



# Multi-locus phylogenetic analysis of lophiostomatoid fungi motivates a broad concept of *Lophiostoma* and reveals nine new species

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## Key words

*Lophiostomataceae*  
*Lophiotremataceae*  
molecular phylogeny  
new taxa  
*Pleosporales*  
taxonomy

**Abstract** Recent studies on the fungal families *Lophiostomataceae* and *Lophiotremataceae* (*Pleosporales*) have provided varying phylogenetic and taxonomic results concerning constituent genera and species. By adding DNA sequences of 24 new strains of *Lophiostomataceae* and nine new strains of *Lophiotremataceae* to a sequence data matrix from international databases, we provide a new understanding of the relationships within these families. Multigene analysis of the four molecular markers ITS, LSU, *TEF1-α*, and *RPB2* reveals that the genera within *Lophiotremataceae* are phylogenetically well supported. *Lophiostoma myriocarpum* is recognised as a species of *Lophiotrema* in contrast to earlier concepts. In *Lophiostomataceae*, we resurrect a broad generic concept of the genus *Lophiostoma* and reduce 14 genera to synonymy: *Alpestrisphaeria*, *Biappendiculispora*, *Capulatispora*, *Coelodictyosporium*, *Guttulispora*, *Lophiohelichrysum*, *Lophiopoacea*, *Neopaucispora*, *Neotrematosphaeria*, *Platystomum*, *Pseudocapulatispora*, *Pseudolophiostoma*, *Pseudoplatystomum*, and *Sigarispora*. Nine new species are described based on molecular data and in most cases supported by morphological characters: *Antealophiotrema populicola*, *Atrocalyx nordicus*, *Lophiostoma carpini*, *Lophiostoma dictyosporium*, *Lophiostoma erumpens*, *Lophiostoma fusi-sporum*, *Lophiostoma jotunheimense*, *Lophiostoma plantaginis*, and *Lophiostoma submuriforme*. *Lophiostoma caespitosum* and *Lophiotrema myriocarpum* are lecto- and epitypified to stabilise their species concepts. High intraspecific variability of several morphological traits is common within *Lophiostomataceae*.

**Citation:** Andreassen M, Skrede I, Jaklitsch WM, et al. 2021. Multi-locus phylogenetic analysis of lophiostomatoid fungi motivates a broad concept of *Lophiostoma* and reveals nine new species. *Persoonia* 46: 240–271. <https://doi.org/10.3767/persoonia.2021.46.09>.  
Effectively published online: 12 June 2021 [Received: 10 January 2021; Accepted: 7 April 2021].

## INTRODUCTION

Species of *Lophiostomataceae* and *Lophiotremataceae* (*Pleosporales*, *Dothideomycetes*, *Ascomycota*) are saprobic and occur on twigs, stems and bark of woody plants and herbs in terrestrial and aquatic environments (Holm & Holm 1988, Ellis & Ellis 1997, Mugambi & Huhndorf 2009). They form attractive study organisms since they are in many cases well defined by crest-like ostioles and are therefore easily recognisable in the field. The two families also share immersed to erumpent ascomata, a carbonaceous peridium, cylindrical or clavate fissitunicate asci and hyaline to dark brown, one- to multiseptate ascospores.

Nitschke (1869) first recognised *Lophiostomataceae*, and Saccardo (1883) formally established the family, based on *Lophiostoma macrostomum* as type species. The genus *Lophiotrema* was traditionally considered in the family *Lophiostomataceae* (Barr 1992, Kirk et al. 2008, Lumbsch & Huhndorf 2009, Hirayama & Tanaka 2011) and was only recently established within the segregate family *Lophiotremataceae*, typified by

*Lophiotrema nucula* (Hirayama & Tanaka 2011). Proposed distinguishing morphological characters between *Lophiostoma* and *Lophiotrema* include ascospore colouration, peridium thickness and textura, ascus shape and stipe length, and mucilaginous layer and terminal appendages of ascospores (Saccardo 1878, Holm & Holm 1988, Barr 1992, Mathiassen 1993, Yuan & Zhao 1994, Tanaka & Harada 2003a, b, Tanaka & Hosoya 2008, Eriksson 2009, Hirayama & Tanaka 2011, Hashimoto et al. 2018). The entangled taxonomic history and superficial morphological resemblance of the two families motivated us to treat them in a common study.

Recent phylogenetic studies using molecular methods have enabled a more natural placement of many families, genera and species within *Pleosporales* (Schoch et al. 2009, Wijayawardene et al. 2020). However, phylogenetic analyses have in several cases used small monophyletic groups or single genera as the basis for new genera without due concern for the remainder of the original genus, thus generating paraphyletic and polyphyletic sister genera (Padamsee et al. 2008, Nuhn et al. 2013, Wu et al. 2014). Tanaka and co-workers (Hirayama & Tanaka 2011, Thambugala et al. 2015, Hashimoto et al. 2017, 2018) have provided a substantial amount of DNA data for numerous representatives of both families, but for other taxa, if at all, often only LSU or ITS is available. By sampling fresh material of mostly European specimens representing the two families, pure culture isolation, DNA sequencing and analyses, we aim to resolve the phylogenetic relationships within the families of *Lophiostomataceae* and *Lophiotremataceae*. Further, we aim

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**Table 1** Overview of PCR and sequencing primers that were used to obtain DNA sequences for specimens from the families *Lophiostomataceae* and *Lophiotremataceae*.

Region <sup>1</sup>	Primer sequence (5'–3')	Direction	Reference
ITS	ITS1: TCCGTAGGTGAACCTGCGG	forward	White et al. (1990)
	ITS4: TCCTCCGCTTATTGATATGC	reverse	White et al. (1990)
LSU	V9G: TTACGTCCCTGCCCTTTGTA	forward	De Hoog & Gerrits van den Ende (1998)
	LR2R: AAGAACTTTGAAAAGAG	forward	Vilgalys & Hester (1990)
	LR5: TCCTGAGGGAAACTTCG	reverse	Vilgalys & Hester (1990)
	LR3: GGTCCGTGTTTCAAG	reverse	Vilgalys & Hester (1990)
TEF1-α	EF1-728F: CATCGAGAAGTTCGAGAAG	forward	Carbone & Kohn (1999)
	TEF1-LLErev: AAC TTGCAGGCAATGTGG	reverse	Jaklitsch et al. (2005)
RPB2	rRPB2-5: GAYGAYMGWGATCAYTTYGG	forward	Novakova et al. (2012)
	rRPB2-7C: CCCATRGCTTGYTTRCCCAT	reverse	Novakova et al. (2012)

<sup>1</sup> ITS: Internal transcribed spacer region (ITS1, 5.8S and ITS2); LSU: 28S large subunit ribosomal RNA; *TEF1-α*: translation elongation factor 1-alpha; *RPB2*: RNA polymerase II, second largest subunit.

to revisit the numerous newly proposed genera of *Lophiostomataceae* based on an extended dataset and to re-evaluate and discuss the suitability of morphological characters used for delimitation of species and genera.

MATERIALS AND METHODS

Taxon selection and sampling

Between September 2018 and September 2019, we collected specimens of *Lophiostomataceae* and *Lophiotremataceae* in the relatively continental to suboceanic eastern parts, the western oceanic lowlands, and alpine areas of southern Norway. Additional collections including type material were loaned from the fungaria in Oslo (O), Tromsø (TROM), and Geneva (G). A subset of unpublished data of strains from countries other than Scandinavia was also included.

Morphological investigation

Ascomata were rehydrated with autoclaved water and investigated using a Nikon SMZ 745T / Zeiss SteREO Discovery V8 dissecting microscope and a Nikon Eclipse Ci-L or a Zeiss Axio Imager A2 compound microscope. Images of ascomata were captured with a NIKON DS-Fi2 or Tucsen DigiRetina 16 camera, using stacking software Lite Helicon Focus 7 v. 7.5.6. The ascomata were dissected with a sterile razor blade or a Leitz 1320 Microtome cutter with a Leitz 1703 Kryomat as freezing element. Micro slides were created with contents of the ascomata mounted in sterile water or 5 % KOH. Indian Ink was used to detect mucilaginous sheaths, and in some cases, cotton blue reagent was added for improved visualization of spores and hymenial structures. Photomicrographs were produced using a Zeiss Axiocam 503 camera and measurements were made with Zeiss AxioVision v. 4.9.1 software (Carl Zeiss AG), and images were processed in GIMP v. 2.8.22 (Kimball & Mattis 1996).

Cultivation techniques

A selection of the collections was used for pure culture isolation from ascospores. Hymenial material was transferred into a sterile water droplet on a micro slide and transferred with a sterile pipette onto Petri dishes containing malt agar (MEA: 3 % malt extract, 1.5 % agar in water) and antibiotics (0.25 % Streptomycin, 0.1 % Tetramycin, 0.5 % Ampicillin). Petri dishes were incubated at 20 °C for spore germination and checked daily for growth under a dissecting microscope. Germinated spores were transferred individually onto MEA plates (without antibiotics), their growth monitored, contaminants removed, and pictures taken. Development of asexual morphs was documented for up to 1.5 yr.

For long time storage, smaller pieces of the cultures were isolated and transferred into Cryovial tubes holding harvesting medium (10 g sucrose, 1 g peptone, 100 mL water, autoclaved) for conservation at -80 °C at Oslo University. Representative isolates have been deposited at the Westerdijk Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands. Freshly collected specimens have been deposited in the Fungarium of Oslo, University of Oslo (O) or the Fungarium of the University of Vienna (WU).

DNA extraction and sequencing

DNA was extracted from cultured mycelia using the Phire Plant Direct PCR Kit (Thermo Scientific, Waltham, USA) following the manufacturer’s manuals for both DNA isolation and Polymerase Chain Reaction (PCR). Efforts were made to amplify the ribosomal DNA regions of internal transcribed spacer (ITS) containing ITS1, 5.8S and ITS2 and a fragment of the 28S large subunit ribosomal (LSU) for all sampled specimens. Subsequent regions of translation elongation factor 1-alpha (*TEF1-α*) and DNA-directed RNA polymerase II subunit (*RPB2*) were also amplified where possible. The primers used for PCR reactions are shown in Table 1.

The following PCR protocols were used to amplify the molecular regions: 2 min at 95 °C, 40 cycles of 15 s (20 s for *TEF1-α*) at 95 °C, denaturation for 15 s at 95 °C (20 s for *TEF1-α*), annealing at 20 s at 53 °C (30 s at 55 °C for *TEF1-α* and *RPB2*) and followed by an elongation for 1 min and 10 s at 70 °C (90 s for *TEF1-α* and 60 s for *RPB2*), with a terminal extension of 3 min at 70 °C. PCR products were checked with electrophoresis on 1.5 % agarose gels. Five µL PCR product was purified with 0.2 µL ExoSAP-IT (GE Healthcare, Waukesha, WI) and 1.8 µL water. Samples were then run on a thermocycler at 37 °C for 15 min, followed by 80 °C for 15 min. Cleaned PCR product was diluted with 45 µL water per sample. Five µL PCR product and 5 µL sequencing primer was added to clean tubes and labelled before sequencing. Sanger sequencing was performed by Eurofins, Luxemburg.

Sequence alignment and phylogenetic analyses

Sequence editing, assembly and concatenations were done using Geneious Prime v. 2020.0.5 (Kearse et al. 2012). Sequence data from Thambugala et al. (2015), Jaklitsch et al. (2016), Hashimoto et al. (2017), Wanasinghe et al. (2018), Bao et al. (2019), Hyde et al. (2019), and Phukhamsakda et al. (2020) were downloaded from GenBank (Table 2, 3). Preliminary alignments were made using Muscle v. 3.8.425 (Edgar 2004), with standard settings as incorporated in Geneious Prime. All alignments were inspected and manually adjusted.

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI). Substitution models

**Table 2** Fungal taxa, strains and GenBank accessions of *Lophiostomataceae* used. The strains and sequences generated in this current study are indicated in **bold**.

Taxa	Family	Strain no.	GenBank accession no.			
			ITS	LSU	<i>TEF1-α</i>	<i>RPB2</i>
<i>Crassiclypeus aquaticus</i>	<i>Lophiostomataceae</i>	KH 104	LC312499	LC312528	LC312557	LC312586
	<i>Lophiostomataceae</i>	KT 970	LC312501	LC312530	LC312559	LC312588
<i>Dimorphiopsis brachystegiae</i>	<i>Lophiostomataceae</i>	CPC 22679	KF777160	KF777213	–	–
<i>Flabellascoma aquaticum</i>	<i>Lophiostomataceae</i>	KUMCC 15-0258	MN304827	MN274564	MN328898	MN328895
<i>Flabellascoma cycadicola</i>	<i>Lophiostomataceae</i>	KT 2034	LC312502	LC312531	LC312560	LC312589
<i>Flabellascoma fusiforme</i>	<i>Lophiostomataceae</i>	MFLUCC 18-1584	MN304830	MN274567	MN328902	–
<i>Flabellascoma minimum</i>	<i>Lophiostomataceae</i>	KT 2013	LC312503	LC312532	LC312561	LC312590
	<i>Lophiostomataceae</i>	KT 2040	LC312504	LC312533	LC312562	LC312591
<i>Lentistoma bipolare</i>	<i>Lophiostomataceae</i>	KT 3056	LC312513	LC312542	LC312571	LC312600
	<i>Lophiostomataceae</i>	CBS 115375	LC312506	LC312535	LC312564	LC312593
<i>Leptoparies palmarum</i>	<i>Lophiostomataceae</i>	KT 1653	LC312514	LC312543	LC312572	LC312601
<i>Lophiostoma arundinis</i>	<i>Lophiostomataceae</i>	KT 606	JN942964	AB618998	LC001737	JN993482
	<i>Lophiostomataceae</i>	KT 651	JN942965	AB618999	LC001738	JN993486
<i>Lophiostoma biappendiculatum</i>	<i>Lophiostomataceae</i>	KT 975P	–	GU205228	–	–
	<i>Lophiostomataceae</i>	KT 1124	–	GU205227	–	–
<i>Lophiostoma caespitosum</i>	<i>Lophiostomataceae</i>	<b>CBS 147391</b>	<b>MW759252</b>	<b>MW750387</b>	<b>MW752404</b>	<b>MW752383</b>
	<i>Lophiostomataceae</i>	MFLUCC 13-0442	KP889134	KP888639	KR075161	–
	<i>Lophiostomataceae</i>	MFLUCC 14-0993	KP889135	KP888640	KR075162	–
<i>Lophiostoma carpini</i>	<i>Lophiostomataceae</i>	<b>CBS 147279</b>	<b>MW759258</b>	<b>MW750386</b>	<b>MW752405</b>	<b>MW752384</b>
<i>Lophiostoma caryophyllacearum</i>	<i>Lophiostomataceae</i>	MFLUCC 17-0749	MG828964	MG829076	MG829238	–
<i>Lophiostoma caudatum</i>	<i>Lophiostomataceae</i>	KT 530	LC001723	AB619000	LC001739	–
<i>Lophiostoma caulium</i>	<i>Lophiostomataceae</i>	MFLUCC 15-0036	MG828965	MG829077	MG829239	–
	<i>Lophiostomataceae</i>	KT 603	LC001724	AB619001	LC001740	–
	<i>Lophiostomataceae</i>	KT 633	LC001725	AB619002	LC001741	–
	<i>Lophiostomataceae</i>	MFLUCC 15-0176	–	KT328493	–	–
<i>Lophiostoma cf. clavatum</i>	<i>Lophiostomataceae</i>	<b>CBS 147278</b>	<b>MW759259</b>	<b>MW750385</b>	<b>MW752406</b>	<b>MW752385</b>
<i>Lophiostoma clavatum</i>	<i>Lophiostomataceae</i>	MFLUCC 18-1316	–	MN274566	MN328901	–
<i>Lophiostoma clematidicola</i>	<i>Lophiostomataceae</i>	MFLUCC 16-0446	MT310609	MT214563	MT394742	–
<i>Lophiostoma clematidis</i>	<i>Lophiostomataceae</i>	MFLUCC 17-2081	MN393004	MT214562	MT394741	MT394689
<i>Lophiostoma clematidis-subumbellatae</i>	<i>Lophiostomataceae</i>	MFLUCC 17-2063	MT310607	MT214560	MT394739	MT394687
<i>Lophiostoma clematidis-vitalbae</i>	<i>Lophiostomataceae</i>	MFLUCC 16-1368	MT310610	MT214564	MT394743	–
<i>Lophiostoma compressum</i>	<i>Lophiostomataceae</i>	<b>CBS 147536</b>	<b>MW759267</b>	<b>MW750388</b>	<b>MW752402</b>	–
	<i>Lophiostomataceae</i>	<b>CBS 147538</b>	<b>MW759268</b>	–	–	–
	<i>Lophiostomataceae</i>	<b>CBS 147537</b>	<b>MW759269</b>	–	<b>MW752399</b>	–
	<i>Lophiostomataceae</i>	<b>CBS 147519</b>	<b>MW759264</b>	<b>MW750393</b>	<b>MW752393</b>	–
	<i>Lophiostomataceae</i>	<b>CBS 147520</b>	<b>MW759266</b>	<b>MW750395</b>	<b>MW752391</b>	–
	<i>Lophiostomataceae</i>	<b>CBS 147521</b>	<b>MW759265</b>	<b>MW750396</b>	<b>MW752390</b>	–
	<i>Lophiostomataceae</i>	<b>CBS 147615</b>	<b>MW759263</b>	<b>MW750397</b>	<b>MW752389</b>	–
	<i>Lophiostomataceae</i>	<b>CBS 147276</b>	<b>MW759272</b>	<b>MW750382</b>	<b>MW752408</b>	<b>MW752381</b>
	<i>Lophiostomataceae</i>	<b>CBS 147390</b>	<b>MW759271</b>	<b>MW750383</b>	–	–
	<i>Lophiostomataceae</i>	<b>TEQ</b>	<b>MW759270</b>	<b>MW750398</b>	–	–
	<i>Lophiostomataceae</i>	IFRD 2014	–	FJ795437	–	FJ795457
	<i>Lophiostomataceae</i>	MFLUCC 13-0343	–	KP888643	KR075165	–
<i>Lophiostoma cornisporum</i>	<i>Lophiostomataceae</i>	KH 322	LC312515	LC312544	LC312573	LC312602
<i>Lophiostoma coronillae</i>	<i>Lophiostomataceae</i>	MFLUCC 14-0941	KT026120	KT026112	–	–
<i>Lophiostoma crenatum</i>	<i>Lophiostomataceae</i>	AFTOL-ID 1581	–	DQ678069	DQ677912	DQ677965
<i>Lophiostoma dictyosporum</i>	<i>Lophiostomataceae</i>	<b>CBS 147389</b>	<b>MW759251</b>	<b>MW750379</b>	<b>MW752411</b>	<b>MW752388</b>
<i>Lophiostoma erumpens</i>	<i>Lophiostomataceae</i>	<b>CBS 147275</b>	<b>MW759262</b>	<b>MW750381</b>	<b>MW752409</b>	<b>MW752386</b>
<i>Lophiostoma fusisporum</i>	<i>Lophiostomataceae</i>	<b>CBS 147891</b>	<b>MW759253</b>	–	<b>MW752401</b>	<b>MW752382</b>
<i>Lophiostoma helichrysi</i>	<i>Lophiostomataceae</i>	IT-1296	KT333435	KT333436	KT427535	–
<i>Lophiostoma heterosporum</i>	<i>Lophiostomataceae</i>	AFTOL-ID 1036	GQ203795	AY016369	DQ497609	DQ497615
<i>Lophiostoma japonicum</i>	<i>Lophiostomataceae</i>	KT 686-1	LC001729	AB619006	LC001745	–
	<i>Lophiostomataceae</i>	MFLUCC 17-2450	MN304829	–	MN328900	–
	<i>Lophiostomataceae</i>	KT 573	LC001728	AB619005	LC001744	–
	<i>Lophiostomataceae</i>	KT 794	LC001730	AB619007	LC001746	–
<i>Lophiostoma jonesii</i>	<i>Lophiostomataceae</i>	GAAZ 54-1	KX687757	KX687753	KX687759	–
	<i>Lophiostomataceae</i>	GAAZ 54-2	KX687758	KX687754	KX687760	–
<i>Lophiostoma jotunheimense</i>	<i>Lophiostomataceae</i>	<b>CBS 147522</b>	<b>MW759261</b>	<b>MW750394</b>	<b>MW752392</b>	–
<i>Lophiostoma junci</i>	<i>Lophiostomataceae</i>	MFLUCC 14-0938	MG828966	MG829078	–	–
<i>Lophiostoma longiappendiculatum</i>	<i>Lophiostomataceae</i>	MFLUCC 17-1452	MT214368	MT214462	MT235783	–
	<i>Lophiostomataceae</i>	MFLUCC 17-1457	MT214369	MT214463	MT235784	MT235821
<i>Lophiostoma macrostomoides</i>	<i>Lophiostomataceae</i>	<b>CBS 147523</b>	<b>MW759256</b>	<b>MW750389</b>	–	–
	<i>Lophiostomataceae</i>	<b>CBS 147277</b>	<b>MW759257</b>	<b>MW750384</b>	<b>MW752407</b>	<b>MW752380</b>
	<i>Lophiostomataceae</i>	CBS 123097	–	FJ795439	GU456277	FJ795458
	<i>Lophiostomataceae</i>	GKM1159	–	GU385185	GU327778	–
	<i>Lophiostomataceae</i>	GKM1033	–	GU385190	GU327776	–
	<i>Lophiostomataceae</i>	GKM224N	–	GU385191	GU327777	–
	<i>Lophiostomataceae</i>	CBS121412	–	MH874664	–	–
	<i>Lophiostomataceae</i>	CBS113435	EU552157	EU552157	–	–
<i>Lophiostoma macrostomum</i>	<i>Lophiostomataceae</i>	KT 508	JN942961	AB619010	LC001751	JN993491
	<i>Lophiostomataceae</i>	KT 709/HHUF 27293	AB433276	AB433274	LC001753	JN993493
	<i>Lophiostomataceae</i>	KT 635/HHUF 27290	AB433275	AB433273	LC001752	JN993484
<i>Lophiostoma medicaginicola</i>	<i>Lophiostomataceae</i>	MFLUCC 17-0681	MG828967	MG829079	–	–
<i>Lophiostoma montanae</i>	<i>Lophiostomataceae</i>	MFLUCC16-0999	MT310611	MT214565	MT394744	–
<i>Lophiostoma multiseptatum</i>	<i>Lophiostomataceae</i>	CBS 623.86	–	GU301833	–	GU371791
	<i>Lophiostomataceae</i>	KT 604/JCM17668	LC001726	AB619003	LC001742	–

Table 2 (cont.)

Taxa	Family	Strain no.	GenBank accession no.			
			ITS	LSU	<i>TEF1-α</i>	<i>RPB2</i>
<i>Lophiostoma neomuriforme</i>	<i>Lophiostomataceae</i>	MFLUCC 13-0744	KY496740	KY496719	–	–
<i>Lophiostoma obtusisporum</i>	<i>Lophiostomataceae</i>	KT 3098	LC312519	LC312548	LC312577	LC312606
	<i>Lophiostomataceae</i>	KT 2838	LC312518	LC312547	LC312576	LC312605
<i>Lophiostoma ononidis</i>	<i>Lophiostomataceae</i>	MFLUCC 14-0613	KU243128	KU243125	KU243127	–
<i>Lophiostoma paramacrostromum</i>	<i>Lophiostomataceae</i>	MFLUCC 11-0463	–	KP888636	–	–
<i>Lophiostoma plantaginis</i>	<i>Lophiostomataceae</i>	<b>CBS 147527</b>	<b>MW759250</b>	<b>MW750378</b>	–	<b>MW752375</b>
<i>Lophiostoma pseudodictyosporium</i>	<i>Lophiostomataceae</i>	MFLUCC 13-0451	KR025858	KR025862	–	–
<i>Lophiostoma pseudomacrostromum</i>	<i>Lophiostomataceae</i>	<b>CBS 147524</b>	<b>MW759249</b>	<b>MW750390</b>	<b>MW752396</b>	–
	<i>Lophiostomataceae</i>	<b>CBS 147525</b>	<b>MW759255</b>	<b>MW750391</b>	<b>MW752395</b>	–
	<i>Lophiostomataceae</i>	<b>CBS 147526</b>	<b>MW759254</b>	<b>MW750392</b>	<b>MW752394</b>	–
<i>Lophiostoma ravennicum</i>	<i>Lophiostomataceae</i>	MFLUCC 14-0005	KP698413	KP698414	–	–
<i>Lophiostoma rosae-ecae</i>	<i>Lophiostomataceae</i>	MFLUCC 17-0807	MG828924	MG829033	MG829217	–
<i>Lophiostoma rosicola</i>	<i>Lophiostomataceae</i>	MFLU 15-1888	MG828968	MG829080	MG829240	–
<i>Lophiostoma sagittiforme</i>	<i>Lophiostomataceae</i>	KT 1934	AB369268	AB369267	LC001756	–
<i>Lophiostoma scabridisporum</i>	<i>Lophiostomataceae</i>	BCC 22835	–	GQ925844	GU479857	GU479830
	<i>Lophiostomataceae</i>	BCC 22836	–	GQ925845	GU479856	GU479829
<i>Lophiostoma scrophulariicola</i>	<i>Lophiostomataceae</i>	MFLUCC 17-0689	MG828969	MG829081	–	–
<i>Lophiostoma semiliberum</i>	<i>Lophiostomataceae</i>	KT 622	JN942966	AB619012	LC001757	JN993483
	<i>Lophiostomataceae</i>	KT 652	JN942967	AB619013	LC001758	JN993485
	<i>Lophiostomataceae</i>	KT 828	JN942970	AB619014	LC001759	JN993489
<i>Lophiostoma spartii-juncei</i>	<i>Lophiostomataceae</i>	MFLUCC 13-0351	KP899136	KP888641	KR075163	–
<i>Lophiostoma submuriforme</i>	<i>Lophiostomataceae</i>	<b>CBS 147274</b>	<b>MW759260</b>	<b>MW750380</b>	<b>MW752410</b>	<b>MW752387</b>
<i>Lophiostoma terricola</i>	<i>Lophiostomataceae</i>	SC-12	JN662930	JX985750	–	–
<i>Lophiostoma thymi</i>	<i>Lophiostomataceae</i>	MFLU 15-2131	MG828970	MG829082	MG829241	–
<i>Lophiostoma tropicum</i>	<i>Lophiostomataceae</i>	KH 352	LC312521	LC312550	LC312579	LC312608
	<i>Lophiostomataceae</i>	KT 3134	LC312522	LC312551	LC312580	LC312609
<i>Lophiostoma vitigenum</i>	<i>Lophiostomataceae</i>	HH 26930	LC001735	AB619015	LC001761	–
	<i>Lophiostomataceae</i>	HH 26931	LC001736	AB619016	LC001762	–
<i>Lophiostoma winteri</i>	<i>Lophiostomataceae</i>	KT 740	JN942969	AB619017	LC001763	JN993487
	<i>Lophiostomataceae</i>	KT 764	JN942968	AB619018	LC001764	JN993488
<i>Neovaginatispora clematidis</i>	<i>Lophiostomataceae</i>	MFLUCC 17-2156	MT310606	MT214559	MT394738	–
<i>Neovaginatispora fuckelii</i>	<i>Lophiostomataceae</i>	MFLUCC 17-1334	MN304828	MN274565	MN328899	MN328896
	<i>Lophiostomataceae</i>	CBS 101952	–	DQ399531	–	FJ795472
	<i>Lophiostomataceae</i>	KH 161	LC001731	AB619008	LC001749	–
	<i>Lophiostomataceae</i>	KT 634	LC001732	AB619009	LC001750	–
<i>Parapaucispora pseudoarmatispora</i>	<i>Lophiostomataceae</i>	KT 2237	LC100021	LC100026	LC100030	–
<i>Paucispora quadrispora</i>	<i>Lophiostomataceae</i>	KH 448	LC001733	LC001722	LC001754	–
	<i>Lophiostomataceae</i>	KT 843	LC001734	AB619011	LC001755	–
<i>Paucispora versicolor</i>	<i>Lophiostomataceae</i>	KH 110	AB918731	AB918732	LC001760	–
' <i>Platystomum</i> ' <i>actinidiae</i>	<i>Lophiostomataceae</i>	KT 521	JN942963	JN941380	LC001747	JN993490
	<i>Lophiostomataceae</i>	KT 534	JN942962	JN941379	LC001748	JN993492
' <i>Platystomum</i> ' <i>crataegi</i>	<i>Lophiostomataceae</i>	MFLUCC 14-0925	KT026117	KT026109	KT026121	–
' <i>Platystomum</i> ' <i>rosae</i>	<i>Lophiostomataceae</i>	MFLUCC 15-0633	KT026119	KT026111	–	–
' <i>Platystomum</i> ' <i>salicicola</i>	<i>Lophiostomataceae</i>	MFLUCC 15-0632	KT026118	KT026110	–	–
<i>Pseudopaucispora brunneospora</i>	<i>Lophiostomataceae</i>	KH 227	LC312523	LC312552	LC312581	LC312610
<i>Vaginatispora amygdali</i>	<i>Lophiostomataceae</i>	KT 2248	LC312524	LC312553	LC312582	LC312611
	<i>Lophiostomataceae</i>	MFLUCC 18-1526	MK085055	MK085059	MK087657	–
<i>Vaginatispora appendiculata</i>	<i>Lophiostomataceae</i>	MFLUCC 16-0314	KU743217	KU743218	KU743220	–
<i>Vaginatispora aquatica</i>	<i>Lophiostomataceae</i>	MFLUCC 11-0083	KJ591577	KJ591576	–	–
<i>Vaginatispora armatispora</i>	<i>Lophiostomataceae</i>	MFLUCC 18-0247	MK085056	MK085060	MK087658	MK087669
	<i>Lophiostomataceae</i>	MFLUCC 18-0213	MN304826	MN274563	MN328897	MN328894
<i>Vaginatispora microarmatispora</i>	<i>Lophiostomataceae</i>	MTCC 12733	MF142592	MF142593	MF142595	MF142596
<i>Vaginatispora scabrisspora</i>	<i>Lophiostomataceae</i>	KT 2443	LC312525	LC312554	LC312583	LC312612
<i>Teichospora rubriostiolata</i>	<i>Teichosporaceae</i>	TR7	KU601590	KU601590	KU601609	KU601599
<i>Teichospora tricolora</i>	<i>Teichosporaceae</i>	C134	KU601591	KU601591	KU601601	KU601600

for each locus were determined based on the AICc model selection criterion (small-sample-size corrected version of Akaike information criterion) as implemented in PartitionFinder v. 1.1.1 (Lanfear et al. 2016). The search was set to 'greedy' and branch lengths set to 'linked'. ML analyses were performed on aligned sequences using RAXML v. 8.2.11 (Stamatakis 2014) as implemented in Geneious. Rapid Bootstrapping and search for best-scoring ML tree algorithms were used and Bootstrap analyses obtained by 1000 bootstrap replications. To examine topological incongruence among datasets, ML bootstrapping analyses were carried out on each of the single-gene datasets. Topological incongruence was assumed if conflicting tree topologies were supported by  $\geq 70$  % ML support. Since topological incongruence could not be observed, maximum likelihood (ML) bootstrapping analyses were carried out on

the concatenated four-locus dataset for both *Lophiostomataceae* and *Lophiotremataceae* using the same settings as for the single-gene analyses. BI analyses were performed with MrBayes v. 3.2.6 (Huelsenbeck & Ronquist 2001) with substitution models for different regions selected with the AICc parameter. Metropolis-coupled Markov chain Monte Carlo (MCMC) runs were performed for 4 M generations with trees sampled every 1000 generations. Convergence of the MCMC procedure was assessed and effective sample (EES) size scores  $> 200$  checked by using the MrBayes build in Tracer v. 1.6 (Rambaut et al. 2018). The first 10 % of trees were discarded as burn-in, and the remaining trees were used to calculate 50 % majority rule trees and to determine posterior probabilities (PP) for individual branches. Output trees were edited with Inkscape v. 0.92.1 (Harrington et al. 2003).

**Table 3** Fungal taxa, strains and GenBank accessions of *Lophiotremataceae* used. The sequences generated in this current study are indicated in **bold**.

Taxa	Family	Strain no.	GenBank accession no.			
			ITS	LSU	<i>TEF1-α</i>	<i>RPB2</i>
<i>Antealophiotrema brunneosporum</i>	<i>Incertae sedis</i>	CBS 123095	LC194474	LC194340	LC194382	LC194419
<i>Antealophiotrema populicola</i>	<i>Incertae sedis</i>	<b>CBS 147528</b>	<b>MW759240</b>	<b>MW750371</b>	–	–
	<i>Incertae sedis</i>	<b>CBS 147529</b>	<b>MW759241</b>	<b>MW750372</b>	<b>MW752398</b>	<b>MW752378</b>
<i>Atrocalyx acutisporus</i>	<i>Lophiotremataceae</i>	KT 2436	LC194475	LC194341	LC194386	LC194423
<i>Atrocalyx asturiensis</i>	<i>Lophiotremataceae</i>	OF	MG912912	MG912912	MG912916	MG912920
<i>Atrocalyx bambusae</i>	<i>Lophiotremataceae</i>	MFLUCC 10-0558	KX672149	KX672154	KX672162	KX672161
<i>Atrocalyx lignicola</i>	<i>Lophiotremataceae</i>	CBS 122364	LC194476	LC194342	LC194387	LC194424
<i>Atrocalyx nordicus</i>	<i>Lophiotremataceae</i>	<b>CBS 147530</b>	<b>MW759244</b>	<b>MW750376</b>	–	–
	<i>Lophiotremataceae</i>	<b>CBS 147531</b>	<b>MW759246</b>	<b>MW750377</b>	–	–
	<i>Lophiotremataceae</i>	<b>CBS 147532</b>	<b>MW759243</b>	<b>MW750374</b>	–	<b>MW752379</b>
	<i>Lophiotremataceae</i>	<b>CBS 147533</b>	<b>MW759245</b>	<b>MW750375</b>	–	<b>MW752376</b>
<i>Crassimassarina macrospora</i>	<i>Lophiotremataceae</i>	KH 152	LC194477	LC194343	LC194388	LC194425
	<i>Lophiotremataceae</i>	KT 1764	LC194478	LC194344	LC194389	LC194426
<i>Cryptoclypeus oxysporus</i>	<i>Lophiotremataceae</i>	KT 2772	LC194479	LC194345	LC194390	LC194427
<i>Cryptoclypeus ryukyuensis</i>	<i>Lophiotremataceae</i>	AH 342	LC194480	LC194346	LC194391	LC194428
	<i>Lophiotremataceae</i>	KT 3534	LC194481	LC194347	LC194392	LC194429
<i>Galeaticarpa aomoriensis</i>	<i>Lophiotremataceae</i>	MAFF 245618	LC194482	LC194366	LC194393	LC194448
<i>‘Lophiotrema’ boreale</i>	<i>Incertae sedis</i>	CBS 114422	LC194491	LC194375	LC194402	LC194457
<i>Lophiotrema ‘eburnoides’</i>	<i>Lophiotremataceae</i>	KT 1424_1	LC001709	LC001707	LC194403	LC194458
<i>Lophiotrema fallopiae</i>	<i>Lophiotremataceae</i>	KT 2748	LC149913	LC149915	LC194404	LC194459
<i>Lophiotrema myriocarpum</i>	<i>Lophiotremataceae</i>	<b>CBS 147534</b>	<b>MW759247</b>	–	<b>MW752403</b>	–
	<i>Lophiotremataceae</i>	<b>CBS 147535</b>	<b>MW759248</b>	–	<b>MW752397</b>	<b>MW752377</b>
<i>Lophiotrema neoarundinariae</i>	<i>Lophiotremataceae</i>	KT 1034	LC194492	AB524598	LC194405	LC194460
	<i>Lophiotremataceae</i>	KT 2200	AB524787	AB524597	AB539110	AB539097
	<i>Lophiotremataceae</i>	KT 856	AB524786	AB524596	AB539109	AB539096
<i>Lophiotrema neohysterioides</i>	<i>Lophiotremataceae</i>	KH 17	LC194493	LC194376	LC194406	LC194461
	<i>Lophiotremataceae</i>	KT 588	LC194494	LC194377	LC194407	LC194462
	<i>Lophiotremataceae</i>	KT 713	LC194495	AB619019	LC194408	LC194463
	<i>Lophiotremataceae</i>	KT 756	LC194496	AB619020	LC194409	LC194464
<i>Lophiotrema nucula</i>	<i>Lophiotremataceae</i>	<b>MAL47</b>	<b>MW759242</b>	<b>MW750373</b>	<b>MW752400</b>	–
	<i>Lophiotremataceae</i>	CBS 627.86	LC194497	AB619021	LC194410	LC194465
<i>Lophiotrema vagabundum</i>	<i>Lophiotremataceae</i>	KH 164	LC194498	AB619022	LC194411	LC194466
	<i>Lophiotremataceae</i>	KH 172	LC194499	AB619023	LC194412	LC194467
	<i>Lophiotremataceae</i>	KT 664	LC194500	AB619024	LC194413	LC194468
	<i>Lophiotremataceae</i>	KT 3310	LC194501	LC194378	LC194414	LC194469
	<i>Lophiotremataceae</i>	CBS 113975	LC194502	AB619025	LC194415	LC194470
<i>Pseudocryptoclypeus yakushimensis</i>	<i>Lophiotremataceae</i>	KT 2186	LC194504	LC194380	LC194417	LC194472

This study follows the guidelines for proposing new genera sensu Vellinga et al. (2015) and adapted by Tulloss et al. (2016). We also follow the concept of Genealogical Concordance Phylogenetic Species Recognition (Taylor et al. 2000, Dettman et al. 2003) and embrace the Consolidated Species Concept (Quaedvlieg et al. 2014).

The following sequences were omitted from our phylogenetic analyses of *Lophiostomataceae*: Three sequences were identified as wrongly labelled and thus not included: *L. viridarium* (IFRDCC 2090) and *L. triseptatum* (SMH 2591, SMH 5287). Also, two taxa from Mugambi & Huhndorf (2009) from Kenya labelled with names of European taxa were considered questionable and were omitted: *L. alpigenum* (GKM 1091b) and *L. quadrinucleatum* (GKM 1233).

RESULTS

Phylogenetic analyses

From 68 strains initially targeted for multi-locus sequencing, a total of 33 ITS, 28 LSU, 23 *TEF1-α*, 14 *RPB2* consensus sequences were produced (Table 2, 3). The protein coding locus *RPB2* proved especially challenging to amplify.

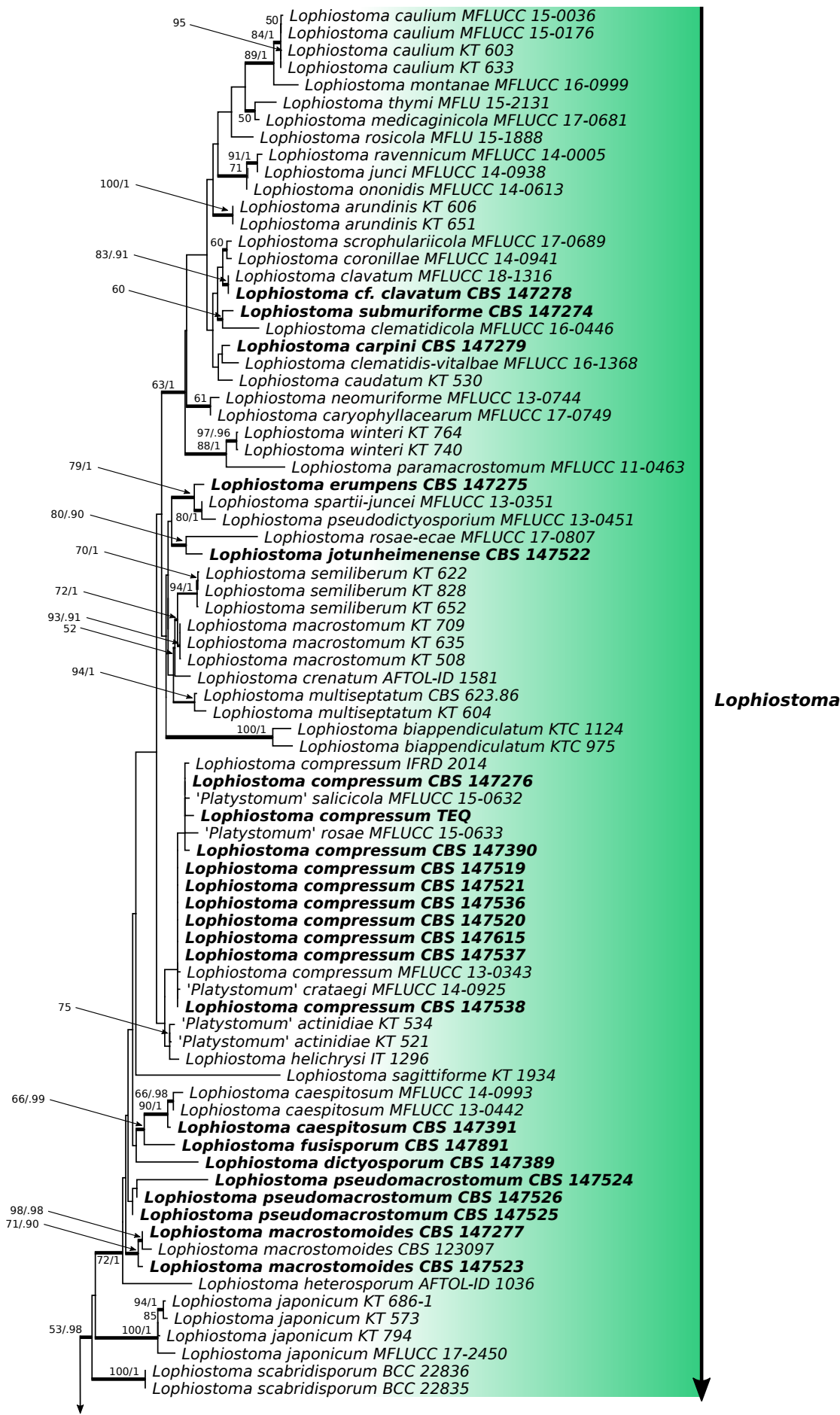
The concatenated alignment for *Lophiostomataceae* comprised 3161 nucleotide characters, including gaps (5.8S and ITS2: 1–410; LSU: 411–1251; *TEF1-α*: 1252–2148; *RPB2*: 2149–3161). The alignment included 24 new strains representing 12 taxa. In total, the alignment was composed of 124 strains of the *Lophiostomataceae*, and the two taxa *Teichospora rubriostiolata*

(TR7) and *Teichospora traticola* (C134) as the outgroup. ITS1 was excluded from the analyses because it contained too many ambiguously aligned regions.

The concatenated alignment for *Lophiotremataceae* comprised 3657 nucleotide characters, including gaps (ITS: 1–463; LSU: 464–1717; *TEF1-α*: 1718–2638; *RPB2*: 2639–3657). The alignment included nine new strains representing four taxa. In total the alignment was composed of 37 strains, including four strains of *Antealophiotrema* (*Antealophiotrema brunneosporum* CBS 123095, *Antealophiotrema populicola* CBS 147528 and CBS 147529, *‘Lophiotrema’ boreale* CBS 114422) as the outgroup taxa.

The maximum likelihood (ML) analysis of the combined datasets yielded the best scoring trees for *Lophiostomataceae* (Fig. 1) and *Lophiotremataceae* (Fig. 2). Also, the Bayesian inference (BI) analysis showed congruence with the topology of the ML analyses, and for simplicity, only the ML trees are shown. Values for both MLB above 50 % and Bayesian posterior probabilities (BPP) higher than 0.90 are given at the nodes. The alignments had 33.31 % and 13.69 % undetermined nucleotide gaps for *Lophiostomataceae* and *Lophiotremataceae*, respectively.

*Pseudopaucispora brunneospora* formed a completely supported clade and sister group to all other *Lophiostomataceae* taxa (MLP 100 % and BPP 1). The genus *Lophiostoma* formed a highly supported sister group to the remaining genera of the *Lophiostomataceae*, viz. *Crassiclypeus*, *Dimorphiopsis*, *Flabellascoma*, *Lentistoma*, *Leptoparies*, *Neovaginatispora*, *Parapaucispora*, *Paucispora*, and *Vaginatispora*. These other



**Fig. 1** Maximum likelihood phylogeny of *Lophiostomataceae* based on ITS2, 5.8S, LSU, *TEF1-α* and *RPB2* combined sequence data. Numbers above branches indicate Maximum likelihood RAxML bootstrap values above 50 % and Bayesian posterior probabilities higher than 0.90 are given at the nodes. Branches supported by ML bootstrap analyses (> 50) are thickened. Newly obtained strains are shown in **bold**. Shorted nodes are marked with crossing lines and indications (x2, x4) of how many times the node has been shortened.



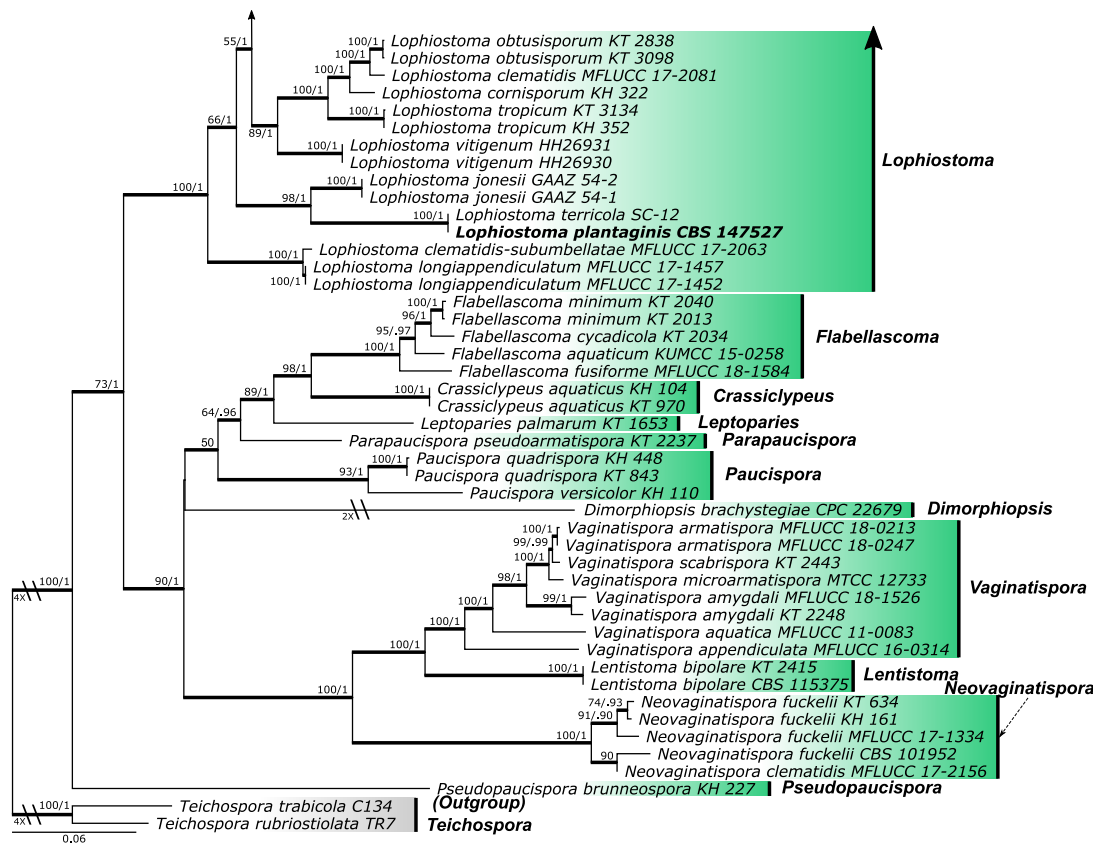


Fig. 1 (cont.)

genera were well supported. Within *Lophiostoma*, most clades containing several taxa were unsupported, and therefore there is no basis for a subdivision into several genera.

Our analyses showed support for seven new species within *Lophiostomataceae*, represented by the strains CBS 147522 (MAL88), CBS 147891 (MAL04), CBS 147527 (MAL92), CBS 147389 (C191), CBS 147274 (C217), CBS 147275 (C220), and CBS 147279 (LQ1), respectively (see Fig. 1). The phylogenetic analyses of the *Lophiotremataceae* revealed a tree, which comprises a well-supported clade of the genus *Lophiotrema* as a sister group of the remaining genera of *Lophiotremataceae*. As a result, *Lophiotrema myriocarpum* is now recognised in *Lophiotrema* after it was considered for a long time to be a species of *Lophiostoma* (Holm & Holm 1988). In the genus *Atrocalyx*, the strains CBS 147530 (MAL20), CBS 147531 (MAL21), CBS 147532 (MAL27), and CBS 147533 (MAL76) formed a strongly supported clade. The morphology of this group is consistent and distinct from the other *Atrocalyx* species, and we therefore described this clade as a new species. Also, within the outgroup, *Antealophiotrema*, a new species represented by the strains CBS 147528 (MAL63) and CBS 147529 (MAL64) is supported by phylogeny and morphology.

**Taxonomy**

The genera *Alpestrisphaeria*, *Biappendiculispora*, *Capulatispora*, *Coelodictyosporium*, *Guttulispora*, *Lophiohelichrysum*, *Lophiopoacea*, *Neopaucispora*, *Neotrematosphaeria*, *Platystomum*, *Pseudocapulatispora*, *Pseudolophiostoma*, *Pseudoplatystomum*, and *Sigarispora* are synonymised with *Lophiostoma* based on molecular phylogeny and morphology.

***Lophiostomataceae*** Sacc., Syll. Fung. (Abellini) 2: 672. 1883 — MycoBank MB 561063

Type genus. *Lophiostoma* Ces. & De Not., Comment. Soc. Crittog. Ital. 1 (4): 219. 1863. MycoBank MB 2933.

***Lophiostoma*** Ces. & De Not., Comment. Soc. Crittog. Ital. 1 (4): 219. 1863 — MycoBank MB 2933

Synonyms. *Alpestrisphaeria* Thambug. & K.D. Hyde, Fungal Diversity 74: 214. 2015. — Index Fungorum IF 551232.

*Biappendiculispora* Thambug. et al., Fungal Diversity 74: 214. 2015. — MycoBank MB 551528.

*Capulatispora* Thambug. & K.D. Hyde, Fungal Diversity 74: 216. 2015. — MycoBank MB 551234.

*Coelodictyosporium* Thambug. & K.D. Hyde, Fungal Diversity 74: 218. 2015. — MycoBank MB 551286.

*Guttulispora* Thambug. et al., Fungal Diversity 74: 220. 2015. — MycoBank MB 551238.

*Lophiohelichrysum* Dayar. et al., Fungal Diversity 75: 85. 2015. — MycoBank MB 551400.

*Lophiopoacea* Ariyaw. et al., Fungal Diversity 74: 220. 2015. — MycoBank MB 551240.

*Neopaucispora* Wanas. et al., Fungal Diversity 89: 65. 2018. — MycoBank MB 554146.

*Neotrematosphaeria* Thambug. et al., Fungal Diversity 74: 223. 2015. — MycoBank MB 551242.

*Platystomum* Trevis., Bull. Soc. Roy. Bot. Belgique 16: 16. 1877. — MycoBank MB 4185.

*Pseudocapulatispora* Mapook & K.D. Hyde, Fungal Diversity 101: 47. 2020. — MycoBank MB 557285.

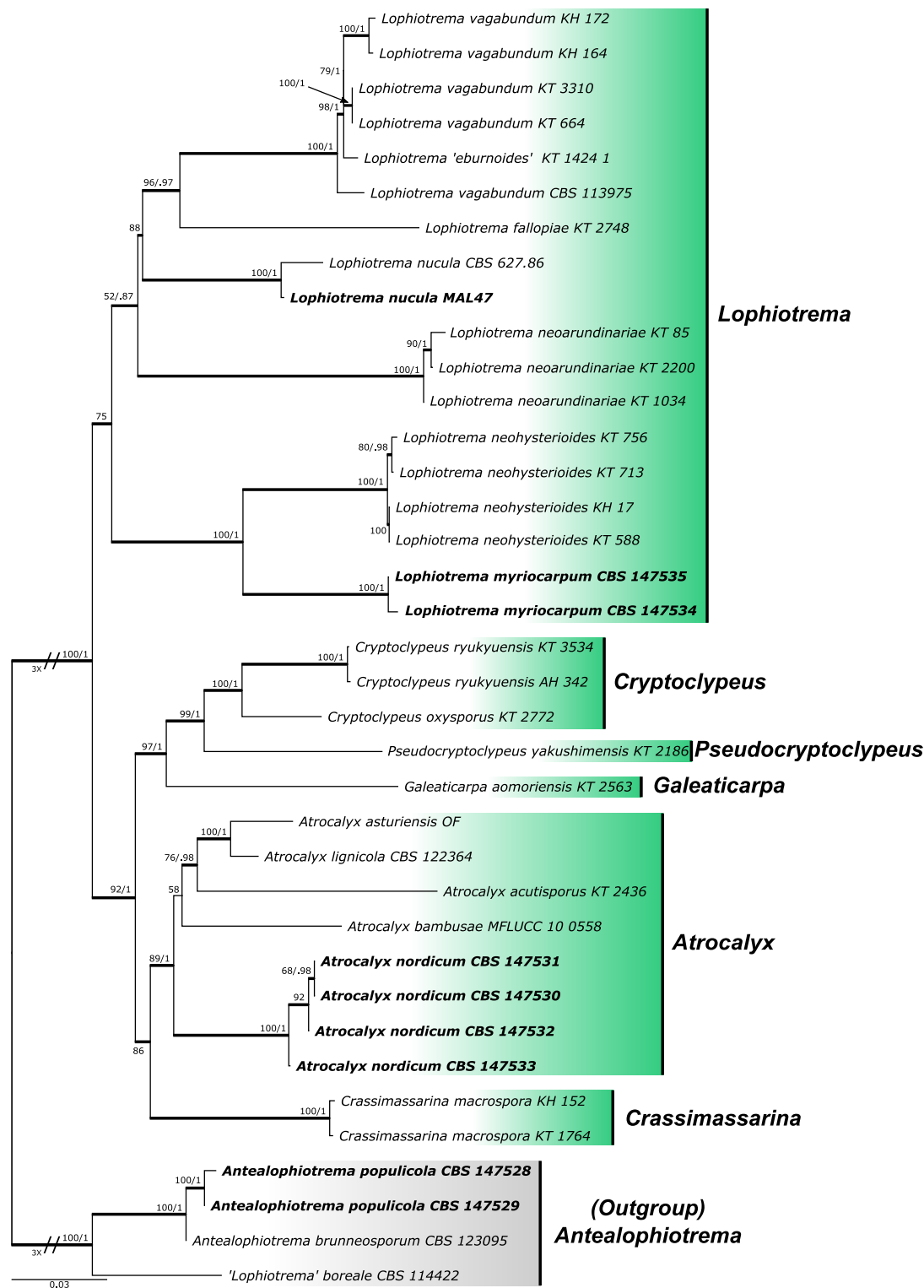
*Pseudolophiostoma* Thambug. et al., Fungal Diversity 74: 235. 2015. — MycoBank MB 551250.

*Pseudoplatystomum* Thambug. & K.D. Hyde, Fungal Diversity 74: 237. 2015. — MycoBank MB 551253.

*Sigarispora* Thambug. & K.D. Hyde, Fungal Diversity 74: 238. 2015. — MycoBank MB 551255.

Type species. *Lophiostoma macrostomum* (Tode) Ces. & De Not., Comment. Soc. Crittog. Ital. 1 (fasc. 4): 219. 1863. — MycoBank MB 422081. (based on *Sphaeria macrostoma* Tode, Fung. Mecklenb. Sel. (Lüneburg) 2: 12. 1791. — MycoBank MB 149287.

*Ascomata* scattered to crowded, immersed to semi-immersed, coriaceous to carbonaceous, dark brown to black, globose to subglobose, ostiolate. *Ascoma* apex crest-like, variable in shape, carbonaceous, with a pore-like ostiole. *Ostiole canal*



**Fig. 2** Maximum likelihood phylogeny of *Lophiostromataceae* based on ITS2, 5.8S, LSU, *TEF1-α* and *RPB2* combined sequence data. Numbers above branches indicate Maximum likelihood RAXML bootstrap values above 50 % and Bayesian posterior probabilities higher than 0.90 are given at the nodes. Branches supported by ML bootstrap analyses (> 50) are thickened. Newly obtained strains are shown in **bold**. Shorted nodes are marked with crossing lines and indications (x3) of how many times the node has been shortened.

rounded, periphysate. *Peridium* thicker at the apex and thinner at the base, composed of several layers of lightly pigmented to dark brown, thin-walled cells of *textura angularis* to *textura prismatica*, cells towards the inside lighter, sometimes fusing with and becoming indistinguishable from the host tissues. *Hamathecium* comprising septate, branched, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 6–8-spored, bitunicate, fissitunicate, clavate to almost cylindrical, short to long pedicellate, rounded at the apex, with an ocular chamber.

*Ascospores* partially biseriate, sometimes uniseriate, hyaline to brown, fusiform to ellipsoid, with narrow acute to rounded ends, 1- to multi-septate or muriform, constricted at the central septum, with or without terminal appendages.

**Ecology** — Saprobic on woody and herbaceous substrates in terrestrial and aquatic habitats.



***Lophiostoma biappendiculatum*** (Kaz. Tanaka et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838972

*Basionym.* *Trematosphaeria biappendiculata* Kaz. Tanaka et al., Fungal Diversity 19: 149. 2005. — MycoBank MB 343643.

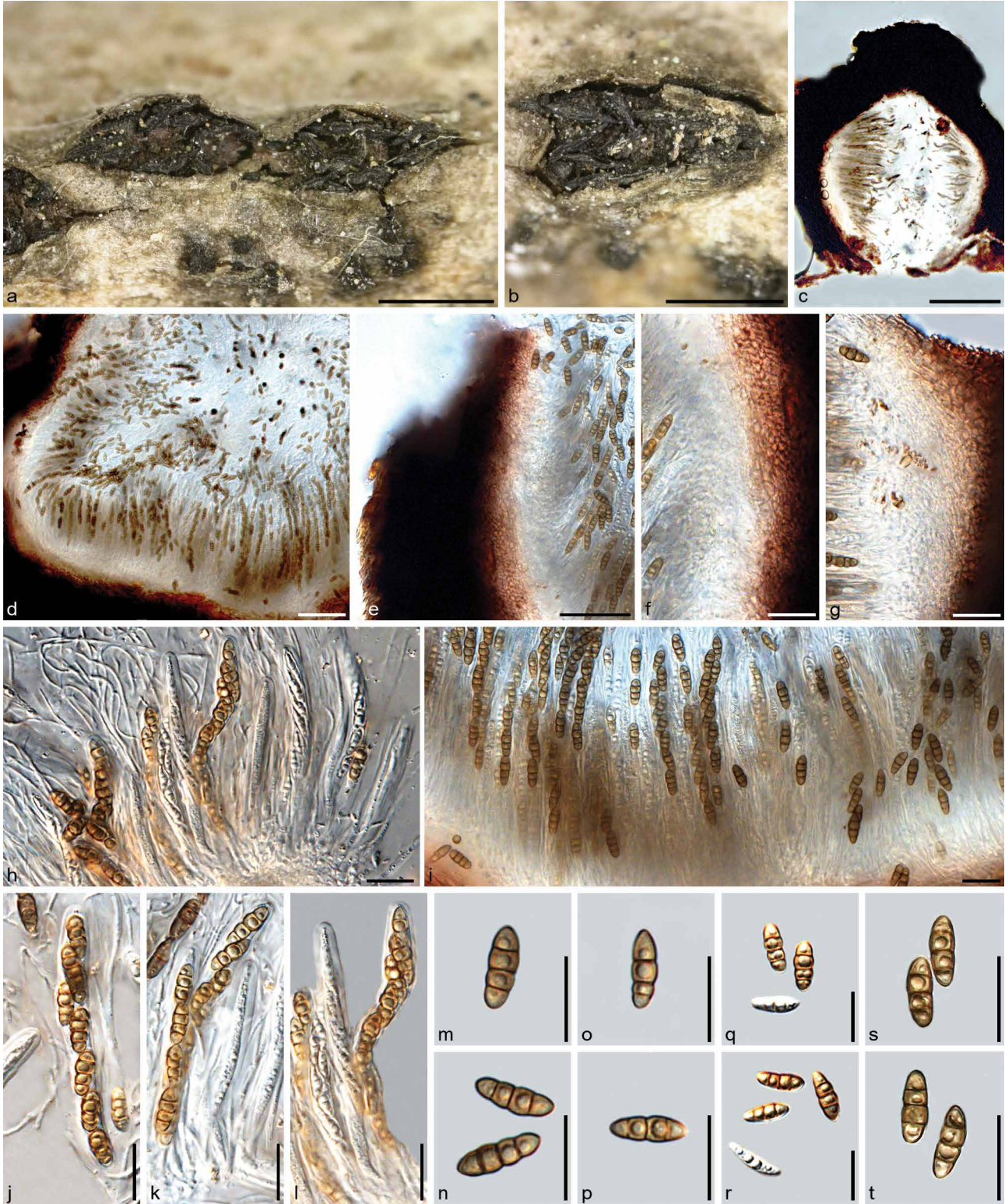
*Synonym.* *Neotrematosphaeria biappendiculata* (Kaz. Tanaka et al.) Thambug. et al., Fungal Diversity 74: 225. 2015. — MycoBank MB 551243.

**Notes** — The overall placement of the strains KTC 975 and KTC 1124 is uncertain due to the absence of the molecular markers of ITS, *TEF1-α*, and *RPB2*. Still, the species clearly

belongs within the genus *Lophiostoma*. When LSU is included in the phylogenetic analyses, *L. biappendiculatum* has been revealed as sister species to *L. pseudodictyosporium* with low support (Thambugala et al. 2015, Bao et al. 2019).

***Lophiostoma caespitosum*** Fuckel, Jahrb. Nassauischen Vereins Naturk. 27–28: 29. 1874 — MycoBank MB 189009; Fig. 3

*Synonym.* *Guttulispora crataegi* Qing Tian et al., Fungal Diversity 74: 220. 2015. — MycoBank MB 551239.



**Fig. 3** *Lophiostoma caespitosum*. a–r. O-F-256902 - epitype. s–t. G00266553 - holotype. a–b. Cespitose ascomatal necks; c–d. section of ascomata; e–g. peridium; h–i. hymenium and pseudoparaphyses; j–l. asci; m–t. ascospores. — Scale bars: a–b = 1000 μm; c = 150 μm; d–e, h–i = 40 μm; f–g = 30 μm; j–t = 20 μm.



*Typus.* SWITZERLAND, Suisse, at Neuchatel, on cortex of *Crataegus*, Feb. 1872, *Morthier* (G00266553- lectotype designated here; MBT 10000314). — AUSTRIA, Vienna, 22nd district, Lobau, Panozzalacke, on attached branches of *Crataegus monogyna*, 20 Nov. 2016, *W. Jaklitsch & H. Voglmayr* (O-F-256902, epitype designated here, MBT 10000315; ex-epitype culture CBS 147391 = LQ2; WU 37933, isoeptype designated here).

**Sexual morph:** *Ascomata* 166–323 µm diam, 150–300 µm high, solitary to most frequently cespitose, immersed, coriaceous to carbonaceous, dark brown to black, globose to subglobose, ostiolate. *Ascoma apex* central, papillate, crest-like, carbonaceous, rather small, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* 30–100 µm thick, composed of several layers, with dark to reddish brown, thick-walled cells of *textura angularis*, becoming lighter towards the inside, somewhat compressed. *Hamathecium* comprising septate, branched, cellular pseudoparaphyses, longer than the asci, embedded in a gelatinous matrix. *Asci* (92–)96–122(–134) × (8–)9–12(–14) µm (n = 20), bitunicate, fissitunicate, narrowly clavate, short-pedicellate, apex rounded, with an ocular chamber, with 8 partially overlapping uniseriate to obliquely biseriate ascospores. *Ascospores* (16–)17–20(–22) × (5–)6–8 µm (n = 62), hyaline when immature and becoming dark brown when mature, ellipsoid to fusiform, 3-septate, constricted at each septum, upper part and particularly the second cell slightly wider, guttulate, smooth-walled, lacking a mucilaginous sheath.

**Ecology** — Saprobiic on dead attached branches of *Crataegus monogyna*.

**Additional materials examined.** AUSTRIA, Vienna, 19th district, Himmelstraße, on attached branches of *Crataegus monogyna*, 18 Mar. 2020, *W. Jaklitsch* (WU 37935); 22nd district, Lobau, between Panozzalacke and the OMV fuel depot, on attached branches of *Crataegus monogyna*, 14 Mar. 2020, *W. Jaklitsch* (WU 37934).

**Notes** — There are several syntypes of *Lophiostoma caespitosum* (e.g., G00266553, S-F-7223), of which we here select collection G00266553 as lectotype. Morphologically, the lectotype material of *L. caespitosum* (G00266553) fully agrees

with our material, and we epitypify the species with collection O-F-256902 to stabilise the species concept. Ascospores of *L. caespitosum* are similar to those of *L. quadrinucleatum*, but are significantly smaller, as already mentioned by Holm & Holm (1988). This species is specific for *Crataegus monogyna* and occurs on dead attached branches and twigs of this host very commonly in the east of Vienna along the river Danube. The host, spore size, shape and colour make this species characteristic within *Lophiostoma*. The name *Guttulispora crataegi* (Thambugala et al. 2015), which was based on two Italian specimens (isolates MFLUCC 13-0442 and MFLUCC 14-0993), is a synonym of *L. caespitosum*, as based on phylogenetic and morphological evidence. *Guttulispora crataegi* is the generic type of *Guttulispora*, which we synonymise with *Lophiostoma*, based on molecular phylogeny and morphology.

***Lophiostoma caryophyllacearum*** (Wanas. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838971

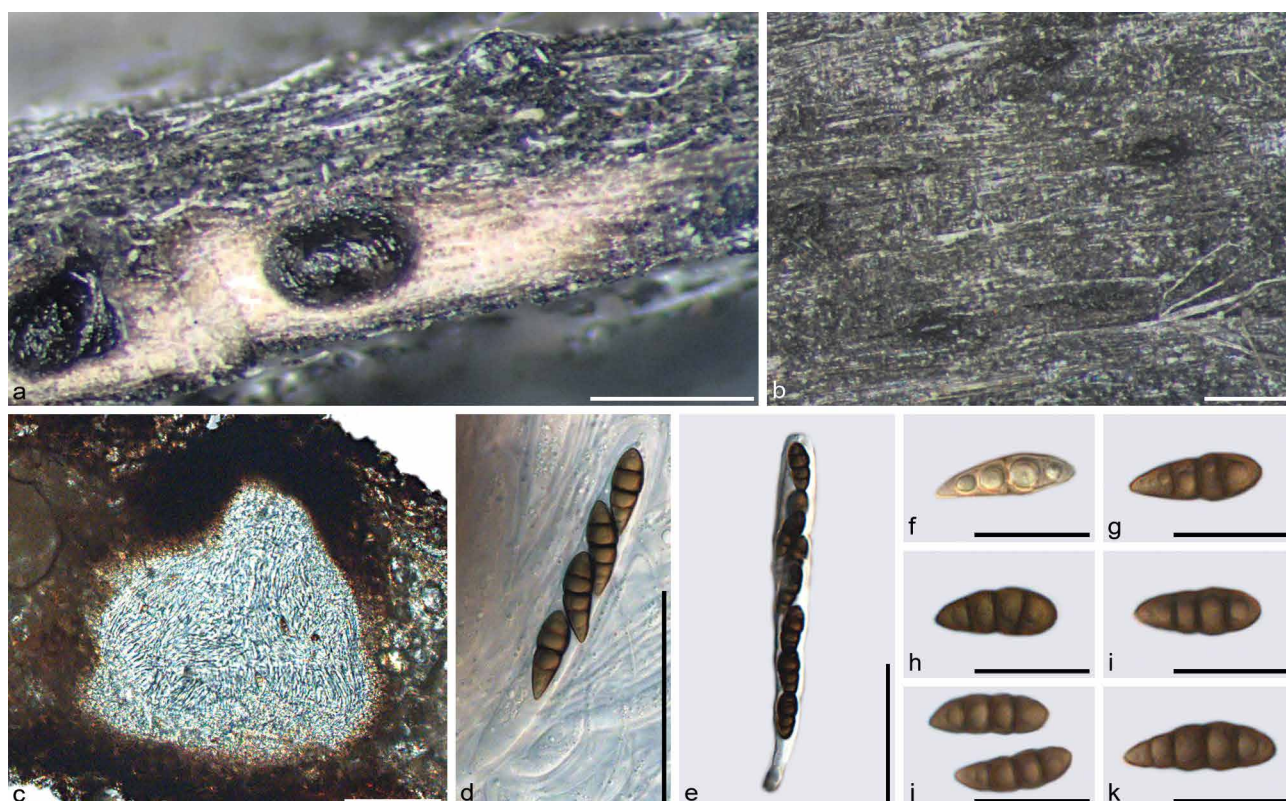
*Basionym.* *Sigarispora caryophyllacearum* Wanas. et al., Fungal Diversity 89: 67. 2018. — MycoBank MB 554148.

***Lophiostoma carpini*** Andreasen, Jaklitsch & Voglmayr, *sp. nov.* — MycoBank MB 838973; Fig. 4

*Etymology.* With reference to its host *Carpinus betulus*.

*Typus.* AUSTRIA, Niederösterreich, Mannersdorf, at a wood pile, on *Carpinus betulus*, 17 Sept. 2015, *W. Jaklitsch & H. Voglmayr* (O-F-256904; ex-holotype culture CBS 147279 = LQ1).

**Sexual morph:** *Ascomata* 295–460 µm diam, semi-immersed to immersed, apex erumpent through host surface, subglobose, coriaceous, black, ostiolate, usually staining the substrate around the ascomata black. *Ascoma apex* crest-like, central, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* 30–65 µm thick, composed of several layers, with dark to reddish brown, thick-walled cells of *textura*



**Fig. 4** *Lophiostoma carpini* (O-F-256904 - holotype). a–b. Ascomata; c. section of ascoma; d–e. asci; f–k. ascospores. — Scale bars: a–b = 400 µm; c = 100 µm; d–e = 50 µm; f–k = 20 µm.



*angularis*, becoming lighter towards the inside, somewhat compressed. *Hamathecium* comprising filiform, hyaline, septate, guttulate, smooth cellular pseudoparaphyses. *Asci* (95–)99–128(–145) × (10–)11–14(–15) µm (n = 18), bitunicate, fissitunicate, clavate, apically rounded, with an ocular chamber, with 4–8 uniseriate to obliquely biseriate ascospores. *Ascospores* (18–)20–24(–27) × (6–)7–9(–10) µm (n = 67), brown to dark brown, fusiform with acute ends, narrower basal cell (4.6–)5–8(–8.8) µm (n = 34) long, usually 3–4-septate, constricted at the central septum, guttulate, smooth-walled, lacking a mucilaginous sheath.

**Ecology** — On decorticated wood of *Carpinus betulus*.

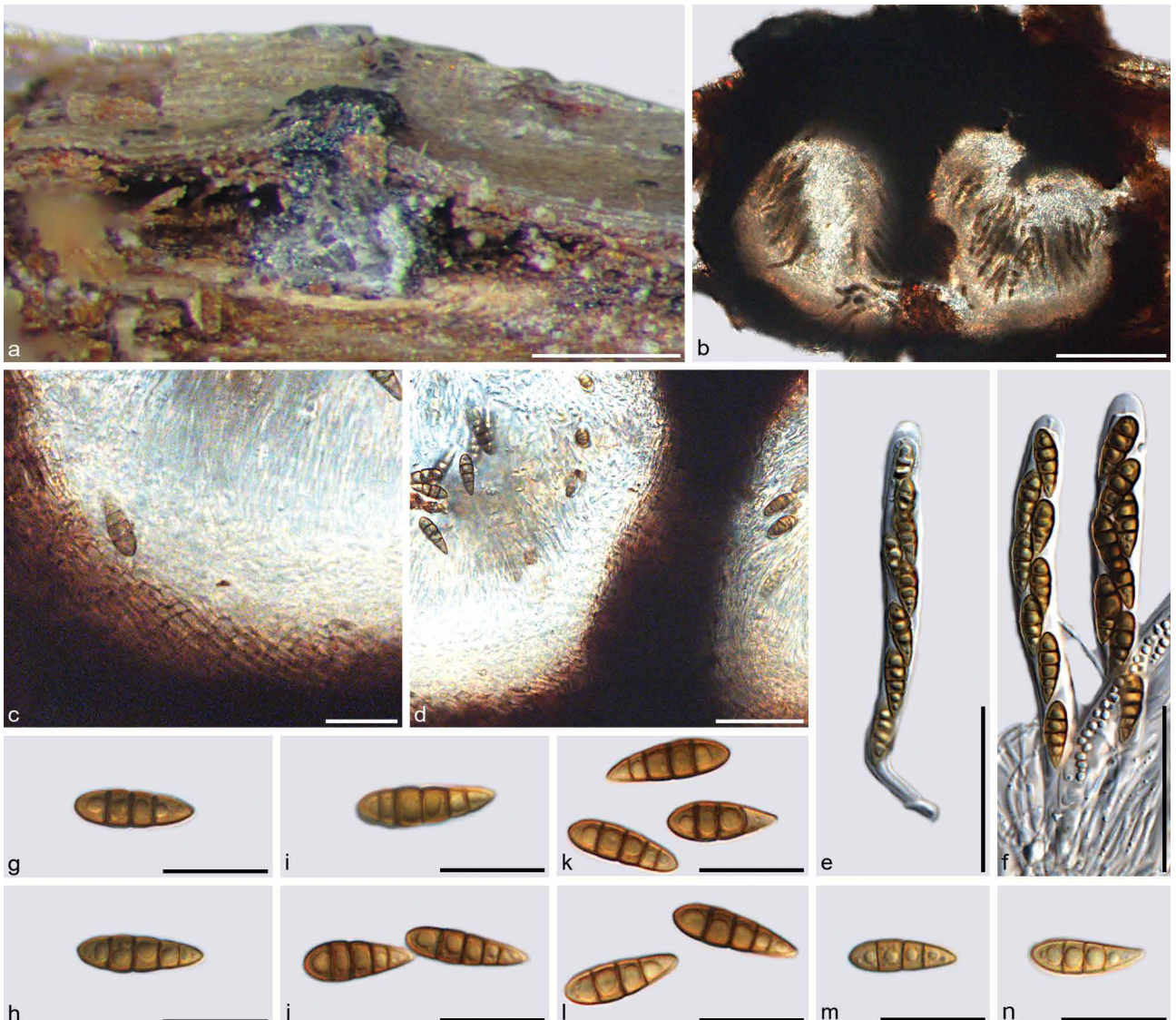
**Notes** — Strain CBS 147279 is nesting with low support in a clade with strain KT 530 named *Lophiostoma caudatum* and as a sister to the strain of *Lophiostoma clematidis-vitalbae* (MFLUCC 16-1368) without significant support. In the phylogenetic tree, there is generally a poor resolution in the upper part of the genus *Lophiostoma* (Fig. 1) due to lack of data for *TEF1-α* and *RPB2*, which is also true for *RPB2* for *Lophiostoma* (cf.) *caudatum* (KT 530) (Thambugala et al. 2015) and *Lophiostoma clematidis-vitalbae* (MFLU 20-0417- holotype; ex-type culture MFLUCC 16-1368) (Phukhamsakda et al. 2020). There are differences in spore size and septation between *L. carpini* O-F-256904 with shorter and wider ascospores of fusiform shape (18–)20–24(–27) × (6–)7–9(–10) µm compared to

KT 530 with more narrowly fusiform ascospores (23.5–34.5 × 5.5–7 µm). Ascospore septation differs with 3–4-septate for *L. carpini* O-F-256904 from (4–)5(–6)-septate for KT 530. As compared to *L. caudatum* KT 530, *L. carpini* O-F-256904 has larger ascomata 295–460 µm diam vs 145–210 µm diam for KT 530, and a thicker peridium. Moreover, the host of our specimen, *Carpinus betulus*, differs from that of *L. caudatum* KT 530, *Dactylis glomerata*. Hence, our material represents a taxon of its own, which we describe as the new species *L. carpini*. It is, however, even unclear whether the material from the monocot *Dactylis glomerata* collected in Japan is truly *L. caudatum*, because this species was originally described from decorticated branches of *Paliurus spina-christi* in France and was compared by Fabre (1879) to *Rebentischia* because of yellowish to pale brownish ascospores having a long, curved and pointed basal appendage cell (Fabre 1879: f. 47).

***Lophiostoma clavatum*** (D.F. Bao et al.) Andreassen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838974; Fig. 5

**Basionym.** *Sigarispora clavata* D.F. Bao et al., *Mycosphere* 10: 1090. 2019. — MycoBank MB 556722.

**Sexual morph:** *Ascomata* 320–590 µm diam, semi-immersed to immersed, subglobose, coriaceous, black, ostiolate. *Ascoma* apex crest-like, central, carbonaceous, with a pore-like ostiole.



**Fig. 5** *Lophiostoma* cf. *clavatum* (O-F-256905). a. Ascoma; b. section of ascomata; c–d. peridium; e–f. asci; g–n. ascospores. — Scale bars: a–b = 150 µm; c–d = 40 µm; e–f = 50 µm; g–n = 20 µm.

*Ostiolar canal* rounded, periphysate. *Peridium* 46–102 µm thick, comprising several layers consisting of brown to dark brown cells of *textura globulosa* to *textura angularis*, becoming lighter towards the inside. *Hamathecium* comprising filiform, hyaline, septate, guttulate, smooth cellular pseudoparaphyses. *Asci* (103–)117–139(–142) × (11–)12–16(–18) µm (*n* = 20), bitunicate, fissitunicate, clavate, apically rounded, with an ocular chamber, with 8 uniseriate to obliquely biseriate ascospores. *Ascospores* (15–)20–24(–25) × (5.5–)7–8(–8.5) µm (*n* = 78), ellipsoid to clavate, obtuse with basal cell (3–)4–7(–8) µm long (*n* = 32), straight or slightly curved, wider at one end, hyaline when young, yellowish brown to dark brown at maturity, 3–4(–5)-septate, slightly constricted at the septa, guttulate, smooth-walled.

**Ecology** — Saprobic on wood including *Rosa canina*.

**Material examined.** AUSTRIA, Niederösterreich, Gumpoldskirchen, vineyards above Melkerhof, on branches of *Rosa canina*, 9 Nov. 2014, W. Jaklitsch, H. Voglmayr & I. Krisai-Greilhuber (O-F-256905; culture CBS 147278 = LQ).

**Notes** — The strain CBS 147278 clusters with the ex-type strain of *Lophiostoma clavatum* (MFLUCC 18-1316) with strong support (Fig. 1). Further phylogenetic investigations should be implemented including additional molecular markers such as *RPB2*, to resolve the position of these strains within the genus *Lophiostoma*. There is a similarity in morphology, which supports that both strains may represent *Lophiostoma clavatum*. Still, there are also differences when comparing our specimen (O-F-256905) to that of Bao et al. (2019) (MFLU 19-0994 - holotype; ex type culture MFLUCC 18-1316), i.e., in larger ascospores (15–)20–24(–25) × (5.5–)7–8(–8.5) µm for our specimen compared to 13–17 × 4–6 µm. We also describe ascospores with up to five-septa and a basal cell of (3–)4–7(–8) µm length, which seems to differ from MFLU 19-0994. *Asci* are also longer and wider with (103–)117–139(–142) × (11–)12–16(–18) µm for our specimen compared to 91–117 × 9–12 µm in the latter. Lastly, our material is collected on branches of *Rosa canina* in Austria, while MFLU 19-0994 was collected on submerged decaying wood in Tibet. There is still a need for a continued sampling of both European and Asian material. Especially the amplification of the molecular markers ITS and *RPB2* for the Asian material is needed to resolve whether these strains represent one or two species.

***Lophiostoma clematidicola*** (Phukhams. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838975

**Basionym.** *Sigarispora clematidicola* Phukhams. et al., Fungal Diversity 102: 53. 2020 — MycoBank MB 557121.

***Lophiostoma clematidis*** (Phukhams. & K.D. Hyde) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838976

**Basionym.** *Pseudolophiostoma clematidis* Phukhams. & K.D. Hyde, Fungal Diversity 102: 51. 2020. — MycoBank MB 557120.

***Lophiostoma clematidis-subumbellatae*** Andreasen, Jaklitsch & Voglmayr, *nom. nov.* — MycoBank MB 838977

**Replaced synonym.** *Pseudocapulatispora clematidis* Phukhams. & K.D. Hyde, Fungal Diversity 102: 47. 2020. — MycoBank MB 557118, non *Lophiostoma clematidis* (Phukhams. & K.D. Hyde) Andreasen et al., this study.

**Etymology.** With reference to its host *Clematis subumbellata*.

**Notes** — As the epithet *clematidis* is already occupied in *Lophiostoma*, a replacement name was necessary.

***Lophiostoma clematidis-vitalbae*** Andreasen, Jaklitsch & Voglmayr, *nom. nov.* — MycoBank MB 838978

**Replaced synonym.** *Sigarispora clematidis* Phukhams. & K.D. Hyde, Fungal Diversity 102: 53. 2020. — MycoBank MB 557122, non *Lophiostoma clematidis* (Phukhams. & K.D. Hyde) Andreasen et al., this study.

**Etymology.** With reference to its host *Clematis vitalba*.

**Notes** — As the epithet *clematidis* is already occupied in *Lophiostoma*, a replacement name was necessary.

***Lophiostoma compressum*** (Pers.) Ces. & De Not., Comment. Soc. Crittog. Ital.: 19. 1861 — MycoBank MB 238397; Fig. 6

**Synonyms.** *Platystomum compressum* (Pers.) Trevis., Bull. Soc. Roy. Bot. Belgique 16: 16. 1877. — MycoBank MB 144522.

*Lophiostoma lojkanum* (Sacc.) Mussat, in Saccardo, Syll. Fung. (Abellini) 15: 198. 1900. — MycoBank MB 241560.

**Sexual morph:** *Ascomata* 400–1000 µm diam, scattered to gregarious, immersed to erumpent, often giving the substrate an intense black colour, globose to subglobose, uniloculate, black, glabrous, ostiolate. *Ascoma* apex crest-like, central, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* 50–120 µm (av. 82 µm, *n* = 80) thick, composed of several layers of dark brown to black, thick-walled pseudoparenchymatous cells, pale inside, more or less fusing with host tissue at the outside. *Hamathecium* comprising branched, cellular pseudoparaphyses, anastomosing between the asci, embedded in a gelatinous matrix. *Asci* 90–230 × (10–)12–20 µm (*n* = 150), bitunicate, fissitunicate, narrowly clavate, pedicellate, apically rounded, with a minute ocular chamber, with 8 overlapping uni- to partly biseriate ascospores. *Ascospores* (15–)16–33(–35) × (6–)7–10(–12) µm (*n* = 300), hyaline when immature, becoming yellow-brown to dark brown at maturity, ellipsoid to oblong, muriform with (3–)4–7-transverse and 1–3-longitudinal septa, constricted at the middle septum, guttulate, smooth-walled.

**Culture characteristics** — Ascospores germinated on MEA within 24 h. Germ tubes produced from several cells. Colonies reaching 2.1–2.3 cm diam after 4 wk at 20 °C, subcircular, with irregular margins, white, turning (irregularly) grey, reverse brown.

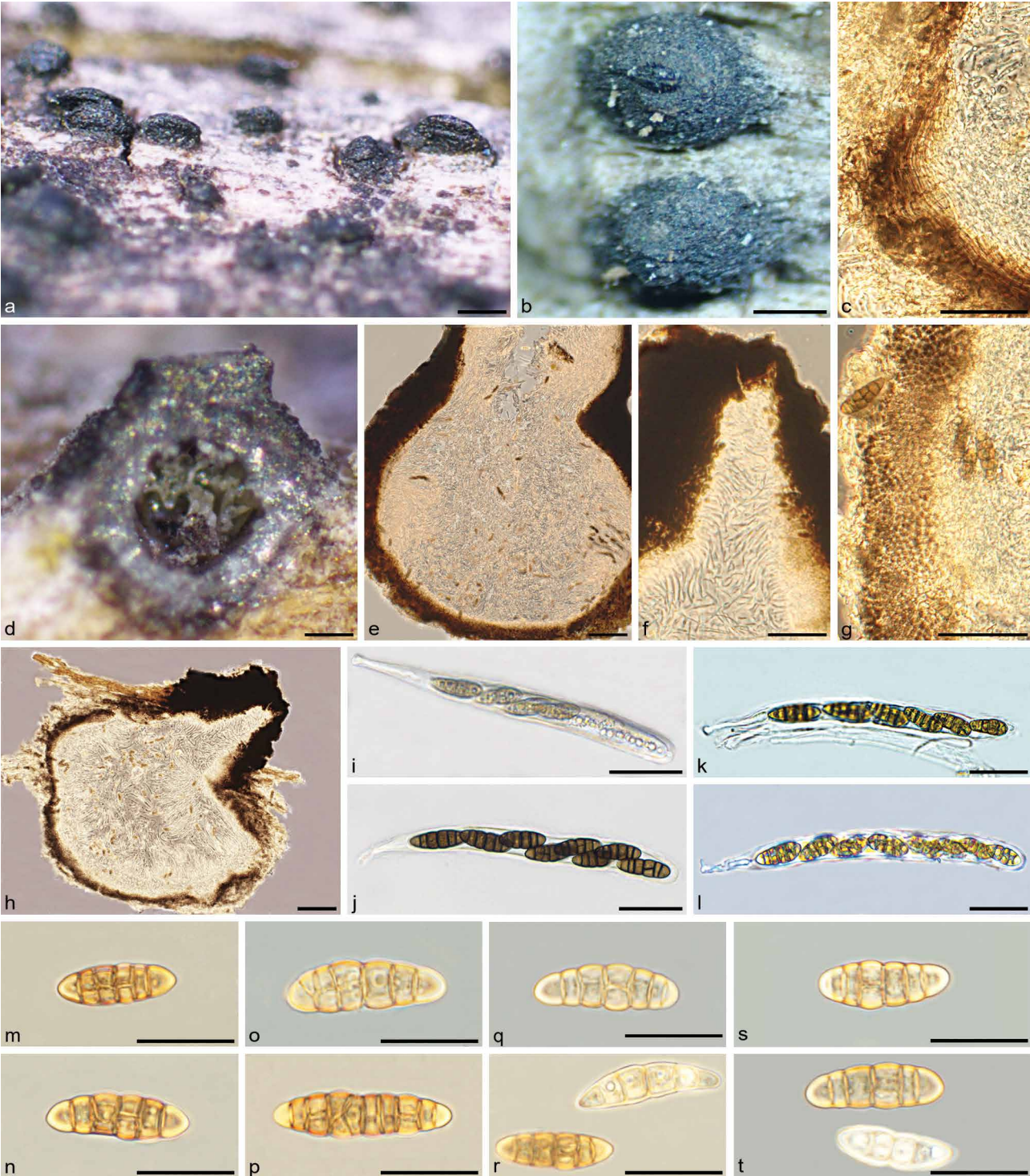
**Ecology** — Saprobic on wood of deciduous trees.

**Materials examined.** AUSTRIA, Kärnten, St. Margareten im Rosental, Triebblach, village area, roadside, at the Bauhof, on *Salix caprea*, 13 Aug. 2016, W. Jaklitsch (O-F-256914; culture CBS 147390 = LC1); Niederösterreich, Pfaffstätten, nature reserve Heferlberg, on *Quercus pubescens*, 1 Nov. 2017, H. Voglmayr (O-F-256915; culture TEQ); Vienna, 21st district, Marchfeldkanalweg, on *Cornus sanguinea*, 22 Aug. 2015, W. Jaklitsch (O-F-256913; culture CBS 147276 = LC). — NORWAY, Møre og Romsdal county, Ålesund municipality, on the bark of living *Populus tremula*, 31 Jan. 2018, O. Olsen (O-F-256906; culture CBS 147536 = MAL02); Viken county, Asker municipality, on bark of living *Populus tremula*, 29 Jan. 2019, M. Andreasen (O-F-256907; culture CBS 147538 = MAL49); Viken county, Asker municipality, on bark of living *Populus tremula*, 29 Jan. 2019, M. Andreasen (O-F-256908; culture CBS 147537 = MAL54); Vestfold county, Tjømø municipality, on dying branch of *Salix* sp., 18 July 2019, M. Andreasen (O-F-256909; culture CBS 147519 = MAL86); Vestfold county, Tjømø municipality, on *Salix* sp., 18 July 2019, M. Andreasen (O-F-256910; culture CBS 147520 = MAL90); Viken county, Asker municipality, on *Salix* sp., 15 Aug. 2019, M. Andreasen (O-F-256911; culture CBS 147521 = MAL93); Viken county, Asker municipality, on *Phragmites australis*, 15 Aug. 2019, M. Andreasen (O-F-256912; culture CBS 147615 = MAL94); unknown location, on *Tilia cordata*, 18th century, N. Green Moe & I. Jørstad (O-F-192124); Oslo county and municipality, on *Quercus robur*, 18th hundred, E. Rostrup (O-F-192125); Viken county, Bærum municipality, on *Corylus*, Jan. 1826, S.C. Sommerfelt & I. Jørstad (O-F-192126); Nordland county, Saltdal municipality, on *Salix phylicifolia*, Jan. 1824, S.C. Sommerfelt & G. Mathiasen (O-F-192128); Oslo county and municipality, on *Pyrus malus*, date unknown, M. Blytt & E. Rostrup (O-F-192129); Oslo county and municipality, on *Salix* sp., 5 Apr. 1912, J. Egeland (O-F-192130); location, host and date unknown, unknown collector (O-F-



192131); location unknown, on *Betula* sp., date unknown, N. Green Moe & E. Rostrup (O-F-192133); Nordland county, Saltdal municipality, on *Salix phylicifolia*, date unknown, S.C. Sommerfelt & G. Mathiassen (O-F-192134); Oslo county and municipality, on *Salix* sp., date unknown, L. Holm (O-F-192136); Trøndelag county, Inderøy municipality, on *Fraxinus excelsior*, 8 May 2014, J.B. Jordal & B. Nordén (O-F-247841); Viken county, Lillestrøm municipality, on *Salix* sp., 29 Sept. 2015, B. Nordén (O-F-305118); Finnmark county, host unknown, Alta municipality, N. Green Moe & G. Mathiassen (O-F-186801); Vestland county, Luster municipality, on branch of living *Ulmus glabra*, 14 June 2012, B. Nordén & J.B. Jordal (O-F-247799); Vestland county, Ullensvang municipality, on *Ulmus glabra*, 3 Oct. 2013, B. Nordén, J.B. Jordal & T. Læssøe (O-F-255564). — SWEDEN, Västergötland county, Vänersborg municipality, on *Viburnum opulus*, 19 June 1898, A.G. Eliasson (O-F-192135).

Notes — *Lophiostoma compressum* is by far the most common species of *Lophiostoma*. It is widespread and occurs on a wide range of host species, and there is a high variability in ascospore size and septation. In our phylogeny, the strains of this species are clustering as a clade within *Lophiostoma* with low support for its overall placement as well as for the clade itself. Possibly, *L. compressum* is a species complex, but currently many strains of *L. compressum* lack specific markers, thus there is need for further phylogenetic investigations including additional molecular markers such as *RPB2* and more fresh material collected in a larger area.



**Fig. 6** *Lophiostoma compressum*. (a, m–n, p, r (O-F-256906). b–j, o, q, s–t (O-F-256909) k–l (O-F-192126). a–b. Ascomata; d–e, h. section of ascomata; f. section of ostiole; c, g. peridium (c. *textura angularis* at the side, g. *textura prismatica* at the base); i–l. asci; m–t. ascospores under Zeiss Axio Imager A2 compound microscope. — Scale bars: a–b = 300 µm; c, g = 40 µm; d–f, h = 50 µm; i–l = 30 µm; m–t = 20 µm.



***Lophiostoma cornisporum*** (A. Hashim. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838979

*Basionym.* *Pseudolophiostoma cornisporum* A. Hashim. et al., *Stud. Mycol.* 90: 173. 2018. — MycoBank MB 823140.

***Lophiostoma coronillae*** (Wanas. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838980

*Basionym.* *Sigarispora coronillae* Wanas. et al., *Fungal Diversity* 74: 241. 2015. — MycoBank MB 551257.

***Lophiostoma dictyosporum*** Andreasen, Jaklitsch & Voglmayr, *sp. nov.* — MycoBank MB 839061; Fig. 7

*Etymology.* With reference to the ascospores having both transverse and longitudinal septa.

*Typus.* Morocco, Agadir, Ait Melloul, behind the Agronomy and Veterinary Institute Hassan II, on a branch of *Argania spinosa*, 6 May 2015, W. Jaklitsch, M. Mokhtari & M. Louay (O-F-256916 - holotype; ex-holotype culture CBS 147389 = C191).

Sexual morph: *Ascomata* 504–985 µm diam, solitary or gregarious, immersed, coriaceous, black, globose to subglobose, ostiolate, apex well-developed. *Ascoma apex* crest-like, central, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* 54–83 µm thick, firm, composed of brown to lightly pigmented cells of *textura prismatica*. *Hamathecium* comprising septate, unbranched, cellular pseudoparaphyses, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* (185–)189–209(–221) × 12–16(–18) µm (n = 10), bitunicate, fissitunicate, cylindrical to subclavate, with a long pedicel, apically rounded, with an ocular chamber, containing 8 uniseriate, sometimes slightly overlapping ascospores. *Ascospores* (18–)20–25(–28) × (9–)10–12(–14) µm (n = 30), hyaline when immature, turning yellowish to brown, finally dark brown when mature, ellipsoid with slightly wider upper part, muriform with 3–7-transverse and 1–3-longitudinal septa, deeply constricted at the middle septum.

*Ecology* — Saprobic in wood of *Argania spinosa*.

*Notes* — Strain CBS 147389 belongs to a clade that also contains strains of *Lophiostoma caespitosum* and *Lophio-*



**Fig. 7** *Lophiostoma dictyosporum* (O-F-256916 - holotype). a. Ascomata; b–c. section of ascomata; d. hymenium; e, g–i. asci; j–r. ascospores. — Scale bars: a–c = 400 µm; d = 40 µm; e–f = 100 µm; g–i = 50 µm; j–r = 20 µm.



*stoma fusisporum*. The characteristic morphology of muriform ascospores in cylindrical to subclavate asci along with the host and phylogenetic placement of the strain within the genus *Lophiostoma* indicates that this is a species new to science.

***Lophiostoma erumpens*** Andreassen, Jaklitsch & Voglmayr, *sp. nov.* — MycoBank MB 838981; Fig. 8

*Etymology.* With reference to the erumpent ascomata.

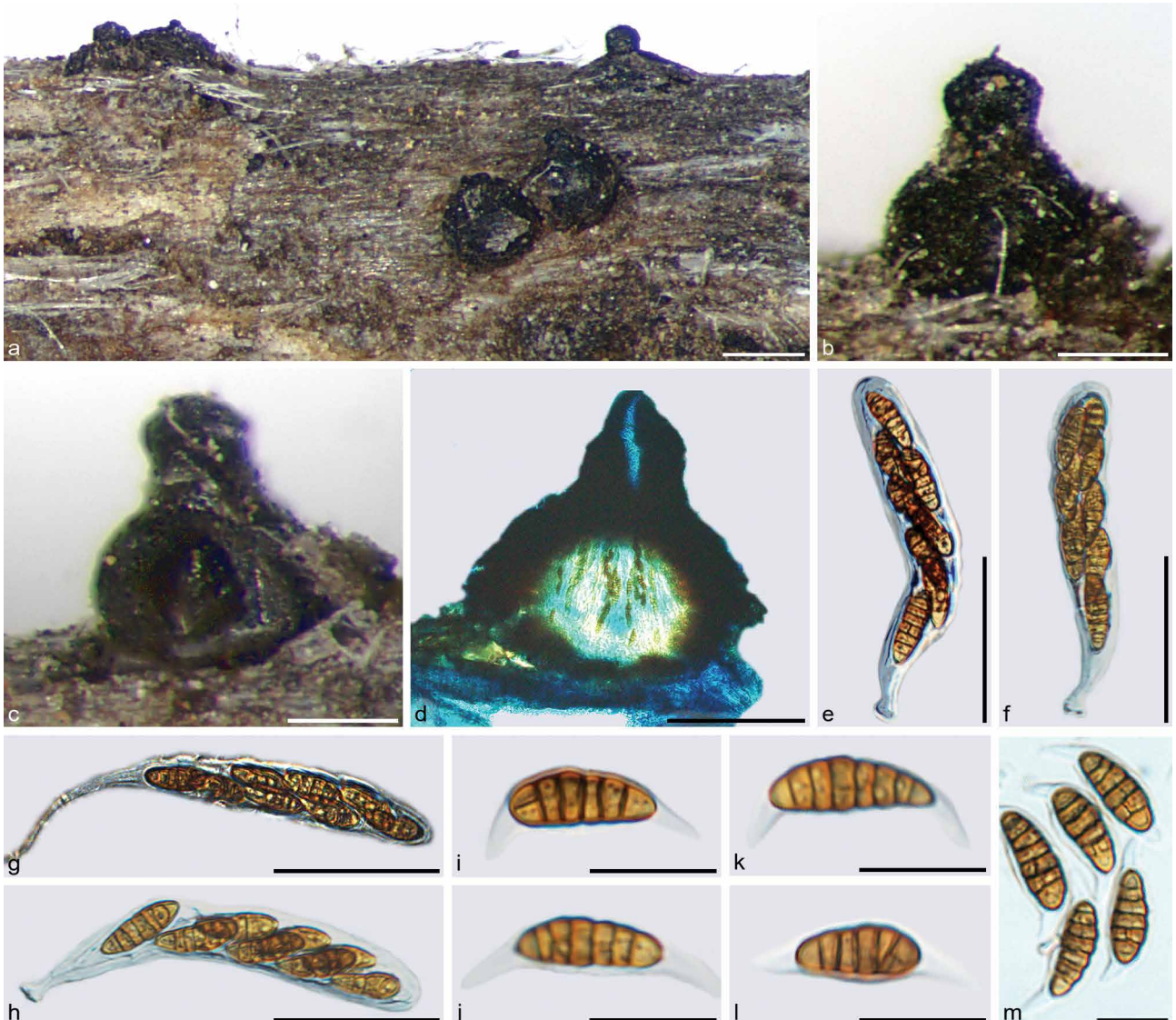
*Typus.* MOROCCO, N Agadir, Aourir, above Alma, N30°29'52.4 W09°33'43.3, elev. 535 m, on branchlets of *Genista* cf. *ferox*, 12 May 2015, W. Jaklitsch (O-F-256921; ex-holotype culture CBS 147275 = C220).

Sexual morph: *Ascomata* 200–400 µm diam, solitary or gregarious, immersed to erumpent, coriaceous, black, globose to subglobose, ostiolate, with well-developed apex. *Ascoma* apex crest-like, central, carbonaceous, with a pore-like ostiole. *Ostiole* rounded, periphysate. *Peridium* 50–82 µm thick, thicker at the apex and thinner at the base, composed of a single layer of dark brown to black, thick-walled cells of *textura angularis*, cells towards the inside lighter, somewhat compressed, laterally fusing with the host tissues, thin at the base. *Hamathecium* comprising septate, unbranched, cellular pseudoparaphyses, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* (95–)98–109(–116) × (15–)16–19(–20) µm (n = 20), bitunicate, fissitunicate, clavate, with

short stipe < 10 µm, apically rounded, with an ocular chamber, containing 8 obliquely uni- to biseriate ascospores. *Ascospores* (19–)21–24(–26) × (7–)8–10(–12) µm (n = 70), yellowish brown to dark brown, ellipsoid to fusiform, muriform with 5–7 transverse and 1–2 vertical septa, constricted at the middle septum, upper part slightly wider, with pointed, (5–)6–10(–15) µm (n = 72) long appendages at both ends.

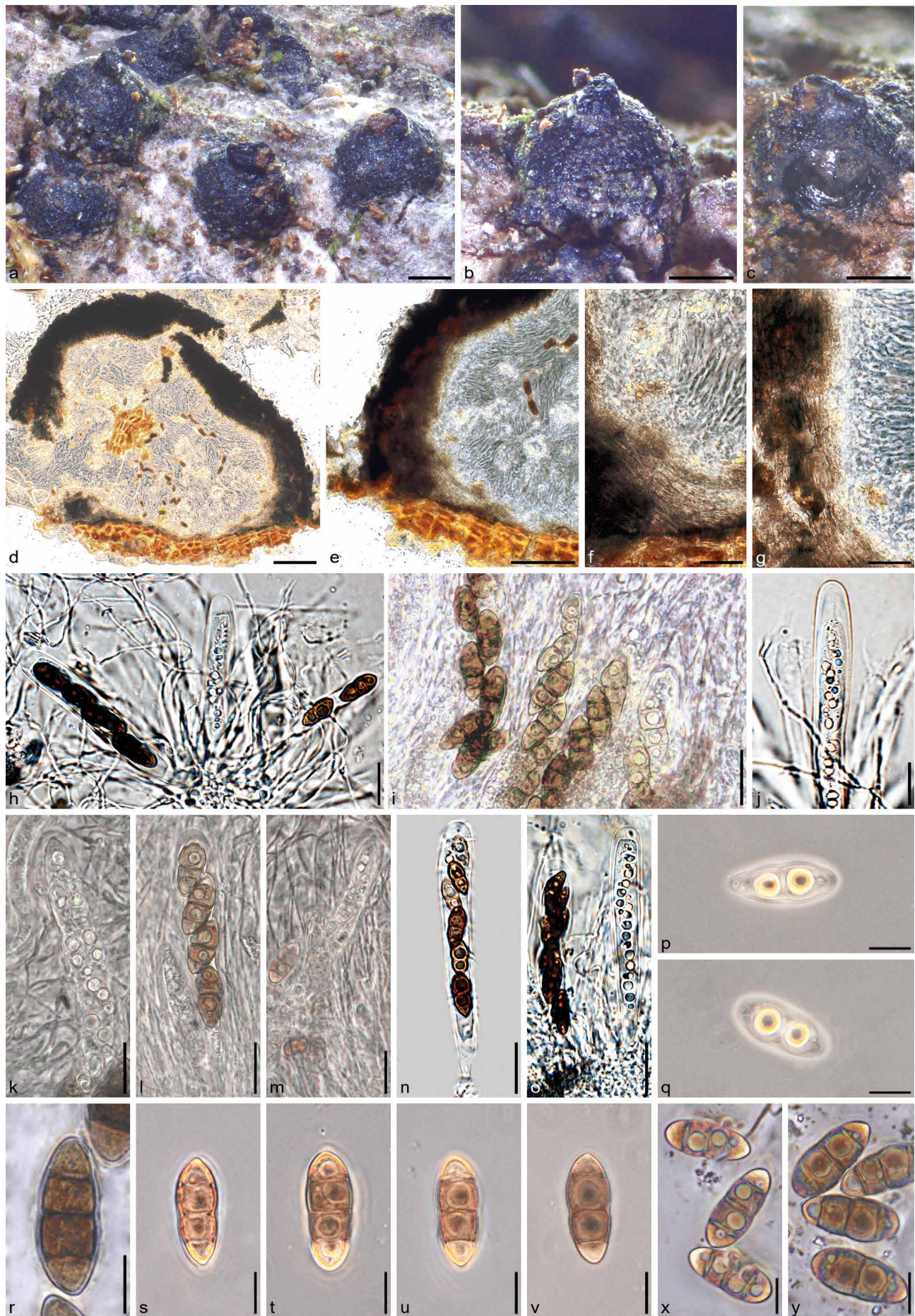
*Ecology* — Saprobic on twigs of leguminous shrubs; collected on *Genista* cf. *ferox*.

*Notes* — We name this species according to its erumpent habit rather than for its more characteristic ascospore appendages, because the epithet *appendiculatum* is occupied in *Lophiostoma*. Phylogenetically and morphologically *L. erumpens* is closely related to *L. pseudodictyosporium* (see Fig. 1), but distinct from that species. This is supported by the genetic distance, which is greater than given for several other species in the tree. Morphologically both species are similar in several respects, but on one hand ascomata of *L. erumpens* (O-F-256921) are smaller, 200–400 µm vs 400–700 µm in *L. pseudodictyosporium*. However, the most striking difference from *L. pseudodictyosporium* are the ascospore appendages, which are very conspicuous and (5–)6–10(–15) µm long in *L. erumpens*, but inconspicuous and small in *L. pseudodictyosporium* (cf. f. 8j–m in Thambugala et al. 2015). In that publication appendage length is not stated in the species description,



**Fig. 8** *Lophiostoma erumpens* (O-F-256921 - holotype). a–b. Ascomata; c–d. section of ascomata; e–h. asci; i–m. ascospores. — Scale bars: a = 350 µm; b–d = 200 µm; e–h = 50 µm; i–m = 20 µm (d in Cotton blue).





**Fig. 9** *Lophiostoma fusisporum* (O-F-256920 - holotype). a–c. Ascomata; d–e. section of ascomata; f–g. peridium; h–i. hymenium; j–o. asci and pseudo-paraphyses; p–q. immature ascospores; r–y. ascospores. — Scale bars: a–c = 200  $\mu$ m; d–e = 100  $\mu$ m; f–g = 40  $\mu$ m; h–o = 20  $\mu$ m; p–y = 10  $\mu$ m.



but the appendages of MFLU 14-0586 are apparently not longer than 5 µm. We also note that both species have fabaceaceous hosts, which belong to different genera, *Genista* vs *Spartium*.

***Lophiostoma fusisporum*** Andreasen & Nordén, *sp. nov.* — MycoBank MB 829060; Fig. 9

*Etymology.* With reference to the fusiform ascospores.

*Typus.* NORWAY, Oslo county and municipality, on the bark of living *Acer platanoides*, 28 Sept. 2018, *M. Andreasen* (O-F-256920 - holotype; ex-holotype culture MAL04).

Sexual morph: *Ascomata* 300–700 µm diam, solitary to scattered, subimmersed, coriaceous to carbonaceous, dark brown to black, globose to subglobose, ostiolate. *Ascoma* apex papillate, crest-like, central, carbonaceous, with a pore-like ostiole. *Ostiole* rounded, periphysate. *Peridium* 75–100 µm thick, composed of a single layer of dark to reddish brown, thick-walled cells of *textura angularis*, cells towards the inside lighter, somewhat compressed, laterally fusing with the host tissues, thin at the base. *Hamathecium* comprising septate, branched, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* (110–)116–146(–155) × (11–)12–17(–20) µm (n = 30), bitunicate, fissitunicate, clavate, short-pedicellate, apically rounded, with an ocular chamber, with 6–8 uniseriate to obliquely biseriate ascospores. *Ascospores* (19.5–)22–26(–28) × (8–)9–10(–11) µm (n = 31), hyaline when immature, brown when mature, end cells remaining lighter to hyaline, fusiform to oblong-ellipsoid, 3-septate, constricted at all septa but more at the middle, upper part slightly wider, guttulate in each cell, two middle cells with a lenticular lumen when overmature, with inconspicuous verruculose ornamentation.

Culture characteristics — Ascospores germinated on MEA within 24 h at 20 °C. Germ tubes produced from end and central cells. Colonies 0.5–1 cm diam after 4 wk, subcircular with somewhat irregular margins; initially pale grey, slightly darkening with time; reverse black.

Ecology — Saprobic on dead bark of living *Acer platanoides*.

Notes — *Lophiostoma fusisporum* clusters with *Lophiostoma caespitosum* with high BPP (0.99) and medium MLB (66 %) support. Interestingly, despite several efforts, it was impossible to produce LSU sequences for this species. Morphologically, *L. fusisporum* differs from *L. caespitosum* in several characters having an overall bigger size of ascomata, peridium wall, asci and having much larger fusiform ascospores with narrowly rounded hyaline end cells. *Asci* have a more clavate form and are wider (up to 20 µm). We also note that it has a different host, *Acer platanoides*, than *L. caespitosum* (*Crataegus*). This species has very characteristic fusiform 3-septate spores with paler end cells and one big oil droplet in each cell.

***Lophiostoma helichrysi*** (Dayar. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838982

*Basionym.* *Lophiohelichrysum helichrysi* Dayar. et al., Fungal Diversity 75: 85. 2015. — MycoBank MB 551401.

***Lophiostoma japonicum*** (Thambug. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838983

*Basionym.* *Biappendiculispora japonica* Thambug. et al., Fungal Diversity 74: 214. 2015. — MycoBank MB 551529.

Notes — The species was earlier named *Lophiostoma caulium* var. f (Tanaka & Harada 2003b).

***Lophiostoma jonesii*** (Ariyaw. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838984

*Basionym.* *Alpestrisphaeria jonesii* Ariyaw. et al., Phytotaxa 277: 261. 2016. — MycoBank MB 552363

***Lophiostoma jotunheimenense*** Andreasen & Nordén, *sp. nov.* — MycoBank MB 838985; Fig. 10

*Etymology.* Referring to the collection site Jotunheimen National Park.

*Typus.* NORWAY, Oppland county, Lom municipality, on *Salix glauca*, 29 June 2019, *M. Andreasen* (O-F-256917 - holotype; ex-holotype culture CBS 147522 = MAL88).

Sexual morph: *Ascomata* large and coarse, 425–1250 µm diam, scattered to gregarious, immersed to erumpent, globose to subglobose, uniloculate, black, glabrous, ostiolate, arranged in a closely aligned layer, giving the substrate an intense black colour. *Ascoma* apex crest-like, central or lateral, carbonaceous, with a pore-like ostiole. *Ostiole* rounded, periphysate. *Peridium* 50–100 µm thick, composed of several layers of dark brown to black, thick-walled pseudoparenchymatous cells, outside fusing with host tissue, paler inward. *Hamathecium* comprising branched, cellular paraphyses, anastomosing among asci, embedded in a gelatinous matrix. *Asci* (125–)135–160(–167) × (11–)12–14(–15) µm (n = 21), bitunicate, fissitunicate, narrowly clavate, with short stipe < 10 µm, apically rounded, with a minute ocular chamber, with 8 uniseriate to obliquely overlapping ascospores. *Ascospores* (18–)20–25(–29) × (7–)8–10(–11) µm (n = 62), first hyaline becoming brown with paler to hyaline end cells, when immature fusiform with acute end cells, becoming oblong-ellipsoid with more or less rounded ends at maturity, upper part wider, 1-septate when immature, becoming muriform with 3–6(–7)-transverse and 1–2-longitudinal septa when mature, distinctly constricted at the middle septum and slightly at other septa, guttulate only when immature, smooth-walled.

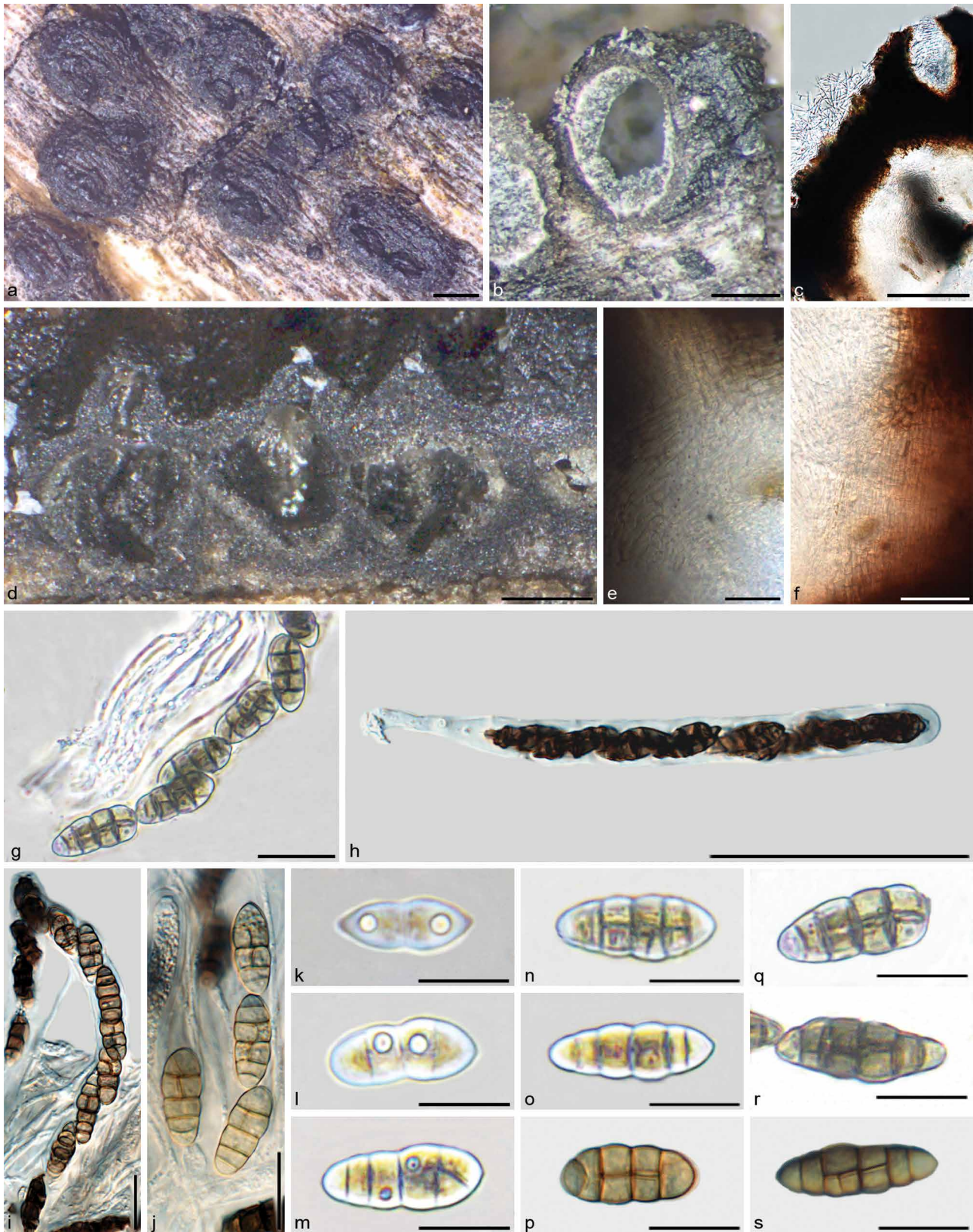
Culture characteristics — Ascospores germinated on MEA within 24 h at 20 °C. Germ tubes produced from end and central cells. Colonies 2.4–5.6 cm diam after 4 wk, subcircular with irregular margins. Colour pale grey, turning dark grey at lower levels; reverse black.

Ecology — Saprobic on dead branches still attached on living *Salix glauca* in an alpine environment.

Notes — We present phylogenetic support for this new species as being a sister species to *Lophiostoma rosae-ecae* (MLP 80 % and BPP 0.90) and nesting in a larger clade composed of *L. crenatum*, *L. macrostomum*, *L. multiseptatum*, and *L. pseudodictyosporium*. The morphology of this species resembles that of *Lophiostoma compressum* in some aspects. Still, it has more oblong ellipsoid muriform spores with pale to hyaline end cells and often with longitudinal septa running parallel to the spore axis. This specimen was found in alpine environments at approximately 1200 m altitude. It differs from *L. rosae-ecae* by oblong-ellipsoid spores vs fusiform spores in *L. rosae-ecae*, overall shorter in size with less acute hyaline end-cells. Spore septation also differs with 3–6(–7)-transverse and 1–2-longitudinal septa, compared to the 3-transversely-septate ascospores of *L. rosae-ecae*. *Asci* are narrower, up to 14(–15) µm wide compared to up to 25 µm wide in *L. rosae-ecae*, and longer, up to 160(–167) µm. Lastly, we note different hosts, *Salix glauca* vs *Rosa ecae* in the latter.

***Lophiostoma junci*** (Wanas. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838987

*Basionym.* *Sigarispora junci* Wanas. et al., Fungal Diversity 89: 69. 2018. — MycoBank MB 554149.



**Fig. 10** *Lophiostoma jotunheimense* (O-F-256917 - holotype). a–b. Ascomata; c–d. section of ascomata; e–f. peridium; g, j. hymenium; h–i. asci; k–l. immature ascospores; m–s. ascospores. — Scale bars: a, b, d = 300  $\mu$ m; c = 100  $\mu$ m; e–g, i–j = 25  $\mu$ m; h = 50  $\mu$ m; k–s = 15  $\mu$ m.

***Lophiostoma longiappendiculatum*** (Mapook & K.D. Hyde)  
Andreassen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank  
MB 838988

*Basionym.* *Pseudocapulatispora longiappendiculata* Mapook & K.D. Hyde,  
Fungal Diversity 101: 48. 2020. — MycoBank MB 557286

***Lophiostoma macrostomoides*** De Not., Comment. Soc.  
Crittog. Ital. 1 (fasc. 4): 219. 1863 — MycoBank MB 241835;  
Fig. 11

Sexual morph: *Ascomata* large and coarse, 400–1200  $\mu$ m  
diam, scattered, gregarious, immersed to erumpent, globose  
to subglobose, black, glabrous, ostiolate. *Ascoma* apex crest-  
like, central, carbonaceous, with a pore-like ostiole. *Ostiolar*



canal rounded, periphysate. *Peridium* (50–)75–125 µm thick, composed of several layers of dark brown to black, thick-walled cells, forming *textura angularis*, tending to be paler inward, fusing with host tissue at the outermost layer. *Hamathecium* comprising branched, cellular pseudoparaphyses, anastomosing above and between the asci, embedded in a gelatinous matrix. *Asci* (120–)130–170(–200) × (6–)8–15(–17) µm (n = 50), bitunicate, fissitunicate, clavate, with a stipe > 10 µm, apically rounded, with a minute ocular chamber, with 8 biserially arranged ascospores. *Ascospores* (30–)32–40(–42) × (8–)9–10(–12) µm (n = 100), brown, with sometimes lighter end cells, oblong-ellipsoid to broadly cylindrical, with (3–)5–7-transverse septa, constricted at the middle septum, at maturity cell above middle septum slightly wider than others, guttulate, smooth-walled.

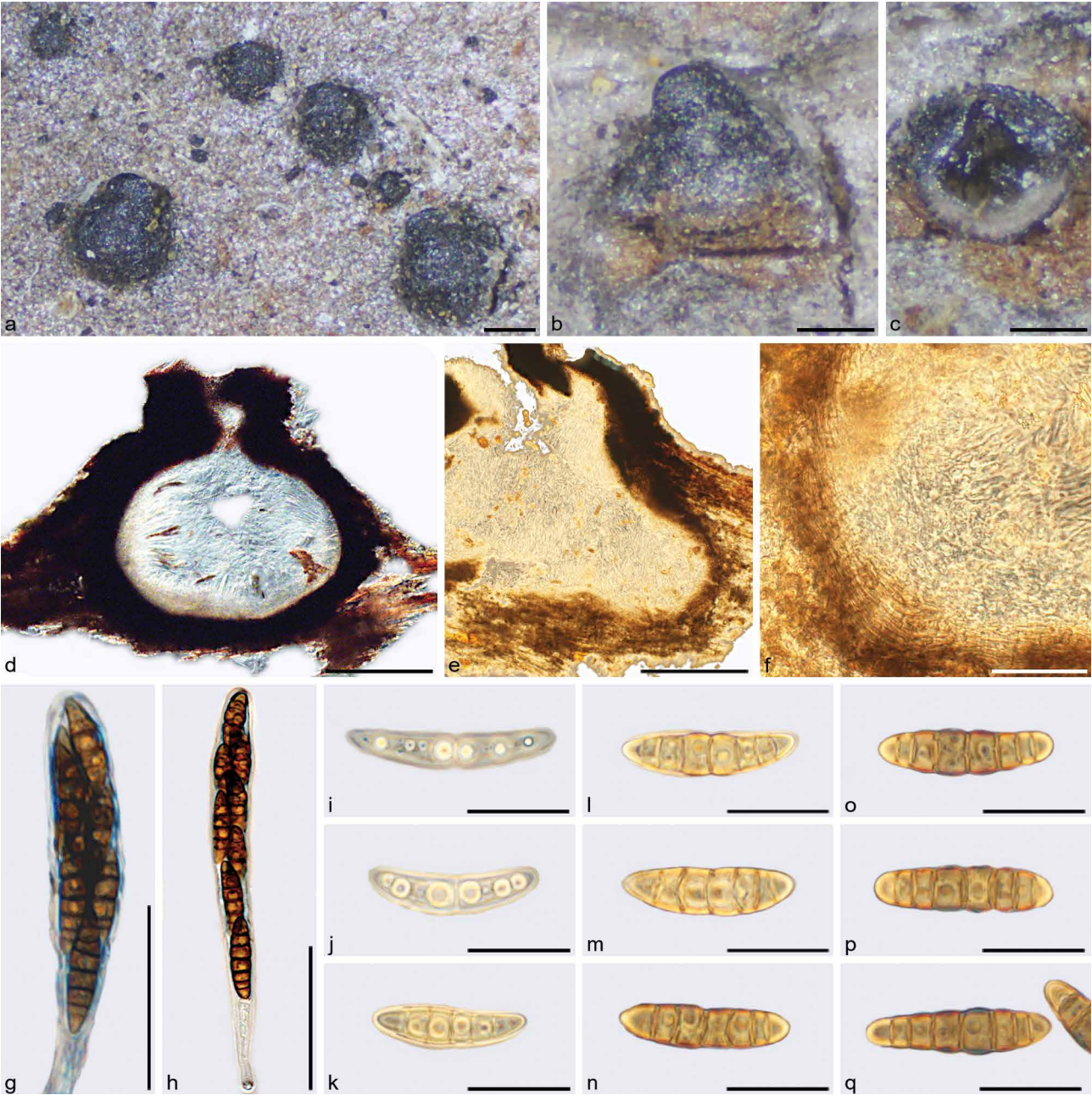
Culture characteristics — Ascospores germinated on MEA within 24 h at 20 °C. Germ tubes produced from end and central cells. Colonies 1.9–2.4 cm diam after 4 wk, more or less circular with somewhat irregular margins; initially whitish

becoming light greyish to dark grey from below, margin dark grey to black; reverse black.

Ecology — Saprobiic in wood of deciduous trees and shrubs such as *Quercus*, *Ulmus*, *Salix*, but also found on the coniferous *Juniperus communis*.

*Materials examined.* AUSTRIA, Kärnten, St. Margareten im Rosental, on *Salix cinerea*, 21 July 2016, W. Jaklitsch (O-F-256919; culture CBS 147277 = LMS). – NORWAY, Viken county, Asker municipality, on *Juniperus communis*, 15 Dec. 2018, M. Andreasen (O-F-256918; culture CBS 147523 = MAL32).

Notes — The strains CBS 147523 and CBS 147277 cluster with high support with strain CBS 123097, representing *L. macrostomoides*, all clustering as a clade with high support. Our specimens are morphologically clearly *L. macrostomoides*. Still, high intraspecific variability in spore shape and septation within each specimen was observed. This variation was observed both between the examined specimens, but also within each specimen. One clear difference in morphology, compared to the closely resembling *L. pseudomacrostromum*, was the



**Fig. 11** *Lophiostoma macrostomoides* (O-F-256918). a–b. Ascomata; c–d. section of ascomata; e–f. peridium (*textura angularis* at the base); g–h. asci; i–k. immature ascospores; l–q. ascospores under Zeiss Axio Imager A2 compound microscope. — Scale bars: a–c = 150 µm; d = 300 µm; e, g = 50 µm; f, i–q = 20 µm; h = 60 µm.

absence of longitudinal septa in any of the ascospores of the examined material.

DNA sequences of two species from Africa misidentified as *Lophiostoma macrostomoides* are present in GenBank. One is from Kenya, represented by the specimens GKM 1033, GKM 1159 and GKM 224N. Both ML and Bayesian analyses placed them outside the *Lophiostomataceae*, thus they were not further considered here. For the other species, represented by the strains CBS 113435 and CBS 121412, originating from South Africa, only ITS and LSU sequences are available. A preliminary LSU analysis placed them outside *L. macrostomoides*. As the correct position of this species could not be determined by multigene analyses, these accessions were not included.

***Lophiostoma medicaginicola*** (Wanas. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838990

*Basionym.* *Sigarispora medicaginicola* Wanas. et al., Fungal Diversity 89: 69. 2018. — MycoBank MB 554150.

***Lophiostoma montanae*** (Phukhams. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838991

*Basionym.* *Sigarispora montanae* Phukhams. et al., Fungal Diversity 102: 55. 2020. — MycoBank MB 557121.

***Lophiostoma neomuriforme*** Andreasen, Jaklitsch & Voglmayr, *nom. nov.* — MycoBank MB 838992

*Replaced synonym.* *Sigarispora muriformis* Tibpromma et al., Fungal Diversity 83: 37. 2017. — MycoBank MB 552696, non *Lophiostoma muriforme* Hazsl., Mathem. Természettud. Közlem. Magg. Tudom. Akad. 25 (2): 84. 1893 '1892'. — MycoBank MB 141107.

**Notes** — As the epithet *muriforme* is already occupied in *Lophiostoma*, a replacement name was necessary.

***Lophiostoma obtusisporum*** (A. Hashim. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838993

*Basionym.* *Pseudolophiostoma obtusisporum* A. Hashim. et al., Stud. Mycol. 90: 173. 2018. — MycoBank MB 823141.

***Lophiostoma ononidis*** (Qing Tian et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838994

*Basionym.* *Sigarispora ononidis* Qing Tian et al., Fungal Diversity 78: 37. 2016. — MycoBank MB 551729.

***Lophiostoma paramacrostromum*** (Ariyaw. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838995

*Basionym.* *Lophiopoacea paramacrostroma* Ariyaw. et al., Fungal Diversity 74: 221. 2015. — MycoBank MB 551241.

***Lophiostoma plantaginis*** Andreasen & Nordén, *sp. nov.* — MycoBank MB 838996; Fig. 12

*Etymology.* With reference to its host *Plantago maritima*.

*Typus.* NORWAY, Viken county, Asker municipality, on the rhizome of *Plantago maritima*, 15 Aug. 2019, M. Andreasen (O-F-256936 - holotype; ex-holotype culture CBS 147527 = MAL92); Vestfold county, Porsgrunn municipality, on the rhizome of *Plantago maritima*, 21 Sept. 2020, M. Andreasen (O-F-256937 - paratype).

**Sexual morph:** *Ascomata* 100–293 µm diam, immersed-erumpent, subglobose, scattered to gregarious, carbonaceous, black. *Ascoma* apex central or lateral crest-like, carbonaceous, with a pore-like ostiole. *Ostiole* rounded, periphysate. *Peridium* 31–51 µm thick, composed of several layers of dark brown to black, thick-walled pseudoparenchymatous cells forming tex-

*tura angularis*, paler inwardly, laterally fusing with host tissue at the outermost layer. *Hamathecium* comprising branched, cellular pseudoparaphyses, anastomosing above and between the asci, embedded in a gelatinous matrix. *Asci* (80–)83–125 × 13–22(–24) µm (n = 20), bitunicate, fissitunicate, clavate, with a stipe < 10 µm long rounded at the apex, apical chamber present, short to long-stalked, with 8 biseriate or partially overlapping, obliquely uniseriate ascospores. *Ascospores* (28–)29–33(–36) × (8–)10–13 µm (n = 30), fusiform to ellipsoid-fusiform with narrowly rounded ends, at first hyaline, smooth and 1-septate, finally brown, verruculose and 1–3-septate, sharply constricted at the median septum.

**Culture characteristics** — Ascospores germinated on MEA within 24 h. Germ tubes produced from one or both ends of ascospores. Colonies 3.4–4.7 cm diam after 4 wk at 20 °C, subcircular with somewhat irregular margins; white with some pale-yellow areas; reverse black.

**Ecology** — Saprobi on rhizomes of *Plantago maritima*, growing in the marine tidal zone of the Oslo-fjord.

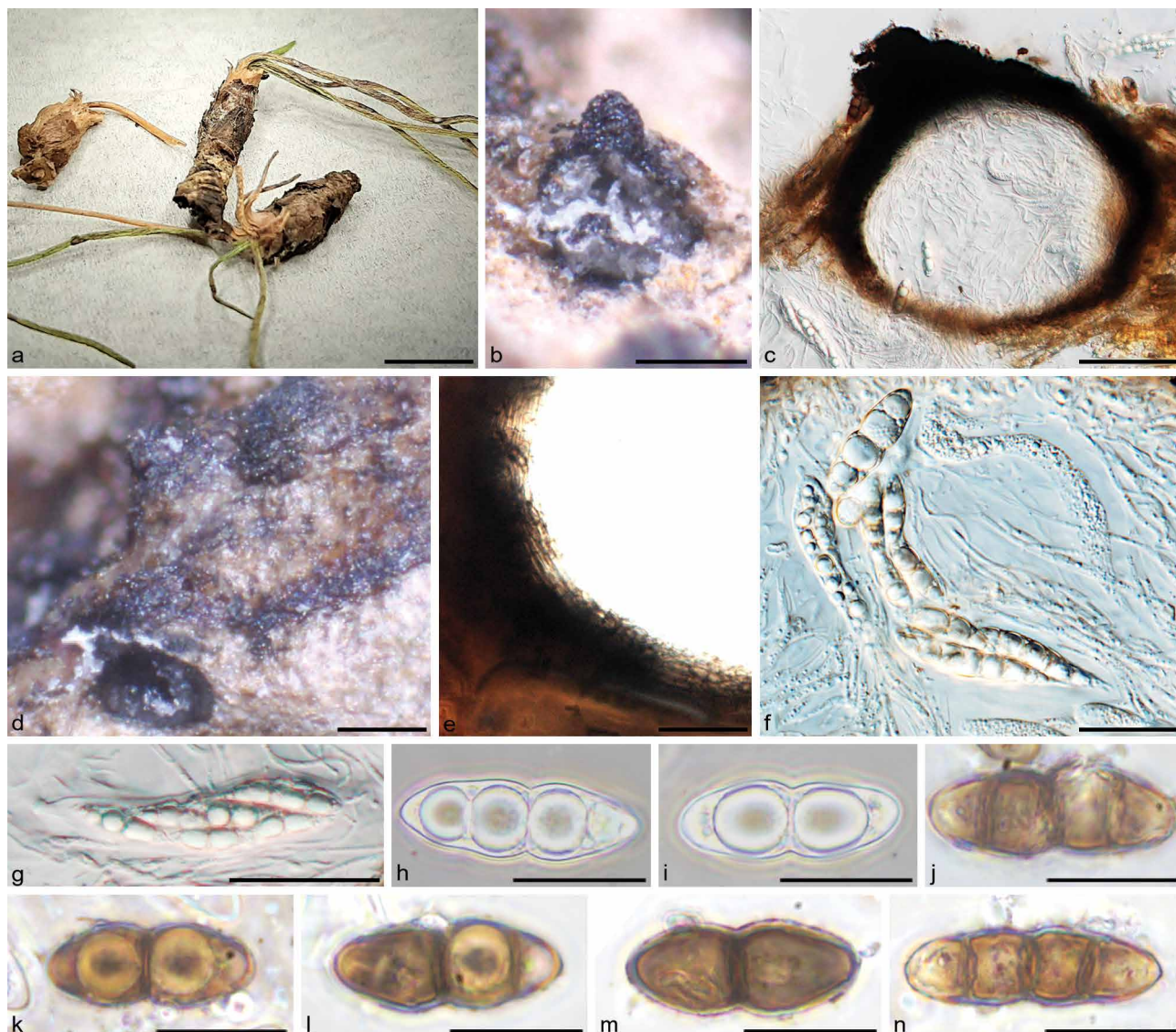
**Notes** — This species forms a strongly supported clade comprising strain CBS 147527 and SC-12 as a sister clade to *Lophiostoma jonesii*. It should be noted that the phylogenetic similarity of these strains is only based on the molecular markers of 5.8S, ITS2 and LSU, unfortunately lacking *RPB2* and *TEF1-α*. Sequence similarities in the ITS region between the two species are relatively low (506/518 = 97.683 %; sequence data not added in the alignment of this study). Our specimens are saprobic on rhizomes of *Plantago maritima* growing in the tidal zone of saltwater in the Oslo fjord. Zhou et al. (2014) described '*Trematosphaeria*' *terricola* = *Lophiostoma terricola* (strain SC-12) based on morphological similarities to *Trematosphaeria*. However, it was shown to cluster with strong support as a sister clade to the remaining taxa of *Lophiostoma* by Thambugala et al. (2015). The strain SC-12 was isolated from ascomata found on alpine soil in China at an altitude of 3177 meters. Our specimens examined were very fragile, rather old and in poor condition, thus making it challenging to present photos of mature asci and mature spores, which were unstable or disintegrated before or during the investigation. Morphological investigations give support for a new species when compared to *L. terricola* (strain SC-12) (Zhou et al. 2014), with an overall smaller size of both ascomata, peridium and ascospores. Mature ascospores also tend to be shorter, are of more oval shape and some remain 1-septate as compared to the more narrowly fusiform, 3-septate mature ascospores of *L. terricola*. Ascospores are overall shorter and broader in *L. plantaginis*, (28–)29–33(–36) µm × (8–)10–13 µm vs 31–42 µm × 7.7–10.7 µm in *L. terricola*. Ascomata of *L. plantaginis* are without a cover of brown septate hyphae, and both ascomata and the peridium are of smaller dimensions, 100–293 µm diam and 31–51.5 µm thick vs 346–632 µm diam and 52–112 µm thick for *L. terricola*, respectively. Asci of our specimen are overlapping in size with a tendency to being slightly shorter with up to 125 µm compared to up to 140 µm for *L. terricola*. Lastly, the alteration in host and environment (marine tidal zone and plant host vs alpine soil), together with the morphological and sequence differences, support the establishment of a new species.

***Lophiostoma pseudodictyosporium*** Qing Tian et al., Fungal Diversity 72: 114. 2015 — MycoBank MB 550887

*Synonym.* *Coelodictyosporium pseudodictyosporium* (Qing Tian et al.) Thambug. & K.D. Hyde, Fungal Diversity 74: 218. 2015. — MycoBank MB 551236.

**Notes** — Based on an asexual morph, *L. pseudodictyosporium* (MFLU 14–0737 - holotype, ex-type culture MFLUCC 13-0451) was first described by Liu et al. (2015). Shortly after-





**Fig. 12** *Lophiostoma plantaginis* (O-F-256936 - holotype). a. Host; b, d ascomata; c. sectioned ascoma; e. peridium; f. hymenium; g. immature ascus; h–i. immature ascospores; j–n. ascospores. — Scale bars: a = 1 cm; b, d = 200 µm; c = 100 µm; e, g = 50 µm; f = 40 µm; h–n = 20 µm.

wards, it was combined in the new genus *Coelodictyosporium* by Thambugala et al. (2015), who simultaneously described the new species *C. muriforme* (MFLU 14-0586, culture MFLUCC 13-0351) based on a sexual morph. Both species were collected on the same host, *Spartium junceum*, in Italy. *Lophiostoma pseudodictyosporium* and *C. muriforme* may represent different morphs of the same fungus, but due to some sequence differences we currently keep them apart, and the new name *Lophiostoma spartii-juncei* is therefore proposed for *C. muriforme*.

***Lophiostoma pseudomacrostromum*** Sacc., *Michelia* 1 (no. 3): 339. 1878 — MycoBank MB 156130; Fig. 13

*Synonyms.* *Navicella pseudomacrostroma* (Sacc.) Kuntze, *Revis. Gen. Pl.* 3 (2): 500. 1898. — MycoBank MB 527378.

*Platystomum compressum* var. *pseudomacrostromum* (Sacc.) Chesters & A.E. Bell, *Mycol. Pap.* 120: 49. 1970. — MycoBank MB 348636.

**Sexual morph:** *Ascomata* large and coarse, (250–)400–1100 µm diam, scattered, gregarious, immersed to erumpent, globose to subglobose, uniloculate, black, glabrous, ostiolate. *Ascoma* apex central or lateral, crest-like, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* (50–)75–125 µm thick, composed of several layers of dark brown to black, thick-walled pseudoparenchymatous cells

forming a *textura angularis*, paler inward, outermost layer fusing with host tissue. *Hamathecium* comprising branched, cellular pseudoparaphyses, anastomosing above and between the asci, embedded in a gelatinous matrix. *Asci* (120–)130–170(–200) × 10–15(–17) µm (n = 75), bitunicate, fissitunicate, clavate, with a stipe > 10 µm long, apically rounded, with a minute ocular chamber, with 4 or 8 biseriate ascospores. *Ascospores* (20–)25–35(–36) × (7–)9–10(–12) µm (n = 120), uniseriate, brown, sometimes with slightly paler end cells, oblong-ellipsoid to broadly cylindric, straight or curved, with (3–)5–8-transverse septa, in a small fraction also with 1 longitudinal septum in 1–2(–3) cells, constricted at the middle septum, upper part often wider than the lower, guttulate, smooth-walled.

**Materials examined.** NORWAY, Vestland county, Kvam municipality, on *Tilia cordata*, 15 May 2019, M. Andreassen (O-F-256922; culture CBS 147524 = MAL73); Viken county, Frogn municipality, on *Tilia cordata*, 4 June 2019, M. Andreassen (O-F-256923; culture CBS 147525 = MAL81); Viken county, Frogn municipality, on *Tilia cordata*, 5 June 2019, M. Andreassen (O-F-256924; culture CBS 147526 = MAL83); Viken county, Frogn municipality, on *Tilia cordata*, 5 June 2019, M. Andreassen (O-F-256925; culture MAL84); Troms county, Bardu municipality, on dead and decaying branches, 9 July 2002, G. Mathiassen (T-F-14733/2, T-F-14792/2, T-F-14911, T-F-14912, T-F-14913, T-F-14914, T-F-14915, T-F-14916); Kv'nangen municipality, Vassnes, on a dead branch, 9 July 2002, G. Mathiassen (T-F-14917).





**Fig. 13** *Lophiostoma pseudomacrostromum*. a–m (O-F-256922). n–s (O-F-256925). t–z (O-F-256924). a–b. Ascomata; c. section of ascoma; d–e. peridium; f–g. asci; h–z. ascospores (immature in n, t–u; h–m under Zeiss Axio Imager A2 compound microscope). — Scale bars: a–b = 500 µm; c, f–g = 50 µm; d–e = 10 µm; h–z = 20 µm.

**Notes** — The strains CBS 147524, CBS 147525 and CBS 147526 form a clade with low support in the phylogenetic analyses (Fig. 1). When we combine phylogenetic and morphological evidence, there is an indication of an independent species. We are therefore able to support Holm & Holm’s (1988) suggestion that *L. pseudomacrostromum* is a distinct species within the genus *Lophiostoma*. There is a need of further sampling and amplification of the marker RPB2 to resolve the overall placement

of the species within *Lophiostoma*. Holm & Holm (1988) and earlier Chesters & Bell (1970) mentioned that collections with morphological similarities to *Lophiostoma macrostromoides* but with the presence of dictyospores could represent the species *L. pseudomacrostromum*. Chesters & Bell (1970) assumed that the species is an intergrading form between *L. compressum* and *L. macrostromoides*, while Holm & Holm (1988) indicated a closer relationship to *L. macrostromoides* than to *L. compressum*.

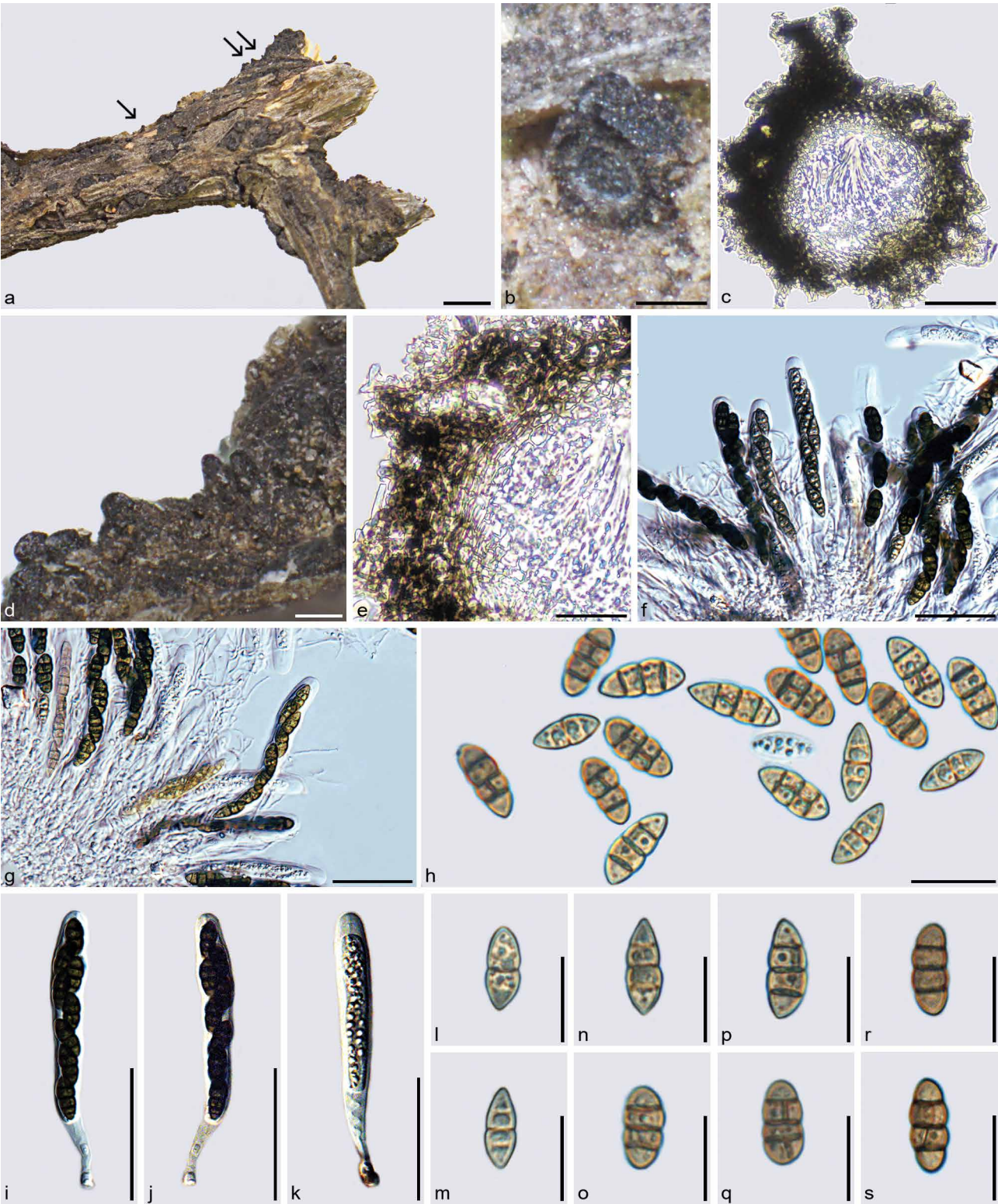


The material of O-F-256922, O-F-256923, O-F-256924 and O-F-256925, representing *L. pseudomacrostromum*, showed morphological similarities of ascomata, peridium, asci, and to some extent ascospores with *L. macrostromoides*, indicating a close relationship. We observed dictyospores in all material of *L. pseudomacrostromum* examined, in some instances dictyospores were few and in others numerous. No dictyospores were seen in the material of *L. macrostromoides*. Still, high intraspecific variability in spore shape and septation was observed within each specimen and even within ascomata, both for *L. pseudomacrostromum* and *L. macrostromoides*.

***Lophiostoma rosae-ecae*** (Wanas. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 838997

*Basionym.* *Neopaucispora rosae-ecae* (as '*rosaecae*') Wanas. et al., Fungal Diversity 89: 65. 2018. — MycoBank MB 554147.

Notes — The host of this specimen is *Rosa ecae* (*Rosa-ceae*), and therefore the correct epithet is *rosae-ecae*, not *rosaecae* as given in the original publication. This species is erroneously listed under the epithet *Neopaucispora rosacearum* (isotype, ex-type MFLUCC 17-0807) in Phukhamsakda et al. (2020).



**Fig. 14** *Lophiostoma submuriforme* (O-F-256926 - holotype). a. Habit; b–c. sections of ascomata; d. ascomatal apices; e. peridium; f–g. hymenium; h, l–s. ascospores; i–k. asci. — Scale bars: a = 2 mm; b = 200 µm; c = 100 µm; d = 300 µm; e, g = 40 µm; f, i–k = 50 µm; h, l–s = 20 µm.



***Lophiostoma scrophulariicola*** Andreasen, Jaklitsch & Voglmayr, *nom. nov.* — MycoBank MB 838998

*Etymology.* Referring to its host *Scrophularia*.

*Replaced synonym.* *Sigarispora scrophulariae* Wanas. et al., Fungal Diversity 89: 79. 2018. — Index Fungorum IF 554152, non *Lophiostoma scrophulariae* Peck, Ann. Rep. N.Y. State Mus. Nat. Hist. 28: 76. 1876 '1875'. — MycoBank MB 151334.

*Notes* — As the epithet is already occupied by *Lophiostoma scrophulariae* Peck, a new epithet was necessary.

***Lophiostoma spartii-juncei*** Andreasen, Jaklitsch & Voglmayr, *nom. nov.* — MycoBank MB 839062

*Replaced synonym.* *Coelodictyosporium muriforme* Thambug. et al., Fungal Diversity 74: 218. 2015. MycoBank MB 551237. — MycoBank MB 362316, non *Lophiostoma muriforme* Hazsl., Mathem. Természettud. Közlem. Magg. Tudom. Akad. 25 (2): 84. 1893 '1892'. — MycoBank MB 141107.

*Notes* — Although *Coelodictyosporium muriforme* may be synonymous with *Lophiostoma pseudodictyosporium*, we currently keep the taxa separate (see Notes of *L. pseudodictyosporium*). The host of these taxa is *Spartium junceum*. As the names *L. muriforme* and *L. spartii* already exist, a new name was necessary for *C. muriforme* in *Lophiostoma*.

***Lophiostoma submuriforme*** Andreasen, Jaklitsch & Voglmayr, *sp. nov.* — MycoBank MB 838999; Fig. 14

*Etymology.* With reference to the submuriform ascospores.

*Typus.* MOROCCO, near the water reservoir between Touaachak and Seisid, N30°02'38.6 W09°05'47.3, elev. 588 m, on twigs of *Genista cf. ferox*, 9 May 2015, W. Jaklitsch (O-F-256926 - holotype; ex-type culture CBS 147274 = C217).

*Sexual morph:* *Ascomata* 140–340 µm diam, solitary to gregarious, immersed to erumpent, coriaceous, black, globose to subglobose, ostiolate, apex well-developed. *Ascoma apex* crest-like, central, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* 92–112 µm thick, strongly developed around the whole hymenium, composed of brown to lightly pigmented cells of tendencies to *textura angularis* but also *textura prismatica* in ostiole regions and at the base. *Hamathecium*, unbranched, pseudoparaphyses, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* (96–)103–126(–139) × (11–)12–15.8(–17) µm (n = 22), bitunicate, fissitunicate, clavate, with a long pedicel, apically rounded, with an ocular chamber, with 8 partly biseriate ascospores. *Ascospores* 18–22(–25) × 8–11 µm (n = 30), yellowish brown to dark brown, fusiform becoming ellipsoid with end cells being first acute becoming more rounded at maturity, with 1–3 transverse septa and in a variable fraction also with 1–2 longitudinal septa, distinctly constricted at the middle septum, upper part slightly wider.

*Ecology* — Saprobic on wood of *Genista cf. ferox*.

*Notes* — Phylogenetically, *L. submuriforme* is situated in a statistically unsupported clade together with *L. caudatum*, *L. clavatum*, *L. coronillae*, and *L. scrophulariicola*. The characteristic morphology with dictyospores having acute end-cells when immature along with phylogenetic evidence indicates that this is a species new to science.

***Lophiostoma terricola*** (G.S. Gong) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 839000

*Basionym.* *Trematosphaeria terricola* G.S. Gong, Mycol. Progr. 13: 38. 2013 '2014'. — MycoBank MB 801031.

*Synonym.* *Alpestrisphaeria terricola* (G.S. Gong) Thambug. & K.D. Hyde, Fungal Diversity 74: 214. 2015. — MycoBank MB 551233.

***Lophiostoma thymi*** (Wanas. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 839001

*Basionym.* *Sigarispora thymi* Wanas. et al., Fungal Diversity 89: 80. 2018. — MycoBank MB 554153.

***Lophiostoma tropicum*** (A. Hashim. et al.) Andreasen, Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB 839002

*Basionym.* *Pseudolophiostoma tropicum* A. Hashim. et al., Stud. Mycol. 90: 175. 2018. — MycoBank MB 823142.

***Lophiotremataceae*** K. Hiray. & Kaz. Tanaka, Mycoscience 52: 405. 2011 — MycoBank MB 561063

*Type genus.* *Lophiotrema* Sacc. — MycoBank MB 2934.

***Antealophiotrema*** A. Hashim. & Kaz. Tanaka, Persoonia 39: 68. 2017 — MycoBank MB 819252

*Type species.* *Antealophiotrema brunneosporum* (Yin. Zhang et al.) A. Hashim. & Kaz. Tanaka. 2017. — MycoBank MB 819253.

*Sexual morph:* *Ascomata* subglobose to depressed ellipsoid. *Ascoma apex* central, carbonaceous, crest-like, elongated, laterally compressed. *Peridium* composed of two layers; outer layer darker red brown to black, inner pale golden brown, forming *textura prismatica* to *textura angularis* on the inner side wall, fusing with host tissue in lower parts. *Hamathecium* of septate, branched and anastomosed, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* bitunicate, fissitunicate, cylindrical to narrowly clavate, apically rounded with an ocular chamber, with 6–8 biseriate ascospores. *Ascospores* narrowly fusiform, with rounded ends, 1–3-septate, hyaline to brown, often hyaline becoming brown at maturity, guttulate when immature, smooth.

*Ecology* — Saprobic on woody plants.

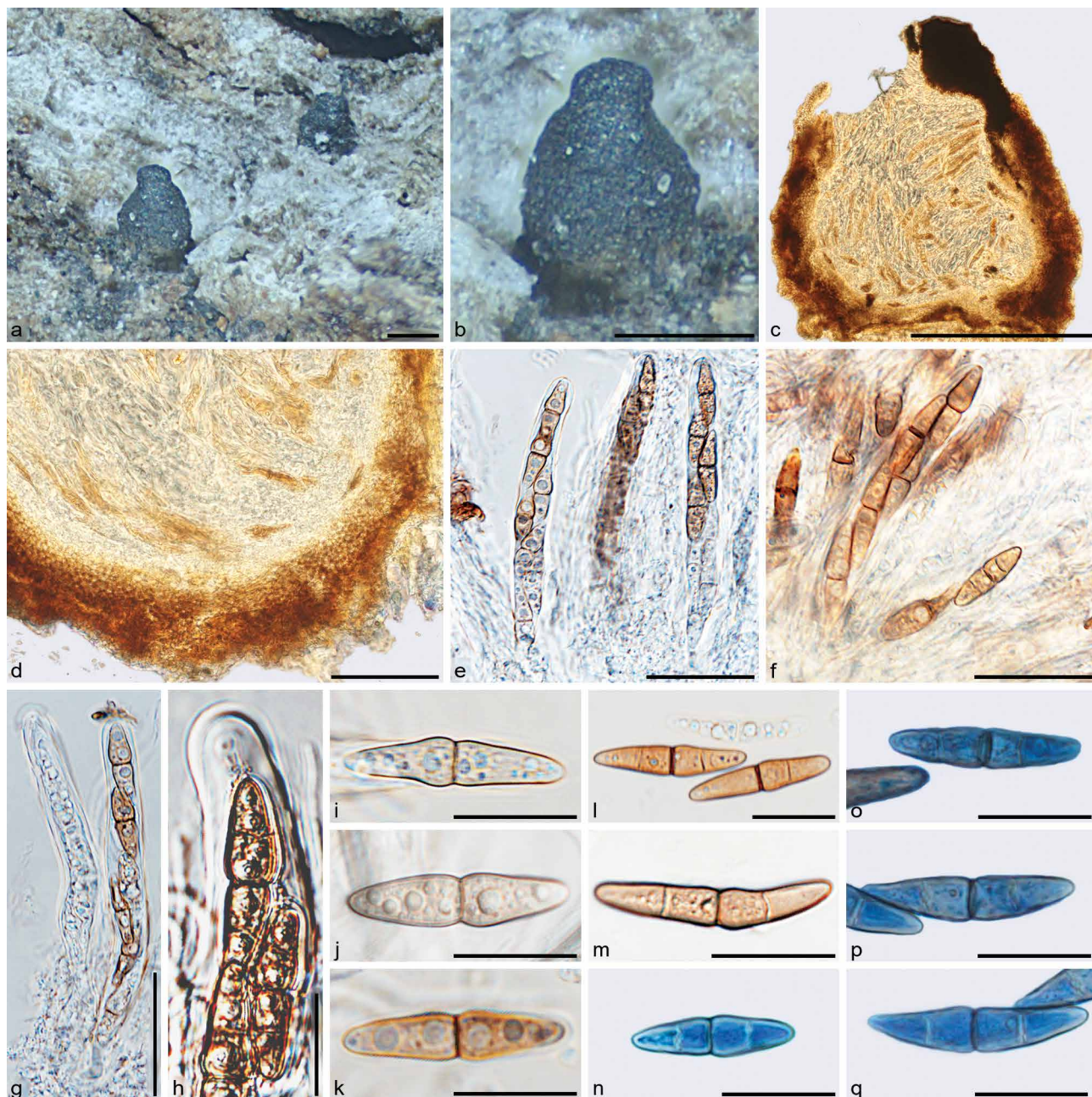
*Notes* — This genus does not belong to the family, but we consider it at this place because we used this genus as outgroup for rooting the phylogenetic tree of the *Lophiotremataceae* and because we describe a new species in the genus.

***Antealophiotrema populicola*** Andreasen, Nordén & J.B. Jordal, *sp. nov.* — MycoBank MB 839003; Fig. 15

*Etymology.* With reference to the host species *Populus tremula*.

*Typus.* NORWAY, Møre og Romsdal county, Tingvoll municipality, on the bark of old living *Populus tremula*, 12 Oct. 2018, J.B. Jordal (O-F-256929 - holotype; ex-holotype culture CBS 147528 = MAL63); Møre og Romsdal county, Aure municipality, on the bark of old living *Populus tremula*, 18 Nov. 2019, J.B. Jordal (O-F-256928 - paratype; ex-paratype culture CBS 147529 = MAL64).

*Sexual morph:* *Ascomata* 160–280 µm diam, subglobose, black. *Ascoma apex* central, crest-like, elongated, laterally compressed, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* 20–40 µm thick, composed of two layers; outer layer darker red brown to black, inner pale golden brown, forming *textura prismatica* to *textura angularis* on the inner sidewall, fusing with host tissue in lower parts. *Hamathecium* comprising septate, branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* (116–)127–144(–161) × (13–)14–17(–18) µm (n = 30), bitunicate, fissitunicate, cylindrical to narrowly clavate, with a stipe < 10 µm, apically rounded, with an ocular chamber, with 6–8 biseriate ascospores, short-stiped. *Ascospores* (23–)25–38(–42) × (7–)8–9(–10) µm (n = 68), narrowly fusiform with narrowly rounded ends, 1–3-septate, strongly constricted at the median septum, first hyaline becoming brown, guttulate, smooth.



**Fig. 15** *Antealophiotrema populicola*. (O-F-256929 - holotype). a–b. Ascomata; c. section of ascoma; d. peridium; e–h. asci; i–q. ascospores (immature in i–k). — Scale bars: a–c = 100  $\mu$ m; d–g = 40  $\mu$ m; h = 20  $\mu$ m; i–q = 20  $\mu$ m (n–q in Cotton blue).

**Culture characteristics** — Ascospores germinated on MEA within 48 h at 20 °C. Germ tubes produced from end and central cells. Colonies 2–2.6 cm diam after 4 wk, subcircular with somewhat irregular margins; initially whitish becoming light greyish to dark grey from below; reverse black.

**Ecology** — Saprobic on the bark of living *Populus tremula*.

**Notes** — In the presented tree (Fig. 2), this species and *Antealophiotrema brunneosporum* (CBS 123095) are used as the outgroup to *Lophiotremataceae*. Hashimoto et al. (2017) noted that *A. brunneosporum* (CBS 123095) and '*Lophiotrema*' *boreale* (CBS 114422) formed a fully supported clade (100 % MLB BP/1.00 BPP) outside the *Lophiotremataceae*. Thus, they recognised them as a distinct lineage named *Antealophiotrema* as a genus *incertae sedis* in the *Pleosporales*. Strains CBS 147528 and CBS 147529 as presented here might shed light on the familiar placement of this family, but an extended dataset is needed. However, we show strong support (100 % ML BP/1.00 Bayesian PP) for our two strains nesting as a sister species next to *A. brunneosporum*. *Antealophiotrema brunneosporum*

and '*Lophiotrema*' *boreale* were misidentified initially as species in *Lophiotrema* based on morphological resemblance to the genus (Mathiassen 1989, 1993, Zhang et al. 2009a). Further examination is needed to clarify the taxonomic placement of '*Lophiotrema*' *boreale* (TROM-F-6942 = GM 218). Morphologically, *A. populicola* differs from '*Lophiotrema*' *boreale* as described by Mathiassen (1989), by having significantly larger asci ((116–)127–144(–161)  $\times$  (13–)14–17(–18))  $\mu$ m vs 70–90(–95)  $\times$  5.5–7  $\mu$ m), and by larger ascospores of slightly different shape ((23–)25–38(–42)  $\times$  (7–)8–9(–10)  $\mu$ m and narrowly fusiform vs 13–16(–17)  $\times$  3–5  $\mu$ m and ellipsoid to fusiform). Both species also have different hosts, *Salix nigricans* ssp. *nigricans* and *Salix nigricans* ssp. *borealis* for '*Lophiotrema*' *boreale* (Mathiassen 1989), and *Populus tremula* for *A. populicola*. However, Holm & Holm (1988) reported '*Lophiotrema*' *boreale* also from *Alnus*, *Betula*, *Corylus*, and *Fraxinus* spp. *Antealophiotrema populicola* differs from *A. brunneosporum* as described by Hashimoto et al. (2017) in smaller ascomata (160–280  $\mu$ m vs 460–530  $\mu$ m diam), thinner peridium (20–40  $\mu$ m vs 42.5–62.5  $\mu$ m thick), and shorter ascospores ((23–)25–38(–42)  $\mu$ m vs 34.5–48  $\times$



6.5–10 µm). Finally, we note that the host of *A. populicola* (*Populus tremula*) differs from that of *A. brunneosporum* (*Salix* sp.).

**Atrocalyx** A. Hashim. & Kaz. Tanaka, Persoonia 39: 59. 2017 — MycoBank MB 819240

*Type species. Atrocalyx acutisporus* A. Hashim. & Kaz. Tanaka. 2017. — MycoBank MB 819241.

Sexual morph: *Ascomata* solitary or scattered to gregarious, semi-immersed to immersed or erumpent, sometimes forming a black continuous crust. *Ascoma apex* crest-like, elongated and laterally compressed, sometimes surrounded by dark brown hyphae. *Peridium* composed of two to several layers of dark brown to black, thick-walled pseudoparenchymatous cells, forming *textura angularis* or *textura prismatica*, paler inwardly, fusing with host tissue at the outermost layer. *Hamathecium* comprising branched, cellular pseudoparaphyses, anastomosing above and between the asci, embedded in a gelatinous matrix. *Asci* bitunicate, fissitunicate, cylindrical to cylindric-clavate, apically rounded with an ocular chamber, containing 6–8 uniseriate to obliquely overlapping ascospores, with short stipe. *Ascospores* broadly fusiform to ellipsoid, with obtuse to acute ends, brown or hyaline, 1–3-septate, guttulate, smooth to verruculose, with or without mucilaginous/gelatinous sheath, with or without appendages.

Ecology — Saprobic on woody plants.

Notes — Hashimoto et al. (2017) stated that the genus *Atrocalyx* 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 nordicus** Andreassen, Nordén & J.B. Jordal, *sp. nov.* — MycoBank MB 839004; Fig. 16

*Etymology.* With reference to its occurrence in a Nordic country (Norway).

*Typus.* NORWAY, Oslo county and municipality, on the bark of old living *Fraxinus excelsior*, 28 Sept. 2018, M. Andreassen (O-F-256932 - holotype; ex-holotype culture CBS 147532 = MAL27); Møre og Romsdal county, Molde municipality, on the bark of old living *Populus tremula*, 3 Sept. 2018, J.B. Jordal (O-F-256930 - paratype; MBT 10000179; ex-paratype culture CBS 147530 = MAL20; O-F-256931 - paratype; ex-paratype culture CBS 147531 = MAL21; O-F-256933 - paratype; ex-paratype culture CBS 147533 = MAL76).

Sexual morph: *Ascomata* 290–450 µm diam, black, scattered to gregarious, immersed or erumpent from the slightly blackened substrate, globose to pyriform. *Ascomata neck* central, crest-like, laterally compressed, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* 40–55 µm thick, composed of several layers of dark brown to black, thick-walled pseudoparenchymatous cells, forming *textura angularis*, paler inwardly, fusing with host tissue at the outermost layer. *Hamathecium* comprising branched, cellular pseudoparaphyses, anastomosing above and between the asci,

embedded in a gelatinous matrix. *Asci* (110–)123–168(–180) × (12–)13–15 µm (n = 80), bitunicate, fissitunicate, cylindric-clavate, with a stipe < 10 µm, apex rounded, with a minute ocular chamber and 8 obliquely uniseriate to biseriate ascospores. *Ascospores* (17–)18–25(–30) × (4–)6–8(–10) µm (n = 100), hyaline, ellipsoid with rather obtuse ends, 3-septate, constricted at the middle septum, with thick (up to 20 µm) diffuse mucilaginous sheath, smooth-walled, with two large globules in each cell, smooth.

Culture characteristics — Ascospores germinated on MEA within 48 h at 20 °C. Germ tubes produced from one or both ends. Colonies 3–3.4 cm diam after 4 wk, subcircular with irregular margins; initially pale grey becoming dark grey to black from below; reverse black.

Ecology — Saprobic on bark of living *Fraxinus excelsior* and *Populus tremula*.

Notes — Although there are some sequence differences between the strains CBS 147530, CBS 147531, CBS 147532 and CBS 147533, they form a clade with strong support within the genus *Atrocalyx*. Morphologically this new species has considerable resemblance to other species of *Atrocalyx* like *A. acutisporus* and *A. lignicola* as described by Hashimoto et al. (2017) (see Table 4). However, *A. nordicus* differs significantly from *A. acutisporus* by larger sizes of ascomata, peridium, asci and ascospores but overlaps with *A. lignicola* on the same characters. Still, the spore shape of *A. nordicus* is more ellipsoid-fusiform and 3-septate at maturity compared to the 1-septate and heteropolar ones of *A. lignicola* as noted by Zhang et al. (2009b).

When first found, the specimens of *A. nordicus* were identified as *Lophiotrema lennartii*, which is very similar in morphological characters, particularly in ascospore shape and size. It still differs in several other aspects such as ascospore septation with up to 3-septate vs 1-septate in *L. lennartii*, more clavate vs strictly cylindrical, thicker-walled and more elongate asci exceeding the maximum observed for *L. lennartii* (140 µm) (Mathiassen et al. 2017). Ascospores have a uniseriate arrangement in asci (vs obliquely uniseriate to uniseriate). Besides, this species is found in Oceanic environments at low altitude collection sites (vs continental high altitude localities). Finally, we note that the hosts of *A. nordicus* (*Fraxinus excelsior* and *Populus tremula*) differ from those of *L. lennartii* (*Aconitum septentrionale* and *Myricaria germanica*).

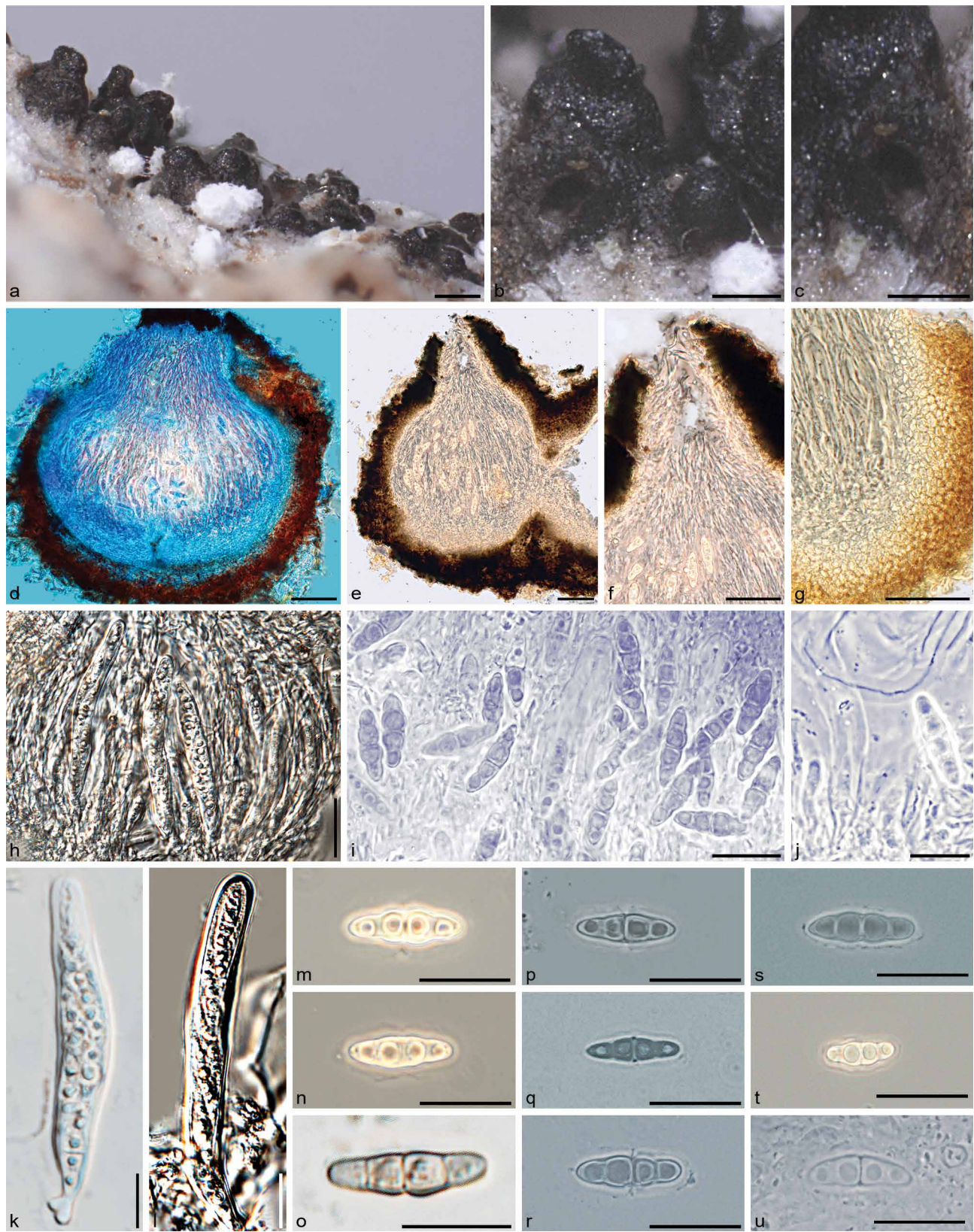
**Lophiotrema** Sacc. emend. Holm & Holm, Symb. Bot. Upsal. 28: 25. 1988 — MycoBank MB 2934

*Type species. Lophiotrema nucula* (Fr.: Fr.) Sacc., Michelia 1: 338. 1878.

Sexual morph: *Ascomata* immersed to erumpent, subglobose. *Ascoma apex* crest-like or rarely papillate, mostly elongated and laterally compressed, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* composed of cells forming *textura angularis* or *textura prismatica*, paler

**Table 4** Comparison of selected morphological characters between *Atrocalyx nordicus*, *Atrocalyx acutisporus* and *Atrocalyx lignicola* as described by this study and by Hashimoto et al. (2017).

	<i>Atrocalyx nordicus</i>	<i>Atrocalyx acutisporus</i>	<i>Atrocalyx lignicola</i>
Ascomata diam	290–450 µm	190–210 µm	350–600 µm
Ascus size	(110–)120–180 × 12–15 µm	(66.5–)75–89.5 × 8–11 µm	100–146 × 12.5–17 µm
Ascospore size	(17–)18–25(–30) × (4–)6–8(–10) µm	13.5–18(–20) × 3–4(–5.5) µm	20–26 × 6.5–9.5 µm
Ascospore shape	ellipsoid with rather obtuse ends	broadly fusiform	broadly fusiform, heteropolar
Ascospore septation	3-septate	1–3-septate	1-septate
Peridium thickness	40–55 µm	20–30 µm	27.5–42 µm
Host	<i>Populus tremula</i> , <i>Fraxinus excelsior</i>	woody plant	<i>Populus</i> sp.



**Fig. 16** *Atrocalyx nordicus* (O-F-256932 - holotype). a–c. Ascomata; d–f. section of ascomata; g. peridium; h–j. hymenium; k–l. immature asci; m–u. ascospores (immature in u). — Scale bars: a–c = 200 µm; d–h = 50 µm; i–u = 20 µm (d, i–k, p–s, u in Cotton blue).

towards the inside, darker at the outside and fusing with host tissues. *Hamathecium* comprising septate, branched, cellular, anastomosing pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. Asci bitunicate, fissitunicate, cylindrical, with a short stipe, 8-spored. Ascospores fusiform, hyaline, smooth, verruculose at maturity.

Ecology — Saprobic on various plants.

Notes — Holm & Holm (1988) distinguished *Lophiotrema* from *Lophiostoma* based on differences in the type of peridium and asci; the peridium in *Lophiotrema* is nearly equal in thickness (c. 25 µm), whereas in *Lophiostoma* it is broader (c. 50 µm). The asci in *Lophiotrema* are cylindrical or oblong, but clavate or oblong in *Lophiostoma*. This definition was accepted by Barr (1992), Yuan & Zhao (1994), Tanaka & Harada (2003b), and Kirk et al. (2008). Hashimoto et al. (2017) 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. With the inclusion of *Lophiotrema myriocarpum* in the genus, the distinction between *Lophiostoma* and *Lophiotrema* by peridium thickness is no longer tenable.

***Lophiotrema myriocarpum*** (Fuckel) Sacc., *Michelia* 1 (no. 3): 338. 1878 — MycoBank MB 173362; Fig. 17

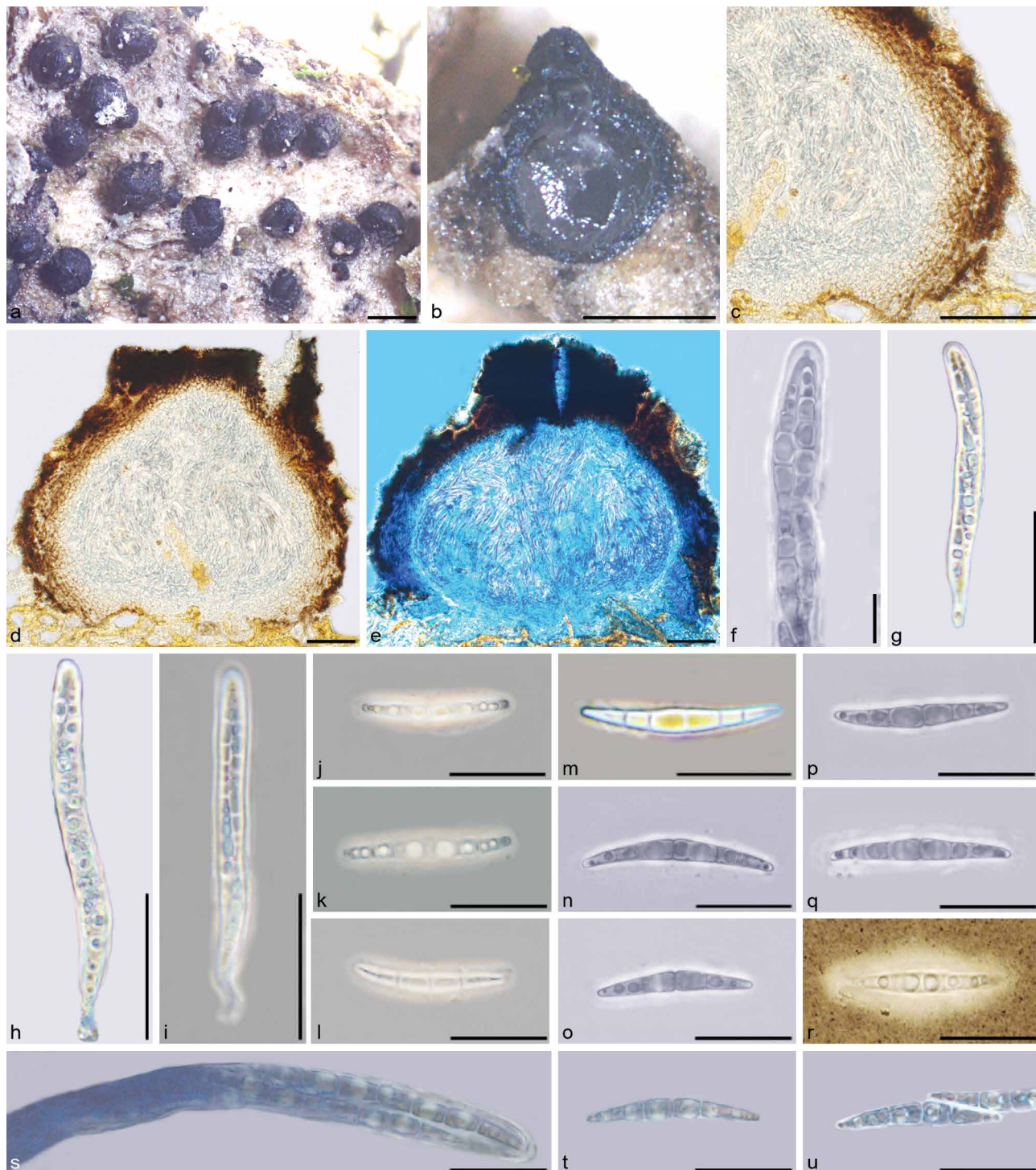
*Basionym.* *Lophiostoma myriocarpum* Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 156. 1870. — MycoBank MB 141605.

*Synonyms.* *Lophiotrema vigheffulense* (Pass.) Berl., *IC. Fung.* 1:4. 1890. — MycoBank MB 206314.

*Lophiosphaera vigheffulensis* Pass., *Erb. Critt. Ital. Ser. 2* no. 1373. 1883. — MycoBank MB 248553.

*Typus.* GERMANY, Hessen, *K.W.G. Fuckel*, in Fuckel, *Fungi Rhen. Exs.* 1807 (G00127825 - lectotype designated here; MBT 10000318). — NORWAY, Rogaland county, Suldal municipality, on the bark of living *Fraxinus excelsior*, 19 Sept. 2018, *J.B. Jordal* (O-F-256934 - epitype designated here; MTB 10000178; ex-epitype culture CBS 147534 = MAL01).

**Sexual morph:** *Ascomata* densely scattered, immersed to erumpent, 170–460 µm diam, black, globose, glabrous. *Ascoma* apex central or lateral, coarse, crest-like, carbonaceous, with a pore-like ostiole. *Ostiole* rounded, periphysate. *Hamathecium* comprising septate, branched, cellular pseudoparaphyses, anastomosing above and between the asci, embedded in a gelatinous matrix. *Peridium* 30–55 µm thick, 2-layered, outer layer composed of several layers of dark brown to black, thick-walled pseudoparenchymatous cells fusing with host tissue at the outermost layer, thick inner layer of similar, but thinner-walled



**Fig. 17** *Lophiotrema myriocarpum*. a–r. (O-F-256934 - epitype), s–u. (G00127825 - lectotype) a. Ascomata; b, d–e. section of ascomata; c. peridium; f–i. s. asci; j–r. t–u. ascospores. — Scale bars: a = 400 µm; b = 250 µm; c–e, g–i = 50 µm; f = 10 µm; j–u = 20 µm (e–f, n–q, s–u in Cotton blue, r in Indian ink).



and pale brown to hyaline cells. Asci (90–)100–115(–120) × 10–12 µm (n = 20), bitunicate, cylindrical with attenuated stipe < 10 µm long, with 6–8 biseriate ascospores. Ascospores (23.5–)26–35(–38) × 4–5(–6) µm (n = 35), hyaline, narrowly fusiform, straight to slightly curved, 3–5-septate, constricted at the median septum, surrounded by inconspicuous mucilaginous sheath 1–4 µm wide, guttulate, with oil drops disappearing when overmature.

Culture characteristics — Ascospores germinated on MEA within 24 h at 20 °C. Germ tubes produced from end and central ascospore cells. Colonies 3.1–4.1 cm diam after 4 wk, subcircular with somewhat irregular margins; initially whitish, becoming pale to dark grey from below, margin dark grey to black; reverse black.

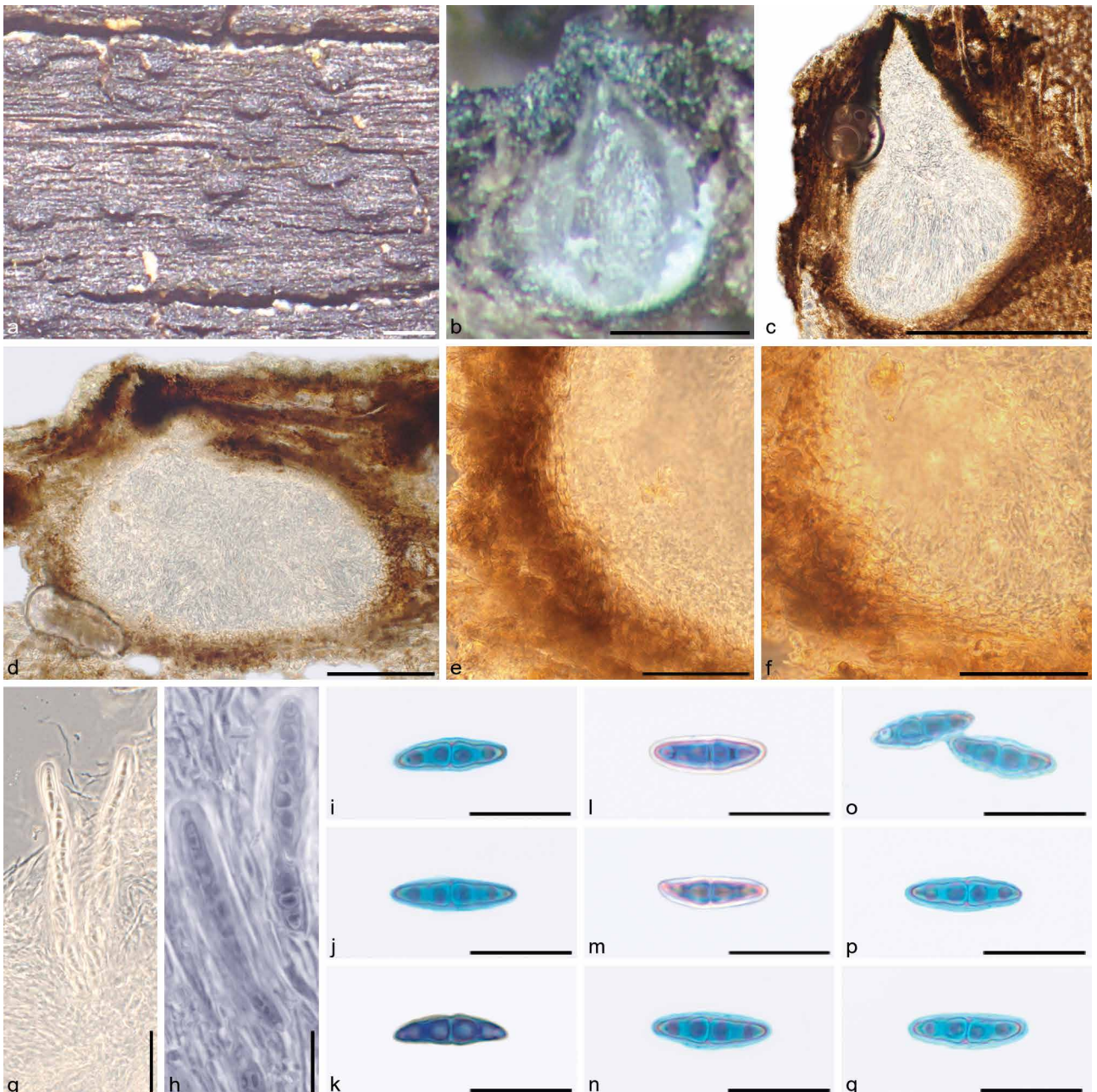
Ecology — Saprobic on wood and bark of deciduous trees and shrubs, also on *Dryas*.

*Additional materials examined.* NORWAY, Vestlandet county, Kvam municipality, on bark of *Ulmus glabra*, 15 May 2019, M. Andreasen (O-F-256935; culture CBS 147535 = MAL71).

Notes — Within *Lophiotrema*, *L. myriocarpum* (strains MAL01 and MAL71) forms a clade with strong support together with *L. neohysterioides* (strains KT 17, KT 588, KT 713, KT 756).

*Lophiotrema neohysterioides* M.E. Barr as re-described by Tanaka & Harada (2003b) has some morphological resemblance, but differs by, e.g., distinctly shorter ((14–)17–25(–26.5) × 3–5 µm) and consistently 3-septate ascospores and smaller ((60–)70–96(–110) × (6–)7–10 µm), narrowly clavate asci.

Holm & Holm (1988) reported that this species is well characterised by the narrow spores, which early have more than one septum. Based on the description of Holm & Holm (1988), the two collections of MAL01 and MAL71 were identified as *Lophiostoma myriocarpum*. The type material of *L. myriocarpum* (G00127825) was investigated, which confirmed the identification, and we reassign this species to the genus *Lophiotrema* and epitypify it with one of our specimens. As there are several syntypes of *L. myriocarpum* (e.g., G00127823, G00127824, G00127825, S-F-71903, S-F-71899, S-F-13329, S-F-267560),



**Fig. 18** *Lophiotrema nucula* (O-F-247790). a. Habit; b–c. ascomata in section; d–f. peridium; g–h. asci; i–q. ascospores. — Scale bars: a–b = 150 µm; c = 200 µm; d = 40 µm; e–f, i–q = 20 µm; g–h = 10 µm (h–q in Cotton blue).



we select collection G00127825 as lectotype, which we epitypify with O-F-256934 to stabilise the species concept.

***Lophiotrema nucula*** (Fr.: Fr.) Sacc., *Michelia* 1: 338. 1878 — MycoBank MB 151729; Fig. 18

*Basionym.* *Sphaeria nucula* Fr., *Kongl. Vetensk. Acad. Handl.* 38: 266. 1817. — MycoBank MB 222550.

*Synonym.* *Lophiostoma nucula* (Fr.) Ces. & De Not., *Comment. Soc. Crittog. Ital.* 1 (4): 222. 1863. — MycoBank MB 244964.

Sexual morph: *Ascomata* 200–450 µm diam, scattered or gregarious, immersed to erumpent, globose to subglobose, black, uniloculate, glabrous, ostiolate. *Ascoma* apex central or lateral, crest-like, carbonaceous, with a pore-like ostiole. *Ostiolar canal* rounded, periphysate. *Peridium* 10–20 µm thick, composed of several layers of dark brown to black, thick-walled pseudoparenchymatous cells forming *textura angularis*, paler inwardly, outermost layer fusing with host tissue. *Hamathecium* comprising branched, cellular pseudoparaphyses, anastomosing above and between the asci, embedded in a gelatinous matrix. *Asci* (70–)80–110(–120) × (8–)9–11.5 µm (n = 120), bitunicate, fissitunicate, cylindrical, with a stipe 15–33 µm long, apically rounded, with a minute ocular chamber, containing (4–)8 (obliquely) uniseriate ascospores. *Ascospores* (18.5–)19–23(–24.5) × (5.5–)6–9.5 µm (n = 180), hyaline becoming brownish when overmature, ellipsoid-fusiform, with rounded ends, 1–3-septate, constricted at the middle septum and slightly at others, slightly narrower towards both ends, surrounded by inconspicuous mucilaginous sheath 0.5–1 µm wide, smooth-walled, sometimes verruculose at maturity, guttulate with one or two guttules in each cell.

Culture characteristics — Ascospores germinated on MEA within 24 h at 20 °C. Germ tubes were produced from one or both ascospore ends. Colonies 2.7–3.5 cm diam after 4 wk, circular with somewhat irregular margin; initially whitish, becoming greyish from the surface; reverse greyish brown.

Ecology — Saprobic on decorticated wood and bark of various deciduous trees such as *Acer pseudoplatanus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Liriodendron tulipifera*, *Populus tremula*, *Quercus* sp., *Salix* sp., *Syringa vulgaris*, and *Ulmus glabra*.

*Materials examined.* NORWAY, Agder county, Arendal municipality, on the bark of living *Populus tremula*, 4 Oct. 2014, Jacques Fournier (O-F-247790; culture MAL47); Viken county, Asker municipality, on branches of living *Salix* sp., 20 Feb. 2019, M. Andreassen (MA19-012); Froland municipality, on *Ulmus glabra*, 3 Oct. 2014, J. Fournier (O-F-247791); same area, on *Populus* cf. *tremula*, 3 Oct. 2014, J. Fournier (O-F-247805); Vestland county, Granvin municipality, on *Ulmus glabra*, 13 May 2014, B. Nordén & J.B. Jordal (O-F-251885).

Notes — The spores of the examined specimens are of a rather fusoid shape compared to the ellipsoid-fusiform (plumper and more obtuse) as presented by Holm & Holm (1988), Tanaka & Harada (2003b) and to some degree to those described by Mathiassen (1989), where the spores are described as broadly ellipsoid to oblong ellipsoid, with rounded ends. Some illustrations by Mathiassen (1989) resemble our spores to a greater extent but with more rounded ends as seen in Fig. 18.

## DISCUSSION

### Phylogenetic reconstruction and genetic markers

Our phylogenetic analyses based on a multigene-matrix of four molecular markers (ITS, LSU, *TEF1-α* and *RPB2*) shed a new light on the relationships within *Lophiostomataceae* and *Lophiotremataceae*. However, the phylogenetic reconstruction, particularly of the *Lophiostomataceae*, is not entirely settled. The four markers inferred more or less stable support for

species and shallow clades. Examples for this in *Lophiotremataceae* are *Lophiotrema myriocarpum* and *L. nucula*, and examples for *Lophiostomataceae* are *Lophiostoma caespitosum*, *L. longiappendiculatum*, *L. macrostomoides*, *L. macrostomum*, *L. obtusisporum*, *L. scabridisporium*, *L. tropicum*, *L. winteri*, and the species of the genera *Flabellascoma* and *Vaginatispora*. However, in the case of the genus *Lophiostoma*, generally little backbone support was found, and *RPB2* sequences are lacking for many taxa. Wherever *RPB2* and *TEF1-α* were present, we observed enhanced resolution and support at the species level. These coding regions gave support to most genera in *Lophiotremataceae*, and of *Crassiclypeus*, *Flabellascoma*, *Lentistoma*, *Neovaginatispora* and *Vaginatispora* in the *Lophiostomataceae*. This tendency of improved support when *RPB2* and *TEF1-α* are present was also reported in other studies of related taxa, e.g., *Teichosporaceae* (Jaklitsch et al. 2016). However, *RPB2* was difficult to amplify in the present study, which may generally be the reason for the low number of available sequences. Thus, this molecular marker, but also *TEF1-α*, albeit less pronounced, are often missing in the alignments. We expect that addition of coding markers such as *TEF1-α* and *RPB2* for all taxa would enhance resolution and support of deeper nodes and probably provide a more stable topology. We see such enhanced resolution and a much better resolved topology in our presented phylogeny of *Lophiotremataceae* for clades where *TEF1-α* and *RPB2* are present for virtually all species, e.g., the clades representing the genera *Atrocalyx*, *Lophiotrema* and *Cryptoclypeus*.

### Lophiostomataceae

The phylogenetic analyses of *Lophiostomataceae* showed stronger support for deeper nodes compared to previous studies (Mugambi & Huhndorf 2009, Hirayama & Tanaka 2011, Zhang et al. 2014, Thambugala et al. 2015, Jaklitsch et al. 2016, Hashimoto et al. 2018, Bao et al. 2019), but with a tendency to weaker support as compared to Phukhamsakda et al. (2020). The phylogeny strongly supports the application of a broad generic concept of *Lophiostoma*, satisfying the criteria of Vellinga et al. (2015) for generic circumscription, in particular a strong statistical support and monophyly.

The validity of *Alpestrisphaeria*, *Coelodictyosporium*, *Guttulispora*, *Lophiohelichrysum*, *Platystomum*, and *Sigarispora*, was earlier questioned by Hashimoto et al. (2018), and we further question the validity of *Biappendiculispora*, *Capulatispora*, *Lophiopoacea*, *Neopaucispora*, *Neotrematosphaeria*, *Pseudocapulatispora*, *Pseudolophiostoma*, and *Pseudoplatystomum*, and synonymise all with *Lophiostoma* based on molecular phylogenetic evidence, thus creating a well-supported genus. Of these synonymised clades *Alpestrisphaeria*, *Biappendiculispora*, and *Pseudolophiostoma*, *Pseudoplatystomum* and the later added *Pseudocapulatispora*, represented as clades A11–A14 by Thambugala et al. (2015), hold strong support in our phylogeny, both for their overall placement and for the species nesting within. If these were accepted as separate genera, also the remaining clades, represented as A1–A10 (i.e., *Platystomum*, *Coelodictyosporium*, *Neotrematosphaeria*, *Sigarispora*, *Lophiopoaceae*, *Lophiohelichrysum*, *Guttulispora*, and *Capulatispora*) by Thambugala et al. (2015), would need separation, but none of them nor an overall clade encompassing all of them received significant statistical support. Only particularly shallow clades of species or sister species hold sufficient support for their placement together (i.e., *L. caespitosum*, *L. crenatum*, *L. fusisporum*, *L. macrostomoides*, and *L. macrostomum*). Lastly, we emphasise the lack of molecular markers such as *TEF1-α* and *RPB2* for these less supported clades within *Lophiostoma*.

In the case of the total alignment of *Lophiostomataceae*, many strains are still lacking sequences of molecular markers repre-

senting ribosomal DNA or protein coding loci, and even some are lacking representatives of both. The result is a persistently unresolved topology, which is made further indistinct in many cases by the lack of support within deeper nodes (Fig. 1).

Concerning morphology and applicable conclusions on general characters used for distinction on generic and species level, we chose to be cautious and only refer to descriptions and photo plates. For both generic and species distinction there is high intraspecific variability of several morphological traits in the genus *Lophiostoma*. Therefore, it has been difficult to assign a name to individual collections safely. Phylogenetic analyses provide an excellent tool for the improvement of this situation. One example is *L. pseudomacrostromum*, which in our phylogenetic analyses is a good species but shares morphological traits with *L. compressum* and *L. macrostromoides*, which make them difficult to distinguish based on morphology alone.

### **Lophiotremataceae**

The results of the phylogenetic analyses show a topology comparable to previous presentations of *Lophiotremataceae* with tendencies to increased support for deeper nodes (Zhang et al. 2009a, b, Hirayama & Tanaka 2011, Hashimoto et al. 2017). One reason for this may be the relatively low number of species, as compared to the *Lophiostomataceae*, another is the scrutinised work of Tanaka and collaborators (Hirayama & Tanaka 2011, Hashimoto et al. 2017), who provided *TEF1-α* and *RPB2* sequences for virtually all species included. One new species is here added to the genus *Atrocalyx* based on four strains (CBS 147530, CBS 147531, CBS 147532, CBS 147533), creating a strongly supported clade within the genus, and further morphological evidence for this species is provided in the species description. Further, we resurrect *Lophiotrema myriocarpum*. This placement is based on strong phylogenetic support and morphological evidence, particularly the cylindrical asci and spore shape, as stated in the notes to the species description.

In earlier studies, only a single strain of *Lophiotrema nucula* (Hirayama & Tanaka 2011, Hashimoto et al. 2017) was included in phylogenetic analyses. The inclusion of our strain now provides strong support for the species as sister to *L. fallopieae*, *L. vagabundum*, and *L. neoarundinariae*.

### **Morphology**

Because of the high intraspecific variability of several morphological traits of the sexual morph within *Lophiostomataceae*, and in particular within the genus *Lophiostoma*, it was challenging to provide a structured presentation of differentiating morphology that reflects both phylogenetic relationships and morphological characters. Thus, no keys for identifying genera nor species are presented here.

This high intraspecific variability of morphology is persistent within many pleosporalean genera such as, e.g., *Teichospora* (Jaklitsch et al. 2016) and the here presented genus *Lophiostoma*. Thus, it is difficult to place pleosporalean fungi in these genera based on morphology alone. It can, therefore, seem attractive to define segregate genera having narrowly defined morphology, such as, e.g., ascospore colour, shape and septation, for those who want to identify fungal species and genera by morphology alone. Still, it is not a workable solution to split these genera, creating small entities with relatively clear morphological characters. This splitting does not increase insight in the evolutionary history of the group in question, but only inflates the taxonomic framework as these genera are no longer distinguishable from other genera in other families within *Pleosporales*. On the other hand, a broad generic concept does not impair the situation with difficulties of identification, but will make it easier to look up the literature. There are morpholo-

gically virtually identical fungi in different genera in different families of the *Pleosporales*, which generally challenges the applicability of a morphological generic classification system by a narrow generic concept.

### **Future perspectives**

Remaining issues concerning the phylogeny and taxonomy of the two families include that several old epithets have never been re-assessed, despite efforts by Chesters & Bell (1970) and Holm & Holm (1988). Materials representing these epithets need recollection at the original collection sites and from the original hosts. Also, for several taxa initially described from Europe in the phylogram, only DNA data of Asian material are present. This even includes the generic type of *Lophiostoma*, *L. macrostromum* alongside many others, e.g., *L. semiliberrum*, *L. caudatum*, or *L. caulium*. These taxa require recollection in Europe for confirmation of the names.

Within the presented topology of the genus *Lophiostoma*, several groupings of strains and taxa are found nesting together without significant support but showing very similar morphological characters. A lack of molecular information (e.g., some strains miss specific molecular markers in the alignment) can in many cases explain some of this missing support, but not always. Cryptic species can be defined as 'morphologically indiscernible biological/phylogenetic units present within taxonomic species' (Knowlton 1993, Balasundaram et al. 2015). *Lophiostoma compressum* is an example of such a morphospecies showing tendencies of being a complex of cryptic species, with molecular differentiation among strains but bearing similar morphological characters. Investigations of their internal relationship, both within strains of the same taxa showing differences in molecular affinities, but also between the different species, could shed further light on this issue. Concerning *Lophiostoma compressum* and the here synonymised genus *Platystomum*, there are many proposed taxa within this species complex, e.g., '*Platystomum*' *rosae*, '*Platystomum*' *salicicola*, and '*Platystomum*' *crataegi* that require further investigation. The strains do not show support for being distinct species and are only represented by ITS and LSU, for the most part.

Another question is the internal relationship between many of the taxa within the genus *Lophiostoma*. Many proposed species are unsupported phylogenetically, others are well supported as species, but their overall relationship within the genus is unclear. The lack of support shows a need for continuous sampling and procurement of sufficient molecular information followed by a thorough morphological investigation. There might even be a need for the identification of new, informative molecular markers to infer better phylogenetic resolution for species and shallow clades. Here, it will be important to select a marker that amplifies well within the group, considering the experience with *RPB2* that does not amplify well in many lophiostomatoid species.

**Acknowledgements** We thank the Norwegian Biodiversity Information Centre for financial support (Biodiversity mapping project on bitunicate ascomycetes, BN, grant no. 2017/33701). The University of Oslo and the Norwegian Institute for Nature Research are acknowledged for funding in connection to the master thesis of the first author. We thank the curators of the fungaria at Oslo, Tromsø, Uppsala, and Geneve for the loan of specimens and permission to study material.

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