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Crassistoma norvegicum (Crassistomataceae), a new genus, species and family of Pleosporales from Norway

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Recent field surveys of Ascomycota in Norway revealed a corticolous pyrenomycetoid species with superficial, black, obpyriform ascomata, bitunicate asci and thick-walled brown, fusoid, bicellular ascospores, somewhat reminding of the North American *Trematosphaeria cariosa*. However, *T. cariosa* differs ecologically by growing on dead wood rather than on bark of a living tree, and comparison with the holotype of *T. cariosa* revealed that the Norwegian fungus differed by rounded-obpyriform vs. conical ascomata, a less carbonaceous peridium with a more uneven/rough surface and often with a basal thin tomentum, non-porate ostiolar papilla with bumpy-warty ridge- to collar-like structures and apically free paraphyses and smaller ascospores with a much narrower and lighter brown equatorial zone at the septum, indicating that both represent distinct species. Molecular phylogenetic analyses of a representative multigene LSU-SSU-*RPB2*-*TEF1* matrix of Pleosporales revealed an isolated position of the Norwegian fungus, remote from the generic type of *Trematosphaeria*, and it is therefore described as a new genus and species, *Crassistoma norvegicum*, within a new family, *Crassistomataceae*.

Key words: Molecular phylogeny, Pleosporales, Taxonomy, *Trematosphaeria*. – 1 new family, 1 new genus, 1 new species.

During a field survey of biodiversity of ascomycetes in Norway, a corticolous pyrenomycetoid species with large, superficial, black obpyriform ascomata, non-porate ostiolar papilla with bumpy-warty ridge- to collar-like structures, bitunicate asci and thick-walled, fusiform, uniseptate ascospores was collected that initially could not be identified. Detailed literature surveys revealed a close match to the North American *Trematosphaeria cariosa*, but the different ecology (corticolous vs. lignicolous) and the different geographic origins casted some doubts about their conspecificity. In addition, also generic classification in *Trematosphaeria* seemed dubious, as the ascomatal and ascospore characters traditionally used for morphological definitions of pleosporalean genera have been shown to be highly polyphyletic in many genera, e.g. *Massarina* (Tanaka et al. 2015), *Cucurbitaria* (Jaklitsch et al. 2018, Jaklitsch & Voglmayr 2017), *Splanchnonema* (Chethana et al. 2015), and also *Trematosphaeria* where e.g. *T. biappendiculata* (=*Lophiostoma biappendiculatum*; Andreasen et al. 2021), *T. hydrela*, *T. lignatilis* (=*Quintaria lignatilis*; Zhang et al. 2012), *T. mangrovei* (=*Rimora mangrovei*; Zhang et al. 2012), *T. terricola* (=*Lophiostoma terricola*; Andreasen et al. 2021) and *T. wegeliniana* have been shown to be phylogenetically distinct from the generic lectotype, *T. pertusa* (Wang et al. 2007, Zhang et al. 2012, Zhou et al. 2014), some of which are still awaiting generic reclassification according to molecular phylogenies.

To resolve the identity of the Norwegian fungus it was morphologically documented and compared with the holotype of *Trematosphaeria cariosa*. To reveal its phylogenetic position, DNA was extracted from a pure culture and SSU-ITS-LSU, *RPB2* and *TEF1* sequences were generated for phylogenetic analyses of a multigene matrix, the results of which are here presented.

Materials and methods

Morphology

Stereomicroscopy photographs were captured with a Nikon SMZ 1500 dissecting microscope equipped with a Nikon DS-U2 digital camera or a Nikon SMZ 745T stereomicroscope with a Tucsen DigiRetina 16 camera. For certain images of ascomata the stacking software Zerene Stacker v. 1.04 (Zerene Systems LLC, Richland, WA, USA) or the Lite Helicon Focus 8 v. 8.2.2 (HeliconSoft, Kharkiv, Ukraine) was used.

For light microscopy, Nomarski differential interference contrast (DIC) was used with the compound microscopes Nikon Eclipse E600 or Zeiss Axio Imager.A1/A2. Images and data were gathered using a Nikon Coolpix 4500, a Nikon DS-U2, a Zeiss Axiocam 506 color digital camera and measured by using the NIS-Elements D v. 3.0 or 3.22.15, or Zeiss ZEN Blue Edition software packages, or Zeiss AxioVision v. 4.9.1 software.

Microscopic observations were made in tap water except where noted, either from hand sections of ascomata, or from squash mounts of ascomatal contents. Measurements are reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of a number of measurements given in parentheses.

Culture preparation, DNA extraction, PCR, and sequencing

Cultures were prepared and maintained as described previously (Jaklitsch 2009) except that CMD (CMA: Sigma, St Louis, Missouri; supplemented with 2 % (w/v) D(+)-glucose-monohydrate) or 2 % malt extract agar (MEA; 2 % w/v malt extract, 2 % w/v agar-agar; Merck, Darmstadt, Germany) was used as the isolation medium.

Isolates were prepared from ascospores as described in Jaklitsch (2009) and grown on 2 % malt extract agar (MEA; 2 % w/v malt extract, 2 % w/v agar-agar; Merck, Darmstadt, Germany) or on CMD (CMA: Sigma, St Louis, Missouri; supplemented with 2 % (w/v) D(+)-glucose-monohydrate). Growth of liquid culture and extraction of genomic DNA was performed as reported previously (Voglmayr & Jaklitsch 2011, Jaklitsch et al. 2012) using the DNeasy Plant Mini Kit (QIAgen GmbH, Hilden, Germany).

The following loci were amplified and sequenced: the terminal 3' end of the small subunit nuclear ribosomal DNA (nSSU rDNA), the complete internally transcribed spacer region (ITS1-5.8S-ITS2) and a ca 900 bp fragment of the large subunit nuclear ribosomal DNA (nLSU rDNA), amplified and sequenced as a single fragment with primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990); a ca. 2.1 kb fragment of the nSSU rDNA with primers SL1 (Landvik et al. 1996) and NS24mod (Voglmayr & Jaklitsch 2011); a ca 1.2 kb fragment of the RNA polymerase II subunit 2 (*RPB2*) gene with primers dRPB2-5f and dRPB2-7r (Voglmayr et al. 2016); and a ca 1.3 kb fragment of the translation elongation factor 1-alpha (*TEF1*) gene with primers EF1-728F (Carbone & Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005). PCR products were purified using an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK), using the same primers used for PCR; additional sequencing primers used were nssu1088 (Kauff & Lutzoni 2002) for SSU and ITS4 (White et al. 1990), LR3 (Hopple & Vilgalys 1994) and LR2R-A (Voglmayr et al. 2012) for LSU. Sequencing was performed on an automated DNA sequencer (ABI 3730xl Genetic Analyzer, Applied Biosystems).

Data analysis

The newly generated sequences were aligned to a representative matrix of Pleosporales, selecting Massariaceae species as outgroups according to Voglmayr & Jaklitsch (2011). The GenBank accession numbers of sequences used in the phylogenetic analyses are given in Tab. 1.

Sequence alignments were produced with the server version of MAFFT v. 7.490 (http://mafft.cbrc.jp/alignment/server/; Katoh et al. 2019), checked and refined using BioEdit v. 7.2.6 (Hall 1999). The SSU and LSU rDNA, *RPB2* and *TEF1* matrices were combined for subsequent phylogenetic analyses. After exclusion of ambiguously aligned regions and long gaps, the final combined data matrix contained 4852 characters (1308 nucleotides of LSU, 1589 nucleotides of SSU, 1035 nucleotides of *RPB2* and 920 nucleotides of *TEF1*). Familial classification of Pleosporales follows Wijayawardene et al. (2020).

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2014) as implemented in raxmlGUI v. 2 (Silvestro & Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1 000 bootstrap replicates. The matrix was partitioned for the different gene regions. For evaluation and discussion of bootstrap support, values below 70 % were considered low, between 70 and 90 % medium/moderate, above 90 % high and 100 % maximum.

Maximum parsimony (MP) bootstrap analyses were performed with PAUP v. 4.0a169 (Swofford 2002), with 1 000 bootstrap replicates using five rounds of heuristic search replicates with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect, COLLAPSE command set to MINBRLEN, each replicate limited to 100 000

Table 1. Isolates and accession numbers used in the phylogenetic analyses. Isolates/sequences in bold were isolated/sequenced in the present study. For species authorities see www.indexfungorum.org.

Taxon	Strain/specimen[1]	GenBank accession numbers[2]			
		LSU	**SSU**	***RPB2***	***TEF1*α**
Acrocordiopsis patilii	BCC 28166	GU479772	GU479736	GU479811	N/A
Acuminatispora palmarum	MFLUCC 18-0460	MH390438	MH390402	MH399252	MH399249
Aigialus grandis	BCC 18419	GU479774	GU479738	GU479813	GU479838
Aigialus mangrovei	BCC 33563	GU479776	GU479741	GU479815	GU479840
Alternaria alternata	CBS 916.96	DQ678082	DQ678031	DQ677980	DQ677927
Amniculicola lignicola	Ying01	EF493861	EF493863	EF493862	N/A
Anteaglonium abbreviatum	ANM 925a	GQ221877	N/A	N/A	GQ221924
Anteaglonium globosum	ANM 925.2	GQ221879	N/A	N/A	GQ221925
Aquasubmersa japonica	KT2862	LC061587	LC061582	LC194421	N/A
Aquasubmersa mircensis	MFLUCC 11-0401	JX276955	JX276956	N/A	N/A
Ascocratera manglicola	BCC 09270	GU479782	GU479747	GU479821	GU479846
Astrosphaeriella fusispora	MFLUCC 10-0555	KT955462	N/A	KT955413	N/A
Astrosphaeriella neofusispora	MFLUCC 11-0161	KT955463	KT955444	KT955418	N/A
Astrosphaeriella stellata	KT998	AB524592	AB524451	N/A	N/A
Astrosphaeriellopsis bakeriana	CBS 115556	GU301801	N/A	N/A	GU349015
Bimuria novae-zelandiae	CBS 107.79	AY016356	AY016338	DQ470917	DQ471087
Byssothecium circinans	CBS 675.92	AY016357	GU205235	DQ767646	GU349061
Caryospora minima	N/A	EU196550	EU196551	N/A	N/A
Caryospora aquatica	MFLUCC 11-0008	MH057847	MH057850	N/A	N/A
Crassistoma norvegicum	**O-F-204539**	**PQ350014**	**PQ350014**	**PQ350091**	**PQ350092**
Cryptocoryneum condensatum	CBS 122629	LC194351	LC194309	LC194433	LC096139
Cryptocoryneum pseudorilstonei	CBS 113641	LC194364	LC194322	LC194446	LC096152
Delitschia chaetomioides	SMH 3253.2	GU390656	N/A	N/A	GU327753
Delitschia didyma	UME 31411	DQ384090	AF242264	N/A	N/A
Delitschia winteri	CBS 225.62	DQ678077	DQ678026	DQ677975	DQ677922
Didymella exigua	CBS 183.55	EU754155	EU754056	XM_033597068	XM_033593527
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023	KJ436586	KJ436588	N/A	N/A
Fissuroma bambusae	MFLUCC 11-0160	KT955468	KT955448	KT955417	KT955430
Hermatomyces iriomotensis	MAFF 245730	LC194367	N/A	LC194449	LC194394
Longicorpus striataspora	MFLUCC 18-0267	MK035988	MK035973	MK034436	MK034428
Lepidosphaeria nicotiae	CBS 101341	DQ678067	N/A	DQ677963	N/A
Leptosphaeria doliolum	CBS 505.75	GU301827	GU296159	N/A	GU349069
Lindgomyces cinctosporae	R56-1	AB522431	AB522430	N/A	N/A
Lindgomyces ingoldianus	ATCC 200398	AB521736	AB521719	N/A	N/A
Lophiostoma macrostomoides	CBS 123097	FJ795439	FJ795482	FJ795458	GU456277
Atrocalyx lignicola	CBS 122364	LC194342	GU296166	LC194424	LC194387
Lophiotrema nucula	CBS 627.86	GU301837	GU296167	GU371792	GU349073
Massaria anomia	CBS 591.78	GU301839	GU296169	GU371769	N/A
Massaria gigantispora	M26	HQ599397	HQ599447	N/A	HQ599337
Massaria inquinans	M19	HQ599402	HQ599444	HQ599460	HQ599342
Massarina eburnea	CBS 473.64	GU301840	GU296170	GU371732	GU349040
Melanomma pulvis-pyrius	CBS 124080	GU456323	GU456302	GU456350	GU456265
Murispora rubicunda	IFRD 2017	FJ795507	GU456308	N/A	N/A
Neoastrosphaeriella krabiensis	MFLUCC 11-0025	JN846729	JN846739	N/A	N/A

Taxon	Strain/specimen[1]	GenBank accession numbers[2]			
		LSU	**SSU**	***RPB2***	***TEF1*α**
Neotestudina rosatii	CBS 690.82	DQ384107	DQ384069	N/A	N/A
Nigrograna fuscidula	CBS 141476	KX650547	KX650509	KX650576	KX650522
Nigrograna mackinnonii	CBS 674.75	GQ387613	GQ387552	KF015703	KF407986
Phaeosphaeria oryzae	CBS 110110	GQ387591	GQ387530	KF252193	N/A
Phoma herbarum	CBS 276.37	DQ678066	DQ678014	DQ677962	DQ677909
Polyplosphaeria fusca	KT1616	AB524604	AB524463	N/A	N/A
Prosthemium betulinum	CBS 279.74	DQ678078	DQ678027	DQ677976	N/A
Prosthemium orientale	KT1669	AB553748	AB553641	N/A	N/A
Pseudoastrosphaeriella africana	MFLUCC 11-0176	KT955474	KT955454	KT955421	KT955436
Pseudoastrosphaeriella thailandensis	MFLUCC 11-0144	KT955478	KT955457	KT955416	KT955440
Pseudotetraploa curviappendiculata	HC 4930	AB524608	AB524467	N/A	N/A
Roussoella nitidula	MFLUCC 11-0182	KJ474843	N/A	KJ474859	KJ474852
Roussoellopsis macrospora	MFLUCC 12-0005	KJ474847	N/A	KJ474862	KJ474855
Salsuginea ramicola	KT2597.1	GU479800	GU479767	GU479833	GU479861
Stemphylium vesicarium	CBS 191.86	DQ247804	DQ247812	DQ247794	DQ471090
Striatiguttula nypae	MFLUCC 18-0265	MK035992	MK035977	MK034440	MK034432
Striatiguttula phoenicis	MFLUCC 18-0266	MK035995	MK035980	MK034442	MK034435
Tetraplosphaeria sasicola	KT563	AB524631	AB524490	N/A	N/A
Trematosphaeria pertusa	CBS 122368	FJ201992	FJ201991	GU371801	N/A
Triplosphaeria maxima	KT870	AB524637	AB524496	N/A	N/A
Ulospora bilgramii	CBS 101364	DQ678076	DQ678025	DQ677974	DQ677921
Verruculina enalia	BCC 18401	GU479802	N/A	GU479835	GU479863
Wicklowia aquatica	CBS 125634	GU045445	GU266232	N/A	N/A
Zopfia rhizophila	CBS 207.26	DQ384104	L76622	JGI	N/A

[1] Abbreviations: ANM: A.N. Miller, University of Illinois, Urbana-Champaign, U.S.A.; ATCC: American Type Culture Collection, Manassas, U.S.A.; BCC: BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Khlong Luang, Thailand; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; KT: K. Tanaka, Hirosaki University, Japan; MAFF: Research Center of Genetic Resources, NARO, Japan; MFLUCC: Mae FahLuang University, Chiang Rai, Thailand; SMH S.M. Huhndorf, Botany Department, Field Museum, U.S.A.; UME: Umeå University, Sweden.

[2] N/A not available; JGI sequences retrieved from JGI-DOE (http://genome.jgi.doe.gov/).

rearrangements) during each bootstrap replicate. All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to MINBRLEN.

Results

Phylogenetic analyses

Of the 4852 nucleotide characters of the combined matrix, 1246 are parsimony informative (253 of LSU, 158 of SSU, 551 of *RPB2*, and 284 of *TEF1*). The best ML tree (-lnL = 44269.441) obtained by RAxML is shown in Fig. 1.

While much of the tree backbone receives no support, the Pleosporalean families commonly receive high support (Fig. 1). Within Pleosporales sensu stricto, the Delitschiaceae are placed as most basal taxon, followed by *Zopfia rhizophila* and the new fungus, which therefore occupies an isolated phylogenetic position as sister species to the residual Pleosporales included; however, this position receives no support. Remarkably, the new fungus is placed remotely from the generic type of *Trematosphaeria*, *T. pertusa* (Trematosphaeriaceae), which is placed in a highly supported sister group position to a highly supported Didymosphaeriaceae-Massarinaceae clade.

Taxonomy

Crassistomataceae Voglmayr, Andreasen, Nordén & Jaklitsch, fam. nov. – MycoBank no.: MB 855856.

Type genus. – *Crassistoma* Voglmayr, Andreasen, Nordén & Jaklitsch

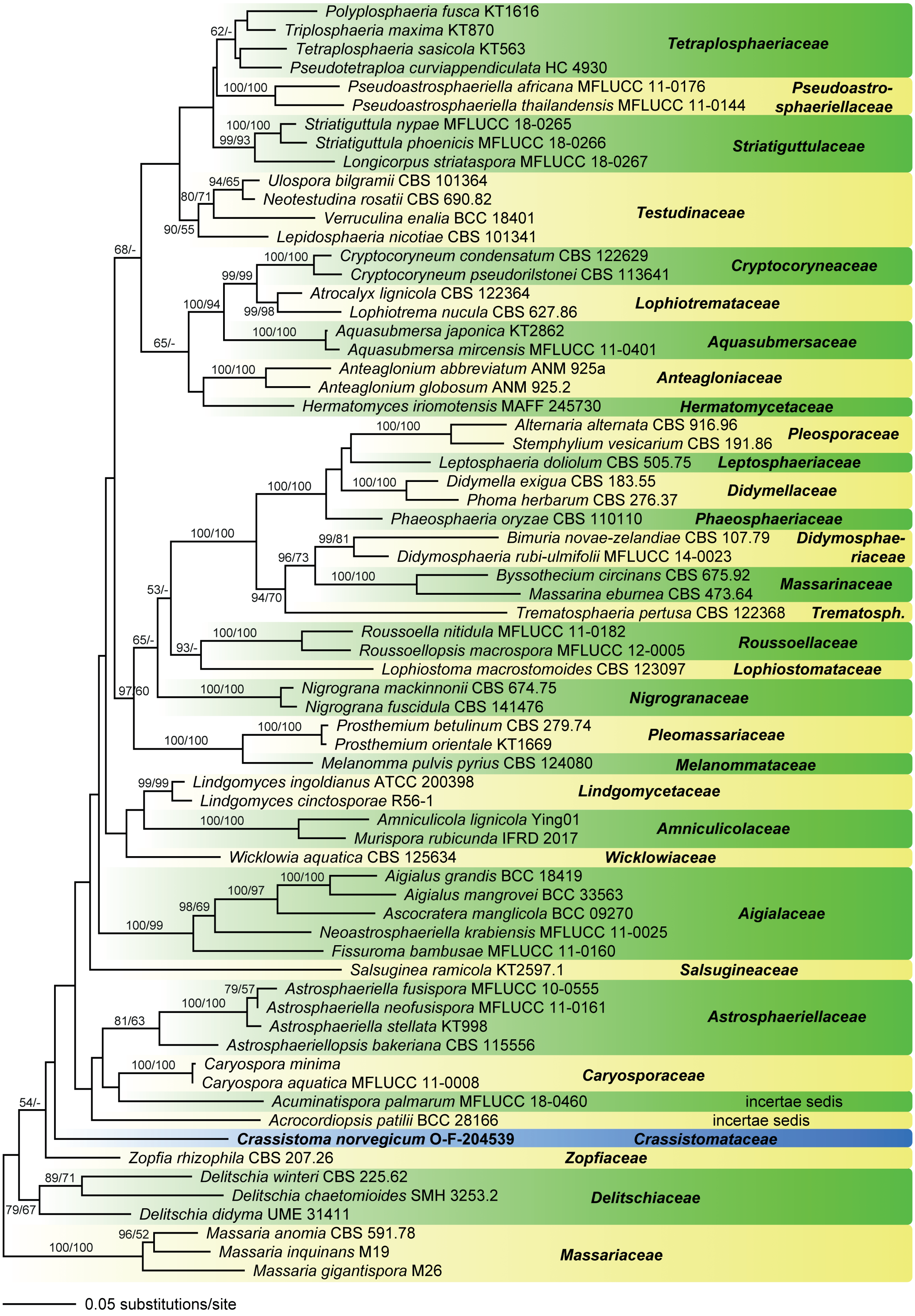


Fig. 1. Phylogram of the ML tree (-lnL = 44269.441) revealed by RAxML from an analysis of the combined SSU-LSU-*RPB2*-*TEF1* matrix of selected Pleosporales. Strain/culture numbers are given following the taxon names. ML and MP bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches.

Description. Family of Pleosporales. Ascomata superficial, sessile, globose-obpyriform with a distinct non-porate ostiolar papilla. Peridium pseudoparenchymatous, of angular, thick-walled cells. Hamathecium of narrow, septate, apically free paraphyses. Asci bitunicate, fissitunicate, with thick endotunica, a small but distinct ocular chamber and an inconspicuous apical ring. Ascospores septate, brown.

Notes: The isolated phylogenetic position at the base of Pleosporales, as well as the unique character combination (trematosphaeria-like ascospores but septate apically free paraphyses and non-porate papilla) justify the establishment of a new family for *Crassistoma* to enable proper familial placement within Pleosporales.

Crassistoma Voglmayr, Andreasen, Nordén & Jaklitsch, gen. nov. – MycoBank no.: MB 855857.

Etymology. Referring to its wide ostiolum.

Type species. — *Crassistoma norvegicum* Voglmayr, Andreasen, Nordén & Jaklitsch

Description. Ascomata, black, superficial, sessile, globose-obpyriform with a distinct, wide ostiolar papilla forming a ridge- to collar-like structure. Peridium pseudoparenchymatous, of angular, thick-walled cells. Hamathecium of narrow, septate, apically free paraphyses. Asci bitunicate, fissitunicate, with thick endotunica, a small but distinct ocular chamber and an inconspicuous apical ring. Ascospores uniseptate, constricted at septum, dark brown.

Notes: Although superficially similar to the genus *Trematosphaeria*, *Crassistoma* differs particularly in a hamathecium of apically free paraphyses and non-porate broad papilla with bumpy-warty ridge- to collar-like structures, while *Trematosphaeria* has a hamathecium of trabeculate pseudoparaphyses and porate narrow papillae.

Crassistoma norvegicum Voglmayr, Andreasen, Nordén & Jaklitsch, **sp. nov.** – Fig. 2. MycoBank no.: MB 855858.

Etymology. – Referring to its occurrence in Norway.

Holotypus. – NORWAY. Møre og Romsdal county, Aure municipality, Todalen, 63.2178838N, 8.7451594E, 96 m, on coarse bark of old living *Populus tremula* L., 11 Oct. 2018, *leg.* O. Olsen, Å. Hasvik, SH. Lien Langmo, Ø. Folden, S. Sjøblom, *det.* H. Voglmayr, WM. Jaklitsch, B. Nordén, M. Andreasen (O-F-204539, ex holotype culture N3 (culture lost)).

Description. – Ascomata (670)780–1290(1600) µm diam (n = 40), (900)660–1270(1400) µm high (n = 20), superficial, sessile, single or aggregated in small groups, globose-obpyriform with a distinct short ostiolar papilla, sometimes laterally fused bearing 2 ostiolar papillae, black, surface irregularly uneven/rough, with a basal thin tomentum. – Ostiolar papilla (300)430–605(660) µm diam (n = 50), non-porate forming a regular or bumpy-warty ridge- to collar-like structure. – Peridium pseudoparenchymatous, somewhat brittle, (70)85–120(125) µm thick (n = 25), consisting of a brown textura angularis of angular, thick-walled cells 3–5 × 3–6(8) µm (n = 20) wide. – Hamathecium forming a dense matrix of narrow septate apically free paraphyses 1.5 µm wide. – Asci 209–255(330) × (14.7)20.8–24.3(24.6) µm (n = 30), oblong to subclavate, bitunicate, fissitunicate, with thick endotunica, a small but distinct ocular chamber and an inconspicuous apical ring not stained in lactophenol cotton blue, Melzer's reagent or Congo Red, a short stipe and containing 8 uniseriate ascospores. – Ascospores (31.5)36–42(49) × (11)12.5–15.5(17.5) µm, l/w = (2.0)2.5–3.2(3.8) (n = 48), fusoid to biconical, equilateral to slightly inequilateral, less commonly broadly ellipsoid, bicellular with upper cell usually slightly wider, strongly constricted at the median to slightly eccentric septum, dark brown, with broadly rounded ends, each ascospore cell containing a large and 0–2 small guttules, ascospore wall finely verruculose. – Asexual morph not seen.

Cultures. – On CMD very slow growing, reaching 3–5 mm diam after 1 mo, black, reverse black, surface mycelium sparse. No sporulation observed.

Habitat. – On coarse bark of old living *Populus tremula*.

Distribution. – Only known from Norway.

Additional material examined. – NORWAY. Vestland county, Øystese municipality, Ørredalsfossen, 60.3999849N, 6.1745715E, 205 m, on coarse bark of old living *Populus tremula* L., 13 May 2019, *leg.* M. Andreasen, *det.* B. Nordén, M. Andreasen (O-F-204540); Møre og Romsdal county, Molde municipality, Eikesdalen, 62.4946695N, 8.1723642E, 105 m, on coarse bark of old living *Populus tremula* L., 8 Sep. 2020, *leg.* JB. Jordal, *det.* JB. Jordal, B. Nordén, M. Andreasen (O-F-204541).

Notes: Although superficially similar to *Trematosphaeria cariosa*, at least in ascospore characters, *Crassistoma norvegicum* differs from the former by ecology (corticolous vs. lignicolous), distribution (Europe vs. North America), ascomatal shape (obpyriform-globose with a distinct non-porate ostiolar papilla and a thin tomentum at the ascomatal

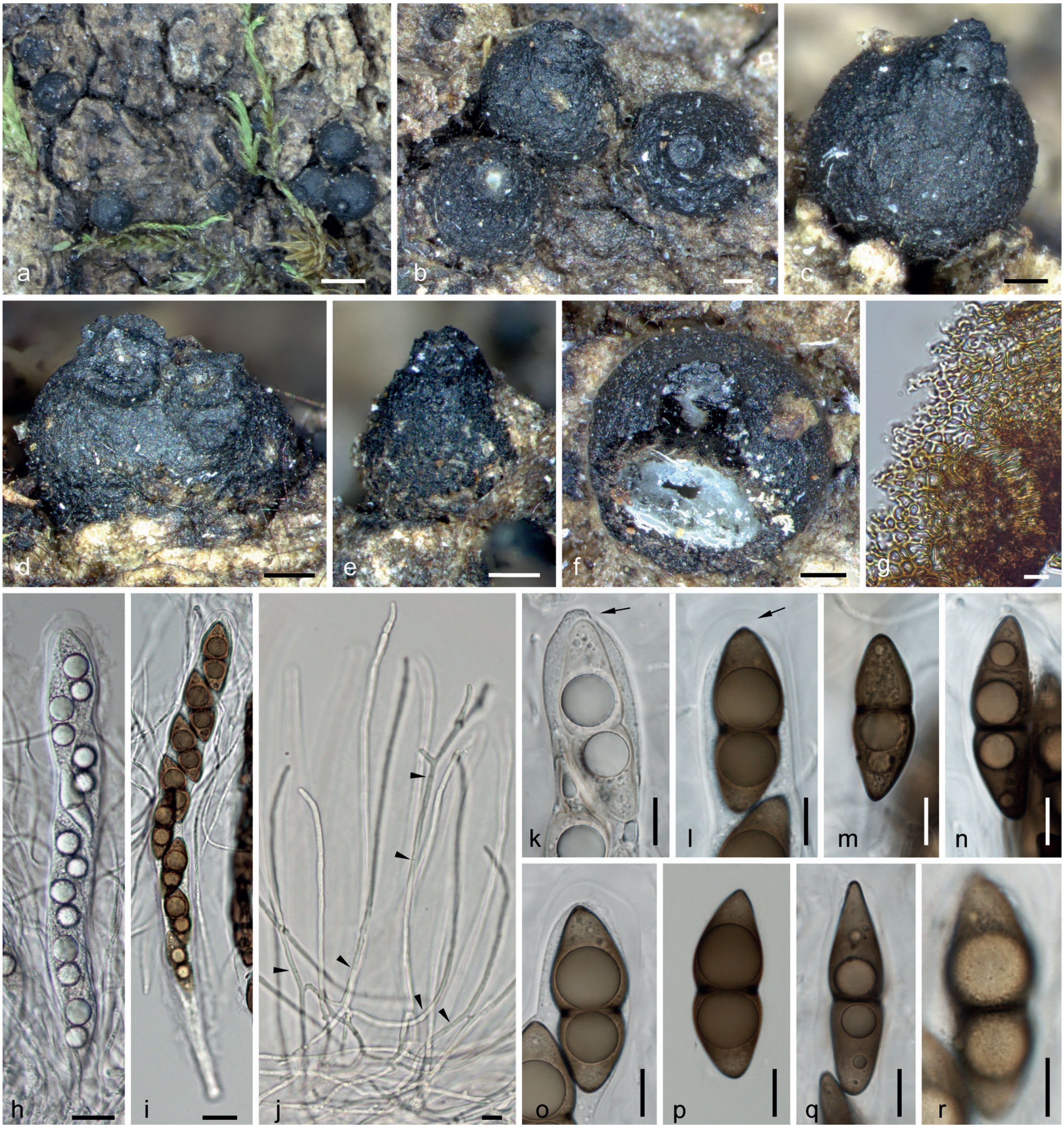


Fig. 2. *Crassistoma norvegicum* (holotype) O-F-204539. **a–e.** Ascomata in surface (a, b) and side (c-e) view; **f.** Ascoma in vertical section; **g.** Peridium in transverse section; **h.** Immature ascus; **i.** Mature ascus; **j.** Filiform septate pseudoparaphyses (septa denoted by arrowheads); **k, l.** Ascus tips with immature (k) and mature (l) ascospores, arrows denoting ocular chamber of the ascus tips; **m–q.** Ascospores; **r.** Verruculose ascospore wall. All microscope mounts in 3% KOH. — Scale bars: a = 1 mm; b–f = 200 µm, g, j–r = 10 µm; h, i = 20 µm.

base vs. conical with indistinct ostiolar papilla and without tomentum), a different hamathecium (apically free paraphyses vs. trabeculate pseudoparaphyses) and smaller ascospores having a much narrower and lighter brown equatorial zone at the septum.

Trematosphaeria cariosa (Fairm.) Boise, Mycologia 77(2): 235 (1985). Fig. 3.

Basionym. – *Caryospora cariosa* Fairm., Proc. Rochester Acad. Sci. 4: 190 (1905).

Description (modified from Boise 1985). – Ascomata ca. 0.5–0.8 mm diam, erumpent to superficial, single or aggregated in small groups, broadly and firmly attached to the wood, conical, without a distinctly separated ostiolar papilla, black, surface dull, smooth, without tomentum. – Ostiolar papilla (65–)75–130(–180) µm diam (n = 30). – Peridium strongly carbonaceous, brittle. – Hamathecium forming a dense matrix of narrow septate trabeculate pseudoparaphyses, 1–2.5 µm wide. – Asci ca. 180–200 × 21–22.5 µm, oblong cylindrical to subclavate, bitunicate, fissitunicate, containing 2–8 ascospores. – Ascospores (39–)41–47.5(–50) × (14–)16–18.3(–19.5) µm, l/w = (2.1–)2.3–2.9(–3.2) (n=30), fusoid to biconical, slightly to distinctly inequilateral, bicellular with one cell usually slightly wider, slightly to strongly constricted at the median to slightly eccentric primary septum, dark brown with 4–5 µm wide distinctly darker equatorial zone at the septum, ascospore wall thick, smooth.

Habitat. – On wood of *Fagus*.

Distribution. – Only known from North America.

Material examined. – USA, New York, Lyndonville, on *Fagus* logs, Oct. 1904, C. E. Fairman (holotype CUP-F-00491(7-13)!).

Notes. – To save the scarce type collection, only minimal material was taken for light microscopy. As the ascomata were very brittle, no ascomatal sections were prepared; therefore, also no peridium characters (thickness, anatomy) could be given. No intact mature asci were present in the microscope mounts; therefore, the measurements given above refer to two immature asci without spores. Ascus size was recorded as 150 × 20 µm in the protologue (Fairman 1905), while Boise (1985) reported ascus size as 110 × 36 µm. Fairman (1905) and Boise (1985) reported up to three septa per ascospore; in our mounts, only uniseptate ascospores were seen.

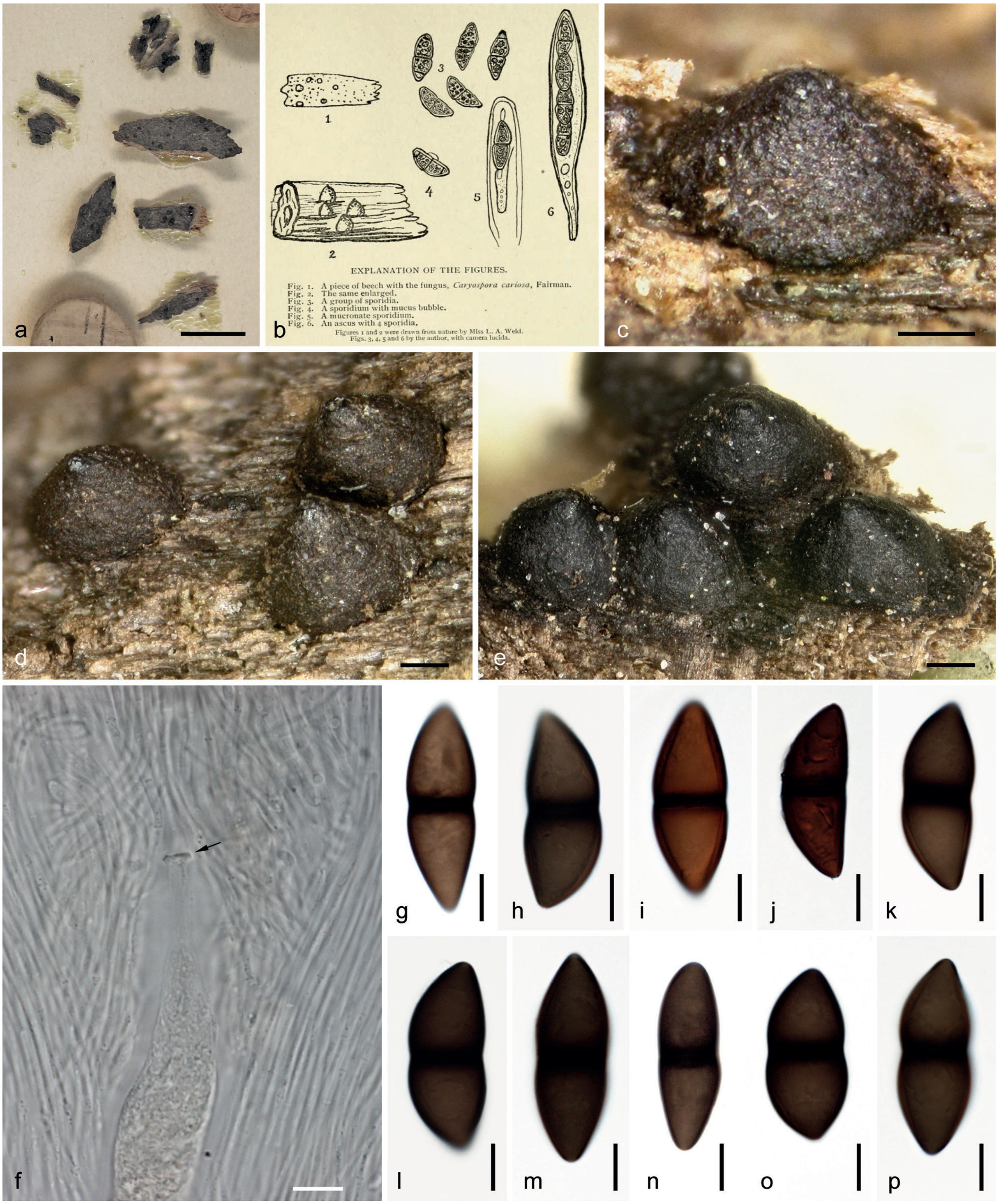
It is very likely that *Trematosphaeria cariosa* is not conspecific with the generic type, *T. pertusa*, but in lack of sequence data phylogenetic affinities currently cannot be assessed.

Discussion

Trematosphaeria cariosa, the fungus being morphologically closest to our new species, was redescribed and discussed in relation to *Crassistoma norvegicum* above. No DNA data are available for *T. cariosa* but it is likely that it does not belong to *Trematosphaeria* phylogenetically. Like with many genera of Pleosporales with a long history dating back to the 19th century, numerous unrelated species have over the times been placed in the genus; more than 200 names have been described in *Trematosphaeria* (Index Fungorum, accessed 12 Aug. 2024). Following Boise (1985), in the late pre-molecular era the concept of *Trematosphaeria* was restricted to few species with medium to large sized ascomata with peridia composed of small compressed cells, clavate to cylindrical bitunicate asci, a trabeculate hamathecium in a gelatinous matrix and reddish-brown ellipsoid to fusoid transversely septate ascospores sometimes with paler end cells (Tanaka et al. 2005). Within this suite of characters, the trabeculate hamathecium was considered to represent a main character distinguishing it from morphologically similar genera in Pleosporales (Tanaka et al. 2005). Based on this morphological genus concept, Boise (1985) accepted five and Tanaka et al. (2005) 15 species in *Trematosphaeria*. However, all species previously classified in *Trematosphaeria* for which sequence data have become available have meanwhile been shown to be not closely related to the generic type species, *T. pertusa* (Wang et al. 2007, Zhang et al. 2012, Zhou et al. 2014). Currently, apart from *T. pertusa*, only *T. grisea*, originally described as a madurella-like asexual morph for which no sexual morph is known, is confirmed in *Trematosphaeria* by DNA data (Ahmed et al. 2014). *Crassistoma* particularly deviates from the morphological concept of *Trematosphaeria* by its free apical paraphyses and non-porate ostiolar papilla. *Crassistoma norvegicum* currently nests among the basal taxa of the Pleosporales, but more taxa are needed in this part of the tree to consolidate a stable position. However, the unique combination of characters (trematosphaeria-like ascospores with septate apically free paraphyses and non-porate papilla) justifies the establishment of a new family Crassistomataceae.

The presence of an ocular chamber and an apical ring represent plesiomorphic characters which are otherwise rare within the Pleosporales (Zhang et al. 2009). On the other hand, Zhang et al. (2009) argue that Pleosporales are most likely derived from a saprotrophic ancestor with later shifts to hemibiotrophic or biotrophic nutritional modes, which indicates a more derived state for the ecological role than for the ascus morphology of our species.

The species belongs to a little-known guild of fungi found on bark of old trees. These fungi have



EXPLANATION OF THE FIGURES.

Fig. 1. A piece of beech with the fungus, *Caryospora cariosa*, Fairman.
Fig. 2. The same enlarged.
Fig. 3. A group of sporidia.
Fig. 4. A sporidium with mucus bubble.
Fig. 5. A mucronate sporidium.
Fig. 6. An ascus with 4 sporidia.

Figures 1 and 2 were drawn from nature by Miss L. A. Weld.
Figs. 3, 4, 5 and 6 by the author, with camera lucida.

Fig. 3. *Trematosphaeria cariosa* (holotype) CUP-F-00491(7-13). **a.** Mounted specimen; **b.** Line drawings of the holotype published in Fairman (1905); **c–e.** Ascomata in inclined side view; **f.** Trabeculate hamathecium of filiform pseudoparaphyses and part of immature bitunicate ascus with apical ring (arrow); **g–p**. Ascospores with thick wall and broad darker brown equatorial zone at the septa. All microscope mounts in 3% KOH. — Scale bars: a = 10 mm; c–e = 200 µm; f–p = 10 µm.

unknown nutritional modes and may be commensals since they do not appear to cause harm to the living tree (Bowd et al. in press).

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