Lophiostomataceae and *Lophiotremataceae*

A phylogenetic and taxonomic study of two ascomycete families

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Abstract

This study shed further light on the phylogenetic and morphological boundaries of the families *Lophiostomataceae* and *Lophiotremataceae* (Pleosporales, Dothideomycetes, Ascomycota) and their encompassing genera and species. By adding 25 new strains of *Lophiostomataceae* and nine new *Lophiotremataceae* strains to a sequence data matrix from international databases, our results provide a new understanding of the relationship within these families. Multigene analysis of the five molecular markers of ITS2, 5.8S, LSU, TEF1-α and RPB2 reveal that in *Lophiotremataceae*, the different genera are phylogenetically well supported. However, in *Lophiostomataceae*, a resurrection of a broad generic concept of the genus *Lophiostoma* is implied. It is argued that the combined support of morphological data and supported monophyletic groups is key to defining genera. Phylogenetic analyses revealed that 11 genera are synonyms of *Lophiostoma* and these genera and their species are thus in need of being combined into this genus. Also, the species "*Guttulispora*" *crataegi* is reported synonym of *Lophiostoma caespitosum* based on both phylogenetic and morphological evidence.

Four new species to science are defined, and one new species combination is presented. All identified species are supported with morphological characters to aid in non-molecular species discrimination. Taxonomical descriptions of families, genera and species of taxa found by this study are accompanied by photo plates. High intraspecific variability of several morphological traits is found within *Lophiostomataceae* and in particular within genus *Lophiostoma*. The species of *Lophiostoma macrostomoides* and *Lophiostoma compressum* are showing morphological and phylogenetic indications of being cryptic species, and it is argued that future analyses on species delimitations of these taxa are needed.

Four species and one genus new to Norway are reported, and collections of more than 300 specimens are added to the national biodiversity mapping project on bitunicate ascomycetes.

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1 Introduction

The diversity of Fungi is enormous and bewildering. Estimates of the total number of fungal species on Earth varies between 2.2 and 5.1 million (Blackwell 2011; Chang et al. 2015; Berbee et al. 2017). From these millions of estimated fungi, 144,000 species have so far been named and classified (Hawksworth and Lücking 2017; Willis et al. 2018), and thus only a few percentages of existing species are yet known to science (Taylor et al. 2014; Willis et al. 2018).

Within Ascomycota, the class Dothideomycetes is the most species rich (Kirk et al. 2008; Schoch et al. 2009), and herein Pleosporales is the largest order, comprising a quarter of all dothideomycetous fungi (Devadatha et al. 2017). Within the Pleosporales, we find the families *Lophiostomataceae* Sacc. and *Lophiotremataceae* K. Hiray. & Kaz. Tanaka.

Nitschke first recognized *Lophiostomatacea*e in 1869, and Saccardo formally established the family in 1883, with type species *Lophiostoma macrostomum* (Tode) Ces. & De Not. The genus *Lophiotrema* Sacc. was traditionally considered nested in the family *Lophiostomataceae* (Barr 1992; Kirk et al. 1995; Lumbsch and Huhndorf, 2009; Hirayama and Tanaka 2011) and has only recently been established and validated as the family *Lophiotremataceae*, typified by *Lophiotrema nucula (Rehm) Mussat* (Hirayama and Tanaka 2011). At present *Lophiostomataceae* encompasses 25 genera with 190 species, and *Lophiotremataceae* encompasses six genera with 28 species (Wijayawardene et al. 2020). During the last four decades increasing work has been put into investigations of diversity within the order Pleosporales in Scandinavia (Eriksson, 1981; Holm and Holm, 1988; Mathiassen 1993; Mathiassen et al. 2017a;b; Nordén, Jäntti and Jordal 2017; Nordén et al. 2019). However, there is still a remaining discrepancy in the number of genera and species reported from Norway compared to international figures. According to the Norwegian Mycological Database, the Norwegian records are limited to one genus with 11 species of *Lophiostomataceae* and one genus encompassing four species within *Lophiotremataceae* (Larsson et al. 2010).

Lophiostomataceae and *Lophiotremataceae* are families of saprobic ascomycetes which occur world-wide on twigs, stems and bark of various plants both in terrestrial and aquatic environments (Holm and Holm 1988; Ellis and Ellis 1997; Mugambi and Huhndorf 2009). The two families share several morphological characters, such as an immersed to erumpent ascomata of 0.1-1.2 mm width, a carbonaceous peridium-wall and a crest-like beak with a slit-

like ostiole. They have cylindrical to clavate fissitunicate asci and hyaline to dark brown, one- to multiseptated ascospores.

The morphological and ecological similarities of the two families lead to species belonging to both families often being collected together in the field and often misplaced in one or the other family. Thus, this study targeted both families, aiming to understand diversity and generic placement of taxa between and within the families. Distinguishing morphological characters between the families of *Lophiostomataceae* and *Lophiotremataceae* have been suggested by many; such as spore colouration of dark spore versus hyaline, peridium thickness and form, ascus shape and stipe size and more recently further characters such as mucilaginous layer and terminal appendages of ascospores have been added to the list of characters determining familiar circumscription (Saccardo 1878; Holm and Holm 1988; Barr 1992; Mathiassen 1993; Yuan and Zhao 1994; Tanaka and Harada 2003a;b; Tanaka and Hosoya 2008; Eriksson 2009; Hirayama and Tanaka 2011; Hashimoto et al. 2018). Fungal taxonomists are often faced with the dilemma of describing taxa with little or none morphological difference and are further challenged by convergent traits (parallel evolution of similar traits) and atavism (lost traits that reappear).

Fortunately, technical advances have opened for comparison of organisms on a molecular level and much altered the science of distinguishing taxa. Recent phylogenetic studies using molecular methods have changed the placement of many families, genera and species within Pleosporales. These approaches have resolved the evolutionary histories in some of these groups and revealed that the previously trusted morphological identification could not be used to recognize all these evolutionary units. The emergence of advanced molecular methods and analyses have allowed a re-evaluation of many characters. However, phylogenetic analyses have probably, to a lesser degree than anticipated, solved the problems of genus delimitation and many genera remain paraphyletic (Padamsee et al. 2008; Nuhn et al. 2013; Wu et al. 2014). These issues have led to two divergent approaches; mirroring the historical distinctions of splitters and lumpers. One pathway has been to take small monophyletic groups as the basis for new genera, without too much concern about the reminder of the original genus, thus allowing polyphyletic sister genera. The second approach embraces a more gestalt view, using a broad genus concept and upkeeping monophyletic groupings and enabling species classification within a binomial classification frame. In this

study, the six guidelines for proposing new genera, proposed by Vellinga et al. (2015) and adapted by Tulloss et al. (2016) will be followed. These guidelines highlight the absolute need for monophyly based on sufficient and strong statistical support, a broad phylogenetic coverage by multimarker phylogeny, a comprehensive discussion and argumentation of data presented and decision-making based on thesis testing. Moreover, Vellinga et al. (2015) emphasises that the importance of monophyly is not only crucial for the grouping in focus, but also the group from which it is separated and the group to which it is added.

For defining species, this study accepts the concept of Genealogical Concordance Phylogenetic Species Recognition (Taylor et al. 2000; Dettman et al. 2003) combining several molecular markers, of diverging conservation-levels, for accurate phylogenetic reconstruction. To fulfil this claim, the of markers from nuclear ribosomal DNA (5.8S, ITS2, LSU) and the two protein coding markers of Translation elongation factor 1-α (TEF1-α) and RNA-polymerase II second largest subunit (RPB2) are used. This study also embraces the Consolidated Species Concept (Quaedvlieg et al. 2014) using a combination of morphological, ecological and phylogenetic species concepts to provide a basis for taxonomic limitations.

Study aims

The aims of this thesis are: (1) To better resolve the phylogenetic relationships within the families of *Lophiostomataceae* and *Lophiotremataceae*, increasing the phylogenetic understanding of these families by adding newly collected taxa; (2) To discuss the numerous newly proposed genera of *Lophiostomataceae* with particular emphasis on the genus *Lophiostoma*, based on an extended dataset; (3) To examine the diversity and distribution of taxa of *Lophiostomataceae* and *Lophiotremataceae* in Norway, to add data to the national biodiversity mapping project on bitunicate ascomycetes; and (4) To investigate if a re-evaluation of morphological characters for species and generic discrimination is possible.

2 Materials and methods

2.1 Taxon selection and sampling

Strains of *Lophiostomataceae* and *Lophiotremataceae* were collected in Norway from September 2018 until September 2019. A total of 17 excursions were carried out with collection sites ranging from southern eastern and western lowlands to central alpine locations. Additional collections of *Lophiostomataceae* were loaned from the fungaria in Oslo (O) and Tromsø (TROM) along with a subset of unpublished data of strains from countries other than Scandinavia.

Small pieces of wood, bark or plant matter with ascomata were collected, dried at room temperature and transferred to fungarium capsules. A subset of these samples was chosen, based on age and viability, to infer phylogeny and to aid in molecular species delimitation.

2.2 Cultivation techniques

A selection of the collections was induced for cultivation by isolation of ascospores. Fertile ascomata were identified, investigated and dissected under a Nikon eclipse 50i compound microscope. Hymenial material was transferred into a sterile water droplet on a micro slide and transferred with a sterile pipette onto Petridisches holding malt agar (MEA plates, 3% malt extract, 1.5% agar in water, autoclaved) and antibiotics (2.5% Streptomycin, 1% Tetramycin, 5% Ampicillin). Plates were incubated at 20 °C for spore germination and daily checked for growth under a Nikon SMZ 745T stereo microscope. Germinating spores were transferred individually onto MEA plates (without antibiotics), their growth monitored, any contaminants removed and pictures taken. Development of asexual morphs was documented for up to 1.5 years.

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.

2.3 DNA extraction and sequencing

DNA was extracted from cultural mycelia using 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 PCR amplify most of the ribosomal

DNA regions of internal transcribed spacer (ITS) ITS1, 5.8S and ITS2 and 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.

Table 1. Overview of primers PCR and sequencing of specimens from family *Lophiostomataceae* and *Lophiotremataceae*.

Region[1]	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
ITS	ITS1[2]: TCCGTAGGTGAACCTGCGG	ITS4[2]: TCCTCCGCTTATTGATATGC
LSU	V9G[3]: TTACGTCCCTGCCCTTTGTA LR2R[4]: AAGAACTTTGAAAAGAG	LR5[4]: TCCTGAGGGAAACTTCG LR3[4]: GGTCCGTGTTTCAAG
TEF1-α	EF1-728F[5]: CATCGAGAAGTTCGAGAAG	TEF1-LLErev[6]: AACTTGCAGGCAATGTGG
RPB2	fRPB2-5[7]: GAYGAYMGWGATCAYTTYGG	fRPB2-7C[7]: CCCATRGCTTGYTTRCCCAT

[1] ITS: The 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.
[2] White et al. 1990
[3] Hoog and Ende 1998
[4] Vilgalys and Hester 1990
[5] Carbone and Kohn 1999
[6] Jaklitsch et al. 2016
[7] Novakova et al. 2012

The following PCR protocols were used to amplify the molecular regions: 2 min at 95 °C, 40 cycles of 15 sec (20 sec for TEF1-α) at 95 °C, denaturation for 15 sec at 95 °C (20 sec for TEF1-α), annealing at 20 sec at 53 °C (30 sec at 55 °C for TEF1-α and RPB2) and followed by an elongation for 1 min and 10 sec at 70 °C (90 sec for TEF1-α and 60 sec for RPB2), ended by 3 min at 70 °C and an indefinite hold at 4 °C. Amplified PCR products were visualized with electrophoresis on 1.5% agarose gels to ensure the presence of amplified product (and only one). 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 five µL primer, same as for amplification, was added to clean tubes and labelled before sequencing. Sanger sequencing was performed by Eurofins, Luxemburg, using the same primers as for the PCR reaction.

2.4 Sequence alignment and phylogenetic analyses

Sequence data for relevant strains were downloaded from GenBank following recent publications and are shown in Table 2 (Thambugala et al. 2015; Jaklitsch et al. 2016;

Hashimoto et al. 2017; Wanasinghe et al. 2018; Hyde et al. 2019; Bao 2019). In some cases, morphological data were not available, and strain names must be regarded as tentative.

Sequence editing, assembly and concatenations were done using Geneious Prime v. 2020.0.5 (Kearse et al. 2012). 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 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 1 000 bootstrap replications.

To examine topological incongruence among data sets, ML bootstrapping analyses were carried out on each of the single-gene data sets. Topological incongruence was assumed if conflicting tree topologies were supported by ≥ 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 and 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 1 000 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.

Some taxa were excluded from the analyses like those of *Neotrematosphaeria biappendiculata* (KTC 975, KTC 1124), *Dimorphiopsis brachystegiae* (CPC 22679) and

Lophiotrema boreale (CBS 114422) due to inconclusive topological placement and very long branch lengths.

Output trees were edited with Inkscape v. 0.92.1 (Harrington et al. 2003).

2.5 Morphological investigation

Ascomata were rehydrated with autoclaved water and investigated using a Nikon SMZ 745T / Zeiss SteREO Discovery V8 stereomicroscope and a Nikon Eclipse Ci-L / Zeiss Axio Imager A2 compound microscope. Images of the fruit bodies were captured with a NIKON DS-Fi2 or Tucsen DigiRetina 16 camera, using stacking software Lite Helicon Focus 7 v. 7.5.6 for giving in depth resolution and precise measurements and scale bar ratios. 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 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 colour 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 and Mattis 1996).

Table 2.1. *Lophiostomataceae*. Fungal names, strains and GenBank accessions used in this study. The sequences generated in this study are indicated in bold, currently with code NNXXXXX until GenBank accession numbers are provided.

Taxa	Original no.	Strain no.	GenBank accession no.			
			ITS	LSU	TEF1-α	RPB2
"Coelodictyosporium" muriforme	MFLUCC 13-0351	MFLUCC 13-0351	KP899136	KP888641	KR075163	-
Crassiclypeus aquaticus	HHUF:30568	KH 104	LC312499	LC312528	LC312557	LC312586
Crassiclypeus aquaticus	HHUF 30569	KH 185	LC312500	LC312529	LC312558	LC312587
Flabellascoma aquaticum	KUMCC15-0258	KUMCC15-0258	MN304827	MN274564	MN328898	MN328895
Flabellascoma cycadicola	CBS 143644	KT 2034	LC312502	LC312531	LC312560	LC312589
Flabellascoma fusiforme	MFLUCC 18-1584	MFLUCC 18-1584	MN304830	MN274567	MN328902	-
Flabellascoma minimum	CBS 143645	KT 2013	LC312503	LC312532	LC312561	LC312590
Flabellascoma minimum	CBS 143646	KT 2040	LC312504	LC312533	LC312562	LC312591
Lentistoma bipolare	HHUF 30573	KT 2415	LC312512	LC312541	LC312570	LC312599
Lentistoma bipolare	HHUF 30574	KT 3056	LC312513	LC312542	LC312571	LC312600
Leptoparies palmarum	HHUF 28983	KT 1653	LC312514	LC312543	LC312572	LC312601
"Lophiohelichrysum" helichrysi	MFLUCC 15-0701	IT-1296	KT333435	KT333436	KT427535	-
"Lophiopoacea" paramacrostoma	MFLUCC 11-0463	MFLUCC 11-0463	-	KP888636	-	-
Lophiostoma aff. macrostomoides	**MA19-036**	**MAL73**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma aff. macrostomoides	**MA19-042**	**MAL81**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma aff. macrostomoides	**MA19-048**	**MAL83**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma aff. macrostomoides	**MA19-049**	**MAL84**	**NNXXXXX**	**NNXXXXX**	**-**	**-**
Lophiostoma alpigenum	GKM 1091b	GKM 1091b	-	GU385193	GU327758	-
Lophiostoma arundis	JCM 13550	KT 606	JN942964	AB618998	LC001737	-
Lophiostoma arundis	JCM 13551/MAFF 239449	KT 651	JN942965	AB618999	LC001738	-
Lophiostoma caespitosum	MFLUCC 13-0442	MFLUCC 13-0442	KP899134	KP888639	KR075161	-
Lophiostoma caespitosum	MFLUCC 14-0993	MFLUCC 14-0993	KP899135	KP888640	KR075162	-
Lophiostoma caespitosum	**LQ2**	**LQ2**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**

Table 2.1. *Lophiostomataceae* (Continued).

Taxa	Original no.	Strain no.	GenBank accession no.			
			ITS	LSU	TEF1-α	RPB2
Lophiostoma caudatum	MAFF 239453	KT 530	LC001723	AB619000	LC001739	-
Lophiostoma caulium	HHUF 27515	KT 686-1	LC001729	AB619006	LC001745	-
Lophiostoma caulium	MFLUCC 17-2450	MFLUCC 17-2450	MN304829	-	MN328900	-
Lophiostoma caulium	HHUF 27313	KT 573	LC001728	AB619005	LC001744	-
Lophiostoma caulium	HHUF 27311	KT 794	LC001730	AB619007	LC001746	-
Lophiostoma caulium	MFLUCC 15-0036	MFLUCC 15-0036	MG828965	MG829077	MG829239	-
Lophiostoma caulium	MAFF 239450	KT 603	LC001724	AB619001	LC001740	-
Lophiostoma caulium	JCM 17669	KT 633	LC001725	AB619002	LC001741	-
Lophiostoma caulium	MFLUCC 15-0176	MFLUCC 15-0176	-	KT328493	-	-
Lophiostoma compressum	**OOL-18.3**	**MAL02**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma compressum	**MA19-003**	**MAL54**	**NNXXXXX**	**-**	**NNXXXXX**	**-**
Lophiostoma compressum	**MA19-056**	**MAL86**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma compressum	**MA19-072**	**MAL90**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma compressum	**MA19-076**	**MAL93**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma compressum	**MA19-077**	**MAL94**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma compressum	**MA19-001**	**MAL49**	**NNXXXXX**	**-**	**-**	**-**
Lophiostoma compressum	HHUF 29192	KT 521	JN942963	JN941380	LC001747	-
Lophiostoma compressum	HHUF:29194	KT 534	JN942962	JN941379	LC001748	-
Lophiostoma compressum	IFRD 2014	IFRD 2014	-	FJ795437	-	FJ795457
Lophiostoma compressum	MFLUCC 13-0343	MFLUCC 13-0343	-	KP888643	KR075165	-
Lophiostoma crenatum	CBS 629.86	AFTOL-ID 1581	-	DQ678069	DQ677912	DQ677965
Lophiostoma heterosporum	CBS 644.86	AFTOL-ID 1036	GQ203795	AY016369	DQ497609	DQ497615
Lophiostoma jonesii	GAAZ 54-1	GAAZ 54-1	KX687757	KX687753	KX687759	-

Table 2.1. *Lophiostomataceae* (Continued).

Taxa	Original no.	Strain no.	GenBank accession no.			
			ITS	LSU	TEF1-α	RPB2
Lophiostoma jonesii	GAAZ 54-2	GAAZ 54-2	KX687758	KX687754	KX687760	-
Lophiostoma macrostomoides	**MA18-072**	**MAL32**	**NNXXXXX**	**NNXXXXX**	**-**	**-**
Lophiostoma macrostomoides	CBS 123097	CBS 123097	FJ795439	FJ795439	GU456277	FJ795458
Lophiostoma macrostomum	HHUF 27288	KT 508	JN942961	AB619010	LC001751	JN993491
Lophiostoma macrostomum	HHUF 27293	HHUF 27293/KT 709	AB433276	AB433274	LC001753	-
Lophiostoma macrostomum	HHUF 27290	HHUF 27290/KT 635	AB433275	AB433273	LC001752	-
Lophiostoma multiseptatum	HHUF 27309	KT 604/JCM17668	LC001726	AB619003	LC001742	-
Lophiostoma pseudodictyosporium	MFLUCC 13-0451	MFLUCC 13-0451	KR025858	KR025862	-	-
Lophiostoma ravennicum	MFLUCC 14-0005	MFLUCC 14-0005	KP698413	KP698414	-	-
Lophiostoma sagatiforme	HHUF 29754	KT 1934	AB369268	AB369267	LC001756	-
Lophiostoma semiliberum	JCM 13548	KT 622	JN942966	AB619012	LC001757	-
Lophiostoma semiliberum	JCM 13547	KT 652	JN942967	AB619013	LC001758	-
Lophiostoma semiliberum	JCM 13549/MAFF 239448	KT 828	JN942970	AB619014	LC001759	-
Lophiostoma semiliberum	CBS 626.86	CBS 626.86	-	FJ795441	-	FJ795460
Lophiostoma sp. nov.	**MA19-068**	**MAL88**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma sp. nov.	**MA18-0001**	**MAL04**	**NNXXXXX**	**-**	**NNXXXXX**	**NNXXXXX**
Lophiostoma terricola	**MAL19-075**	**MAL92**	**NNXXXXX**	**NNXXXXX**	**-**	**NNXXXXX**
Lophiostoma terricola	SC-12	SC-12	JN662930	JX985750	-	-
Lophiostoma triseptatum	SMH 2591	SMH 2591	-	GU385183	-	-
Lophiostoma triseptatum	SMH 5287	SMH 5287	-	GU385187	-	-
Lophiostoma vitigenum	JCM 13534/MAFF 239459	HH26930	LC001735	AB619015	LC001761	-
Lophiostoma vitigenum	JCM 17676	HH26931	LC001736	AB619016	LC001762	-
Lophiostoma winteri	JCM 17648	KT 740	JN942969	AB619017	LC001763	JN993487

Table 2.1. *Lophiostomataceae* (Continued).

Taxa	Original no.	Strain no.	GenBank accession no.			
			ITS	LSU	TEF1-α	RPB2
Lophiostoma winteri	MAFF 239454	KT 764	JN942968	AB619018	LC001764	JN993488
Lophiostoma sp.	**LMS**	**LMS**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma sp.	**LQ**	**LQ**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma sp.	**LQ1**	**LQ1**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma sp.	**LC**	**LC**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma sp.	**LC1**	**LC1**	**NNXXXXX**	**NNXXXXX**	**-**	**-**
Lophiostoma sp.	**C191**	**C191**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma sp.	**C217**	**C217**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma sp.	**C220**	**C220**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiostoma sp.	**TEQ**	**TEQ**	**NNXXXXX**	**NNXXXXX**	**-**	**-**
Neovaginatispora fuckelii	MFLUCC 17-1334	MFLUCC 17-1334	MN304828	MN274565	MN328899	MN328896
Neovaginatispora fuckelii	CBS 101952	CBS 101952	-	DQ399531	-	FJ795472
Neovaginatispora fuckelii	HHUF 30076	KH 161	LC001731	AB619008	LC001749	-
Neovaginatispora fuckelii	HHUF 27325	KT 634	LC001732	AB619009	LC001750	-
Parapaucispora pseudoarmatispora	HHUF 30497	KT 2237	LC100021	LC100026	LC100030	-
Paucispora quadrispora	HHUF 30455	KH448	LC001733	LC001722	LC001754	-
Paucispora quadrispora	HHUF 27321	KT 843	LC001734	AB619011	LC001755	-
Paucispora versicolor	HHUF 30448	KH 110	AB918731	AB918732	LC001760	-
"Platystomum" crataegi	MFLUCC 14-0925	MFLUCC 14-0925	KT026117	KT026109	KT026121	-
"Platystomum" rosae	MFLUCC 15-0633	MFLUCC 15-0633	KT026119	KT026111	-	-
"Platystomum" salicicola	MFLUCC 15-0632	MFLUCC 15-0632	KT026118	KT026110	-	-
"Pseudolophiostoma" obtusisporum	HHUF 30171	KT 3098	LC312519	LC312548	LC312577	LC312606
"Pseudolophiostoma" obtusisporum	HHUF 30189	KT 3119	LC312520	LC312549	LC312578	LC312607

Table 2.1. *Lophiostomataceae* (Continued).

Taxa	Original no.	Strain no.	GenBank accession no.			
			ITS	LSU	TEF1-α	RPB2
"Pseudolophiostoma" tropicum	HHUF 30586	KH 352	LC312521	LC312550	LC312579	LC312608
"Pseudolophiostoma" tropicum	HHUF 30202	KT 3134	LC312522	LC312551	LC312580	LC312609
"Pseudopaucispora brunneospora	CBS 143661	KH 227	LC312523	LC312552	LC312581	LC312610
"Pseudoplatystomum" scabridisporum	BCC 22835	BCC 22835	-	GQ925844	GU479857	GU479830
"Pseudoplatystomum" scabridisporum	BCC 22836	BCC 22836	-	GQ925845	GU479856	GU479829
"Sigarispora" caryophyllacearum	MFLUCC 17-0749	MFLUCC 17-0749	MG828964	MG829076	MG829238	-
"Sigarispora" clavata	MFLUCC 18-1316	MFLUCC 18-1316	-	MN274566	MN328901	-
"Sigarispora" coronillae	MFLUCC 14-0941	MFLUCC 14-0941	KT026120	KT026112	-	-
"Sigarispora" junci	MFLUCC 14-0938	MFLUCC 14-0938	MG828966	MG829078	-	-
"Sigarispora" medicaginicola	MFLUCC 17-0681	MFLUCC 17-0681	MG828967	MG829079	-	-
"Sigarispora" muriformis	MFLUCC 13-0744	MFLUCC 13-0744	KY496740	KY496719	-	-
"Sigarispora" ononidis	MFLUCC 14-0613	MFLUCC 14-0613	KU243128	KU243125	KU243127	-
"Sigarispora" rosicola	MFLU 15-1888	MFLU 15-1888	MG828968	MG829080	MG829240	-
"Sigarispora" scrophulariae	MFLUCC 17-0689	MFLUCC 17-0689	MG828969	MG829081	-	-
"Sigarispora" thymi	MFLU 15-2131	MFLU 15-2131	MG828970	MG829082	MG829241	-
Teichospora rubriostiolata	WU 33594/CBS 140734	TR7	KU601590	KU601590	KU601609	KU601599
Teichospora trabicola	WU 33582/CBS 140730	C134	KU601591	KU601591	KU601601	KU601600
Vaginatispora amygdali	HHUF 30588	KT 2248	LC312524	LC312553	LC312582	-
Vaginatispora amygdali	MFLUCC 18-1586	MFLUCC 18-1586	MK085055	MK085059	MK087657	-
Vaginatispora appendiculata	MFLUCC 16-0314	MFLUCC 16-0314	KU743217	KU743218	KU743220	-
Vaginatispora aquatica	MFLUCC 11-0083	MFLUCC 11-0083	KJ591577	KJ591576	-	-
Vaginatispora armatispora	MFLUCC 18-0247	MFLUCC 18-0247	MK085056	MK085060	MK087658	MK087669
Vaginatispora armatispora	MFLUCC 18-0213	MFLUCC 18-0213	MN304826	MN274563	MN328897	MN328894

Table 2.1. *Lophiostomataceae* (Continued).						
Vaginatispora fuckelii	HHUF 30076	KH 161	LC001731	AB619008	LC001749	-
Vaginatispora fuckelii	HHUF 27325	KT 634	LC001732	AB619009	LC001750	-
Vaginatispora microarmatispora	PUFD62	MTCC 12733	MF142592	MF142593	MF142595	MF142596
Vaginatispora scabrispora	HHUF 30589	KT 2443	LC312525	LC312554	LC312583	LC312612

Table 2.2. *Lophiotremataceae*. Fungal names, strains and GenBank accessions used in this study. The sequences generated in this study are indicated in bold, currently with code NNXXXXX until GenBank accession numbers are provided.

Taxa	Original no.	Strain no.	GenBank accession no.			
			ITS	LSU	TEF1-α	RPB2
Antealophiotrema sp. nov.	**JB18DurP9-1**	**MAL63**	**NNXXXXX**	**NNXXXXX**	**-**	**-**
Antealophiotrema sp. nov	**JB18Vikp7-1**	**MAL64**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**
Antealophiotrema brunneosporum	CBSH 20222	CBS 123095	LC194474	LC194340	LC194382	LC194419
Atrocalyx acutisporus	HHUF 30504	KT 2436	LC194475	LC194341	LC194386	LC194423
Atrocalyx asturiensis	CBS 143912	OF	MG912912	MG912912	MG912916	MG912920
Atrocalyx bambusae	MFLU11-0150	MFLUCC 10-0558	KX672149	KX672154	KX672162	KX672161
Atrocalyx Lignicola	CBSH 20221	CBS 122364	LC194476	LC194342	LC194387	LC194424
Atrocalyx sp. nov.	**JB18-502**	**MAL20**	**NNXXXXX**	**NNXXXXX**	**-**	**-**
Atrocalyx sp. nov.	**JB18-506**	**MAL21**	**NNXXXXX**	**NNXXXXX**	**-**	**-**
Atrocalyx sp. nov.	**JB18-509**	**MAL22**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Atrocalyx sp. nov.	**MA18-0003**	**MAL27**	**NNXXXXX**	**NNXXXXX**	**-**	**NNXXXXX**
Atrocalyx sp. nov.	**JB18-505**	**MAL76**	**NNXXXXX**	**NNXXXXX**	**-**	**NNXXXXX**
Crassimassarina macrospora	HHUF 30512	KH 152	LC194477	LC194343	LC194388	LC194425
Crassimassarina macrospora	HHUF 29084	KT 1764	LC194478	LC194344	LC194389	LC194426
Cryptoclypeus oxysporus	HHUF 30507	KT 2772	LC194479	LC194345	LC194390	LC194427
Cryptoclypeus ryukyuensis	HHUF 30510	AH 342	LC194480	LC194346	LC194391	LC194428
Cryptoclypeus ryukyuensis	HHUF 30509	KT 3534	LC194481	LC194347	LC194392	LC194429
Lophiotrema eburnoides	HHUF 30079	KT 1424_1	LC001709	LC001707	LC194403	LC194458

Table 2.2. *Lophiotremataceae* (Continued).

Taxa	Original no.	Strain no.	GenBank accession no.			
			ITS	LSU	TEF1-α	RPB2
Lophiotrema fallopiae	HHUF 30506	KT 2748	LC149913	LC149915	LC194404	LC194459
Lophiotrema myriocarpum	**JB17-513**	**MAL01**	**NNXXXXX**	**-**	**NNXXXXX**	**-**
Lophiotrema myriocarpum	**MA19-034**	**MAL71**	**NNXXXXX**	**-**	**NNXXXXX**	**NNXXXXX**
Lophiotrema neoarundinaria	HHUF 27547	KT 856	AB524786	AB524596	AB539109	AB539096
Lophiotrema neoarundinaria	HHUF 30015	KT 1034	LC194492	AB524598	LC194405	LC194460
Lophiotrema neoarundinaria	HHUF 30014	KT 2200	AB524787	AB524597	AB539110	AB539097
Lophiotrema neohysterioides	HHUF 30511	KH 17	LC194493	LC194376	LC194406	LC194461
Lophiotrema neohysterioides	HHUF 27368	KT 588	LC194494	LC194377	LC194407	LC194462
Lophiotrema neohysterioides	HHUF 27328	KT 713	LC194495	AB619019	LC194408	LC194463
Lophiotrema neohysterioides	HHUF 27330	KT 756	LC194496	AB619020	LC194409	LC194464
Lophiotrema nucula	**HUO F247790**	**MAL47**	**NNXXXXX**	**NNXXXXX**	**NNXXXXX**	**-**
Lophiotrema nucula	JCM14132	CBS 627.86	LC194497	AB619021	LC194410	LC194465
Lophiotrema vagabundum	HHUF 30077	KH 164	LC194498	AB619022	LC194411	LC194466
Lophiotrema vagabundum	HHUF 30078	KH 172	LC194499	AB619023	LC194412	LC194467
Lophiotrema vagabundum	HHUF 27323	KT 664	LC194500	AB619024	LC194413	LC194468
Lophiotrema vagabundum	HUF 30508	KT 3310	LC194501	LC194378	LC194414	LC194469
Lophiotrema vagabundum	F-634236	CBS 113975	LC194502	AB619025	LC194415	LC194470
Pseudocryptoclypeus yakushimensis	HHUF 30503	KT 2186	LC194504	LC194380	LC194417	LC194472

3 Results

3.1 Phylogenetic analyses

From 68 strains initially targeted for multi-locus sequencing, a total of 34 ITS, 29 LSU, 24 TEF1-α, 6 RPB2 consensus sequences were produced (Table 2, taxa from this study in bold). The protein coding locus RPB2 proved especially challenging to amplify.

The concatenated alignment for *Lophiostomataceae* comprised 3160 nucleotide characters, including gaps (5.8S and ITS2: 1-410; LSU: 411-1250; TEF1-α: 1251-2147; RPB2: 2148-3160). The alignment included 25 new strains representing more than 12 taxa. In total the alignment was composed of 117 strains of the *Lophiostomataceae*, and the two taxa *Teichospora rubriostiolata* (TR7) and *Teichospora trabicola* (C134) as the outgroup. ITS1 was excluded from the analyses because it contained too many ambiguously aligned regions.

The concatenated alignment for *Lophiotremataceae* comprised 3650 nucleotide characters, including gaps (5.8S and ITS2: 1-461; LSU: 462-1710; TEF1-α: 1711-2631; RPB2: 2632-3650). The alignment included nine new strains representing two taxa. In total the alignment was composed of 33 strains, including three strains of *Antealophiotrema brunneosporum* (CBS 123095, MAL63, MAL64) as the outgroup taxa. ITS1 was excluded from the analyses because it contained too many ambiguously aligned regions.

Topological incongruence was assumed if conflicting tree topologies were supported by ≥ 70 % maximum likelihood RAxML bootstrap support (MLB). Since topological incongruence was not observed between the single-gene data sets (not shown), maximum likelihood (ML) phylogenetic analyses with bootstrap were carried out on concatenated five-locus datasets for both *Lophiostomataceae* and *Lophiotremataceae*. ML analyses of the combined datasets provided higher bootstrap support values for the genus level than did those of the individual gene trees. The ML analysis of the combined datasets yielded the best scoring tree for *Lophiostomataceae* (Figure 1) and *Lophiotremataceae* (Figure 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) greater than 0.90 are given at the nodes. The alignments had 35.29% and 14.77% undetermined nucleotide gaps for *Lophiostomataceae* and *Lophiotremataceae*, respectively.

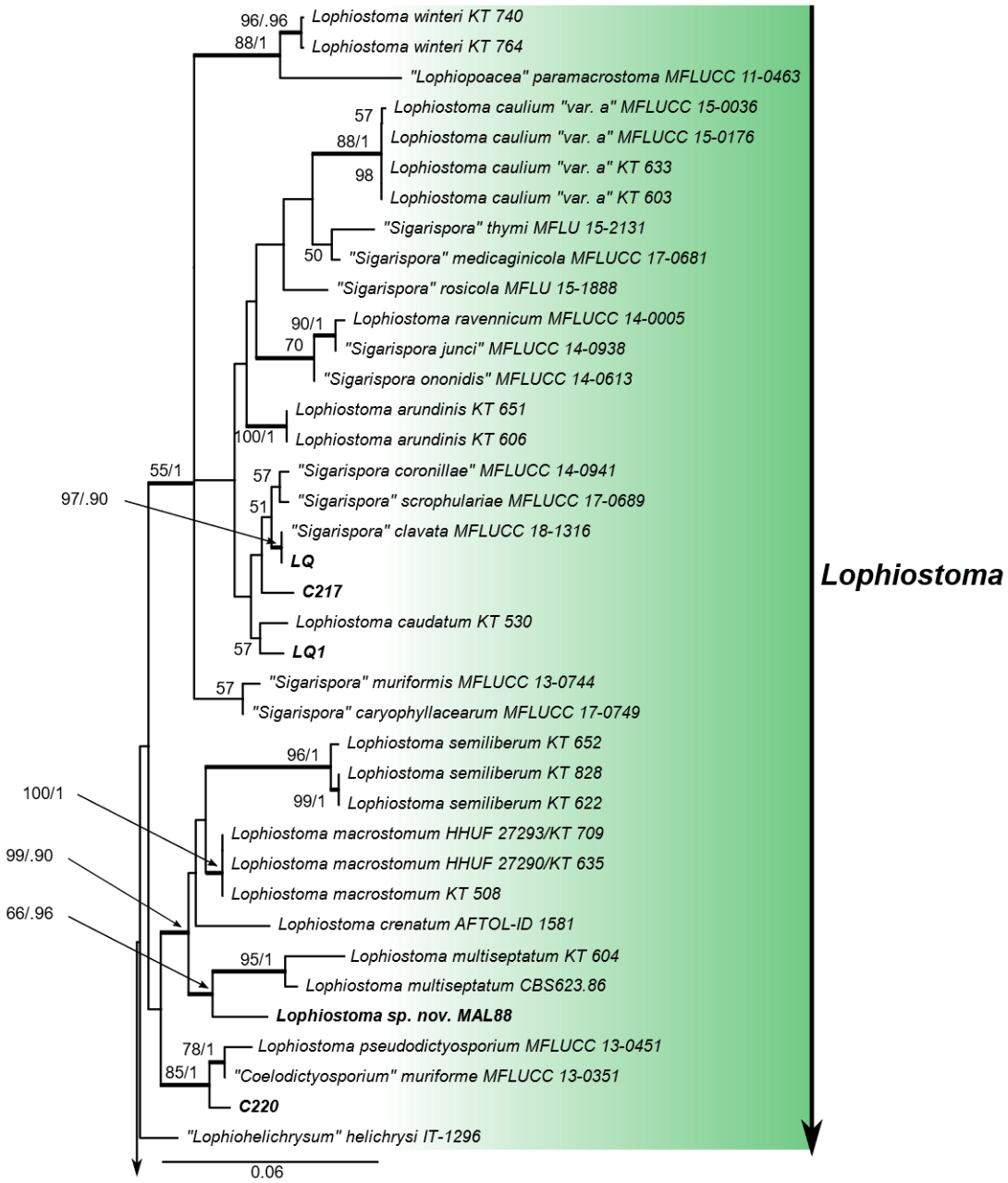

Figure 1. Maximum likelihood phylogeny of *Lophiostomataceae* based on ITS2, 5.8S, LSU, TEF1-α and RPB2 combined sequence data. Numbers above branches indicate Maximum likelihood RAxML bootstrap values above 50% and Bayesian posterior probabilities greater than 0.90 are given at the nodes. Newly obtained strains are shown in bold. Shorted nodes are marked with crossing lines and indications (x5, x6) of how many times the node has been shortened.

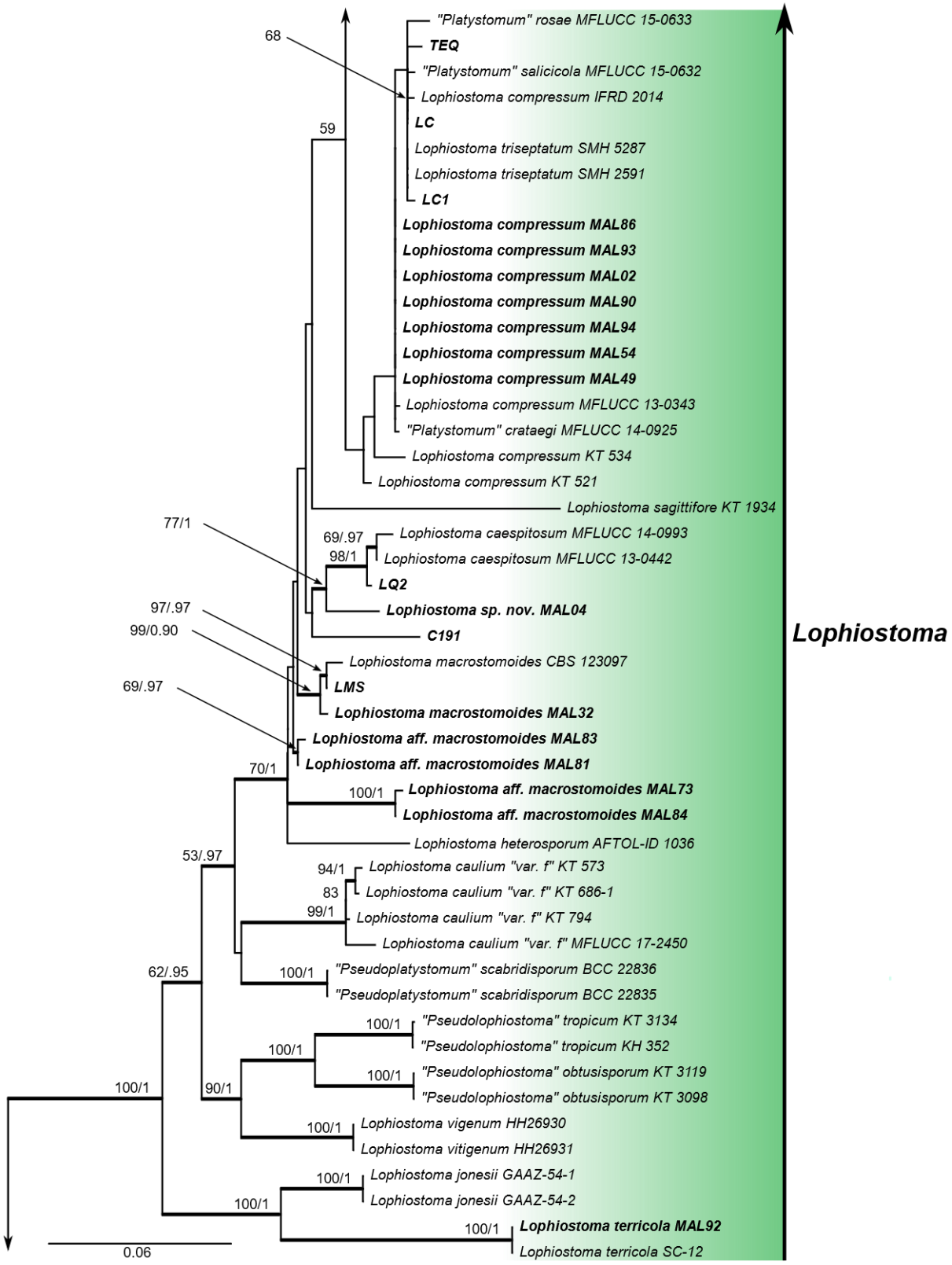

Figure 1. Continued

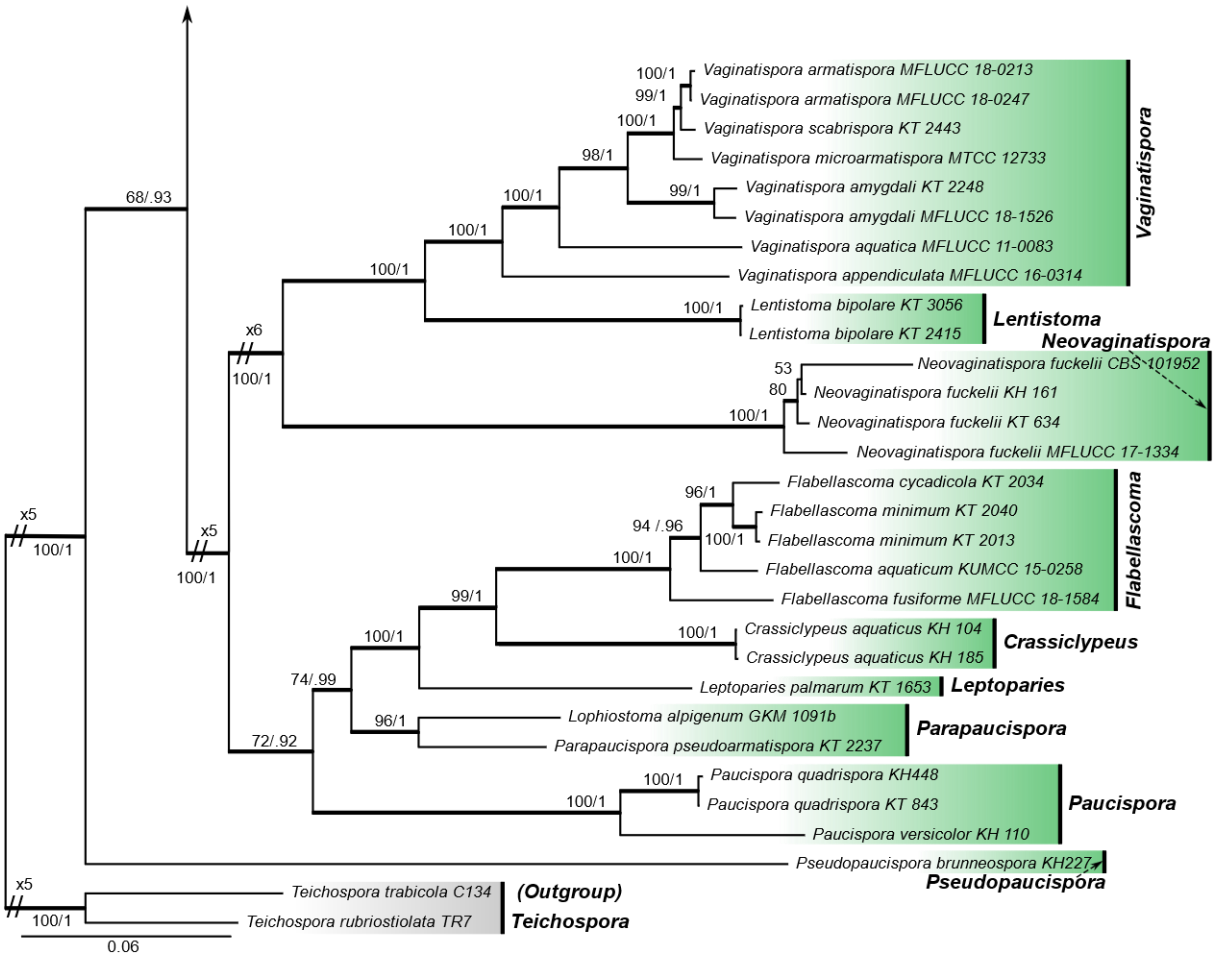

Figure 1. Continued. Shorted nodes are marked with crossing lines and indications (x5, x6) of how many times the node has been shortened.

For *Lophiostomataceae* the phylogenetic analyses of the present study showed that taxa *Pseudopaucispora brunneospora* formed a sister group to all other *Lophiostomataceae* taxa, with low MLB and medium BPP support. The genus *Lophiostoma* formed a clade with high support for nesting as a sister group to the remaining genera of *Paucispora*, *Parapaucispora*, *Leptoparies*, *Crassiclypeus*, *Flabellascoma*, *Neovaginatispora*, *Lentistoma* and *Vaginatispora*. These other genera were all supported. Many species within *Lophiostoma* were dispersed throughout the genus without significant support. Still, several highly supported clades were revealed (Figure 1). The results from the molecular analyses showed support for two new species for science within *Lophiostomataceae*, represented by the strains MAL88 and MAL04 respectively. In the taxonomical section (below) morphological support for these new species were indicated in the notes to each species. The strains C191, C220 and C217, may represent additional new species, but morphological investigation is needed before a taxonomic decision is made.

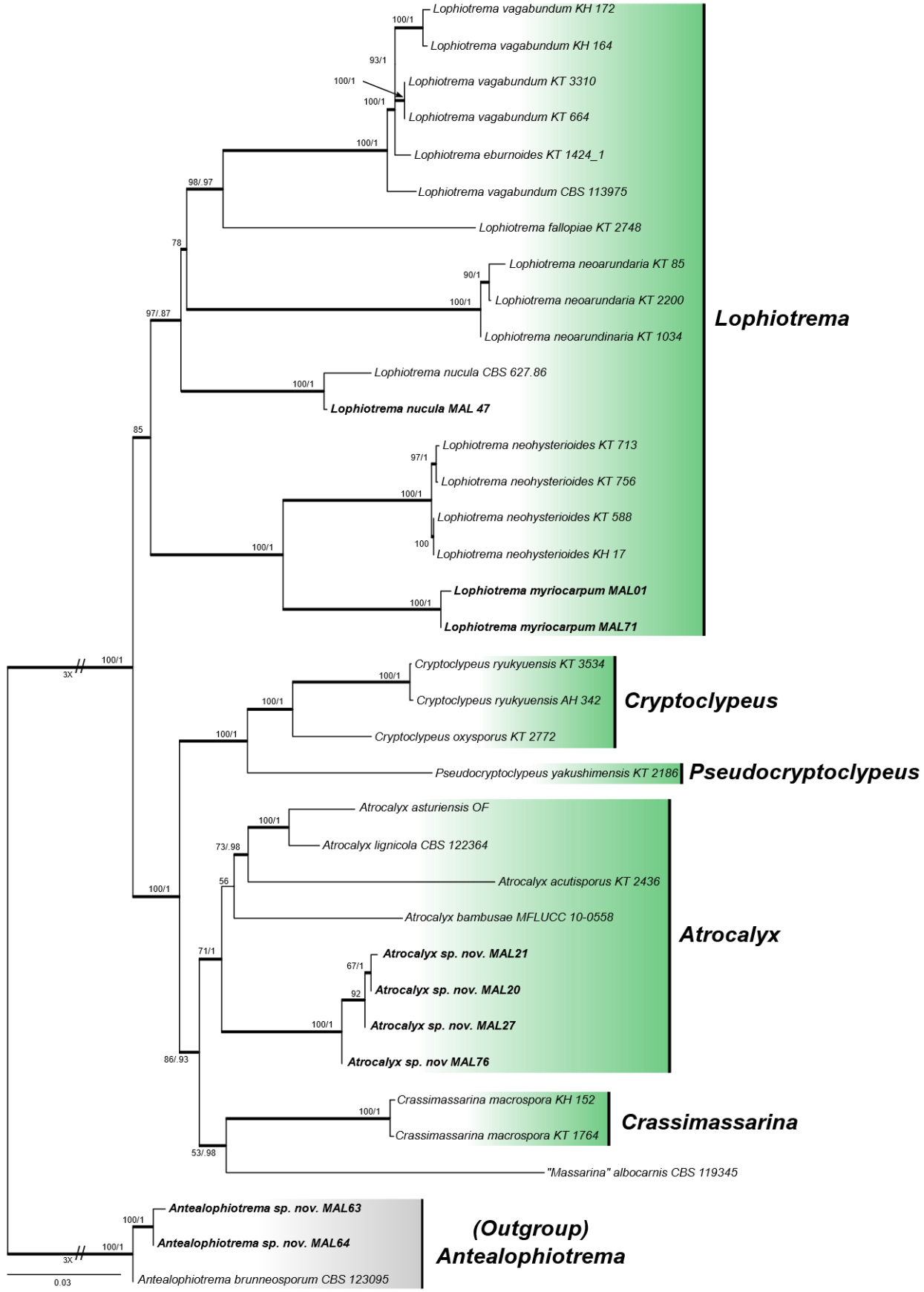

Figure 2. Maximum likelihood phylogeny of *Lophiotremataceae* based on ITS2, 5.8S, LSU, TEF1-α and RPB2 combined sequence data. Numbers above branches indicate Maximum likelihood RAxML bootstrap values above 50% and Bayesian posterior probabilities greater than 0.90 are given at the nodes. Newly obtained strains are shown in bold.

The genera *Alpestrisphaeria* Thambug. & K.D. Hyde, *Biappendiculispora* Thambug. & Hyde, *Capulatispora* Thambug., Kaz. Tanaka & K.D. Hyde, *Coelodictyosporium* Thambug. & Hyde, *Guttulispora* Thambug., Qing Tian & K.D. Hyde, *Lophiohelichrysum* Dayar., Camporesi & K.D. Hyde, *Lophiopoacea* Ariyaw., Thambug. & K.D. Hyde, *Platystomum* Trevis*, Pseudolophiostoma* Thambug., Kaz. Tanaka & K.D. Hyde, *Pseudoplatystomum* Thambug. & Hyde and *Sigarispora* Thambug. & Hyde were suggested synonymized with *Lophiostoma* based on argumentations that include molecular phylogenetic considerations and morphology. Where prior combinations of *Lophiostoma* naming exists, these were used in our study. Generic names in need of new combinations and renaming were marked with quotation marks (Figure 1, Table 2). Arguments for the resurrection of a broader circumscribed *Lophiostoma* genus can be found in the discussion.

For *Lophiotremataceae* the phylogenetic analyses of this study showed a topology comprising a clade of genus *Lophiotrema* with strong support for it being a sister group to the remaining genera of *Lophiotremataceae*. *Lophiotrema* includes species *L*. *eburnoides*, *L*. *fallopiae*, *L*. *neoarundinaria*, *L*. *neohysterioides*, *L*. *nucula*, *L*. *vagabundum* and *L*. *myriocarpum,* which all showed moderate to strong support. The species *Lophiotrema myriocarpum* is transferred from *Lophiostoma* and resurrection of the species epithet *Lophiotrema myriocarpum* (Fuckel) Sacc is proposed. In the genus *Atrocalyx*, the strains MAL20, MAL21, MAL27 and MAL76 formed a strongly supported clade. Morphology also shows evidence of these strains resembling the same species as indicated in the taxonomical notes (below). Thus, these strains represent a new species for science. The genera *Crassimassarina* is nesting as a sister clade to genus *Atrocalyx* with low MLB and high BPP support.

3.2 Taxonomy

Of 94 induced collections, 58 successfully grew into viable sterile mycelia. Photographies of the cultures from representative strains of the different species are shown in figure 3.

A total of 76 collections were identified by morphology to *Lophiostomataceae* and 18 as *Lophiotremataceae*. The identifications was subsequent guided by the molecular results and resulted in changes of species and even genus. Collections were also acquired from Oslo herbarium (O) and Tromsø herbarium (TROMS) for morphological investigations, along with additional taxa attained from the two national biodiversity mapping projects associated with the thesis.

Data of registered taxa of *Lophiostomataceae* and *Lophiotremataceae* from the Norwegian Mycological Database (Larsson et al. 2010) and from this study, are listed next to the worldwide accepted genera as by Wijayawardene et al. 2020 in Table 3.

Table 3. List of genera and species within *Lophiostomataceae* and *Lophiotremataceae* registered in The Norwegian Mycological Database[1] and genera accepted worldwide as per Wijayawardene et al. 2020[2].

Norwegian recorded taxa[1] (number of specimens recorded in the database)	**Worldwide accepted genera**[2] genera marked with red colours are suggested synonymized by this study (number of accepted species)
Lophiostomataceae	***Lophiostomataceae***
Lophiostoma Ces. & De Not.	*Alpestrisphaeria* Thambug. & K.D. Hyde (2)
appendiculatum (1)	*Biappendiculispora* Thambug., Kaz. Tanaka & K.D. Hyde (1)
caulium (3)	*Capulatispora* Thambug. & K.D. Hyde (1)
compressum (279)	*Coelodictyosporium* Thambug. & K.D. Hyde (3)
curtum (171)	*Decaisnella* Fabre (13)
fusiforme (1)	*Crassiclypeus* A. Hashim., K. Hiray. & Kaz. Tanaka (1)
holmiorum (9)	*Dimorphiopsis* Crous (1)
macrostomoides (54)	*Flabellascoma* A. Hashim., K. Hiray. & Kaz. Tanaka (2)
macrostomum (7)	*Guttulispora* Thambug., Qing Tian & K.D. Hyde (1)
pseudomacrostomum (44)	*Lentistoma* A. Hashim., K. Hiray. & Kaz. Tanaka (1)
quadrinucleatum (68)	*Leptoparies* A. Hashim., K. Hiray. & Kaz. Tanaka (1)
semiliberum (1)	*Lophiohelichrysum* Dayar., Camporesi & K.D. Hyde (1)
sp. (24)	*Lophiopoacea* Ariyaw., Thambug. & K.D. Hyde (2)
	Lophiostoma Ces. & De Not. (c. 100)
	Neopaucispora Wanas., Gafforov & K.D. Hyde (1)
	Neotrematosphaeria Thambug., Kaz. Tanaka & K.D. Hyde (1)
	Neovaginatispora A. Hashim., K. Hiray. & Kaz. Tanaka (1)
	Parapaucispora A. Hashim., K. Hiray. & Kaz. Tanaka (1)
	Paucispora Thambug., Kaz. Tanaka & K.D. Hyde (3)
	Platystomum Trevis. (c. 20)
	Pseudolophiostoma Thambug., Kaz. Tanaka & K.D. Hyde (5)
	Pseudopaucispora A. Hashim., K. Hiray. & Kaz. Tanaka (1)
	Pseudoplatystomum Thambug. & K.D. Hyde (1)
	Quintaria Kohlm. & Volkm.-Kohlm (3)
	Sigarispora Thambug. & K.D. Hyde (14)
	Vaginatispora K.D. Hyde (8)
Lophiotremataceae	***Lophiotremataceae***
Lophiotrema Sacc.	*Atrocalyx* A. Hashim. & Kaz. Tanaka (6)
boreale (12)	*Crassimassarina* A. Hashim. & Kaz. Tanaka (1)
myriocarpum (5)	*Cryptoclypeus* A. Hashim. & Kaz. Tanaka (2)
nucula (129)	*Galeaticarpa* A. Hashim. & Kaz. Tanaka (1)
vagabundum (4)	*Lophiotrema* Sacc. (17)
sp. (10)	*Pseudocryptoclypeus* A. Hashim. & Kaz. Tanaka (1)

1 Larsson et al. 2010

2 Wijayawardene et al. 2020

Below are descriptions of families, genera and species that were collected during this study. In cases where no new or additional information have been found, descriptions are based on the preceding description (Holm and Holm 1988; Mathiassen 1993; Thambugala et al. 2015; Hashimoto et al. 2018; Bao 2019).

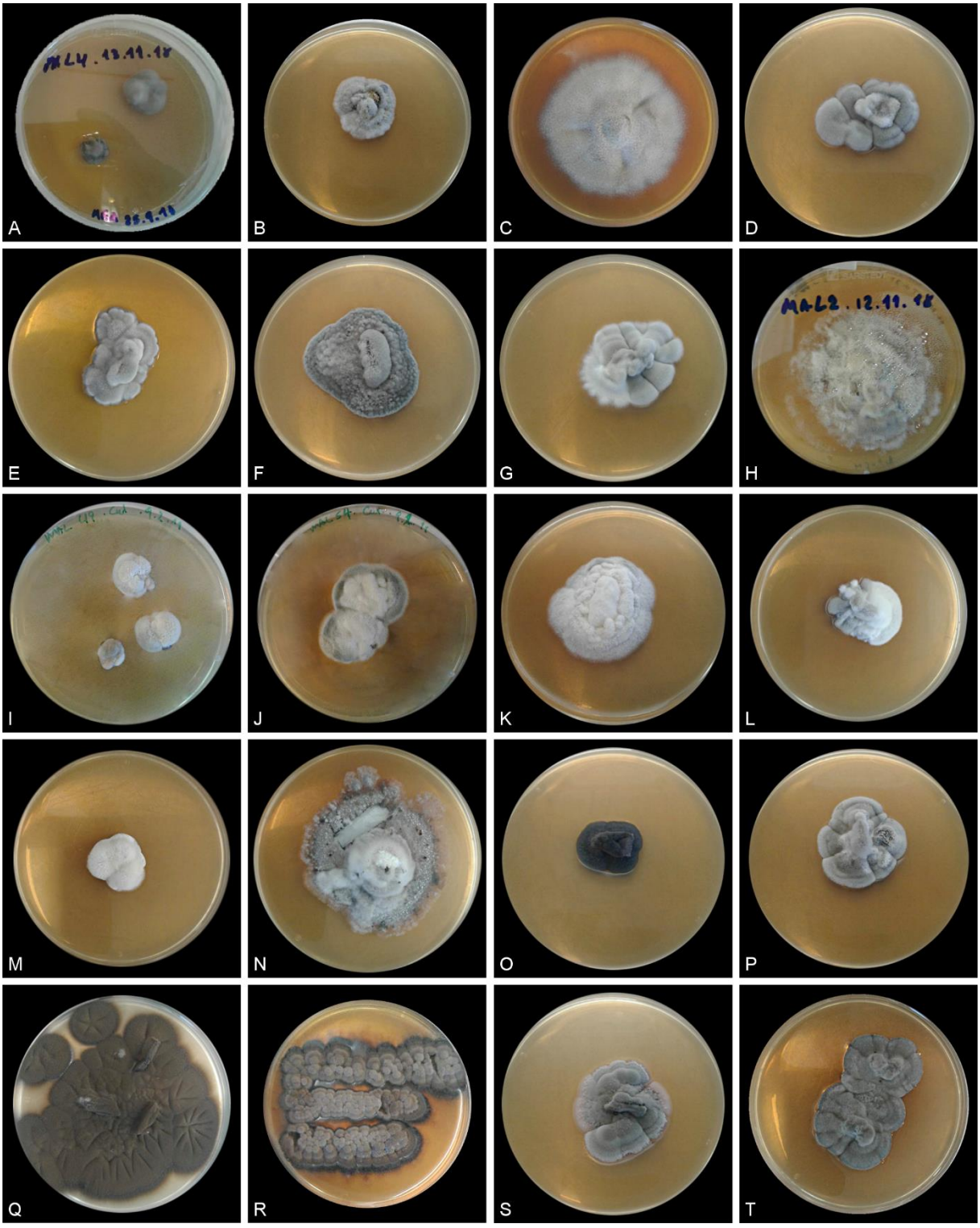

Figure 3. Cultures of *Lophiostomataceae* and *Lophiotremataceae* on 9 cm diam. Petri dishes holding MEA medium. **A** *Lophiostoma sp. nov.* [Strain: MAL04] (Specimen no.: MA18-01) after two weeks at 20 °C. **B** *Lophiostoma sp. nov.* [MAL88] (MA19-068) after three weeks at 20 °C. **C** *Lophiostoma macrostomoides* [MAL32] (MA19-068) after one month at 20 °C. **D** *Lophiostoma aff. macrostomoides* [MAL73] (MA19-036) after three weeks at 20 °C. **E** *Lophiostoma aff. macrostomoides* [MAL81] (MA19-042) after three weeks at 20 °C. **F** *Lophiostoma aff. macrostomoides* [MAL83] (MA19-048) after three weeks at 20 °C. **G** *Lophiostoma aff. macrostomoides* [MAL84] (MA19-049) after three weeks at 20 °C. **H** *Lophiostoma compressum* [MAL02] (OOL-198.3) after one month at 20 °C. **I** *Lophiostoma compressum* [MAL49] (MA19-001) after two weeks at 20 °C. **J** *Lophiostoma compressum* [MAL54] (MA19-003) after three weeks at 20 °C. **K** *Lophiostoma compressum* [MAL86] (MA19-056) after one month at 20 °C. **L** *Lophiostoma compressum* [MAL90] (MA19-072) after two weeks at 20 °C. **M** *Lophiostoma compressum* [MAL93] (MA19-076) after two weeks at 20 °C. **N** *Lophiostoma compressum* [MAL94] (MA19-077) after three months at 20 °C.

Figure 3. text continued. O *Antealophiotrema sp. nov.* [MAL63] (JB18DurP9-1) after two weeks at 20 °C. **P** *Antealophiotrema sp. nov.* [MAL64] (JB18Vikp7-1) after two weeks at 20 °C. **Q** *Atrocalyx sp. nov.* [MAL21] (JB18-506) after three months at 20 °C. **R** *Atrocalyx sp. nov.* [MAL20] (JB18-502) after 3 months at 20 °C. **S** *Atrocalyx sp. nov.* [MAL27] (MAL18-003) after three weeks at 20 °C. **T** *Lophiotrema myriocarpum* [MAL01] (JB17-513) after one month at 20 °C.

Lophiostomataceae Sacc., Syll. Fung. (Abellini) 2: 672 (1883)

Mycobank no.: MB561063

Type genus: *Lophiostoma* Ces. & De Not., Commentario della Società Crittogamologica Italiana 1 (4): 219 (1863) [MB#2933].

Ecology: Saprobic on twigs or bark of various woody plants and herbaceous plants in terrestrial and aquatic environments.

Sexual morph: *Ascomata* solitary or scattered to gregarious, immersed to erumpent, coriaceous to carbonaceous, dark brown to black, globose to subglobose, uni-loculate, ostiolate. *Ostiole* rounded or slit-like, with a small to large, compressed, periphysoid or not, crest or slot-like apex, which may be poorly developed or lacking, variable in shape and composed of pseudoparenchymatous cells. *Peridium* thick at the sides, broad at the apex and thinner at the base, one to several layers, composed of small to medium, dark brown or lightly pigmented to hyaline, thin walled cells of *textura prismatica* or *textura angularis*, fusing and indistinguishable from the host tissues. *Hamathecium* comprising numerous, septate or aseptate, anastomosing and branched or unbranched, cellular or filamentous pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* 2–8-spored, bitunicate, fissitunicate, cylindrical to clavate, pedicellate, rounded at the apex, with an ocular chamber. *Ascospores* uni- to biseriate, partially overlapping, hyaline or yellowish to brown, sometimes with hyaline end cells, fusiform or ellipsoid to fusiform, with acute or rounded ends, 1 to multi-septate or muriform, and constricted at the central septum, often guttulate, smooth-walled or verrucose, sometimes with terminal appendages, with or lacking a mucilaginous sheath.

Lophiostoma Ces. & De Not., Commentario della Società Crittogamologica Italiana 1 (4): 219 (1863)

MycoBank no.: MB2933

= *Alpestrisphaeria* Thambug. & K.D. Hyde, Fungal Diversity 74: 214 (2015) [MB#551232]

= *Guttulispora* Thambug., Qing Tian & K.D. Hyde, Fungal Diversity 74: 220 (2015) [MB#551238]

= *Biappendiculispora* Thambug., Kaz. Tanaka & K.D. Hyde, Fungal Diversity 74: 214 (2015) [MB#551528]

= *Capulatispora* Thambug. & K.D. Hyde, Fungal Diversity 74: 216 (2015) [MB#551234]

= *Pseudolophiostoma* Thambug., Kaz. Tanaka & K.D. Hyde, Fungal Diversity 74: 235 (2015) [MB#551250]

= *Pseudoplatystomum* Thambug. & K.D. Hyde, Fungal Diversity 74: 237 (2015) [MB#551253]

= *Platystomum* Trevis., Bulletin de la Société Royale de Botanique de Belgique 16: 16 (1877) [MB#4185]

= *Coelodictyosporium* Thambug. & K.D. Hyde, Fungal Diversity 74: 218 (2015) [MB#551286]

= Sigarispora Thambug. & K.D. Hyde, Fungal Diversity 74: 238 (2015) [MB#551255]

= *Lophiopoacea* Ariyaw., Thambug. & K.D. Hyde, Fungal Diversity 74: 220 (2015) [MB#551240]

= Lophiohelichrysum Dayarathne, Camporesi & K.D. Hyde, Fungal Diversity 75: 85 (2015) [MB#551400]

Type species: *Lophiostoma macrostomum* (Tode, Fungi mecklenberg. sel. (Lüneburg) 2: 12 (1791) Ces. & De Not., Comm. Soc. Critt. Ital. 1(4): 219, 1863, Mycobank no.: MB149287.

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

Ascomata scattered to gregarious, immersed to semi-immersed, papilla erumpent through host surface, coriaceous to carbonaceous, dark brown to black, globose to subglobose, ostiolate. *Ostiole* slit-like, variable in shape, with crest-like apex, usually opening apically with a pore, plugged by gelatinous tissue and occasionally lighter coloured. *Peridium* wider at the apex and thinner at the base, composed of a single stratum, comprising several layers of lightly pigmented to dark brown, thin-walled cells of *textura angularis* to *textura prismatica*, cells towards the inside, lighter, at the outside, darker, fusing and indistinguishable from the host tissues. *Hamathecium* comprising 1–2 µm wide, septate, branched, cellular pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short to long pedicellate, rounded at the apex with an ocular chamber. *Ascospores* uniseriate or partially biseriate, hyaline to yellowish brown, fusiform with narrow, acute to rounded ends, 1- to multi-septate, sometimes with 3–5-eusepta, constricted at the central septum, with or without terminal appendages.

Lophiostoma caespitosum Fuckel, Jahrbücher des Nassauischen Vereins für Naturkunde 27-28: 29 (1874)

Figure 4.

Mycobank no.: MB189009

≡ *Guttulispora crataegi* Qing Tian, Thambug., Camporesi & K.D. Hyde, Fungal Diversity 74: 220 (2015) [MB#551239]

Ecology: Saprobic on a dead and branches of *Crataegus sp*.

Sexual morph: *Ascomata* (165-)176–325.5(-389)x150–300 µm (n = 10), solitary or scattered, immersed, coriaceous to carbonaceous, dark brown to black, globose to subglobose, ostiolate. *Ostiole* central, papillate, with a small crest-like apex and an irregular pore-like opening, plugged by gelatinous tissue, made up of lightly pigmented, pseudoparenchymatous cells. *Peridium* (25-)34.1–89.9(-100) µm wide (n = 10), composed of a single stratum, with dark to reddish brown, thick-walled cells of *textura angularis*, cells towards the inside lighter, at the outside, darker, somewhat compressed, fusing and indistinguishable from the host tissues. *Hamathecium* comprising 1–2 µm (n = 10) wide, septate, branched, cellular pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* (96.7-)97.3-120.1(-124.8)×(8.5-)8.7–11.1(-11.9) µm (n = 10), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short-pedicellate, apically rounded with an ocular chamber, uniseriate to oblique biseriate. *Ascospores* (17-)17.6-19.7(-20.1)×(5.6-)5.8-7(-7.8) µm (n = 30), obliquely uniseriate, partially overlapping, hyaline when immature and becoming brown when mature, ellipsoid to fusiform, 3-septate, constricted at each septum, upper part slightly wider, guttulate at each cell, smooth-walled, lacking a mucilaginous sheath.

Material examined: LQ2

Notes: This species has characteristic tri-septate spores with dark brown pigmentation, it grows on the host species of *Crataegus sp*. and have relatively wide cylindrical to clavate asci that appear more clavate in shape than many other *Lophiostoma* species. Especially the spore form and colourations make this species rather characteristic within *Lophiostoma*.

Here further molecular and morphological data is added to the existing two Italian specimens collected by Erio Camporesi in 2013 and 2014 (MFLUCC 13-0442, MFLUCC 14-0993), and treated in the publication of Thambugala et al. 2015. The two Italian specimens were proposed as a new species to science and found even to compile a new genus. Morphologically investigated the type material of *Lophiostoma caespitosum* identified the

specimen LQ2 as being this species. Strain LQ2 is nesting in a strongly supported clade together with strain M++FLUCC 13-0442, MFLUCC 14-0993 and we find equally strong morphological evidence of them being the same species. The species epithet "*Guttulispora*" *crataegi* as basionym to *L. caespitosum* is therefore proposed.

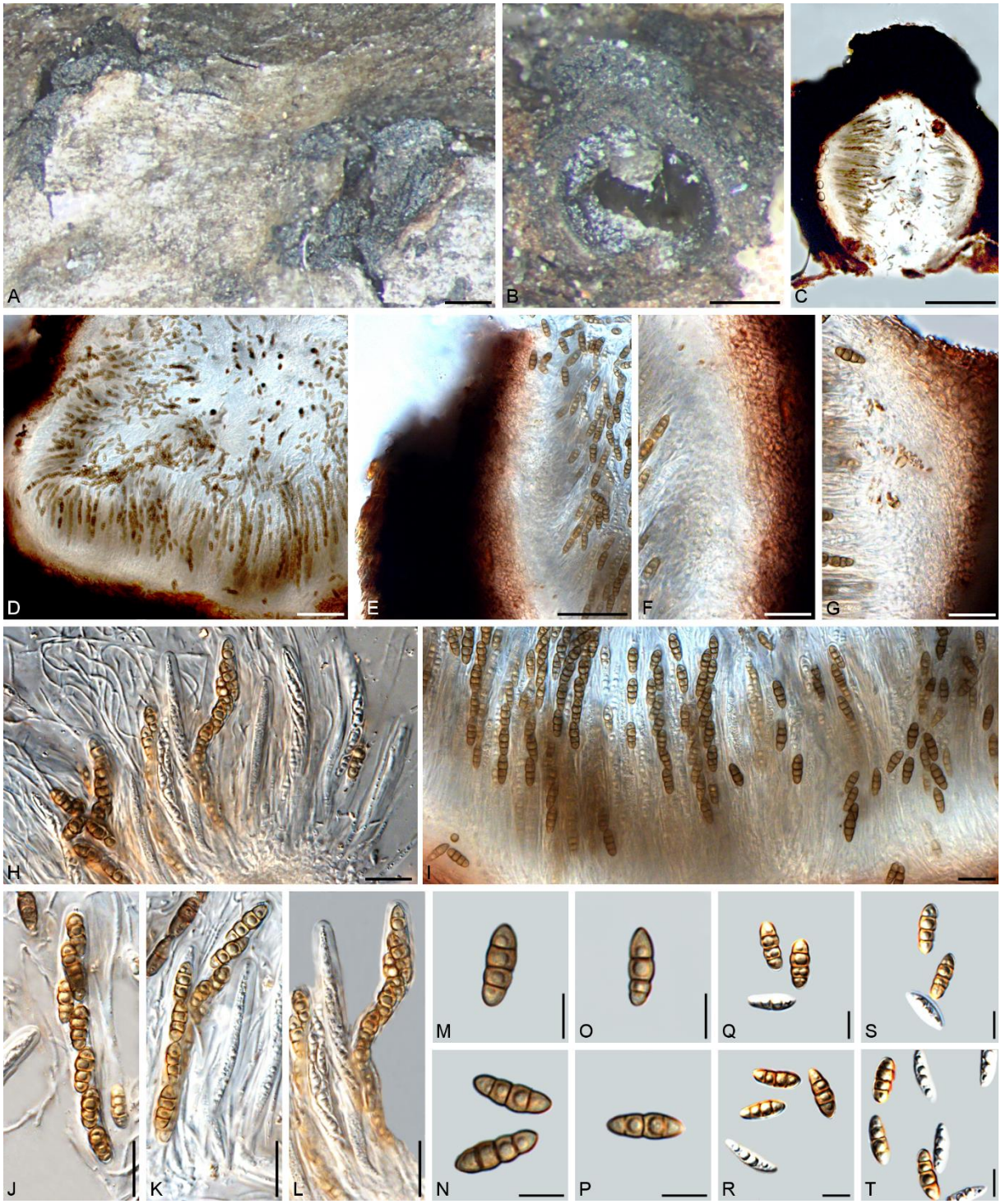

Figure 4. *Lophiostoma caespitosum* [LQ2]. **A, B** Ascomata. **C, D** Section of ascoma. **E-G** Peridium. **H-I** Hymenium and paraphyses. **J-L** Asci. **M-T** Ascospores. Scale bars: **A-C** = 150 µm, **D-E** = 40 µm, **F-G** = 30 µm, **H-I** = 40 µm, **J-T** = 20 µm.

Lophiostoma compressum (Pers.) Ces. & De Not., Comm. Soc. crittog. Ital.: 19 (1861)

Figure 5.

Mycobank no.: MB238397

≡ *Platystomum compressum* (Pers.) Trevis., Bulletin de la Société Royale de Botanique de Belgique 16: 16 (1877), [MB#144522]

= *Lophiostoma lojkanum* (Sacc.) Mussat, in Saccardo, Syll. fung. (Abellini) 15: 198 (1900)

Ecology: Saprobic on the wood of frondose trees such as *Ulmus glabra, Alnus, Corylus*, *Betula, Salix, Tilia* and *Quercus*.

Sexual morph: *Ascomata* 200-1000 µm wide (n = 20), black, scattered, gregarious, immersed to erumpent, globose to subglobose, uni-loculate, glabrous, ostiolate. *Ostiole* central or lateral, carbonaceous, generally with a pore-like to slit-like opening. *Peridium* 50-120 µm (n = 80) composed of several layers of dark-brown to black, thick-walled pseudoparenchymatous cells, arranged in a *textura angularis*, fusing with host tissue at the most outside layer and pale inwardly, lower layers of *textura prismatica* type. *Hamathecium* comprising 1–2.5 µm (n = 30) wide, branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* 90-230 × 8-20 µm (n = 150), 8-spored, bitunicate, fissitunicate, cylindric-clavate, with longer stipe >10 µm, pedicellate, apically rounded with a minute ocular chamber, uniseriate. *Ascospores* (15.5-)16-33(-35) × (6-)7-10(-12) µm (n = 300), hyaline as immature, becoming brown at maturity, ellipsoid to fusiform and slightly muriform, (3-)4-7-transverse septae, 1-3-longitudinal septae, constricted at the middle septum, guttulate, smooth-walled.

Culture characters: Ascospores germinated in MEA within 24h. Germ tubes produced from one or both ends of ascospore and central cells. Colonies were growing unregular circular, reaching 2.1-2.3 cm in diam. after four weeks at 20 °C, with somewhat irregular margins. White, some with light greyish areas, deeper light grey, reverse brown.

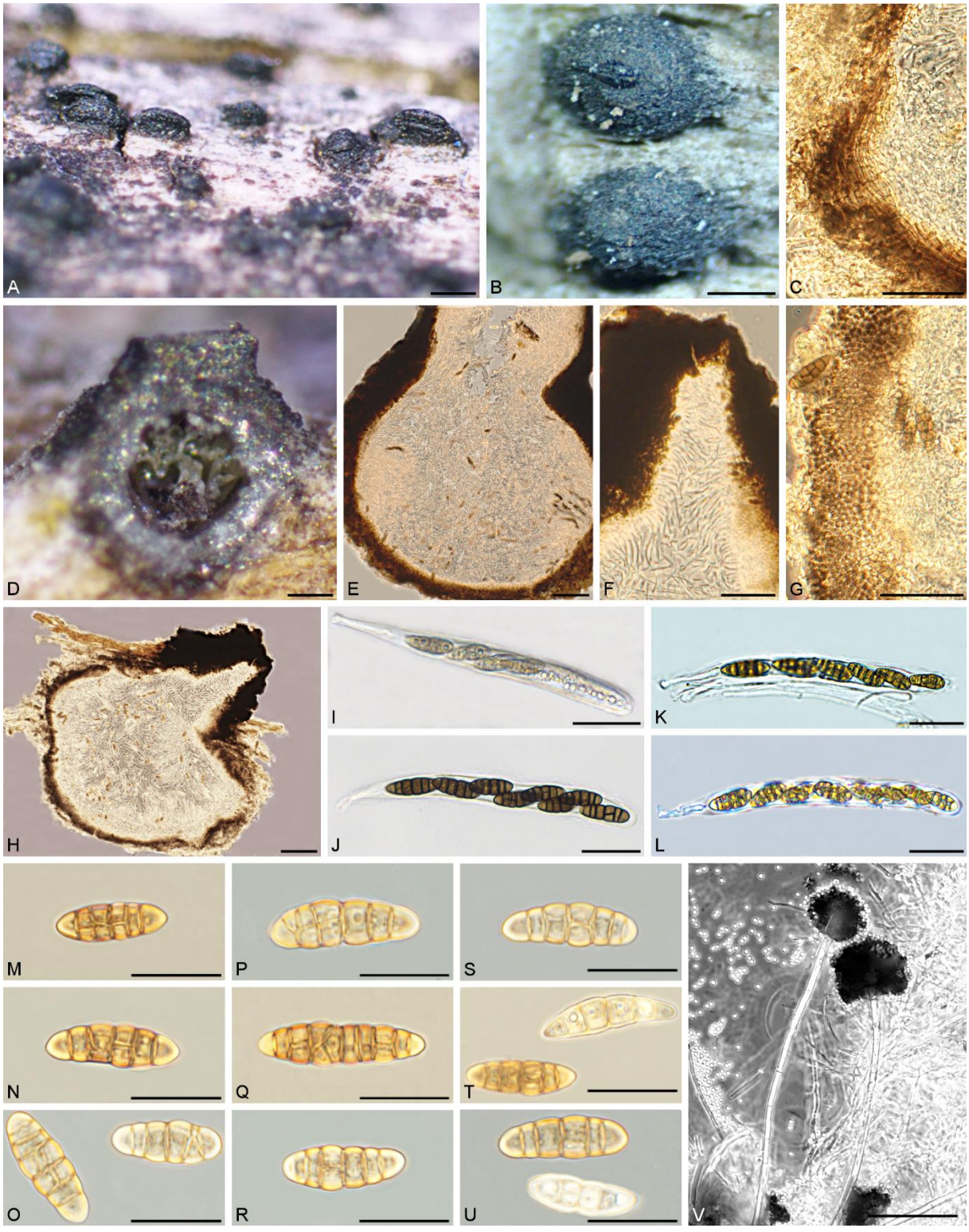

Figure 5. *Lophiostoma compressum*. **A-B** Ascomata. **D, E, H** Section of ascoma. **F** Section of ostiole. **C, G** Peridium, **C** with textura angular cell form in sidewalls, **G** with *textura prismatica* cell form in basal wall. **I-L** Asci. **M-U** Ascospores. **V** Conidiophore. Scale bars: **A-B** = 300 µm, **C, G =** 40 µm **D, E-F, H** = 50 µm, **I-L** = 30 µm, **M-U** = 20 µm, **V** = 10 µm.

Material examined: **Norway**, Møre og Romsdal county, Ålesund municipality, on bark of living *Populus tremula*, 31 January 2018, *Oddvar Olesen* , [MAL02] (OOL-18.3); Viken county, Asker municipal, on bark of living *Populus tremula*, 29 January 2019, *Mathias Andreasen* [MAL49] (MA19-001); Viken county, Asker municipal, on bark of living *Populus tremula*, 29 January

2019, *Mathias Andreasen* [MAL54] (MA19-003); Vestfold county, Tjøme municipal, on dying branch of *Salix sp.*, 18 July 2019*, Mathias Andreasen,* [MAL86] (MA19-056); Vestfold county, Tjøme municipal, on *Salix sp*., 18 July 2019, *Mathias Andreasen*, [MAL90] (MA19-72); Viken county, Asker municipal, on *Salix sp*., 15 August 2019, *Mathias Andreasen*, [MAL93] (MA19-76); Viken county, Asker municipal, on *Phragmites australis,* 15 August 2019*, Mathias Andreasen*, [MAL94] (MA19-77); Unknown location, on *Tilia cordata*, 18th century, *Nils Green Moe (1812–1892) & Ivar Jørstad*, (O-F192124); Oslo county and municipality, on *Quercus robur*, 18th hundred, *Emil Rostrup (1831–1907)*, (O-F192125); Viken county, Bærum municipality, on *Corylus sp*., January 1826, *Søren Chr. Sommerfelt* &*Ivar Jørstad*, (O-F192126); Nordland county, Saltdal municipality, on *Salix phylicifolia*, January 1824, *Søren Chr. Sommerfelt* & *Geir Mathiassen*, (O-F192128); Oslo county and municipality, on *Pyrus malus*, date unknown, *Mathias Blytt* & *Emil Rostrup*, (O-F192129); Oslo county and municipal, on *Salix sp*., 05 April 1912, *John Egeland*, (O-F192130); Location, host and date unknown, unknown collector, (O-F192131); Location unknown, on *Betula sp*., date unknown, *Nils Green Moe* & *Emil Rostrup*, (O-F192133); Nordland county, Saltdal municipality, on *Salix phylicifolia*, date unknown, *Søren Chr*. *Sommerfelt* & *Geir Mathiassen*, (O-F192134); Oslo county and municipality, on *Salix sp*., date unknown, *Lennart Holm*, (O-F192136); Trøndelag county, Inderøy municipality, on *Fraxinus excelsior*, 08 May 2014, *John Bjarne Jordal* & *Björn Nordén*, (O-F247841); Viken county, Lillestrøm municipality, on *Salix sp*., 29 September 2015, *Björn Nordén*, (O-F305118); Finmark county, host unknown, Alta municipality, *Nils Green Moe & Geir Mathiassen*, (O-F186801). Vestland county, Luster municipality, On the branch of living *Ulmus glabra,* 14 June 2012, *Björn Nordén* & *John Bjarne Jordal*, (O-F247799). Vestland county, Ullensvang municipal, On *Ulmus glabra*, 03 October 2013, *Björn Nordén* & *John Bjarne Jordal* & *Thomas Læssøe*, (O-F255564); **Sweden**, Västregötland county, Vänersborg municipality, on *Viburnum opulus*, 19 June 1898, *A*. *G*. *Eliasson*, (O-F192135).

Lophiostoma sp. nov. *M.* Andreasen & B. Nordén **sp. nov.**

Figure 6.

Mycobank no.: *MBXXXXXX*

Holotype: (MA18-01) [MAL04]

Ecology: Saprobic on bark of living *Acer platanoides*.

Sexual morph: *Ascomata* 300–700 µm diam. (n = 10), solitary or scattered, subimmersed, coriaceous to carbonaceous, dark brown to black, globose to subglobose, ostiolate. *Ostiole*

central, papillate, with a small crest-like apex and an irregular pore-like opening, plugged by gelatinous tissue, made up of lightly pigmented, pseudoparenchymatous cells. *Peridium* 75–100 µm wide (n = 20), composed of a single stratum, with dark to reddish brown, thick-walled cells of *textura angularis*, cells towards the inside lighter, at the outside, darker, somewhat compressed, fusing and indistinguishable from the host tissues, very thin layer at base towards the host tissue. *Hamathecium* comprising 1–2 µm wide (n = 20), septate, branched, cellular pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* 91-108(111) x 17.5-20(-22) µm (n = 30), 6-spored, bitunicate, fissitunicate, clavate, short-pedicellate, apically rounded with an ocular chamber, uniseriate to oblique biseriate. *Ascospores* 25-28(-30) x 9.5-11 µm (n = 30), hyaline when immature and becoming brown when mature, endcells lighter and remaining hyaline, fusiform to oblong-ellipsoid, 3-septate, constricted at all septae but more at the middle, upper part slightly wider, guttulate in each cell, at over-maturity the two middle cells with a lenticular lumen, lacking a mucilaginous sheath, with verruculose ornamentation.

Culture characteristics: Ascospores germinated in MEA within 24 h at 20 °C. Germ tubes produced from one or both ends of ascospore and very often more central cells. Colonies growing unregular circular, reach 0.5-1 cm in diam. after four weeks, with somewhat irregular margins, growing very slow. Initially, light grey becoming slightly lighter in outer layers and dark greyish from below, margin and deeper strata light grey, reverse black.

Material examined: **Norway**, Oslo county and municipality, on the bark of living *Acer Platanoides*, 28 September 2018, *Mathias Andreasen*, [MAL04] (MA18-01).

Notes: This species has very characteristic fusiform 3-septate spores with one big oil droplets in each cell, constricted at the middle septum and hyaline endcells.

The strain MAL04 show support for a placement as a sister species to *Lophiostoma caespitosum* (high BPP 0.9965 and medium MLB 77%). Morphological strain MAL04, specimen MA18-01, differs from *Lophiostoma crataegi* in several characters having an overall bigger scale of ascomata, peridium wall, asci and having much bigger fusiform spores with acute hyaline end cells. Asci has a more clavate form, are wider (up to 22 µm) and only 6-spored. The host of *Acer Platanoides* also differs from the obligate *Crataegus sp*. for *L*. *caespitosum*. Great efforts were made to retrieve sequences of the molecular marker LSU for the specimen but without result.

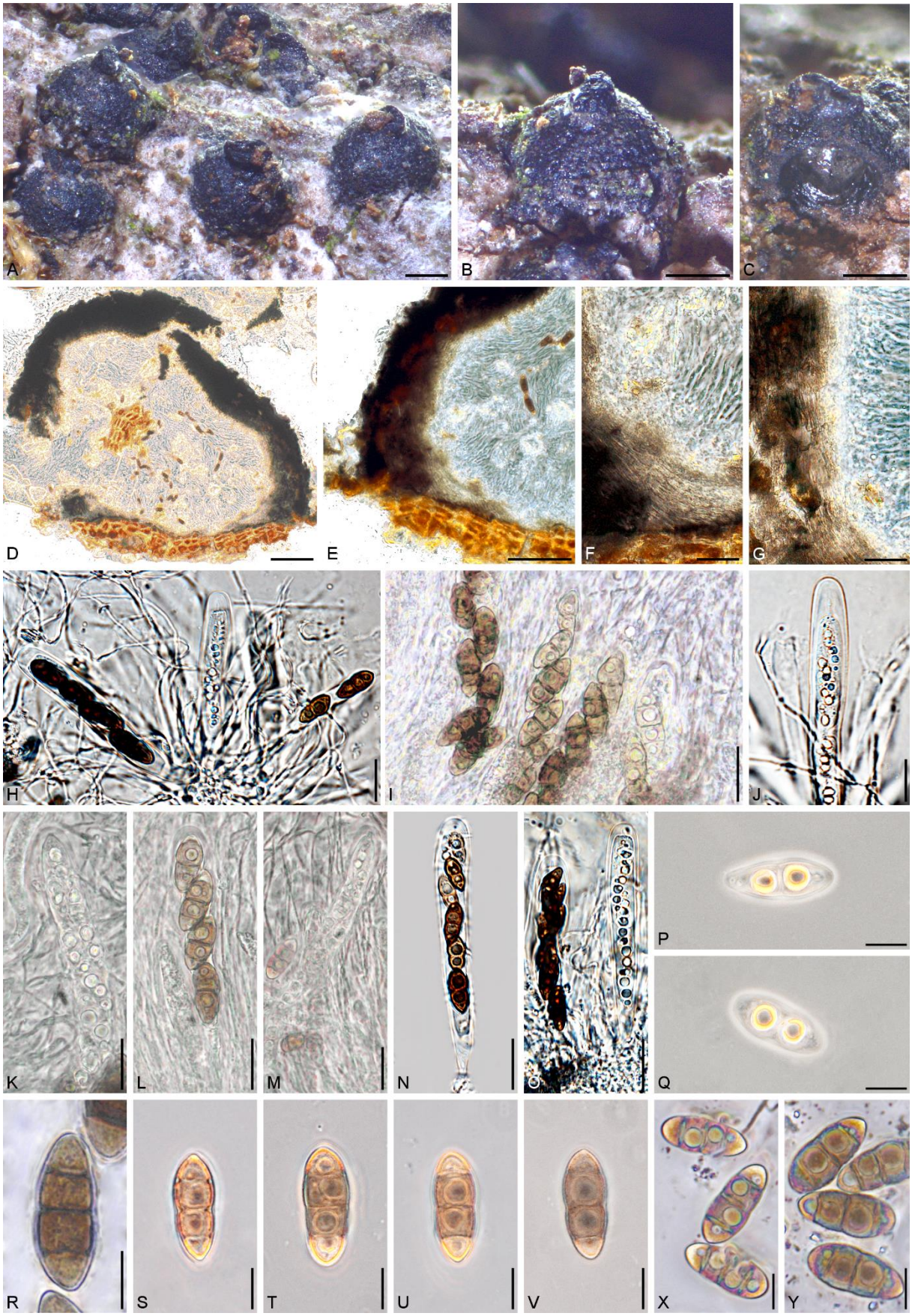

Figure 6. *Lophiostoma sp. nov*. holotype [MAL04] (MA18-01). **A-C** Ascomata. **D-E** Section of ascoma. **F-G** Peridium. **H-I** Hymenium. **J-O** Asci and paraphyses. **P-Q** Immature ascospores. **R-Y** Ascospore. Scale bars: **A-C** = 200 µm, **D-E** = 100 µm, **F-G** = 40 µm, **H-O** = 20 µm, **P-Y** = 10 µm.

Lophiostoma sp. nov. M. Andreasen & B. Nordén **sp. nov.**

Figure 7.

Mycobank no.: *MBXXXXXX*

Holotype: [MAL88] (MA19-068).

Ecology: Saprobic on dead branches still attached on living *Salix glauca* growing in alpine environment at app. 1200 meters altitude.

Sexual morph: *Ascomata* 400-800 µm (n = 10), black, scattered, gregarious, immersed to erumpent, large and coarse, globose to subglobose, uni-loculate, glabrous, ostiolate. *Ostiole* central or lateral, carbonaceous, with a slit-like opening. *Peridium* 50-100 µm (n = 10) composed of several layers of dark-brown to black, thick-walled pseudoparenchymatous cells, arranged in a *textura angularis*, fusing with host tissue at the most outside layer and pale inwardly but having a black wall encircling the whole hymenium. *Hamathecium* comprising 1–2.5 µm (n = 30) wide, branched, cellular paraphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* 120-125 x 10-13 µm (n = 10), 8-spored, bitunicate, fissitunicate, clavate, with short stipe <10 µm, pedicellate, apically rounded with a minute ocular chamber, uniseriate to obliquely uniseriate. *Ascospores* 23-29 x 6-9 µm (n = 30), first hyaline becoming brown, when immature fusiform with acute endcells becoming oblong-ellipsoid at maturity and ends up as almost muriform with rounded endcells when overmatured, upper part wider, hyaline endcells, 1-septate when immature becoming 3-5(-7)-transverse septate and 1-3(-4)-longitudinal septae when mature, clearly constricted at the middle septum and slightly at remaining septae, guttulate when immature later not, smooth-walled.

Culture characters: Ascospores germinated in MEA within 24 h at 20 °C. Germ tubes produced from one or both ends of ascospore and very often more central cells. Colonies growing unregular circular, reach 2.4-5.6 cm in diam. after four weeks, with somewhat irregular margins. Initially, light grey becoming lighter in colours and dark greyish from below, margin light grey, deeper strata dark grey to black, reverse black.

Material examined: **Norway**, Oppland county, Lom municipal, on *Salix glauca*, 29 June 2019, *Mathias Andreasen*, [MAL88] (MA19-068).

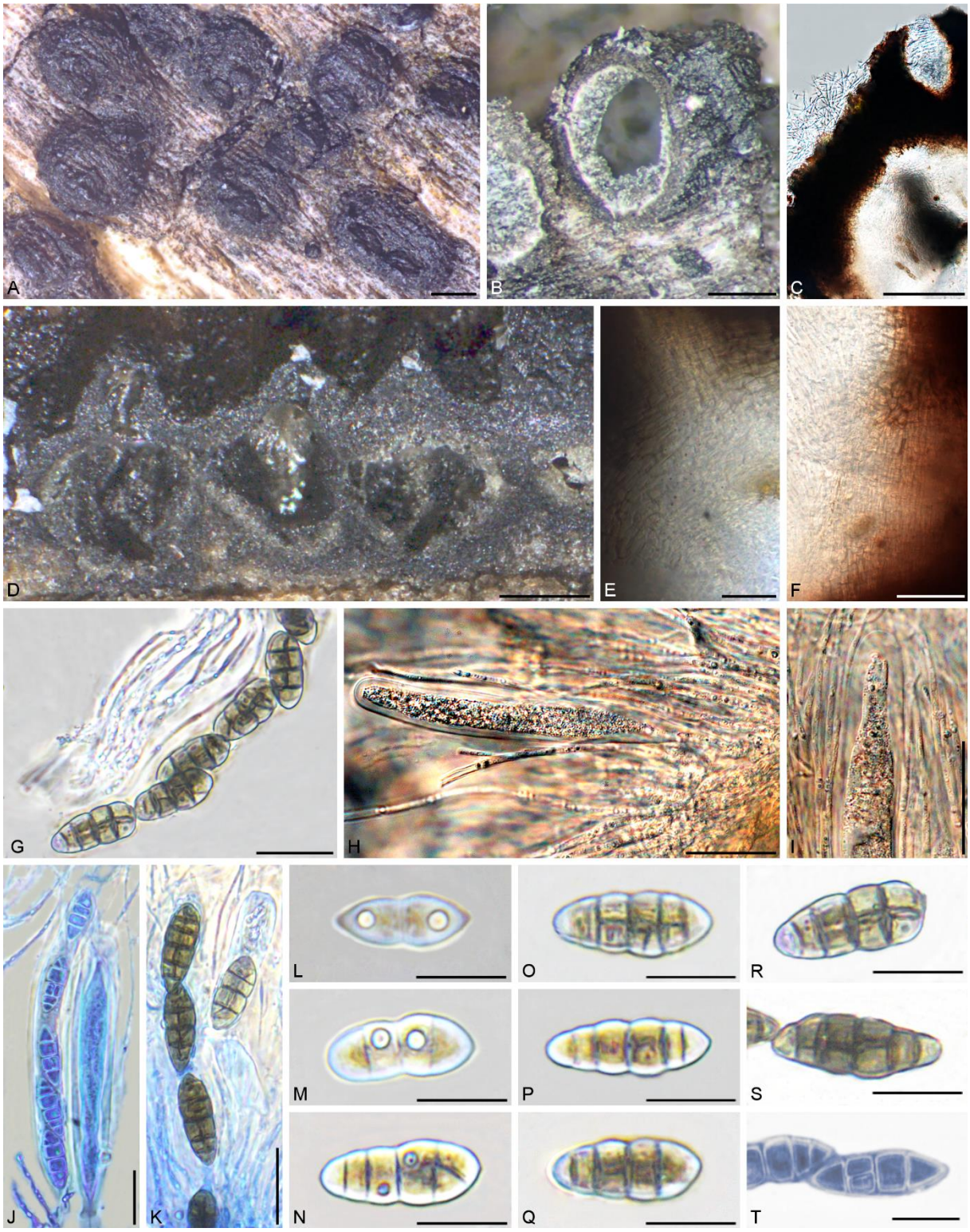

Figure 7. *Lophiostoma sp. nov.* Holotype [MAL88] (MA19-068). **A-B** Ascomata. **C-D** Section of ascoma. **E-F** Peridium. **G** Hymenium. **H-K** Asci and paraphyses. **L-M** Immature ascospores. **N-T** Ascospore. Scale bars: **A, B, D** = 300 µm, **C** = 100 µm, **E-K** = 25 µm, **L-T** = 15 µm. **J-T** in cotton blue.

Note: The morphology of this species resembles that of *Lophiostoma compressum* in many aspects. Still, it has more oblong ellipsoid to muriform spores with hyaline endcells and often longitudinal septae running parallel to the middle of the spore towards each end, not

stretching into the hyaline end cells. This spore form also differentiates from its other closely related species of *L*. *macrostomum* and *L*. *semiliberum* which all have fusiform spores.

This study presents phylogenetic support for this new species as being a sister species to *Lophiostoma multiseptatum* (MLP 66% and BPP 0.96). Ascomata are arranged in a closely aligned layer, giving the substrate a bright black colour. This specimen was found in alpine environments at app. 1200 meters altitude. It differs in many aspects from *L*. *multiseptatum* having overall shorter and a bit wider muriform ascospores (compared to fusiform) with less acute hyaline end-cells and without appendages. Spore septation is also different with both 3-5(-7)-transverse and 1-3(-4)-longitudinal clearly constriction at middle septae and biseriate, compared to 7-transverse-septate, 1-2-seriate for *L*. *multiseptatum*. Asci are of more cylindrical and narrower form and up to 13 µm wide, compared to clavate and up to 20 µm wide in *L*. *multiseptatum*. Peridium cells are of *textura angularis* form compared to *textura prismatica*.

Lophiostoma jonesii (Ariyawansa, K.D. Hyde & Z.Y.) M. Andreasen, I. Skrede, W. Jaklitsch, H. Voglmayr & B. Nordén. **comb. nov.**

Mycobank no.: *MB#XXXXXX*

= *Alpestrisphaeria jonesii* Ariyawansa, K.D. Hyde & Z.Y. Liu, Phytotaxa 277 (3): 261 (2016) [MB#552363]

Lophiostoma macrostomoides (De Not.) Ces. & De Not., Schem. di Classif. Sferiacei: 219 (1863)

Figure 8.

Figure 9. (*affinis macrostomoides*)

Mycobank no.: MB241835

Ecology: Saprobic on the wood of deciduous trees of frondose trees and shrubs such as *Quercus, Ulmus, Salix* but also found on coniferous *Juniperus communis*.

Sexual morph: *Ascomata* (150-)400-1000(-1200) µm (n = 20), black, scattered, gregarious, immersed to erumpent, large and coarse, globose to subglobose, uni-loculate, glabrous, ostiolate. *Ostiole* central or lateral, carbonaceous, generally with a pore-like to slit-like opening. *Peridium* (50-)75-200 µm (n = 75) composed of several layers of dark-brown to black, thick-walled pseudoparenchymatous cells, arranged in a *textura angularis*, fusing with host tissue at the most outside layer and pale inwardly. *Hamathecium* comprising 1–2.5 µm (n =

75) wide, branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* (120-)130-170(-200) × (6-)8-15(-17) µm (n = 100), 8-spored, bitunicate, fissitunicate, clavate, with longer stipe bigger than 10 µm, pedicellate, apically rounded with a minute ocular chamber, biseriate. *Ascospores* (30-)32-40(-42) × (8-)9-10(-12) µm (n = 100), uniseriate, brown, tendencies to hyaline endcells, oblong-ellipsoid to broadly cylindric, uniform, (3-)5-7-transverse septae, sometimes with constricted at the middle septum, at maturity first cell above middle septum slightly wider than remaining, guttulate, smooth-walled.

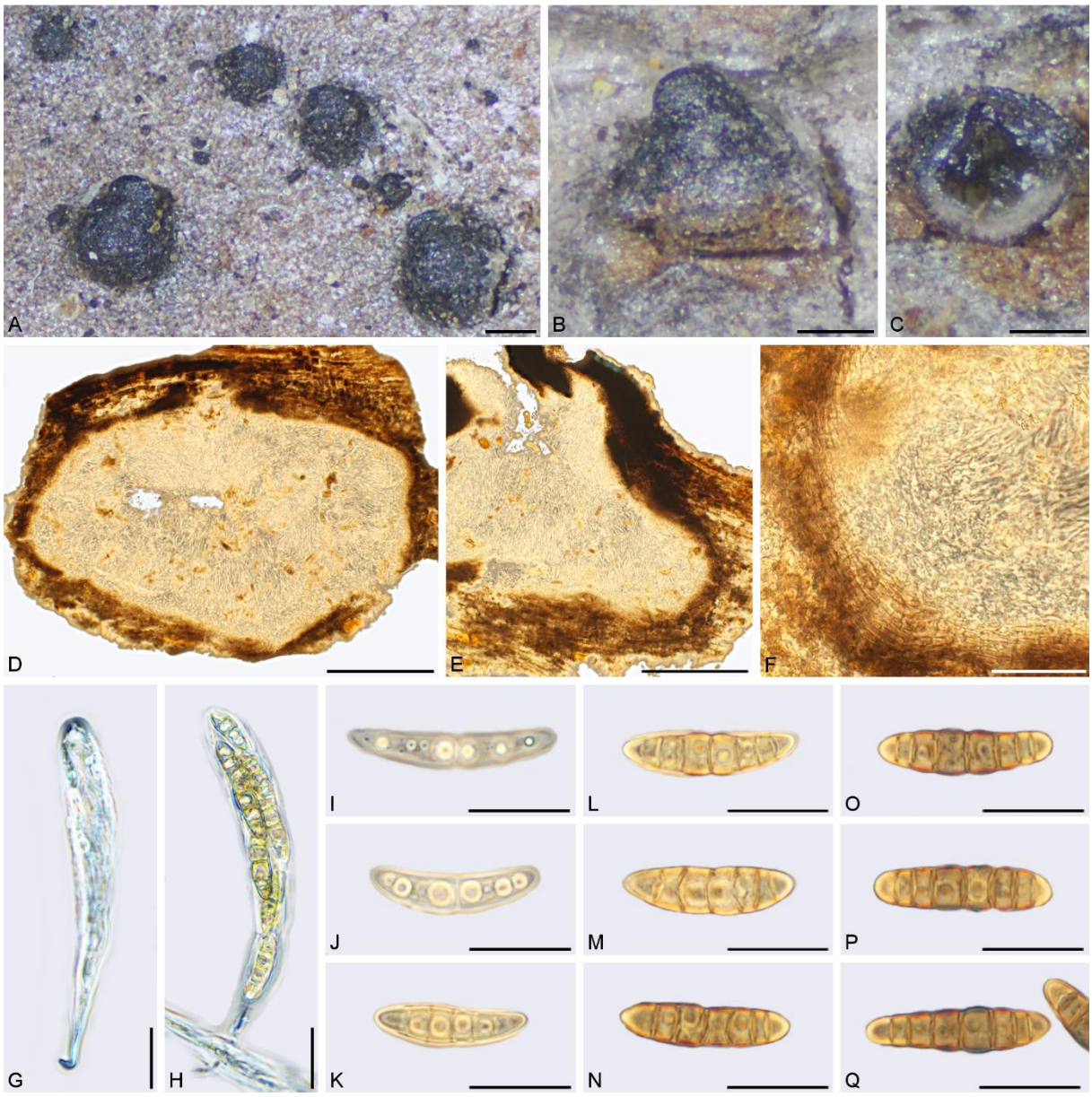

Figure 8. *Lophiostoma macrostomoides* [MAL32] (MA18-072). **A-B** Ascomata. **C** Section of ascoma. **D-F** Peridium, F with *textura angularis* cell form in basal wall. **G-H** Asci. **I-K** Immature ascospores. **L-Q** Ascospores. Scale bars: **A-D** = 150 µm, **E** = 50 µm, **F** = 20 µm, **G-H** = 40 µm, **I-Q** = 20 µm.

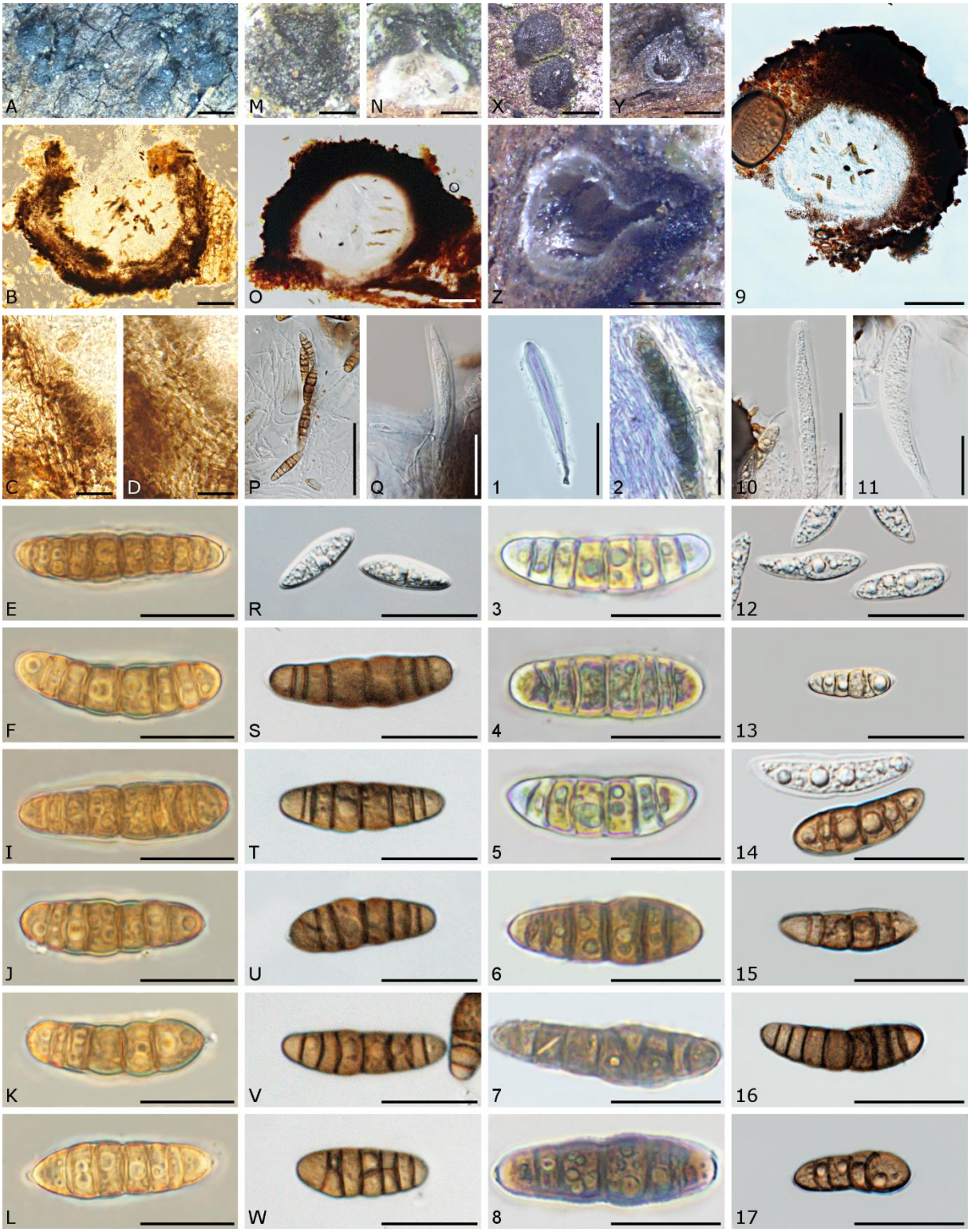

Figure 9. *Lophiostoma aff. macrostomoides*. **A-L** [MAL73] (MA19-036). **M-W** [MAL84] (MA19-049). **X-8** [MAL81] (MA19-042). **9-17** [MAL83] (MA19-048). **A, M, N** Ascomata. **B, O, Y, Z, 9** Section of ascoma. **C, D** Peridium. **P, Q, 1, 2, 10, 11** Asci. **R, 12-14** Immature ascospores. **E-L, R-W, 3-8, 12-17** Ascospores. Scale bars: **A, X-Z** = 500 µm, **M, N** = 200 µm, **B** = 100 µm, **O-Q, 1, 9-11** = 50 µm, **E-L, R-W, 2-8, 12-17** = 20 µm, **C, D** = 10 µm. **1-5** in Cotton blue.

Culture characters: Ascospores germinated in MEA within 24 h at 20 °C. Germ tubes produced from one or both ends of ascospore and very often more central cells. Colonies growing unregular circular, reach 1.9-2.4 cm in diam. after four weeks, with somewhat irregular

margins. Initially, whitish becoming light greyish to dark greyish from below, margin and deeper strata dark grey to black, reverse black.

Material examined: **Norway**, Viken county, Asker municipal, on *Juniperus communis*, 15 December 2018, *Mathias Andreasen*, [MAL32] (MA18-072). **Norway**, Vestland county, Kvam municipal, on Tilia cordata, 15 May 2019, *Mathias Andreasen* [MAL73] (MA19-036); Viken county, Frogn municipal, on *Tilia cordata*, 04 June 2019, *Mathias Andreasen*, [MAL81] (MA19-042); Viken county, Frogn municipal, on *Tilia cordata*, 05 June 2019, *Mathias Andreasen*, [MAL83] (MA19-048); Viken county, Frogn municipal, on *Tilia cordata*, 05 June 2019, *Mathias Andreasen*, [MAL84] (MA19-049).

Notes: The strains of MAL32, MAL73, MAL81, MAL83, MAL84 showed similar morphology within ascomata, peridium, asci, and to some extent spores, indicating a close relationship. Still, high intraspecific variability in spore form and septation within each specimen was observed. This variation was observed both in between the strains, but also within each specimen and even within each ascomata. A hypothesis using spore septation of longitudinal orientation as an indication of an intergrading form of *L*. *pseudomacrostomum*, between *L*. *compressum* and *L*. *macrostomoides*, might be a suitable solution. Longitudinal septae were found in some, but not all strains of MAL84 and MAL73, MAL81 and MAL83, respectively forming two strong supported clades in the topology (Figure 1). Longitudinal septae was not observed in MAL32 which is nesting within a highly supported clade with two other strains (CBS 123097, LMS) of *Lophiostoma macrostomoides*. This study, therefore, names the strongly supported clade compiling strain CBS 123097, MAL32 and LMS as the true *L*. *macrostomoides*, while marking the clades compiling strain MAL83, MAL81 and MAL73, MAL84 as *Lophiostoma* aff. *macrostomoides*.

Lophiostoma terricola (G.S. Gong) (Thambug. & K.D. Hyde) M. Andreasen, I. Skrede, W. Jaklitsch, H. Voglmayr & B. Nordén. **comb. nov.**

Fig. 10.

Mycobank no.: *MB#XXXXXX*

= *Alpestrisphaeria terricola* (G.S. Gong) Thambug. & K.D. Hyde, Fungal Diversity 74: 214 (2015) [MB#551233]

Sexual morph: *Ascomata* (159.4-)169-208.6(-222.9) µm diameter (n = 10), erumpent, subglobose, scattered to gregarious, carbonaceous. *Ostiole* central or lateral, carbonaceous, normally with a pore-like to slit-like opening. *Peridium* (44.8-)50.5-70.9(-75) µm (n = 10)

composed of several layers of dark-brown to black, thick-walled pseudoparenchymatous cells, arranged in a *textura angularis*, fusing with host tissue at the most outside layer and pale inwardly. *Hamathecium* comprising 1.7-2.4 µm (n = 10) wide, branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* 74.5-140.1 x 14.2-22.5 µm (n = 10), 8-spored, bitunicate, fissitunicate, clavate, rounded at the apex, apical chamber present, short to long-stalked, biseriate or obliquely partially overlapping in asci. *Ascospores* (26.1-)29.9-35.2(-36.1) x (10.7-)11.5-13.3(-14.1) (n = 30), fusiform with narrowly rounded ends, often slightly curved, at first hyaline, smooth and 1 septum, finally brown, verruculose and 3 septa, sharply constricted at the median septum.

Culture characters: Ascospores germinated in MEA within 24h. Germ tubes produced from one or both ends of ascospore. Colonies growing unregular circular, reach 3.4-4.7 cm in diam. after four weeks at 20 °C, with somewhat irregular margins. White, some with light yellow areas, deeper light grey, reverse black.

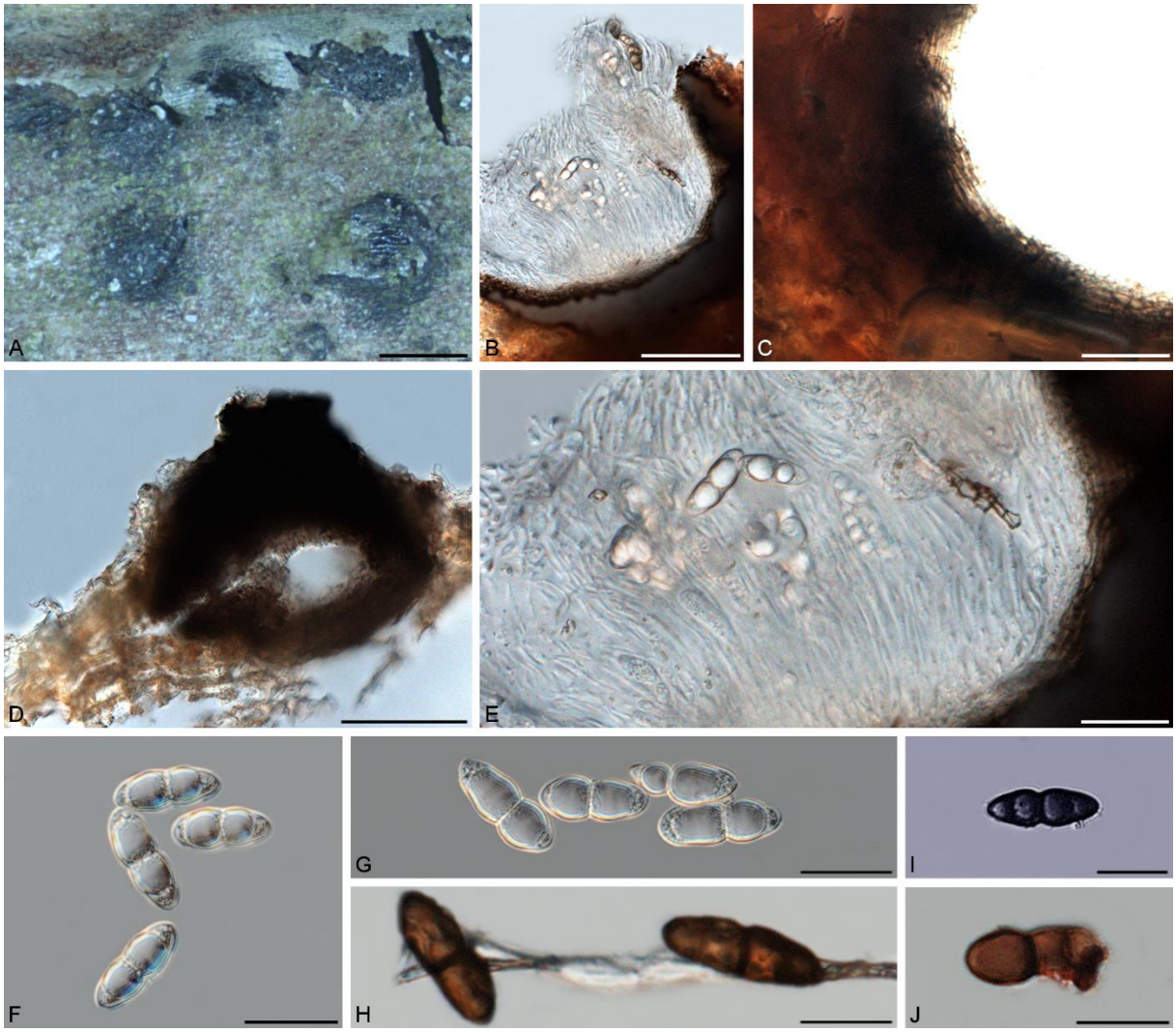

Figure 10. *Lophiostoma terricola*. **A** Ascomata. **B-D** Section of ascoma. **E** Hymenium. **F-G** Immature ascospores. **H-J** Ascospores. Scale bars: **A** = 150 µm, **B, D** = 100 µm, **C** = 50 µm, **E** = 40 µm, **F-J** = 20 µm.

Material exanimated: **Norway**, Viken county, Asker municipal, on the rhizome of *Plantago maritima*, 15 August 2019, *Mathias Andreasen* [MAL92] (MA19-075).

Notes: The specimen is morphologically identical to SC-12 following the description and photographies of Zhou et al. (2014).

This species forms a strongly supported clade comprising strain MAL92 and SC-12 nesting as a sister species 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. The specimen collected by this study are saprotrophic on rhizomorphs of *Plantago maritima* growing in the tidal zone of saltwater in the Oslo fiord. Zhou et al. (2014) described this species (SC-12) *Trematosphaeria terricola* mainly based on its morphological affinities with the generic concept of *Trematosphaeria*. However, it was shown to be nesting with strong support as a sister clade to the remaining taxa of *Lophiostoma* by Thambugala et al. 2015. The strain (SC-12) was extracted from ascomata found on alpine soil in China at an altitude of 3,177 meters. The finding of this species in marine environments is therefore surprising. Also, the morphological investigations support that this might be the same species, but with slightly shorter (29.9-35.2 vs 31.1-41.9 µm) and wider spores (11.5-13.3 vs 7.7-10.7 µm) and ascomata without a cover of brown septate hyphae.

Lophiotremataceae K. Hiray. & Kaz. Tanaka, Mycoscience 52: 405 (2011)
MycoBank no.: MB 561063
Type genus: *Lophiotrema* Sacc.

Ecology: Saprotrophic on various plants, bark and wood of shrubs and trees.

Sexual morph: *Ascomata* subglobose to globose, scattered to crowded. *Ostiole* compressed, slit-like. *Peridium* composed of pale brown, small, thin-walled cells of *textura prismatica* or *textura angularis* form. *Hamathecium* comprising pseudoparaphyses, filamentous, numerous, septate, branched, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* fissitunicate, cylindrical, with a short stipe or sessile, rounded at the apex, with an apical chamber. *Ascospores* fusiform to cylindrical, 1- to multiseptated, hyaline to brown, with or without an entire gelatinous sheath.

Antaelophiotrema *A. Hashim. & Kaz. Tanaka, Persoonia 39: 68 (2017)*
Mycobank no.: MB819252

Saprobic on woody plants.

Sexual morph: *Ascomata* subglobose to depressed ellipsoidal. *Ostiolar* central, carbonaceous, neck crest-like, elongated, laterally compressed. *Peridium* ununiform composed of 2 zones; outer zone darker red brown to black, inner light golden brown, composing of *textura prismatica* cells with tendencies to *textura angularis* cells on the inner sidewall, fusing with host tissue in lower parts. *Hamathecium* septate, branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* bitunicate, fissitunicate, cylindrical to clavate, 4–8-spored. *Ascospores* narrowly fusiform, 1- to 3-septate, hyaline to brown, guttulate, smooth.

Antealophiotrema sp. nov. M. Andreasen, B. Nordén & J.B. Jordal. **sp. nov.**

Figure 11.

Mycobank no.: MB819253

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

Holotype: [MAL63] (JB18DurP9-1)

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

Sexual morph: *Ascomata* subglobose, 100-250 µm diam. *Ostiole* central, carbonaceous, neck crest-like, elongated, laterally compressed. *Peridium* 20–40 µm (n = 10), ununiform composed of 2 zones; outer zone darker red brown to black, inner light golden brown, composing of *textura prismatica* cells with tendencies to *textura angularis* cells on the inner sidewall, fusing with host tissue in lower parts. *Hamathecium* comprising 0.5–1.5 µm (n = 10) wide, septate, branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* bitunicate, fissitunicate, cylindrical to clavate, 80–120 × 10–13 µm (n = 10), with a short, apically rounded with an ocular chamber, with biseriate 4–8 ascospores. *Ascospores* 24–40 × 7–10 µm (n = 30), narrowly fusiform with slightly rounded ends, 1- or 3-septate, strongly constricted at the septum, hyaline becoming brown, guttulate, smooth.

Culture characteristics: Ascospores germinated in MEA within 48 h at 20 °C. Germ tubes produced from one or both ends of ascospore and very often more central cells. Colonies growing unregular circular, reach 2-2.6 cm in diam. after four weeks, with somewhat irregular margins. Initially, whitish becoming light greyish to dark greyish from below, margin and deeper strata dark grey to black, reverse black.

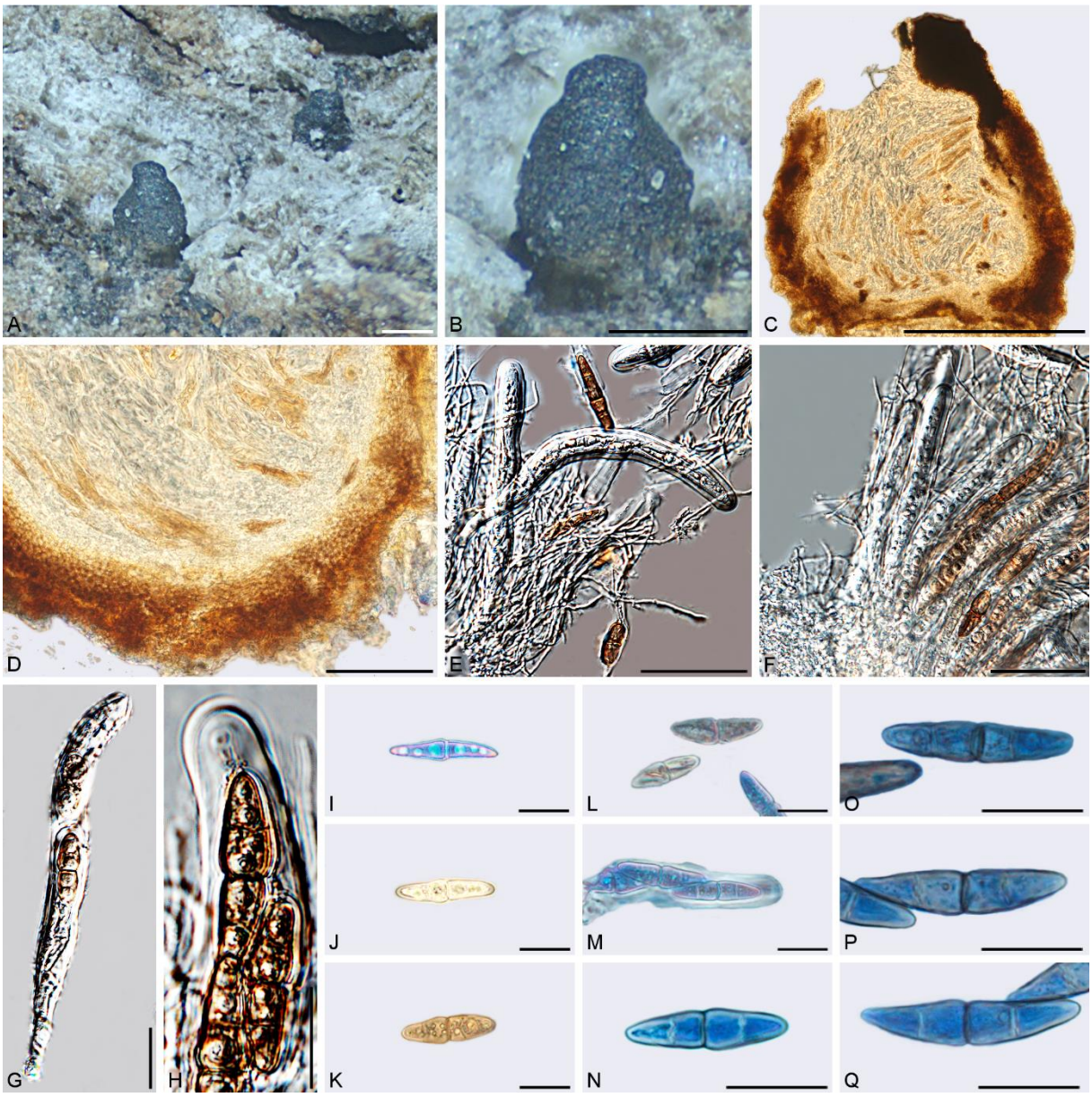

Figure 11. *Antealophiotrema sp. nov.* **A-B** Ascomata. **C** Section of ascoma. **D** Peridium. **E-H** Asci. **I-J** Immature ascospores. **K-Q** Ascospores. Scale bars: **A-C** = 100 µm, **D** = 40 µm, **E-F** = 60 µm, **G-H** = 20 µm, **I-Q** = 20 µm. **I-Q** in Cotton blue.

Material examined: **Norway**, Møre og Romsdal county, Tingvoll municipal, on the bark of living *Populus tremula*, 12 October 2018, *John Bjarne Jordal* [MAL63] (JB18DurP9-1); Møre og Romsdal county, Aure municipal, on the bark of living *Populus tremula*, 18 November 2019, *John Bjarne Jordal* [MAL64] (JB18Vikp7-1)

Notes: In the presented topology, this species and *Antealophiotrema brunneosporum* (CBS 123095) are used as the outgroup and comment on their familiar placement is therefore not possible other than referring to Hashimoto et al. 2017. They noted that *A. brunneosporum* (CBS123095) and '*Lophiotrema' boreale* (CBS 14136) formed a fully supported clade (100% MLB BP/1.00 BPP) outside of *Lophiotremataceae* and recognised them as a lineage distinct

from *Lophiotrema sensu stricto*. Thus, treating *Antealophiotrema* as *incertae sedis* in Pleosporales. Strains MAL63 and MAL64 as presented here might be able to shed light on the familiar placement of this family, but an extended dataset is needed. However, the results of this study show strong support (100 % ML BP/1.00 Bayesian PP) for our two strains nesting as a sister species next to *A*. *brunneosporum*.

Morphologically this species differs in overall scale compared to *A. brunneosporum*. Ascospores are starting as hyaline and becoming brown in contrast to just brown, and they are shorter up to 40 µm and 1-3-septate in contrast to up to 48 µm and 1-septate. Asci are also shorter and narrower, and peridium cells are of form *textura prismatica* and less of a rectangular form as present in *A*. *brunneosporum*. Lastly, the host of *Populus tremula* of *Antealophiotrema sp*. *nov*. differs from that of *Salix sp.* for *A. brunneosporum*.

Atrocalyx *A. Hashim. & Kaz. Tanaka, Persoonia 39: 59 (2017)*

Mycobank no.: MB819240

Type species. *Atrocalyx acutisporus* A. Hashim. & Kaz. Tanaka. (2017).

Ecology: Saprobic on woody plants.

Sexual morph: *Ascomata* solitary to gregarious, semi-immersed to immersed. *Ostiole* crest-like, elongated and laterally compressed, surrounded by dark brown hyphae. *Peridium* composed of two zones at the side. Pseudoparaphyses septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* broadly fusiform, hyaline, 1-septate, smooth.

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

Atrocalyx sp. nov. M. Andreasen, B. Nordén & J.B. Jordal. **sp. nov.**

Figure 12.

MycoBank no.: MB#XXXXXX

Holotype: (MA18-003) [MAL27]

Ecology: Saprobic on the bark of *Fraxinus excelsior* and *Populus tremula*.

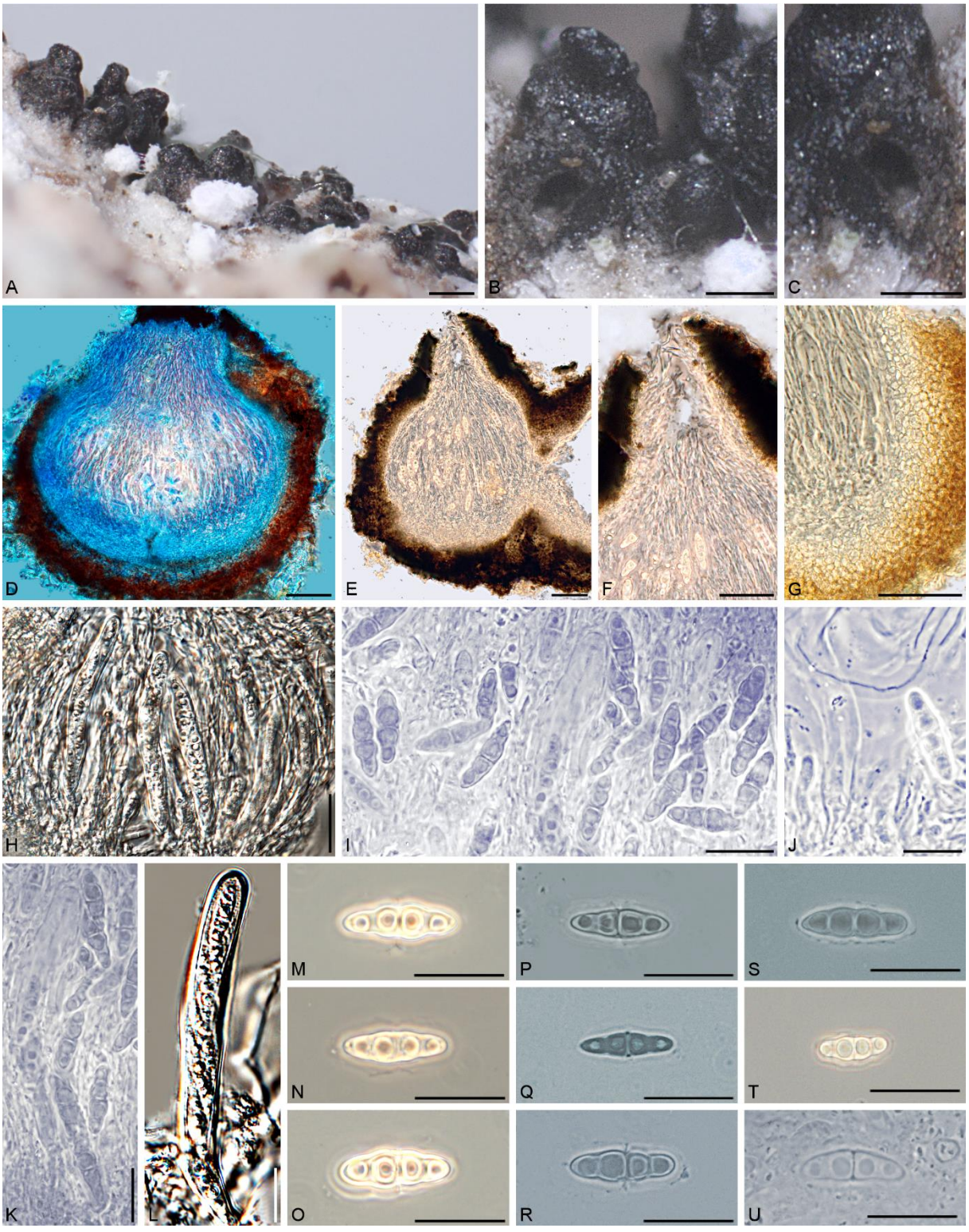

Figure 12. *Atrocalyx sp. nov.* Holotype (MAL27) (MA18-003). **A-C** Ascomata. **D-F** Section of ascoma. **G** Peridium. **H-J** Hymenium and paraphyses. **K-L** Asci. **M-T** Ascospores. **U** Immature ascospore. Scale bars: **A-C** = 200 µm, **D-H** = 50 µm, **I-U** = 20 µm. **D, I-K, P-S, U** in Cotton blue.

Sexual morph: *Ascomata* 300–400 µm diam., black, scattered to gregarious, immersed or erumpent from the slightly blackened substrate, globose to pyriform. *Ostiole* up to 150 µm long, central, carbonaceous, neck crest-like, elongated, laterally compressed. *Peridium* 40–55 µm thick (n = 20), composed of several layers of dark-brown to black, thick-walled

pseudoparenchymatous cells, arranged in a *textura angularis*, fusing with host tissue at most outside layer and pale inwardly, with a peridium wall at the base. *Hamathecium* comprising (0.7)1.0–1.5(2.0) µm wide (n = 60), branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* (110)120-180 × 12—15 µm (n = 80), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short-stiped, pedicellate, apically rounded with a minute ocular chamber, uniseriate. *Ascospores* (17)20-25(30) x (4-)6-8(-10) µm (n = 100), hyaline, ellipsoid with rather obtuse ends, two-celled, constricted at septae, with thick (up to 20 µm) diffuse mucilaginous sheath, smooth-walled, with two large globules in each cell, 1-2-seriate, smooth.

Culture characteristics: Ascospores germinated in MEA within 48 h at 20 °C. Germ tubes produced from one or both ends. Colonies growing unregular circular, reach 3-3.4 cm in diam. after four weeks, with somewhat irregular margins. Initially, light grey becoming dark grey to black from below, margin and deeper strata dark grey to black, reverse black.

Material examined: **Norway**, Møre og Romsdal county, Molde municipality, on *Populus tremula.*, 03 September 2018, *John Bjarne Jordal*, [MAL20] (JB18-502); Møre og Romsdal county, Molde municipality, on *Populus tremula.*, 03 September 2018, *John Bjarne Jordal*, [MAL21] (JB18-506); Oslo county and municipality, on the bark of living *Fraxinus excelsior*, 28 September 2018, *Mathias Andreasen*, [MAL27] (MA18-003); Møre og Romsdal county, Molde municipality, on the bark of living *Populus tremula*, 03 September 2019, *John Bjarne Jordal,* [MAL76] (JB18-505).

Notes: Morphologically this new species bare considerable resemblance to other species of *Atrocalyx* like *A*. *acutisporus*, *A*. *lignicola*. Still, there are differentiating characters in an overall bigger scale of both ascomata, peridium, asci and ascospores. The strains MAL20, MAL21, MAL27 and MAL76 representing this species, create a strongly supported clade within the genus *Atrocalyx*. There is phylogenetic support for variation within this clade between the strains, but morphology suggests that they are one species. When first found and investigated, these specimens were identified as *Lophiotrema lennartii* Math., Granmo & Stensrud and resembled this species, especially in spore form and size. It still differs in several other aspects such as spore septation with up to 3-septate which has not been observed above 1-septae for *L*. *lennartii*. Asci of more clavate form (vs strictly cylindrical), very thick-walled and length are exceeding the maximum observed 140 µm for *L*. *lennartii*. Ascospores have uniseriate placement in asci (vs obliquely uniseriate to uniseriate), and this species is found in Oceanic

environments at low altitude collection sites (vs continental and high altitude locality). Lastly the hosts of *Populus* and *Fraxinus* for *Atrocalyx sp. nov.* differs from *Myricaria germanica* and *Aconitum septentrionale* for *L. lennartii*.

Lophiotrema *Sacc. emend. Holm & Holm, Symb. Bot. Ups. 28(2):25, 1988.*

Mycobank no.: MB2934

Type species: *Lophiotrema nucula* (Rehm) Mussat, Sylloge Fungorum 15: 199 (1900),

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

Ecology: Saprobic on various plants.

Sexual morph: *Ascomata* immersed, erumpent at the apex, subglobose. *Ostiole* crest-like or rarely papillate, mostly elongated and laterally compressed. *Peridium* globose in outline, composed of a few layers, comprising rectangular to globose cells of angularis-textura globose form with uniform size, cells towards the inside lighter, at the outside, darker, fusing and indistinguishable from the host tissues. *Hamathecium* comprising 1–2 µm wide, septate, branched, cellular, anastomosed pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* bitunicate, fissitunicate, cylindrical, with a short stipe, 8-spored. *Ascospores* fusiform to broadly fusiform, hyaline, smooth.

Notes: Hashimoto et al. 2017 found that *Lophiotrema sensu stricto* 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.

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

Figure 13.

MycoBank no.: MB151729

≡ *Lophiosphaera nucula* (Fr.) Cooke (?) [MB#465456]

≡ *Sphaeria nucula* Fr., Kongliga Svenska Vetenskapsakademiens Handlinger 38: 266 (1817) [MB#222550]

≡ *Lophiostoma nucula* (Fr.) Ces. & De Not., Commentario della Società Crittogamologica Italiana 1 (4): 222 (1863) [MB#244964]

Ecology: Saprobic on the bark of *Salix*, *Populus*, *Acer*, *Quercus*, and *Ulmus,* also *o*n branches of *Liriodendron tulipifera*.

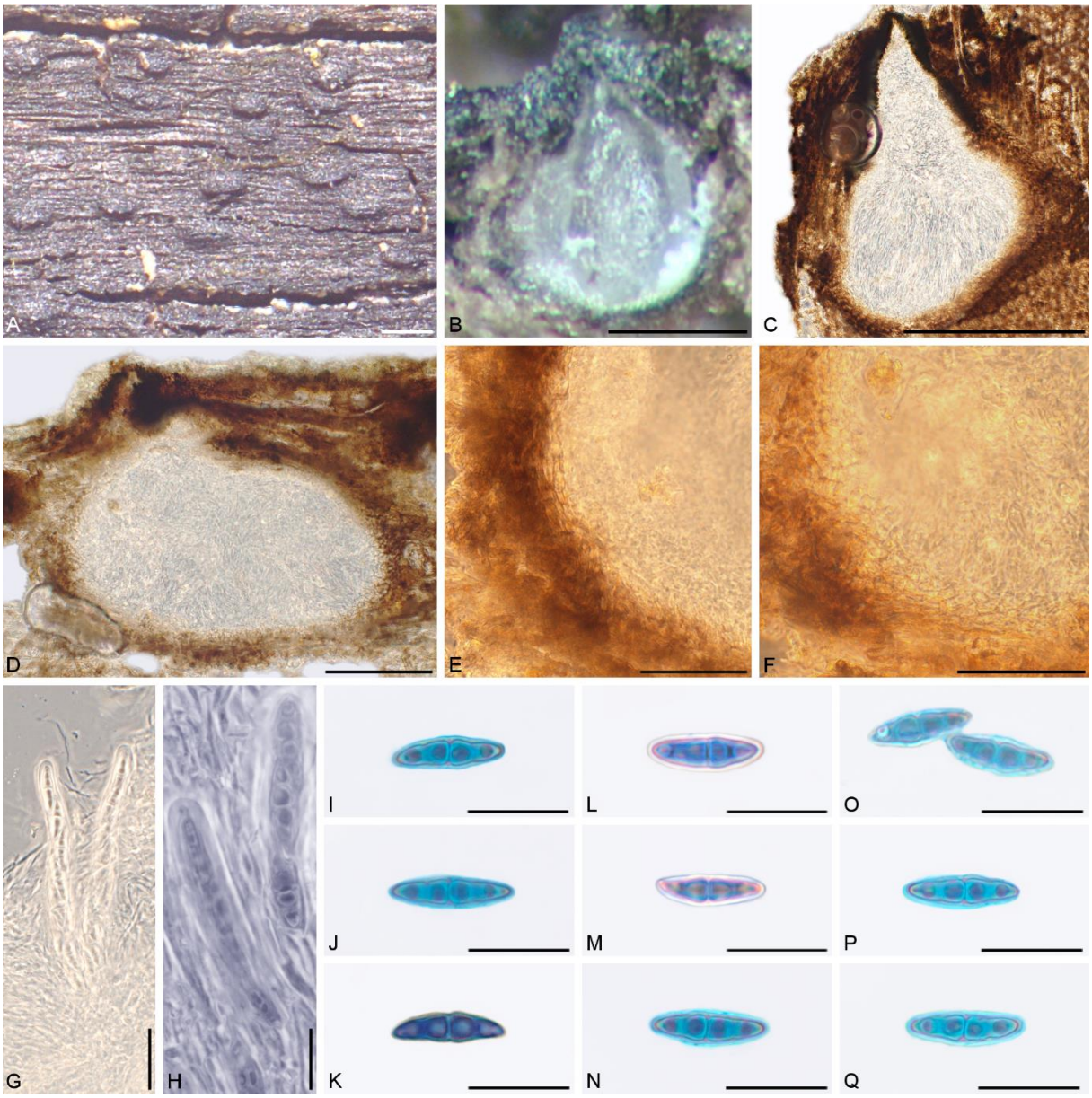

Figure 13. *Lophiotrema nucula.* **A-B** Ascomata. **C** Section of ascoma. **D-F** Peridium. **G-H** Asci. **I-Q** Ascospores. Scale bars: **A, B** = 150 µm, **C** = 200 µm, **D** = 40 µm, **E-F, I-Q** = 20 µm. **G, H** = 10 µm. **H-Q** in Cotton blue.

Sexual morph: *Ascomata* 300–360 µm high, 300–435 µm diameter, globose to subglobose, black, scattered, gregarious, immersed to erumpent, globose to subglobose, uni-loculate, glabrous, ostiolate. *Ostiole* central or lateral, carbonaceous, normally with a pore-like to slit-like opening. *Peridium* 10-20 µm (n = 90) wide, composed of several layers of dark-brown to black, thick-walled pseudoparenchymatous cells, arranged in a *textura angularis*, fusing with host tissue at the most outside layer and pale inwardly. *Hamathecium* comprising 1–2 µm wide (n = 120), branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Asci* (70-)80-110(-120) x (8-)9-11.5 µm (n = 120), (4-)8-spored, bitunicate, fissitunicate, cylindrical to clavate, with short stipe 15-33 µm, pedicellate, apically rounded with a minute ocular chamber. *Ascospores* 19-

24.5 × 6-9.5 µm (n = 180), hyaline becoming brown at germination/at over-maturity, elliptic-fusiform, with rounded ends, 1- or 3-septate, constricted at the middle septum, slightly narrower towards both ends, inconspicuous mucilaginous sheath 0.5-1 µm wide, smooth-walled, guttulate with one or two guttules in each cell.

Culture characteristics: Ascospores germinated in MEA within 24 h at 20 °C. Germ tubes produced from one or both ends of ascospore. Colonies growing circular, and reach 2.7-3,5 cm in diam. after four weeks, with a somewhat irregular margin. Initially whitish, and become greyish from above, reverse greyish-brown.
Material examined: **Norway**, Agder county, Arendal municipality, on the bark of living trunk of *Populus tremula*, 04 October 2014, *Jacques Fournier*, [MAL47] (O-F247790); Viken county, Asker municipality, on branches of living *Salix sp.*, 20 February 2019, *Mathias Andreasen*, (MA19-012). Agder county, Froland municipality, on *Ulmus glabra*, 03 October 2014, *Jacques Fournier*, (O-F247791); Agder county, Froland municipality, on *Populus cf. tremula*, 03 October 2014, *Jacques Fournier*, (O-F247805); Vestland county, Granvin municipality, on *Ulmus glabra*, 13 May 2014, *Björn Nordén* & *John Bjarne Jordal*, (O-F251885).

Lophiotrema myriocarpum **(Fuckel) (Sacc)** M. Andreasen, I. Skrede, W. Jaklitsch, H. Voglmayr and B. Nordén. **comb. nov.**
Figure 14.
≡ *Lophiostoma myriocarpum* Fuckel, Jahrbücher des Nassauischen Vereins für Naturkunde 23-24: 156 (1870) [MB#141605].
= *Lophiotrema vigheffulense* (Pass.) Berl., IC. Fung. 1:4 (1890) [MB#206314].
= *Lophiosphaera vigheffulensis* Pass., Erb. Critt. Ital. Ser. 2 no. 1373 (1883) [MB#248553].

Ecology: Saprobic on wood and bark of frondose trees and also on *Dryas*.

Sexual morph: *Ascomata* densely scattered, immersed to erumpent, 300-400(-600) µm wide, with coarse papilla. *Ostiole* 200 µm, central or lateral, carbonaceous, generally with a slit-like opening. *Hamathecium* comprising 0.5–1.5 µm (n = 10) wide, septate, branched, cellular pseudoparaphyses, anastomosing among and between the asci, embedded in a gelatinous matrix. *Peridium* 25-50 µm, composed of several layers of dark-brown to black, thick-walled pseudoparenchymatous cells, arranged in a *textura angularis* in the innermost and median layers along with *textura prismatica* in the outer, lower and cells situated towards the ostiole, fusing with host tissue at the most outside layer and pale inwardly strongly developed. *Asci*

almost cylindric 100-120 x 10-12 µm, 6-spored, bitunicate, biseriate. *Ascospores* (24-)28-36 x 4-6 µm, hyaline, narrowly fusiform, slightly curved, 3- or 5-septate, constricted at middle septae, inconspicuous mucilaginous sheath 1-4 µm wide, guttulate, oil drops disappearing when overmatured.

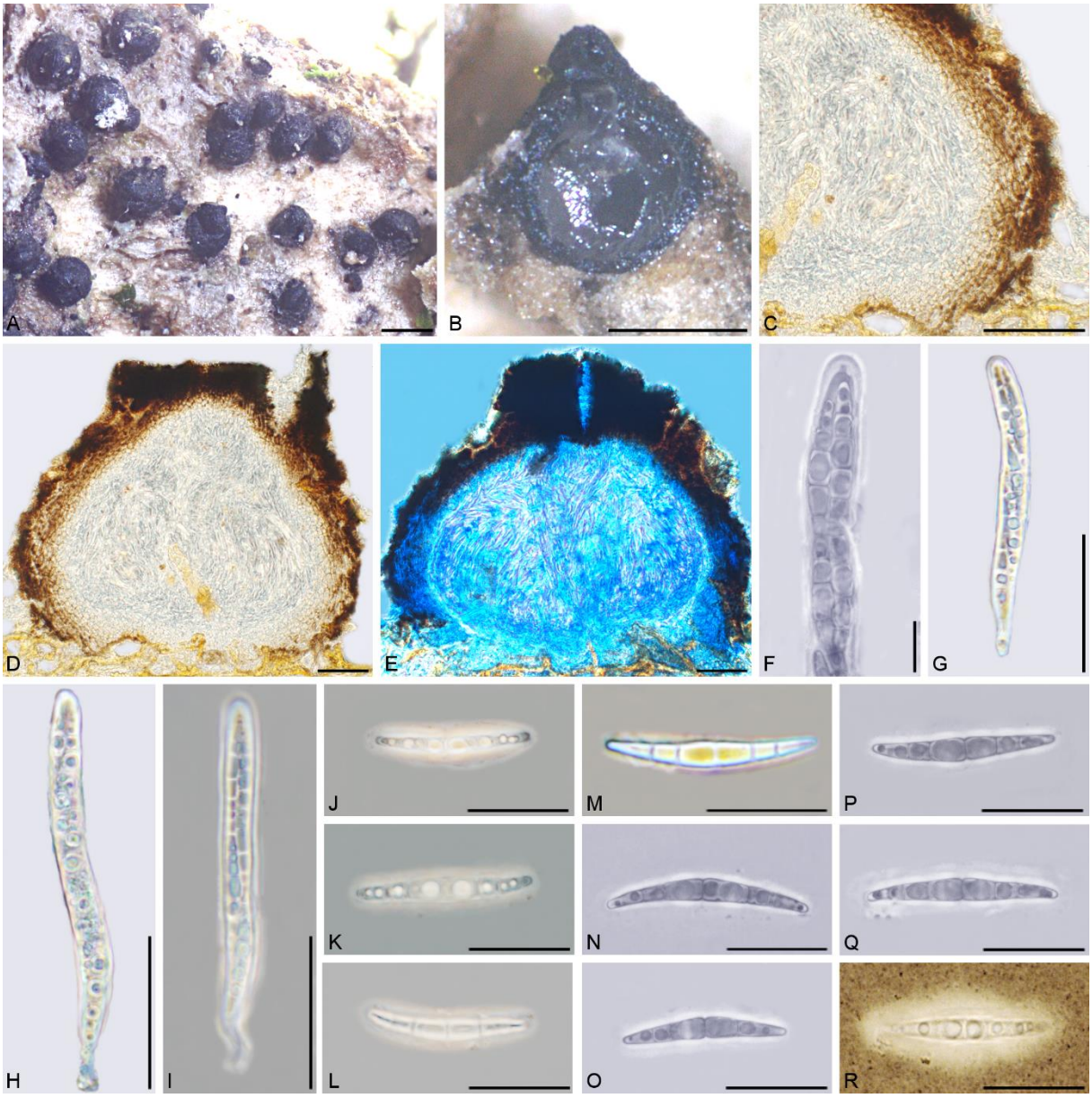

Figure 14. *Lophiotrema myriocarpum.* **A** Ascomata. **B, D, C** Section of ascoma. **C** Peridium. **F-I** Asci. **J-R** Ascospores. Scale bars: **A** = 400 µm, **B** = 250 µm, **C-E, G-I** = 50 µm, **F** = 10 µm, **J-R** = 20 µm. **E-F, N-Q** In cotton blue, **R** in Indian ink.

Culture characteristics: Ascospores germinated in MEA within 24 h at 20 °C. Germ tubes produced from one or both ends of ascospore and very often more central cells. Colonies growing unregular circular, reach 3.1-4.1 cm in diam. after four weeks, with somewhat irregular margins. Initially, whitish becoming light greyish to dark greyish from below, margin and deeper strata dark grey to black, reverse black.

Material examined: **Norway**, Rogaland county, Suldal municipal, on the bark of living *Fraxinus Excelsior*, 19 September 2018, *John Bjarne Jordal*, [MAL01] (JB17-513); Vestlandet county, Kvam municipal, on the bark of *Ulmus glabra*, 15 May 2019, *Mathias Andreasen*, [MAL71] (MA19-034).

Notes: Holm and Holm (1988) reported that this species is well characterized by the narrow spores, which early have more than one septum. Based on the description of Holm and Holm (1988), the two collections of MAL01 and MAL71 are here identified as *Lophiostoma myriocarpum*. The similarity to the description was noted within both ascomata, peridium, paraphyses, asci and ascospores, but for lack of spore appendages in contrast to the presence of a thin mucilaginous sheath. The type material of *Lophiostoma myriocarpum* should be investigated as final evidence of the identification. This study suggests a combination for *Lophiostoma myriocarpum* with *Lophiotrema myriocarpum* and thereby the resurrection of the species epithet *Lophiotrema myriocarpum* (Fuckel) Sacc, which was proposed by Fuckel in 1866 and synonymised with *Lophiostoma myriocarpum* in 1878 by Saccharo.

NCBI BLAST results of the molecular markers of strain MAL01 and MAL71 showed relatively high identity percentage (MAL01: 96.26 % for ITS, 95.52 % for TEF1-α; MAL71: 95.74 % for ITS, 94.98 % for TEF1-α and 89.83 % for RPB2) with strains identified as *Lophiotrema neohysterioides* and morphological similarities to this species were noticed. Still, a row of differences in morphology were also noticed, e.g. spore septation with 3 or 5 (vs only 3 septae in *L. neohysterioides*), bigger more oblong ascospore and longer cylindrical asci with longer stipe (vs clavate asci with very short stipe). These differencing characters are backed up by phylogenetic evidence here (Figure 2) showing the two strains nesting as a sister species to *L. neohysterioides* with strong support.

4 Discussion

This study presents an enhanced phylogenetic dataset for the families of *Lophiostomataceae* and *Lophiotremataceae* based on the five molecular markers of ITS2, 5.8S, LSU, TEF1-α and RPB2. It introduces taxonomic and phylogenetic data supporting new findings which include several new species to science, new combinations and resurrection of a broad generic concept to genus *Lophiostoma*.

4.1 Molecular markers

The choice of a combination of molecular markers that provide information about the phylogenetic relationships among groups should be evaluated individually for each specific group under study. Both the number of markers selected and their evolutionary rate may affect the results. Datasets including hundreds of loci, like those produced by next generation sequencing techniques, may provide high resolution phylogenies, but they may also create many conflicting trees (Lemmon and Lemmon 2013; Philippe et al. 2011). Moreover, recent studies point out that the choice of genetic markers is more critical than the number of markers and their length (Aguileta et al. 2008; Balasundaram et al. 2015; Stielow et al. 2015). The five markers (5.8S, ITS2, LSU, TEF1-α, RPB2) inferred in this study, stable support for species and shallow clades, but also for the topology of certain deep branches of the tree (Figure 1, Figure 2). Still, in the case of genus *Lophiostoma* little support is found for species and shallow clades. In this case, there is a need for a revaluation of markers needed to infer support at the species level. For both the molecular markers RPB2 and TEF1-α loci this study observed both high variation, inferring support for species and shallow clades, but also more highly conserved regions, inferring support for deeper nodes. This tendency is also observed by other studies working with related groupings of fungi (Jaklitsch et al. 2016). In the present study, the molecular markers of TEF1-α and RPB2 were difficult to amplify. Thus, these molecular markers are often missing in the alignment. It is reasonable to assume that this lack of highly informative markers in the alignment might be one of the reasons for the lack of support at the deeper nodes and to some extent also an explanation for the lack of support for species and shallow clades. If additional markers with higher variability such as ITS, TEF1-α and RPB2 were added for all taxa, the topology might change substantially, especially in the many nodes with low support, but this may also be the case when additional taxa are added.

In this study, the ITS1 region was unalignable and omitted form the phylogenetic analyses of both the families of *Lophiostomataceae* and *Lophiotremataceae*. ITS is the universal barcode for fungal species recognition (Schoch et al. 2012) and is informative by showing considerable variation between related species within many, but not all, fungal groups (Bruns 2001). However, this large variation may make it impossible to align properly, thus uninformative for phylogenetic reconstruction at family and genus level, as shown here for *Lophiostomataceae* and *Lophiotremataceae*.

4.2 *Lophiostomataceae*

The results of phylogenetic analyses for *Lophiostomataceae* as presented here, show increased support for deeper nodes of the topology compared to previously published phylogenies of this family (Mugambi and Huhndorf 2009; Hirayama and Tanaka 2011; Zhang et al. 2014; Thambugala et al. 2015; Jaklitsch et al. 2016; Hashimoto et al. 2018; Bao 2019). The phylogeny presented in this study strongly supports to apply a broad generic concept to the genus *Lophiostoma*. The presented broad concept of the genus *Lophiostoma* satisfies the criteria of Vellinga et al. (2015) for generic circumscription in particular by having strong statistical support and being monophyletic. Further, morphological support is also present and comply to the traditional characters for generic circumscription within the family: (1) ascomatal size, (2) thickness of ascomatal peridium, (3) peridial cell type, (4) ascus shape, (5) ascospore colour, (6) ascospore septation, and (7) ascospore appendages (Hirayama and Tanaka 2011; Hirayama et al. 2014). Following the application of a broad generic concept of genus Lophiostoma, there is a need for synonymising of genera *Alpestrisphaeria*, *Biappendiculispora*, *Capulatispora*, *Coelodictyosporium*, *Guttulispora*, *Lophiohelichrysum*, *Lophiopoacea*, *Platystomum, Pseudolophiostoma*, *Pseudoplatystomum* and *Sigarispora* with *Lophiostma*.

With the here proposed broad generic concept for genus *Lophiostoma*, we touch upon the philosophical perspective of "*lumping or splitting*" within generic circumscription, as outlined in the introduction. The boundaries for generic circumscription are to some extent open to interpretation and genera are artificial units which primarily enable species classification within a binomial classification frame. Nevertheless, the presented criteria of Vellinga et al. (2015) and Tulloss et al. (2016) on the matter, give a documented foundation for decision-making. Vellinga et al. (2015) discuss the issue of single clades holding few strains

being split into several genera and states: “This does not increase insight in the evolutionary history of the group in question, only inflates the taxonomic framework. From a formal phylogenetic perspective it may not matter whether we have one family, with more than a hundred genera, or whether we have one genus with many infrageneric units, formally named or not”, and they further state a clear recommendation “We strongly advocate that different options are explored and discussed, instead of using a boilerplate model in which every monophyletic clade is translated into a genus.”. By maintaining a genus *Lophiostoma*, with a broad generic circumscription, we maintain monophyly and avoid a splitting snowball motion where small monophyletic groupings result in the formation of paraphyletic genera. Another essential criterion for generic circumscription as outlined by Vellinga et al. (2015) and Tulloss et al. (2016), is the need of several ribosomal DNA gene markers and additional markers of protein coding genes as the basis for decision making. Their study also recognizes the concept of Genealogical Concordance Phylogenetic Species Recognition for taxonomy of species, expressing the absolute need of combining several molecular markers, of diverging conservation-levels, for accurate phylogenetic reconstruction. It could, therefore, be argued that the fulfilment of this criteria and concept should precede any proposal of the splitting of existing genera. In the case of the total alignment of *Lophiostomataceae,* many strains are still lacking sequences of molecular markers representing ribosomal DNA or protein coding loci, and even some are lacking representatives of both. The result is a persisting unresolved topology, which is made further indistinct in many cases by the lack of support within deeper nodes (Figure 1). The argument of a persistent lack of sequences of molecular markers and the lack of support underpins the proposal of continued use of a broad generic concept for genus *Lophiostoma*.

Concerning morphology and applicable conclusions on general characters of both familiar, generic and species level distinction, the choice of this study has been to be very cautious and only refer to descriptions and photo tables. For familiar and generic distinction, there are seemingly good characters, such as the ones presented above. Still, it is found that there are high intraspecific variability of several morphological traits in genus *Lophiostoma*. Thus, several morphological characters (e.g. spore form, septation, pigmentation, presence of mucilaginous sheet; ascomata form and placement, ostiole form, and peridium cell of form *textura angularis* vs *textura prismatica*) can at best be used for distinction at the species level and in some cases not at all.

Lophiostoma caespitosum forms a strongly supported phylogenetic clade, and the species is further supported by morphological evidence. The newly proposed species epithet "*Guttulispora" crataegi* Thambug., Qing Tian & K.D. Hyde is therefore synonymized with *Lophiostoma caespitosum*. Further, the strain MAL04 is a supported sister taxon to *Lophiostoma caespitosum*. MAL04 show phylogenetic and morphological support for being an independent species and the evidence is presented in the species description. The strain C191 is also found in the same clade as MAL04 and *Lophiostoma caespitosum*, but without the support and the specimen requires further morphological investigation. The topology of the node containing LQ2, MFLUCC 14-0993, MFLUCC 13-0442, MAL04 and C191 are unsupported and in need of further investigation.

The strongly supported clade compiling strain CBS 123097, MAL32 and LMS is named as the true *L. macrostomoides*, while the two closely related clades compiling strain MAL83, MAL81 and MAL73, MAL84 are marked as *Lophiostoma* aff. *macrostomoides*. These three clades share morphological characters that suggest that these taxa are closely related. Still, there is no support for them nesting together. It is noted that for most of these strains, sequences of the molecular marker RPB2 are missing, a marker which could have resolved the relationship of these strains further. Representations of the morphology of the strains can be found in figure 8 and 9.

4.3 *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. 2009; Hirayama and Tanaka 2011; Hashimoto et al. 2017). One species is introduced to genus *Atrocalyx* based on four strains (MAL20, MAL21, MAL27, MAL76), creating a strongly supported clade within the genus, and further morphological evidence for this species is provided in the species description. Further, a resurrection of the species epithet *Lophiotrema myriocarpum* (Fuckel) Sacc. (1878) is indicated and thus a synonymising of *Lophiostoma myriocarpum* with *Lophiotrema myriocarpum*. This combination is based on strong phylogenetic support and further morphological evidence, as stated in the notes to the species description. Unfortunately, it has so far been impossible to obtain the type species from Uppsala fungarium, and therefore this recombination remains putative until the type material has been investigated.

The taxon *Lophiotrema nucula* is strongly supported as a sister species to *L*. *fallaopiae*, *L*. *vagabundum* and *L*. *eburnoides* and the status of this species as an independent species is thereby strengthened. Earlier studies including the strain of CBS 627.86 did show same topology, but lower support for the species (Hirayama and Tanaka 2011; Hashimoto et al. 2017).

4.4 Morphology

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

This high intraspecific variability of morphology is persistent within many Pleosporalean genera, in particular within genera holding a broad generic description such as, e.g. *Teichospora* (Jaklitsch et al. 2016) and the here presented genus *Lophiostoma*. Thus, making it very 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 viable 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.

4.5 Ecology and distribution

Species of *Lophiostomataceae* and *Lophiotremataceae* are found on an extensive range of plant species where their ecological function are indicated as being saprotrophic (Holm and Holm, 1988; Mathiassen 1993; Ellis and Ellis, 1997). The majority of the species occur on wood and bark, plant stems, trunks or rhizomes, and some occur on branches that are immersed in fresh or saltwater (Holm and Holm, 1988; Mathiassen 1993; Ellis and Ellis 1997; Tanaka and Harada 2003a;b; Mugambi and Huhndorf 2009; Thambugala et al. 2015; Hashimoto et al. 2017). The families are distributed worldwide in all biomes and occur on a great variety of

plant hosts from both angiosperms and gymnosperms, some seemingly host specific while others are not.

In this study, many findings have been made of sporulating ascomata on living bark of trunks of frondose trees. Examples of such are *Lophiotrema nucula*, *Lophiotrema myriocarpum* and *Lophiotrema* sp. *nov*. (MAL20, MAL21, MAL27, MAL76) on *Ulmus*, *Fraxinus* and *Populus*. But also findings of *Lophiostoma aff. macrostomoides* on *Tilia cordata* and the new species of MAL04 on *Acer platanoides*. These findings on living host tissue raise questions on the ecological role of these fungi as saprotrophs. These species are saprotrophic of nature (Mathiassen 1989; Mathiassen 1993), but the observations made in this study may suggest that these fungal groupings might have an additional role as endophytes or latent-infecting fungi (Redlin and Carris 1996).

Recent investigative studies on secondary metabolites (polyketides) isolated from specimens of *Lophiostomataceae* from both terrestrial and marine environment have shown the presence of antibacterial and antifungal agents alongside with metabolites of nematicidal properties, in addition to cytotoxicity towards cancerous and non-cancerous animal cells (Shushni et al. 2013; Intaraudom et al. 2015; Rupcic et al. 2018). These secondary metabolites are found in the genera of *Lophiostoma* and *Vaginatispora*, and also other pleosporean genera such as *Massarina* and *Keissleriella*. Perhaps these polyketides could be seen as factors underpinning ecological roles of saprotrophic, endophytic, parasitic or even latent-infecting life cycles. Also, these metabolites could maybe, in the future, be used as supporting taxonomical characters. The fact that these organisms host secondary metabolites with prominent and selective biological properties, agrochemical pesticides and cytotoxic compounds, underline the applied potential these fungal taxa holds.

In Scandinavia efforts have been made to investigate the distribution of these fungal families within the order Pleosporales, e.g. Eriksson 1981; Holm and Holm 1988; Mathiassen 1993; Mathiassen et al. 2017a, b; Nordén et al. 2017; Nordén et al. 2019. Nevertheless, this study postulates that an increased effort and a search of a greater variety of plant hosts alongside with studying submerged wood in both fresh- and saltwater, would inevitably reveal new species and new combinations. This study shows that there is high diversity within the families of *Lophiostomataceae* and *Lophiotremataceae* in Norway, a tendency also shown to be true in both tropical and temperate regions worldwide (Tanaka and Hosoya 2008; Hirayama et al. 2014; Zhang et al. 2014; Thambugala et al. 2015; Hashimoto et al. 2018; Bao 2019).

4.6 Future perspective

Many unsolved questions of both taxonomical and ecological character for *Lophiostomataceae* and *Lophiotremataceae* remains to be answered. One 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 unclear. The lack of support shows demand for continued sampling and procurement of sufficient molecular information followed by a thorough morphological investigation. There might even be a need for the identification of a new, informative molecular marker to infer better phylogenetic resolution for species and shallow clades.

Within the presented topology of the genus *Lophiostoma,* several groupings of strains and taxa are found nesting together without sufficient support, but showing very similar morphological characters. On the other hand, strains showing support of nesting together but not showing morphological similarities were also observed. A lack of molecular information (e.g. some strains miss specific molecular markers in the alignment) can in many cases answer for 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). The taxa of *Lophiostoma compressum* and *Lophiostoma macrostomoides* are examples of such species showing tendencies of being complexes of cryptic species, showing differences in molecular affinities in between strains but bearing similar morphological characters. These taxa have long been suggested as closely related. Even the species of *L*. *pseudomacrostomum*, sharing morphology with *L*. *macrostomoides* but with the presence of muriform spores approaching *L*. *compressum*, has been suggested as an intergrading form between *L*. *compressum* and *L*. *macrostomoides* (Holm and Holm 1988, Mathiassen 1993). Their placement as taxa nesting closely together are also presented in the topology presented here, but they lack basal node support. Investigations of their internal relationship, both within strains of the same taxa showing differences in molecular affinities, but also in between the different species, could shed further light on this historical issue. Methods and software for delimiting species based on multilocus data such as the analytic tool of STACEY (Jones 2017) based on multispecies coalescent theory, might be a way of estimating gene trees, the species tree and species delimitations simultaneously. Also, an investigation of the phylogenetic placement of taxa

Lophiostoma pseudomacrostomum Sacc. is needed, a species which is morphologically very similar to *L. macrostomoides*. Concerning *Lophiostoma compressum* and the here proposed synonymised genus of *Platystomum* Fries, there are many proposed taxa within this species complex, e.g. "*Platystomum*" *rosea*, "*Platystomum salicicola*, "*Platystomum crataegi* and *Lophiostoma triseptatum* that require further investigation. The strains of these taxa are not showing support as being independent species, but hold only the molecular markers of ITS and LSU, for the most part.

This study shed further light on the phylogenetic and morphological boundaries of family *Lophiostomataceae* and *Lophiotremataceae* and their encompassing genera and species. By combining sampling of fresh material, cultivation and phylogenetic analyses of molecular markers, new species and combinations for science are revealed. These new data and their following analyses can be used to investigate and resolve both new and persisting phylogenetic relationships. Describing species and investigating evolutionary relationships are the cornerstone for our understanding of fungal diversity and consequently, their ecological roles. Adequate description of species is the very foundation for making decisions of conservational needs for species in their natural ecosystems.

The author hopes that the considerations in this thesis give fuel for thought and encouragement for further studies of these beautiful and interesting fungal families.

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