaticarpa aomoriensis</i>	Lophiotremataceae	KT 2563	HHUF 30505 ^H	MAFF 245618 = NBRC 112319	LC194324	LC194366	LC194393	LC194448
<i>Hermatomyces trifoliotensis</i>	Hermatomyceaceae	KH 361	HHUF 30518 ^H	MAFF 245730 = NBRC 112471	LC194325	LC194367	LC194394	LC194449
<i>H. tectoriae</i>	Hermatomyceaceae	KT 2450	HHUF 30520	MAFF 245731	LC194326	LC194368	LC194395	LC194450
	Hermatomyceaceae	AH 276	HHUF 30521	MAFF 245732	LC194327	LC194369	LC194396	LC194451
	Hermatomyceaceae	AH 314	HHUF 30522	MAFF 245733	LC194328	LC194370	LC194397	LC194452
	Hermatomyceaceae	KH 329	HHUF 30523	MAFF 245734	LC194329	LC194371	LC194398	LC194453
	Hermatomyceaceae	KH 356	HHUF 30524	MAFF 245735	LC194330	LC194372	LC194399	LC194454
	Hermatomyceaceae	KH 390	HHUF 30525	MAFF 245736	LC194331	LC194373	LC194400	LC194455
	Hermatomyceaceae	KH 409	HHUF 30526	MAFF 245737	LC194332	LC194374	LC194401	LC194456
<i>'Lophiotrema' boreale</i>	incentae sedis	–	–	CBS 114422 = JCM 14136	LC194333	LC194375	LC194402	LC194457
<i>L. eburnoides</i>	Lophiotremataceae	KT 1424-1	HHUF 30079 ^H	JCM 17826 = MAFF 242970	LC001706 ³	LC001707 ³	LC194403	LC194458
<i>L. fallopiae</i>	Lophiotremataceae	KT 2748	HHUF 30506 ^H	MAFF 245612	LC149911 ⁴	LC149915 ⁴	LC194404	LC194459
<i>L. necaerundinaria</i>	Lophiotremataceae	KT 856	HHUF 27547	MAFF 239461	AB524455 ⁵	AB524596 ⁵	AB539109 ⁶	AB524786 ⁵
	Lophiotremataceae	KT 1034	HHUF 30015	NBRC 106239	AB524457 ⁵	AB524598 ⁵	LC194405	LC194460
	Lophiotremataceae	KT 2200	HHUF 30014	NBRC 106238	AB524456 ⁵	AB524597 ⁵	AB539110 ⁶	AB524787 ⁵
	Lophiotremataceae	KH 17	HHUF 30511	MAFF 245619	LC194334	LC194376	LC194406	LC194461
	Lophiotremataceae	KT 588	HHUF 27368	MAFF 245620	LC194335	LC194377	LC194407	LC194462
	Lophiotremataceae	KT 713	HHUF 27328	JCM 17673	AB618701 ⁷	AB619019 ⁷	LC194408	LC194463
	Lophiotremataceae	KT 756	HHUF 27330	MAFF 239457	AB618702 ⁷	AB619020 ⁷	LC194409	LC194464
<i>L. nucula</i>	Lophiotremataceae	–	–	CBS 62786 = JCM 14132	AB618703 ⁷	AB619021 ⁷	LC194410	LC194465
<i>L. vagabundum</i>	Lophiotremataceae	KH 164	HHUF 30077	JCM 17674	AB618704 ⁷	AB619022 ⁷	LC194411	LC194466
	Lophiotremataceae	KH 172	HHUF 30078	JCM 17675	AB618705 ⁷	AB619023 ⁷	LC194412	LC194467
	Lophiotremataceae	KT 664	HHUF 27323	MAFF 239456	AB618706 ⁷	AB619024 ⁷	LC194413	LC194468
	Lophiotremataceae	KT 3310	HHUF 30508	MAFF 245621	LC194336	LC194378	LC194414	LC194469
	Lophiotremataceae	–	F-634236	CBS 113975 = JCM 14138	AB618707 ⁷	AB619025 ⁷	LC194415	LC194470
<i>'Massarina albocanis'</i>	Lophiotremataceae	–	–	CBS 119345	LC194337	LC194379	LC194416	LC194471
<i>Pseudocryptoclypeus yakushimensis</i>	Lophiotremataceae	KT 2186	HHUF 30503 ^H	MAFF 245622 = NBRC 112320	LC194338	LC194380	LC194417	LC194472
<i>Pseudolophiotrema elymicola</i>	incentae sedis	KT 1450	HHUF 28994 ^H	JCM 13090 = MAFF 239600	LC194339	LC194381	LC194418	LC194473

^a H = holotype; P = paratype.^b Sequences obtained in this study are shown in bold. Sequences with ¹ from Ariyawansa et al. (2015), ² from Hashimoto et al. (2016), ³ from Liu et al. (2015), ⁴ from Hyde et al. (2016), ⁵ from Tanaka et al. (2009), ⁶ from Schoch et al. (2009), ⁷ from Hirayama & Tanaka (2011).

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	tef1	rpb2	ITS
<i>Amniculicola immersa</i>	Amniculicolaceae	CBS H-20226 ^H	CBS 123083	GU456295	FJ795498	GU456273	GU456358	–
<i>A. parva</i>	Amniculicolaceae	CBS H-20227 ^H	CBS 123092	GU296134	GU301797	GU349065	–	–
<i>Anteaglonium abbreviatum</i>	Anteagloniaceae	ANM 925.1	–	–	GQ221877	GQ221924	–	–
<i>A. globosum</i>	Anteagloniaceae	ANM 925.2 ^H	–	–	GQ221879	GQ221925	–	–
	Anteagloniaceae	SMH 5283 ^P	–	–	GQ221911	GQ221919	–	–
<i>A. parvulum</i>	Anteagloniaceae	MFLU 16-0473	MFLUCC 14-0815	KU922912	KU922911	KU922919	–	–
	Anteagloniaceae	MFLU 16-0472	MFLUCC 14-0817	KU922914	KU922913	–	–	–
	Anteagloniaceae	MFLU 16-0474	MFLUCC 14-0821	KU922916	KU922915	KU922921	–	–
	Anteagloniaceae	MFLU 16-0470	MFLUCC 14-0823	KU922918	KU922917	KU922922	–	–
<i>A. thailandicum</i>	Anteagloniaceae	MFLU 16-0471 ^H	MFLUCC 14-0816	KU922910	KU922909	KU922920	–	–
<i>Aquasubmersa mircensis</i>	Aquasubmersaceae	MFLU 111001 ^H	MFLUCC 11-0401 = IFRDCC 2572	JX276956	JX276955	–	–	JX276954
<i>Byssolophis sphaeroides</i>	incertae sedis	–	IFRDCC 2053	GU456296	GU456318	GU456263	GU456348	–
<i>Hermatomyces tectonae</i>	Hermatomycetaceae	MFLU 15-3437 ^H	MFLUCC 14-1140	KU712465	KU764695	KU872757	KU712486	KU144917
	Hermatomycetaceae	MFLU 15-3438 ^P	MFLUCC 14-1141	KU712466	KU764696	KU872758	–	KU144918
	Hermatomycetaceae	MFLU 15-3439 ^P	MFLUCC 14-1142	KU712467	KU764697	–	KU712487	KU144919
<i>H. thailandica</i>	Hermatomycetaceae	MFLU 15-3440 ^H	MFLUCC 14-1143	KU712468	KU764692	KU872754	KU712488	KU144920
	Hermatomycetaceae	MFLU 15-3441 ^P	MFLUCC 14-1144	KU712469	KU764693	KU872755	KU712489	KU144921
	Hermatomycetaceae	MFLU 15-3442 ^P	MFLUCC 14-1145	KU712470	KU764694	KU872756	KU712490	KU144922
<i>Lepidosphaeria nicotiae</i>	Testudinaceae	–	CBS 101341	–	DQ678067	DQ677910	DQ677963	–
<i>Lophiostoma arundinis</i>	Lophiostomatataceae	–	CBS 621.86	DQ782383	DQ782384	DQ782387	DQ782386	AJ496633
' <i>Lophiostoma bambusae</i>	Lophiostomatataceae	MFLU 11-0150	MFLUCC 10-0558	KX672159	KX672154	KX672162	KX672161	KX672149
<i>L. crenatum</i>	Lophiostomatataceae	–	CBS 629.86	DQ678017	DQ678069	DQ677912	DQ677965	–
<i>Polyposphaeria fusca</i>	Tetraplosporaaceae	HHUF 29399 ^H	JCM 13175 = MAFF 239685	AB524463	AB524604	–	–	AB524789
<i>Pseudocastrospora tiella bambusae</i>	Pseudocastrosporaellaceae	MFLU 11-0155 ^H	MFLUCC 11-0205	KT955455	KT955475	KT955437	KT955414	–
<i>P. longicolla</i>	Pseudocastrosporaellaceae	MFLU 11-0207 ^H	MFLUCC 11-0171	–	KT955476	KT955438	KT955420	–
<i>P. thailandensis</i>	Pseudocastrosporaellaceae	MFLU 11-0145 ^H	MFLUCC 10-0553	KT955456	KT955477	KT955439	KT955411	–
<i>Pseudotetraploa curvicaulis</i>	Tetraplosporaaceae	HHUF 28582 ^H	JCM 12852 = MAFF 239495	AB524467	AB524608	–	–	AB524792
<i>Quadrifaria septentrionalis</i>	Tetraplosporaaceae	HHUF 28781 ^P	CBS 125429	AB524474	AB524615	–	–	AB524799
<i>Tetrapla sasicola</i>	Tetraplosporaaceae	HHUF 27566 ^H	JCM 13167 = MAFF 239677	AB524490	AB524631	–	–	AB524807
<i>Triplospora maxima</i>	Tetraplosporaaceae	HHUF 29390 ^H	JCM 13172 = MAFF 239682	AB524496	AB524637	–	–	AB524812
<i>Ulospora bilgramii</i>	Testudinaceae	–	CBS 101364	DQ678025	DQ678076	DQ677921	DQ677974	–
<i>Verruculina enalia</i>	Testudinaceae	–	BCC 18402	GU479771	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 10× 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*, *Pseudoastrophaeriellaceae*, *Testudinaceae*, and *Tetraplosphaeriaceae* were also phylogenetically analysed (Table 2). *Lophiostoma crenatum* and *L. arundinis* (*Lophiostomataceae*) 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. neohysterioides*, *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* (Mathiasen 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.

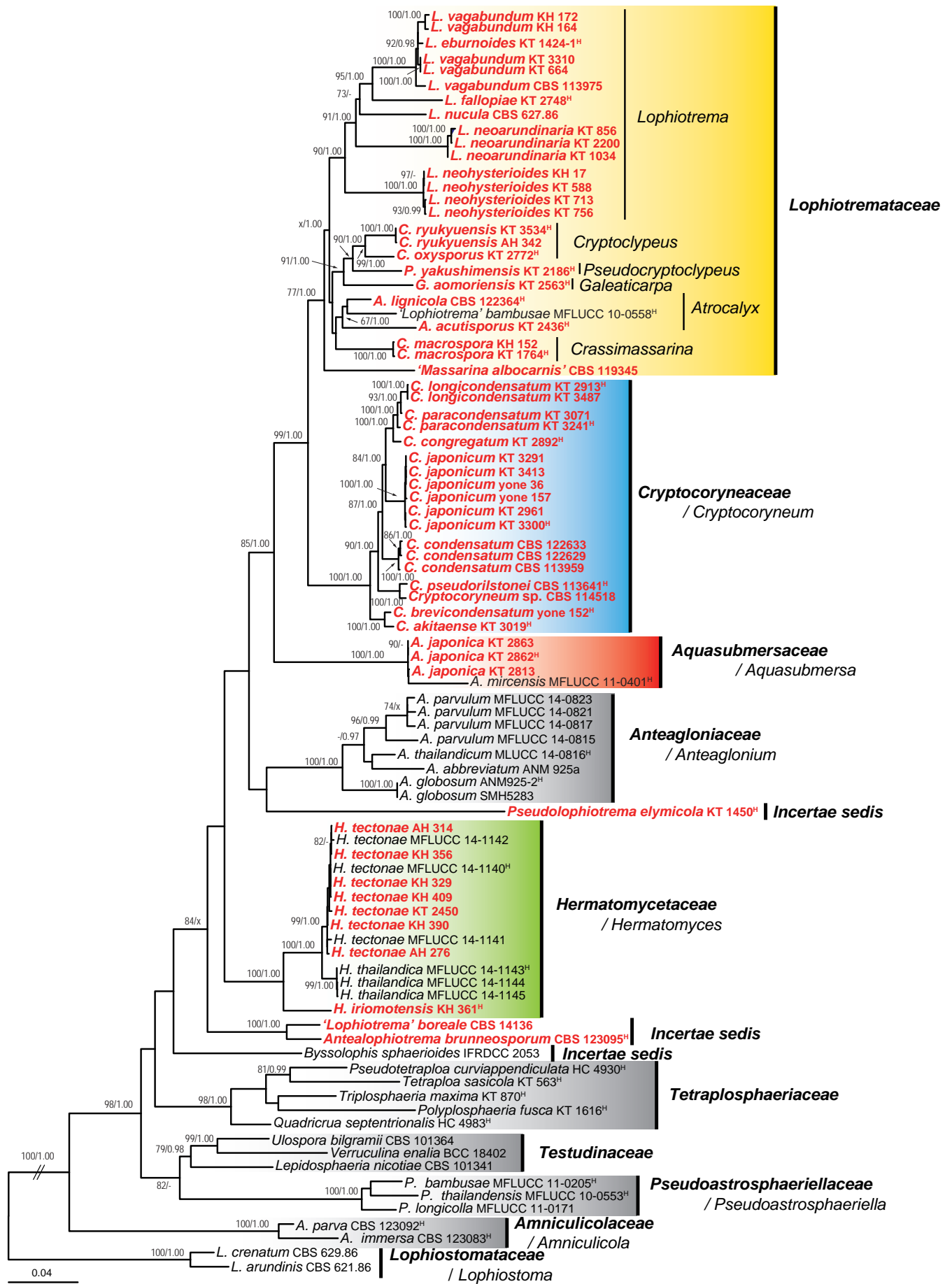


Fig. 1 Maximum-likelihood (ML) tree of *Aquasubmersaceae*, *Cryptocoryneaceae*, *Hermatomyetaceae*, and *Lophiotremataceae* based on SSU-ITS-LSU-*tef1-rpb2* sequences. ML bootstrap proportion (BP) > 60 % and Bayesian posterior probabilities (PP) > 0.95 are presented at the nodes as ML BP/Bayesian PP. 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. *Asci* 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 hysterioides* (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.

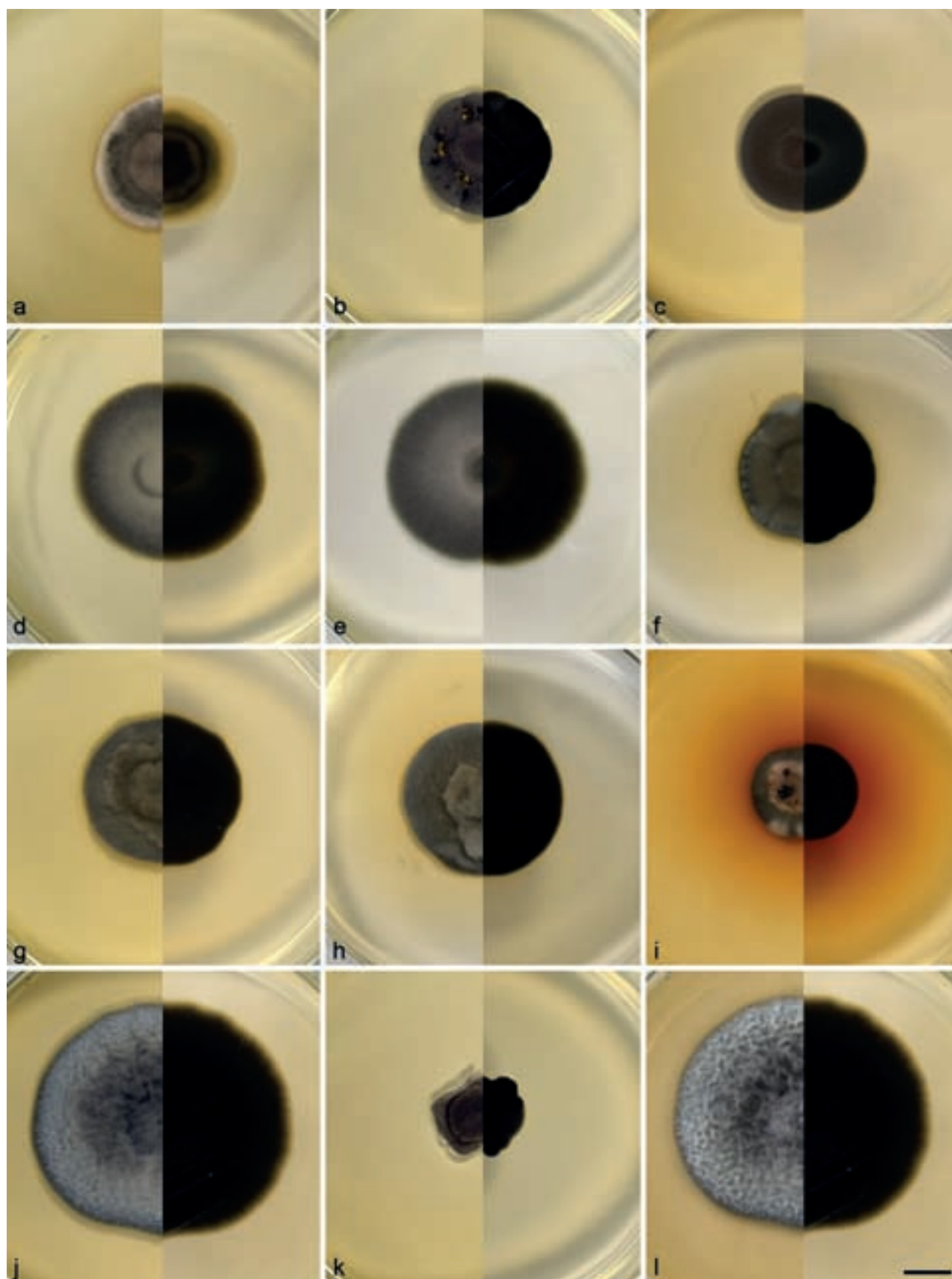


Fig. 2 Colony characters of lophiotremataceous species used in this study on PDA within 3 wk at 20 °C in the dark (left: upper, right: reverse). a. *Hermatomyces iriomotensis* (MAFF 245730 = NBRC 112471, ex-holotype culture); b. *Atrocalyx acutisporus* (MAFF 245613 = NBRC 112316, ex-holotype culture); 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. *Asci* 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 ampiform, hyaline. *Conidia* ellipsoidal to cylindrical with rounded ends, hyaline, smooth, aseptate or multi-septate.

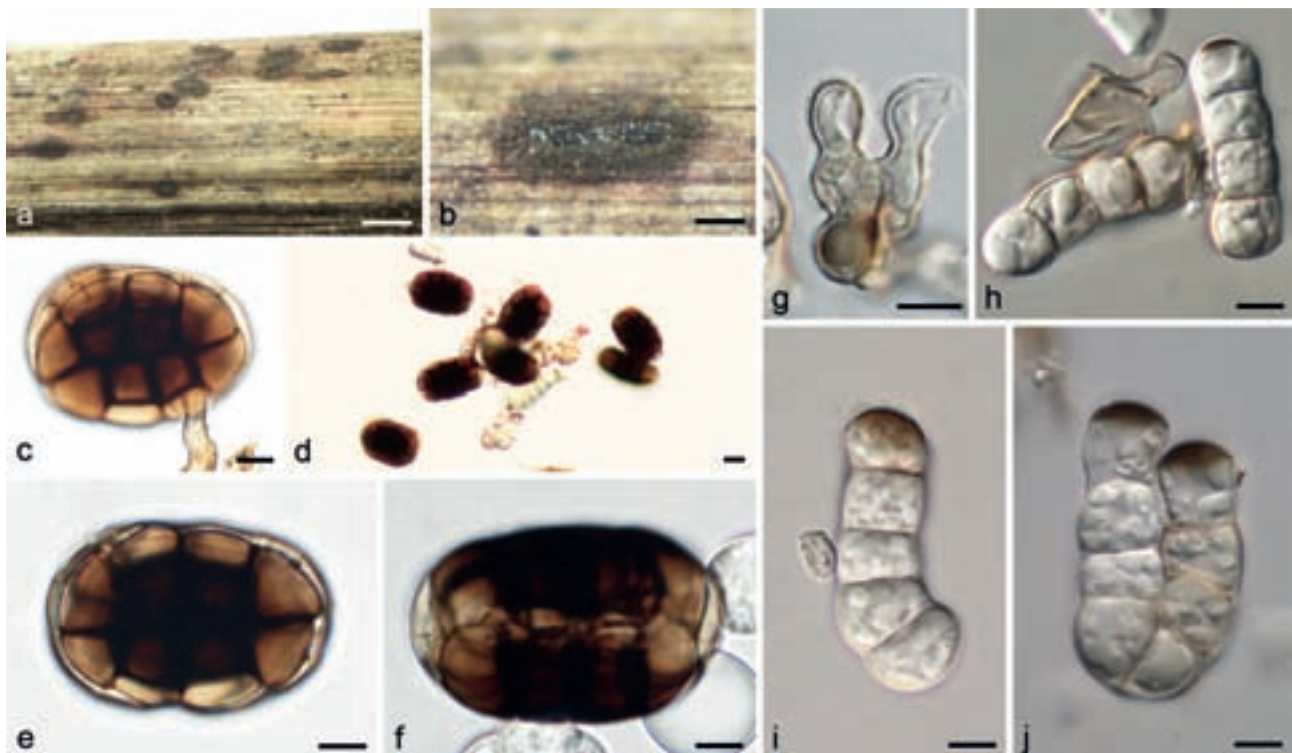


Fig. 3 *Hermatomyces iriomotensis*. a–b. Conidiomata on substrate; c. conidiogenous cell and immature lenticular conidium; d–f. lenticular conidia (f. lateral view); g. conidiogenous cells and immature cylindrical conidium; h–j. cylindrical conidia (all: HHUF 30518, holotype). — Scale bars: a = 1 mm; 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 ascomata with a slit-like ostiole and an ascomatal wall of uniform thickness, asci 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: *Ascomata* 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. *Asci* 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: *Ascomata* 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. *Asci* 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.

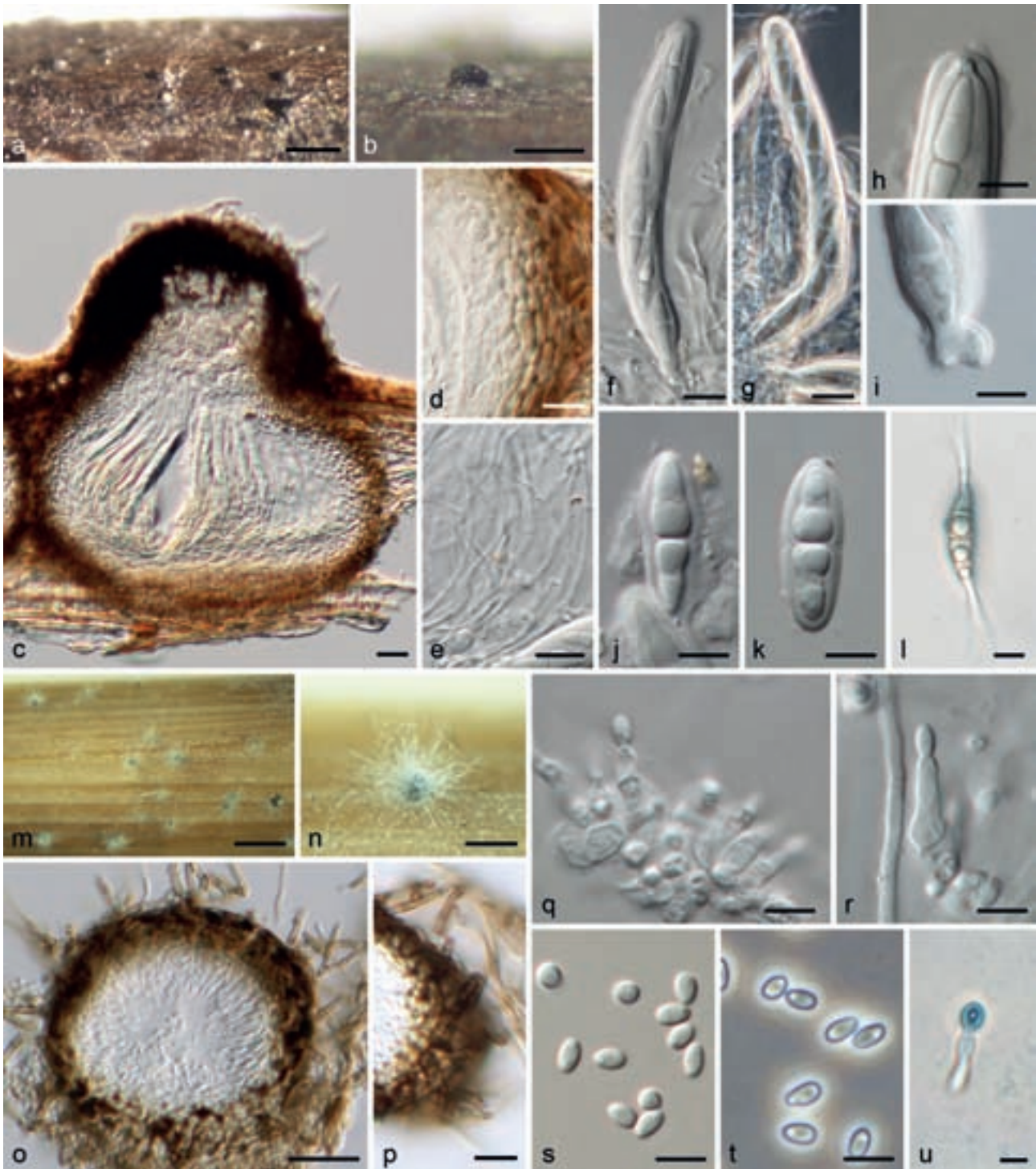


Fig. 4 *Atrocalyx acutisporus*. a–b. Appearance of ascomata on substrate; c. ascoma in longitudinal section; d. peridium of ascoma; e. pseudoparaphyses; f–g. asci; h. ascus apex; i. ascus stipe; j–k. ascospores; l. germinating ascospore; m–n. conidiomata in culture; o. conidioma in longitudinal section; 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 μ m; b = 200 μ m; c, o = 20 μ m; d–g, p = 10 μ m; h–l, q–u = 5 μ m; m = 1 mm; n = 250 μ m.

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: *Ascomata* ellipsoidal, 330–380 μ m high, 350–600 μ m long, 230–400 μ m 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 μ m thick at side, composed of 2 zones; outer zone 15–20 μ m thick, composed of elongated, thin-walled, 10–12.5 \times

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

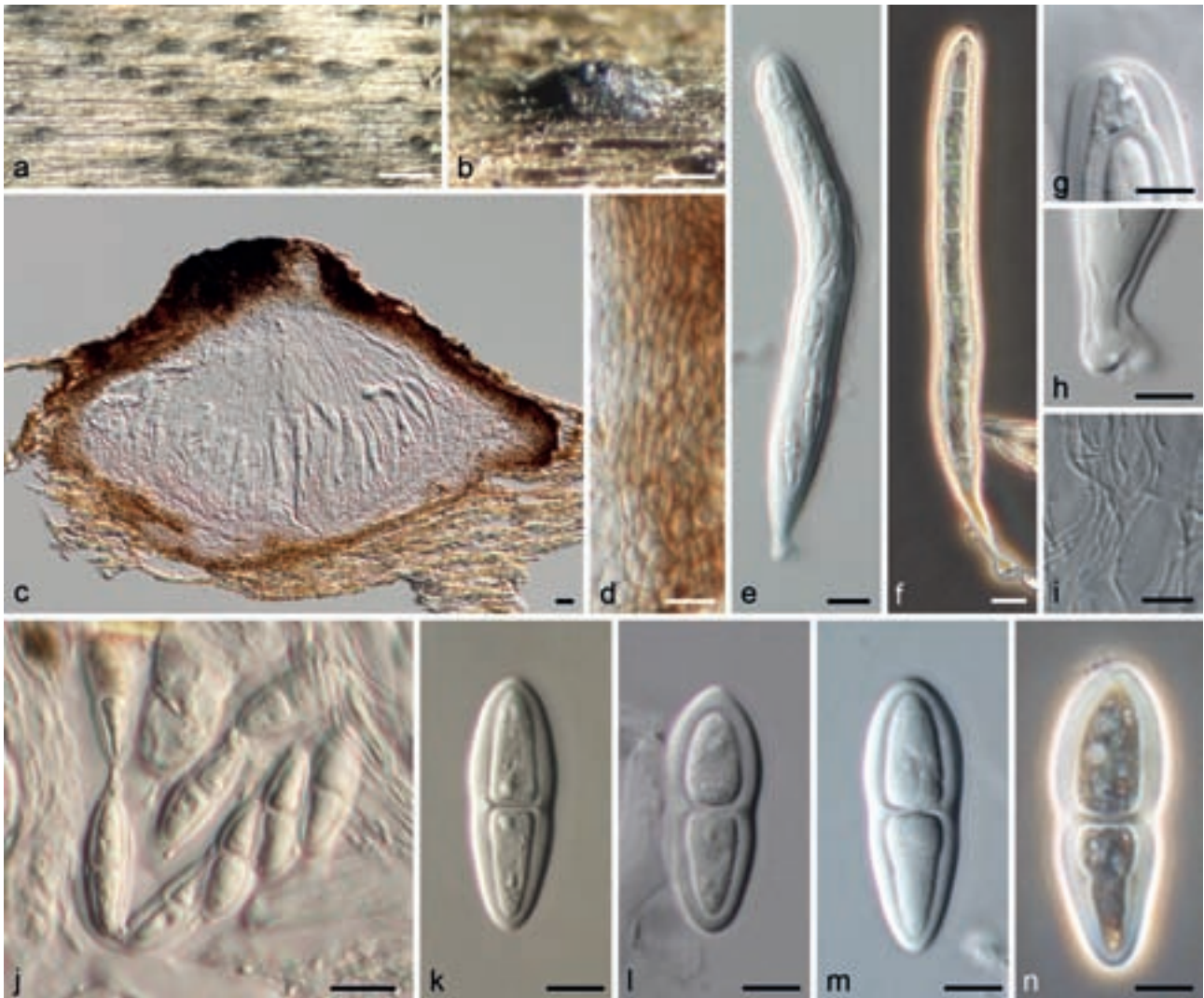


Fig. 5 *Atrocalyx lignicola*. a–b. Appearance of ascomata on substrate; c. ascoma in longitudinal section; d. peridium of ascoma; e–f. asci; g. ascus apex; h. ascus stipe; i. pseudoparaphyses; j–n. ascospores (all: CBS H-20221, holotype). — Scale bars: a = 1 mm; b = 250 μ m; c = 20 μ m; d–f, i = 10 μ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

entire gelatinous sheath up to 2 μ m 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 ascomata 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 ascumatal 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. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *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 μ m high, 380–700 μ m diam. *Ostiolar neck* papillate, without slit-like ostiole, composed of carbonaceous, thick-walled, black cells. *Peridium* 25–60 μ m thick, composed of 6–8 layers of rectangular, thin-walled, 5–12.5 μ m diam cells, surrounded by brown

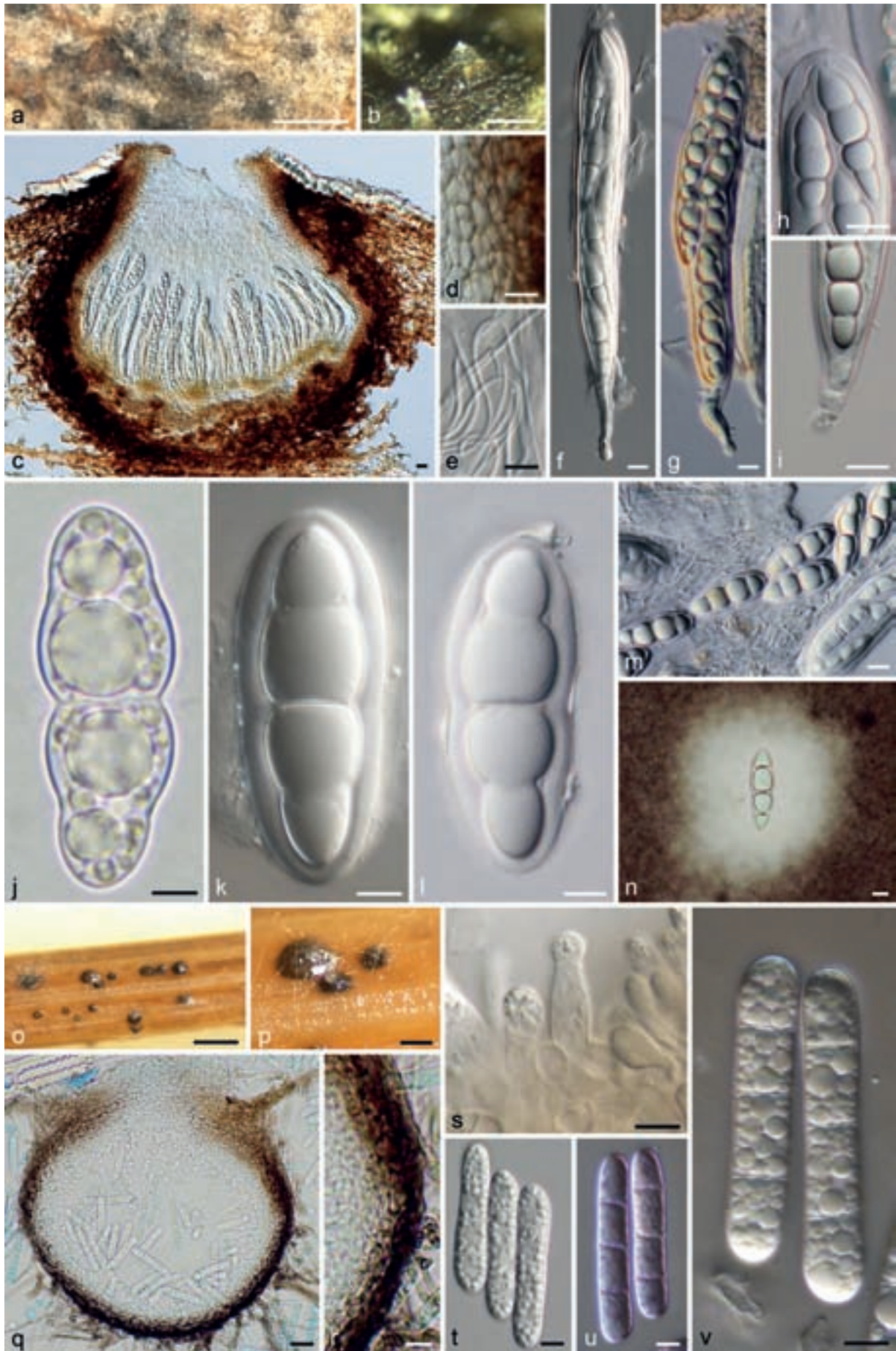


Fig. 6 *Crassimassarina macrospora*. a–b. Appearance of ascomata on substrate; c. ascoma in longitudinal section; d. peridium of ascoma; e. pseudoparaphyses; f–g. asci; h. ascus apex; i. ascus stipe; j–m. ascospores; n. ascospore with a gelatinous sheath (in India ink); o–p. conidiomata in culture; q. conidioma in longitudinal section; r. peridium of conidioma; s. conidiogenous 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.lat. (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 ascomatal 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 ascomatal 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* (*Tetraplophaeriaceae*), 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*

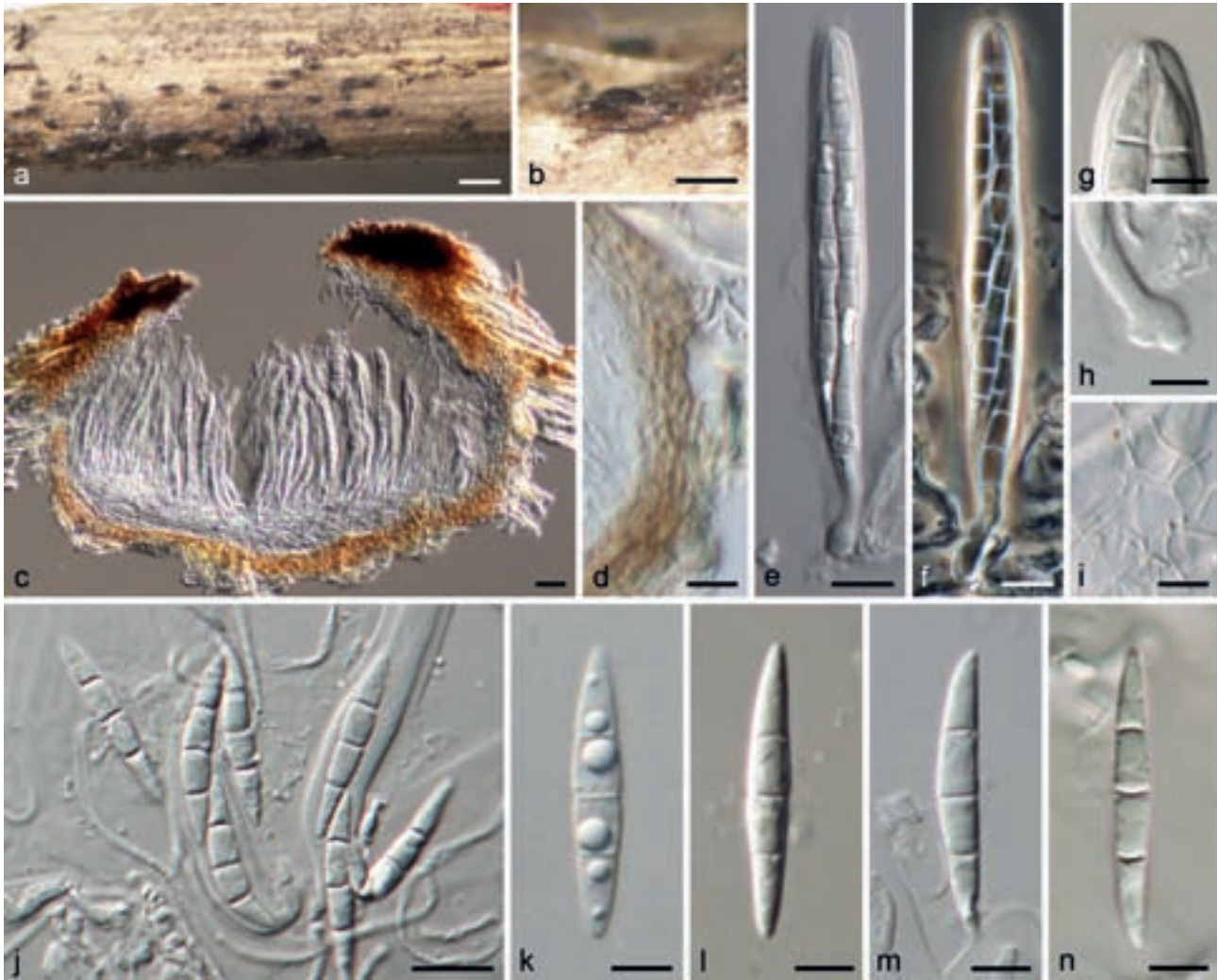


Fig. 7 *Cryptoclypeus oxysporus*. a–b. Appearance of ascomata on substrate; c. ascoma in longitudinal section; d. peridium of ascoma; e–f. asci; g. ascus apex; h. ascus stipe; i. pseudoparaphyses; j–n. ascospores (all: HHUF 30507, holotype). — Scale bars: a = 1 mm; b = 250 μ m; c = 20 μ m; d–f, i = 10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

crest-like, elongated, laterally compressed, with less-developed black clypeus (380–450 μ m wide). *Peridium* uniform, 22.5–25 μ m thick at side, composed of rectangular, thin-walled, 10–22.5 \times 5–6.5 μ m, pale brown cells. *Pseudoparaphyses* numerous, 1–1.5 μ m wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 72.5–100.5 \times 6–10 μ m (av. = 87.0 \times 7.8 μ m, n = 20), with a short stipe (4–9 μ m long, av. = 6.5 μ m, n = 20), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with rounded ends, straight, 15–24 \times 3–4.5 μ m (av. = 19.7 \times 3.8 μ m, 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 μ m high, 230–340 μ m diam, 3–5-grouped, immersed. *Ostiolar neck* up to 50 μ m high, carbonaceous, papillate. *Peridium* 17.5–27.5 μ m thick, composed of 7–10 layers of 4–8 \times 2.5–6.5 μ m, subglobose to rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, 7–12 \times 2–3 μ m, ampliform to cylindrical, hyaline, smooth. *Conidia* cylindrical with slightly angular ends, 11.5–15 \times 2–2.5 μ m (av. = 12.9 \times 2.1 μ m, 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 $^{\circ}$ 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. ryukyensis* 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*

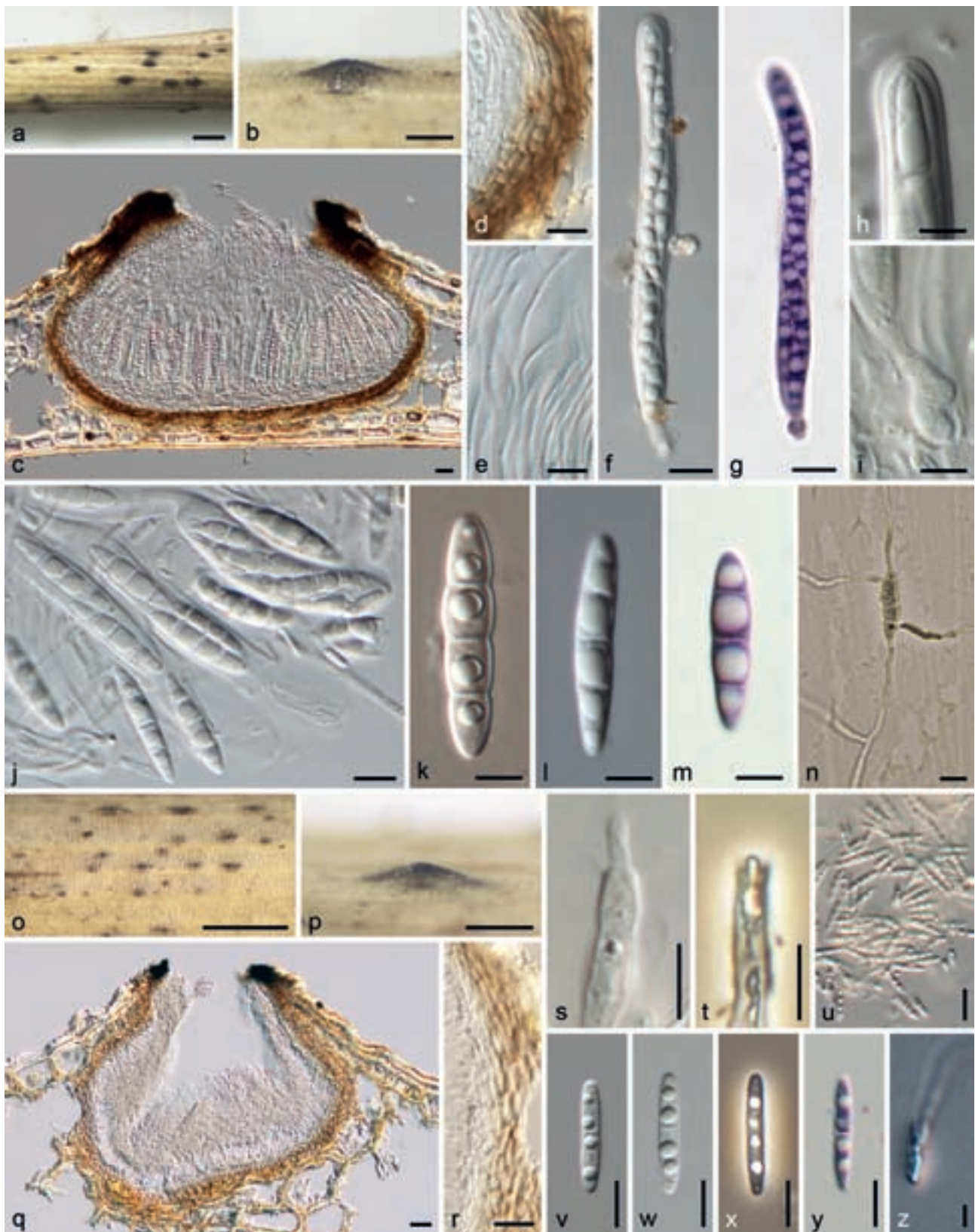


Fig. 8 *Cryptoclypeus ryukyuensis*. a–b. Appearance of ascomata on substrate; c. ascoma in longitudinal section; d. peridium of ascoma; e. pseudoparaphyses; f–g. asci (g in Trypan Blue); h. ascus apex; i. ascus stipe; j–m. ascospores (m in Trypan Blue); n. germinating ascospore; o–p. conidiomata on substrate; 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 μ m; c, q = 20 μ m; d–g, j, n, r, u = 10 μ m; h–i, k–m, s–t, v–z = 5 μ m.

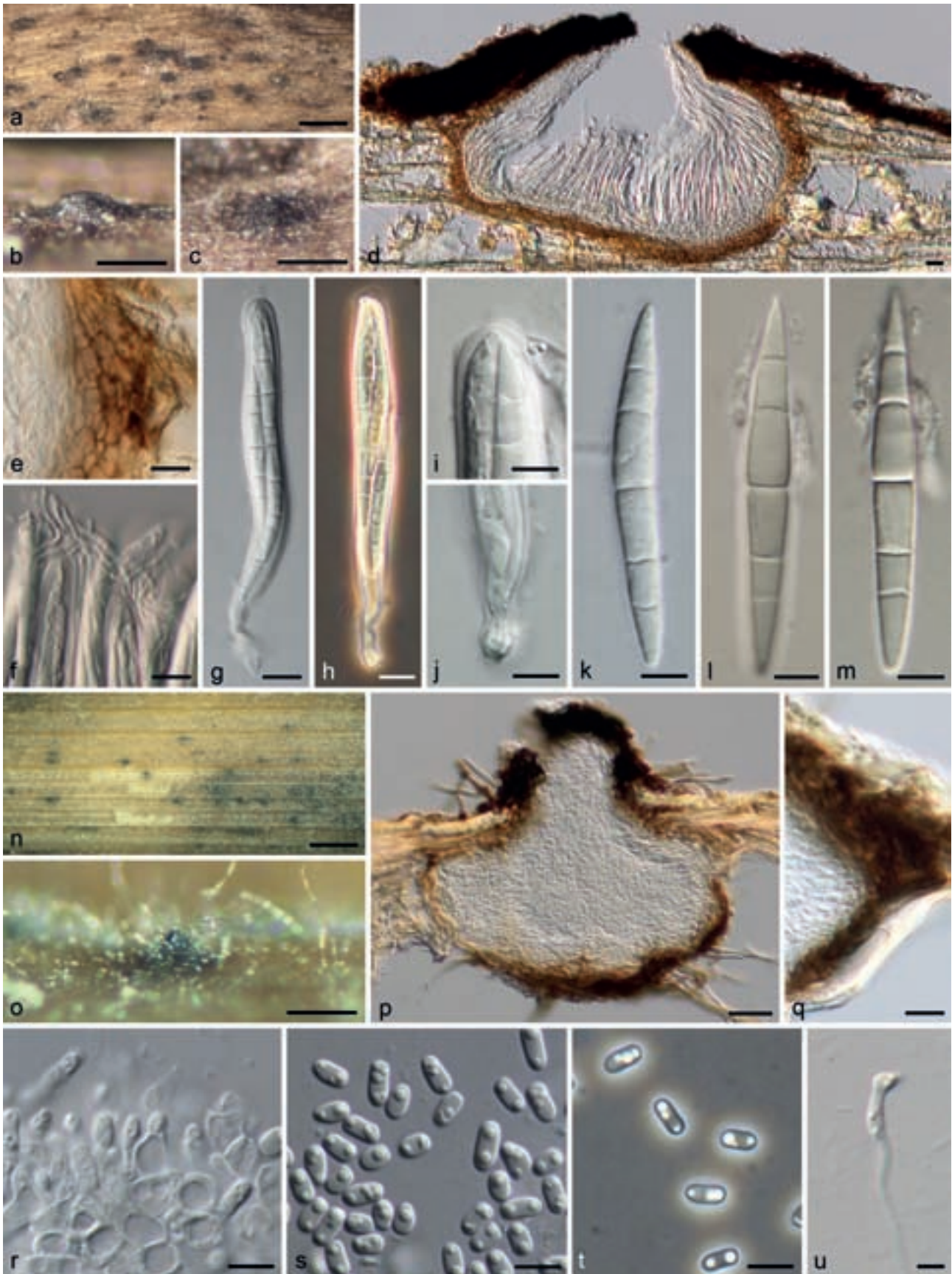


Fig. 9 *Galeaticarpa aomoriensis*. a–c. Appearance of ascomata on substrate; d. ascoma in longitudinal section; e. peridium of ascoma; f. pseudoparaphyses; g–h. asci; i. ascus apex; j. ascus stipe; k–m. ascospores; n–o. conidiomata in culture; p. conidioma in longitudinal section; q. peridium of conidioma; 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 μ m; d, p = 20 μ m; e–h, q = 10 μ m; i–m, r–u = 5 μ m.

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 *Astrosphaeriella*, but *Galeaticarpa* can be distinguished from *Astrosphaeriella* by its crest-like ostiolar neck and uniformly developed peridium (vs a poorly developed peridium at the base in *Astrosphaeriella*; Chen & Hsieh 2004, Phookamsak et al. 2015). These two genera are distantly related and belong to the families *Lophiotremataceae* and *Astrosphaeriellaceae*, 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.

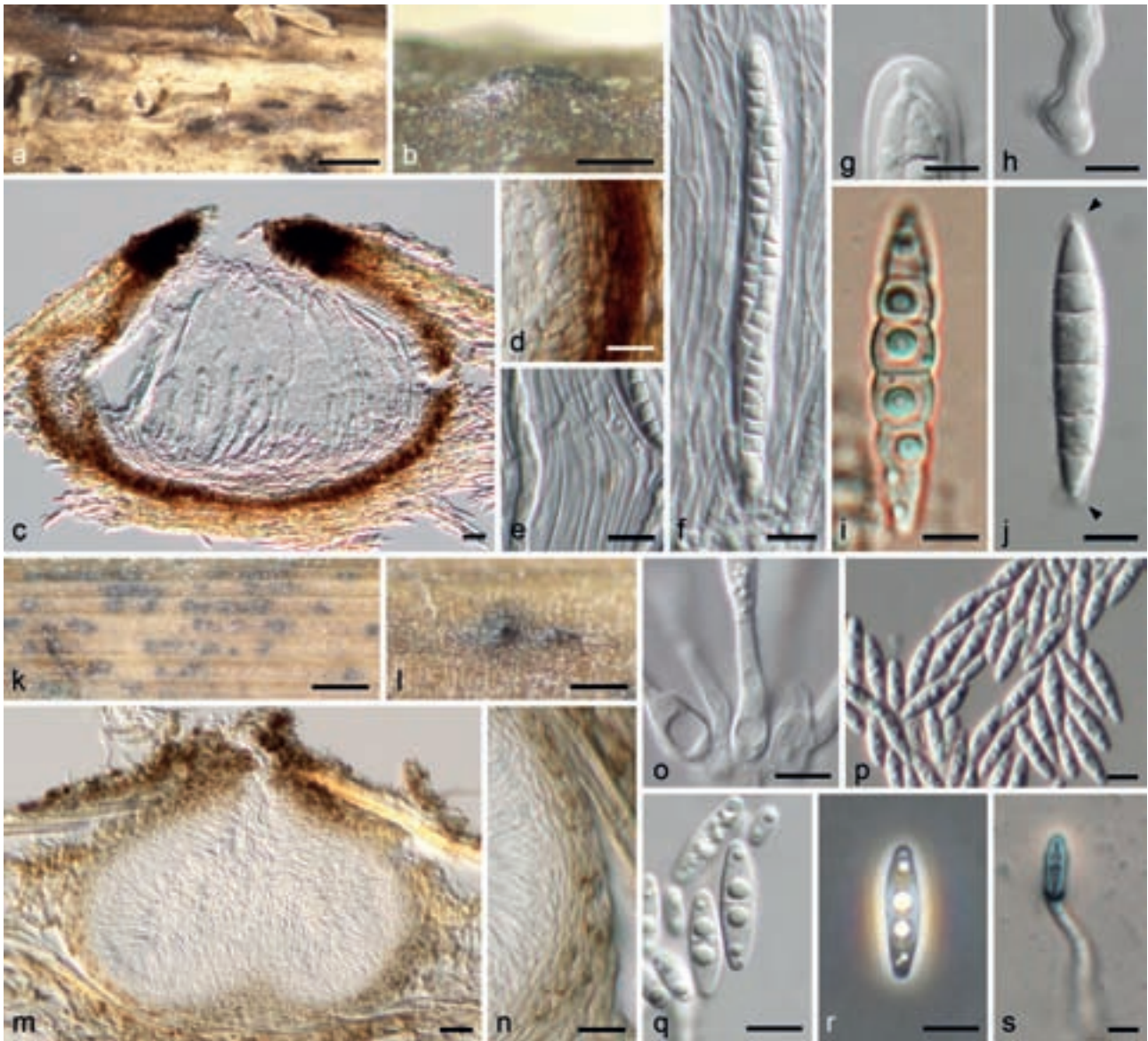


Fig. 10 *Pseudocryptoclypeus yakushimensis*. a–b. Appearance of ascomata on substrate; c. ascoma in longitudinal section; d. peridium of ascoma; e. pseudoparaphyses; f. ascus; g. ascus apex; h. ascus stipe; i–j. ascospores (j arrowheads indicate gelatinous pad); k–l. conidiomata in culture; m. conidioma 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 μ m; c, m = 20 μ m; d–f, n = 10 μ m; g–j, o–s = 5 μ m; l = 250 μ m.

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 ascomata, multi-septate ascospores, pycnidial conidiomata, and 1-septate, hyaline conidia. *Pseudocryptoclypeus* has an ascomatal peridium composed of 2 zones and holoblastic conidiogenous cells (Fig. 10d, o) rather than the ascomatal 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 MB819252

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. *Asci* 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 ob-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.

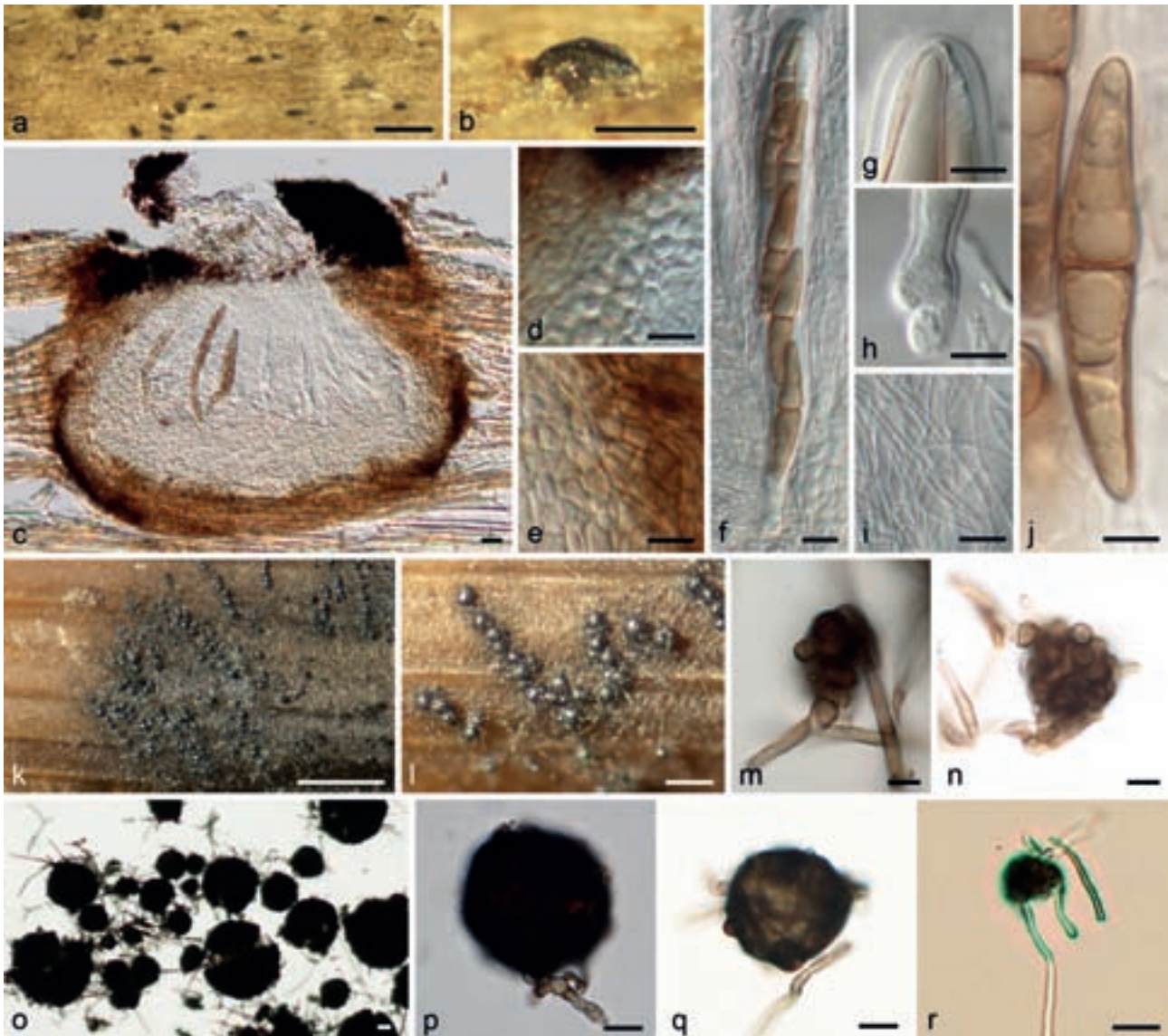


Fig. 11 *Antealophiotrema brunneosporum*. a–b. Appearance of ascomata on substrate; c. ascoma in longitudinal section; d. peridium of ascoma near ostiole; e. peridium of ascoma at side; f. ascus; g. ascus apex; h. ascus stipe; i. pseudoparaphyses; j. ascospore; k–l. conidia in culture; m–n. conidiogenous 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 μ m; c, o, r = 20 μ m; d–f, i, p–q = 10 μ m; g–h, j, m–n = 5 μ m.

Sexual morph: *Ascomata* subglobose to depressed ellipsoidal, up to 380 μ m high, 460–530 μ m diam. *Ostiolar neck* crest-like, elongated, laterally compressed. *Peridium* ununiform, 42.5–62.5 μ m thick, composed of 2 zones; outer zone 22.5–25 μ m thick, composed of moderately thick-walled, rectangular, (5–)7–9 \times 5–7.5 μ m, brown cells, with brown hyphae; inner zone 20–32.5 μ m thick of thin-walled, rectangular, 7–9 \times 4–7.5 μ m, hyaline to pale brown cells; at base 27.5–35 μ m thick, of globose to rectangular, 3.5–7 \times 3.5–4 μ m, pale brown cells. *Pseudoparaphyses* numerous, 0.8–1.5 μ m wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical to clavate, 119–148 \times 13–15 μ m (av. = 138.2 \times 14.3 μ m, n = 8), with a short stipe (8.5–16 μ m long, av. = 10.9 μ m, n = 8), apically rounded with an ocular chamber, with biseriate 4–8 ascospores. *Ascospores* narrowly fusiform with slightly rounded ends, 34.5–48 \times 6.5–10 μ m (av. = 39.8 \times 8.0 μ m, n = 32), l/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 μ m diam (av. = 52.0 μ m, 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 μ m 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. *Asci* 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. 2l, 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. *Asci* 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-

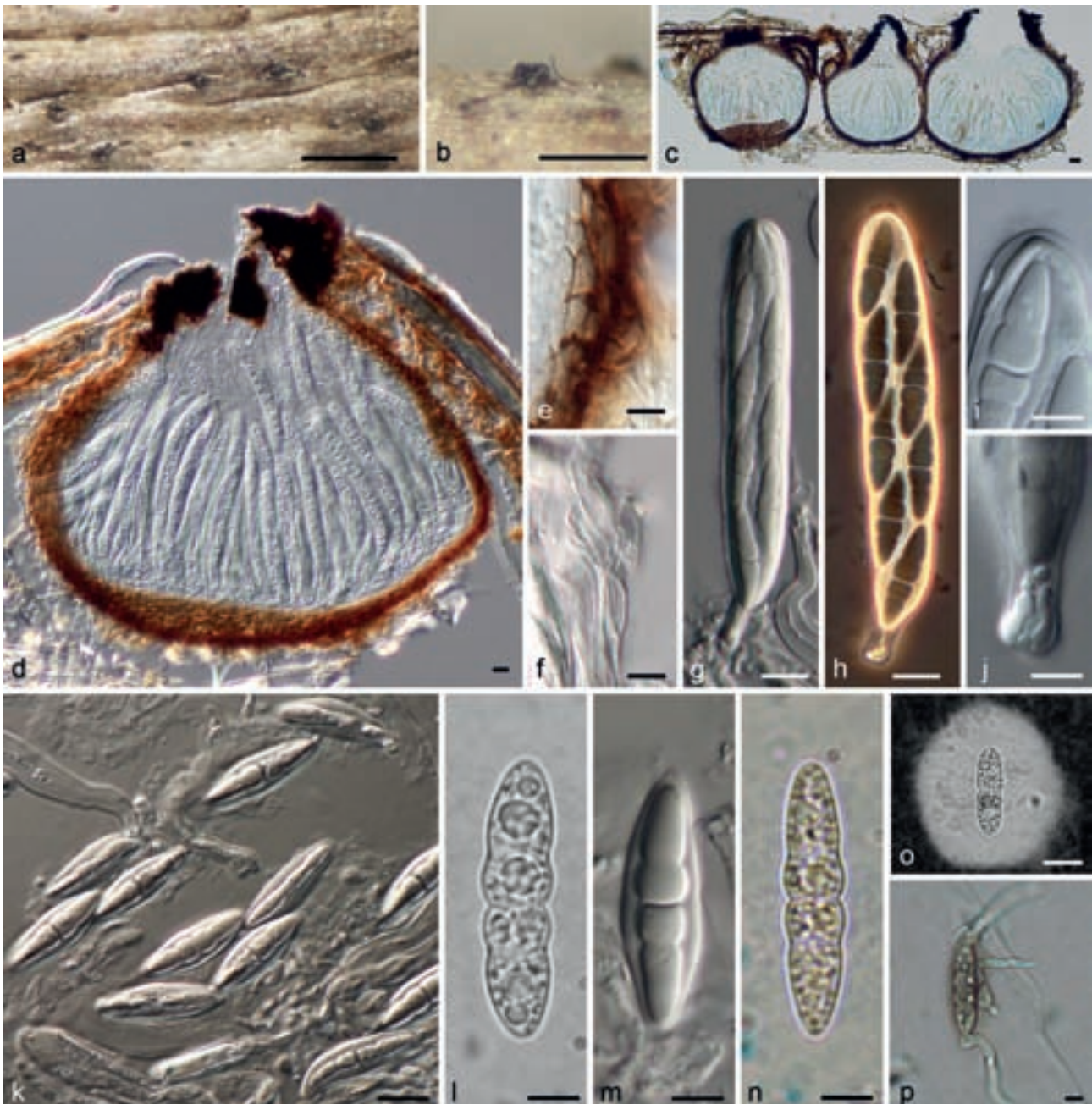


Fig. 12 *Pseudolophiotrema elymicola*. a–b. Appearance of ascomata on substrate; c–d. ascomata in longitudinal section; e. peridium of ascoma; f. pseudoparaphyses; g–h. asci; i. ascus apex; j. ascus stipe; k–n. ascospores; o. ascospore with a gelatinous sheath (in India ink); p. germinating ascospore (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 asci 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 *Pleomassarinae* 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 ascomata with slit-like ostioles in *Lophiotremataceae*, such as ascomata with a distinct clypeus around the ostiolar neck (in *Cryptoclypeus*, *Galeaticarpa* and *Pseudocryptoclypeus*, Fig. 7c, 8c, 9d, 10c), ascomata with a well-developed peridium (*Atrocalyx*, Fig. 4c, 5c), and ascomata 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 ascomata has been especially emphasised as a useful character for familial circumscription in ascomycetes (Chesters & Bell 1970). In earlier studies, *Lophiotrema* was placed in *Lophiostomataceae* 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 *Lophiostomataceae* 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 ascomata 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 ascomata 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 hysterothecial ascomata in *Anteagloniaceae* (Mugambi & Huhndorf 2009), *Gloniaceae* (Boehm et al. 2009), *Hysteriaceae* (Boehm et al. 2009), *Lophiostomataceae* (Thambugala et al. 2015), and *Mytiliniaceae* (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 *Botryosphaeriales*, 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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