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Species of *Peziza s. str.* on water-soaked wood with special reference to a new species, *P. nordica*, from central Norway

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Peziza oliviae, P. lohjaoensis, P. montirivicola and a new species from Norway form a well-supported clade within the *Peziza s. str.* group based on study of the internal transcribed spacer + 5.8S rRNA gene, large subunit rRNA gene and the 6–7 region of the DNA-dependent RNA polymerase II gene. Like *P. oliviae* and *P. montirivicola,* the new species, *P. nordica*, is distinctly stipitate and occurs on wood that has been inundated by fresh water. These species also have paraphyses with yellow vacuolar inclusions. They fruit early in the season or at high elevations and are presumed to be saprobic. A discussion of application of the name *Peziza* is given.

Keywords: Ascomycota, molecular phylogeny, Pezizales, taxonomy.

The present work was begun to determine the identity of a collection made by one of us (RK) in August 2014. This large, orange brown to brown, stipitate discomycete grew on water-soaked logs and debris of dead birch and willow at the southern shore of Strandavatn (lake), in the Hallingskarvet National Park in central Norway, ca 1000 m asl. In August of 2015 this fungus was found again in the same location. The water level in this lake is regulated and the normal waterlevel is between 950 and 1000 meters, depending on the inflow of the annual snow melt from the mountains surroundings the lake. In the upper level of the inundated zone there is significant accumulation of dead, water-soaked sticks and branches of willow (*Salix* spp.) and birch (*Betula pubescens* subsp. *tortuosa)*.

The species was found in a small stream that was deeply covered by snow during the long winter of at least 4–5 months (Fig. 1). During the spring logs and branches are submerged in continuous running water and the spot where the fungus was found seems to be continuously moist during the summer. These conditions supported other lignicolous aquatic discomycetes: *Adelphella babingtonii* (Sacc.) Pfister, Matočec & I. Kušan, *Vibrissea truncorum* (Alb. & Schwein.) Fr., *Cudoniella clavus* (Alb. & Schwein.) Dennis and frequently *Scutellinia scutellata* (L.) Lambotte. The habitat and vernal fruiting suggest similarities to snow bank fungi, such as *Peziza nivalis* (R. Heim & L. Rémy) M.M. Moser.

Our morphological and molecular phylogenetic study of this material led us to examine several species within the large and heterogenous genus *Peziza* Fr. These studies reveal it to be an undescribed species allied to *P. oliviae* J.L. Frank, *P. lohjaoensis* Harmaja and the recently described *P. montirivicola* Perić.

These species have a boreal distribution and seem to fruit early in the season or as the temperatures moderate at high elevations or latitudes. *Peziza oliviae* was found in Oregon, USA, fruiting underwater (Frank 2013). *Peziza lohjaoensis* was described from southern Finland in the spring on plant litter, mull and sometimes on decaying deciduous wood (Harmaja 1986). *Peziza montirivicola* was collected in July and September on wood along streams in mountainous areas at 1662 and 1388 m in Montenegro, Switzerland and Bosnia-Herzegovina (Perić & Grebenc 2015). In our study these species group together with high support in the *Peziza s. str.* group.

Materials and methods

Sampling

Specimens # G07/14, and # G01/15 were used in this study. They were collected by one of the authors (RK) in Norway and sent to D. Pfister for examination. Samples of *P. oliviae* from Oregon, USA, and *P. lohjaoensis* from Finland, were included in this study for morphological and phylogenetic comparison to the *Peziza* specimens from Norway. These collections are summarized in Tab. 1.

Morphological studies

Material of specimens G07/14 and G01/15 were studied in fresh condition by RK who did preliminary measurements and observations. Dried material of all species was studied as follows: material was rehydrated in water and sectioned by hand using a razor blade. Measurements were made in water mounts using an Olympus BX50 microscope equipped with a XC50 digital camera using the MicroSuite Five software system for measurements and for digitization. Iodine reaction was checked with Melzer's reagent (MLZ) and spore ornamentation was observed using Cotton Blue in Lactic acid.

Molecular techniques

The Qiagen DNeasy Plant Mini Kit (Qiagen, Germany; cat. no. 69104) was used to extract genomic DNA from herbarium specimens of the stipitate *Peziza* species examined in this study (Tab. 1). A 1/10 and 1/100 dilution of the DNA was used for PCR amplification of the ITS rDNA region (internal transcribed spacer + 5.8S rRNA gene), LSU rDNA region (large subunit rRNA gene) and the 6–7 region of RPB2 (DNA-dependent RNA polymerase II gene) (Hansen et al. 2005). The ITS was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). The LSU rDNA region was amplified using primers LROR and LR5 (Moncalvo et al. 2000). The 6–7 region of the RPB2 gene was amplified using the primers RPB2-P6Fa and RPB2-P7Ra as designed by Hansen et al. (2005). PCR parameters were as previously described (RPB2 conditions as in Hansen et al. 2005, and ITS and LSU PCR conditions as in LoBuglio et al. 1993). All PCR reactions were done in a Peltier Thermal Cycler PTC–200 (MJ Research, Watertown, MA). The ITS PCR reactions used EconoTaq DNA Polymerase (Lucigen, Middleton, WI) whereas the RPB2 6–7 region was amplified using Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen, Life technologies, Carlsbad, California, USA). PCR purification and sequencing techniques were as described in Hansen et al. (2005). Sequencher 4.6 (GeneCodes, Ann Arbor, Michigan) was used to edit the DNA sequences obtained. The ITS, LSU and RPB2 DNA sequences determined in this study were deposited in GenBank and are listed in Tabs. 1 and 2.

Tab. 1. *Peziza* specimens and GenBank sequences used in phylogenetic analyses.

Species [a]	Collection Number (Herbarium) [b]	Collector	ITS [c]	LSU	RPB2
P. nordica, **H**	G01/15 (FH)	Kristiansen	KU898045*	KU898046*	KU898054*
P. nordica	G07/14 (FH)	Kristiansen	KU898044*	KU898047*	KU898055*
P. lohjaoensis	20.V.1982 (H)	Harmaja & Simojoki	KU898041*	KU898052*	KU898060*
P. lohjaoensis	4.VI.2003 (H)	Hoijer	KU898040*	KU898053*	KU898061*
P. lohjaoensis, **H**	30.V.1982 (H)	Harmaja	AF491576	NA	NA
P. oliviae, **H**	JLF2091 (OSU)	Frank	JX415340	KU898048*	KU898056*
P. oliviae	JLF2538 (OSU)	Frank	KU898043*	KU898050*	KU898058*
P. oliviae	JLF2140 (OSU)	Frank	KU898042*	KU898051*	KU898059*
P. oliviae	JLF2088 (OSU)	Frank	JX415339	KU898049*	KU898057*
P. oliviae	JLF2479 (OSU)	Frank	KC916729	NA	NA
P. oliviae	JLF2474 (OSU)	Frank	KC916728	NA	NA
P. montirivicola, **H**	2011-MNE (M)	Peric	LN881741	NA	NA
P. montirivicola	2010-MNE (M)	Peric	LN881740	NA	NA
P. montirivicola	2014-CH (M)	Peric	LN881742	NA	NA

[a] **H =** Holotype of the species

[b] Herbarium designations: FH = Farlow Herbarium; H = Helsinkii Herbarium; OSU = Oregon State University; and M = Botanische Staatssammlung München

[c] Sequences obtained in this study are designated with an asterisk (*); NA = not available

DNA sequence alignment

Alignment of the DNA sequences was done using MAFFT through http://mafft.cbrc.jp/alignment/server/index.html, and then manually adjusted with Se-Al v 2.0a11 (Rambaut 2002). Two data sets were constructed: a combined data set of LSU rDNA and RPB2 DNA sequences (which included representative taxa from the Pezizaceae, see Tabs. 1 and 2) and a second data set which included ITS rDNA sequences of only the *Peziza* species under study (Tab. 1). The LSU and RPB2 DNA sequences determined in this study were aligned with the data matrix constructed by Hansen et al. (2005) (TreeBase legacy study ID M1330). Sequences from two isolates of each of the three species, *P. oliviae*, *P. lohjaoensis* and the specimens from Norway, were included in the LSU and RPB2 analyses to determine where in the Pezizaceae these species fall. The outgroup taxa were *Ascobolus crenulatus* P. Karst., *A. carbonarius* Karst. and *A. denudatus* Fr.

The ITS data set comprised sequences of *P. oliviae* (five isolates), *P. lohjaoensis* (three isolates), the new species (two isolates) and three sequences of the recently described *P. montirivicola* (Tab.1). The outgroup taxa were *P. ammophila* and *P. vesiculosa*. Phylogenetic analyses of the data sets used Maximum Parsimony (MP) run on PAUP 4.0b10 (MP; Swofford 2002); Maximum Likelihood (ML) run on RAxML–HPC2 through the Cipres Science Gateway (ML; Miller et al. 2010); and Bayesian posterior probabilities (PP) as determined using MrBayes v3.0b4 (Huelsenbeck & Ronquist 2001) and as previously described (LoBuglio & Pfister 2010). Branch support for MP and ML analyses was determined by 1000 bootstrap replicates.

Species delimitation analyses

The ITS region has been used as the barcoding marker for fungi (Schoch et al. 2012), and we used this locus for species delimitation analyses. We calculated the intra- and interspecific genetic distance of these *Peziza* species from ITS rDNA sequence alignments using PAUP 4.0b10 (MP; Swofford 2002) to determine the genetic diversity in this group of fungi and help define species boundaries.

In addition to recognizing putative species based on monophyletic, well-supported clades in ITS phylogenies, two other methods were used to delimit species boundaries: the Automatic Barcode Gap Discovery Method (ABGD, Puillandre et al. 2012); and a Maximum Likelihood Poisson Tree Processes model (PTP, Zhang et al. 2013). The default parameters for the ABGD online method (http://wwwabi.snv.jussieu.fr/public/abgd/) were used (Leavitt et al. 2015, Puillandre et al. 2012): the JC69 model, Pmin = 0.001, Pmax = 0.01, steps = 10, and Nb bins = 20. We tested a range of values for the gap width (X), between 0.1 and 1.5. The PTP method was carried out through the online site http://species.h-its.org/ptp/ by submitting the Maximum-Likelihood tree obtained with RAxML–HPC2 using the GTR-GAMMA model on XSEDE through the Cipres Science Gateway (ML; Miller et al. 2010). The default parameters from the online site were used for this analysis: 100000 MCM generations, 100 Thinning, and 0.1 burn-in.

Results

Molecular phylogenetic and species delimitation analyses

The combined LSU rDNA and RPB2 DNA sequence data included 72 taxa from the Pezizaceae. This data set consisted of 1390 total characters, of which 550 were parsimony informative. Bayesian, MP and ML analyses placed the three stipitate *Peziza* species from USA, Finland and Norway together in a highly supported clade (Fig. 2) sister to *P. vesiculosa*, and within *Peziza s. str.* No sequences were available for *P. montirivicola*. Furthermore, each of the three stipitate species is on a well-supported, monophyletic clade in the LSU rDNA and RPB2 DNA phylogenetic tree (Fig. 2).

The ITS rDNA sequence data set consisted of 814 characters from 16 samples of which 44 characters were parsimony informative. Phylogenetic analyses of the ITS data set, which included *P. montirivicola*, identified four well-supported clades for these species: *Peziza lohjaoensis, P. oliviae, P. montirivicola* and the new species (Fig. 3). The greatest intra-specific ITS divergence was observed among the five isolates of *P. oliviae,* 0–0.8 % (with *P. oliviae*, JLF2474, having the greatest genetic distance compared to the other isolates of this species). The intra-specific ITS divergences for the other species were as follows: *Peziza lohjaoensis* 0–0.4 %*, P. montirivicola* 0 %, and the new species 0 %.

Peziza montirivicola and the new species are the most closely related species (Fig. 3) with only 0.8 % genetic divergence. *Peziza lohjaoensis* had the greatest ITS sequence inter-specific divergence which ranged up to 2.0 %.

The ABGD and PTP Maximum Likelihood method, used to further delimit species boundaries, recognized five taxonomic groups: 1) *Peziza lohjaoensis;* 2) *P. oliviae;* 3) *P. oliviae* JLF2474; 4) *P. montirivicola;* and 5) *P. nordica* based on the ITS

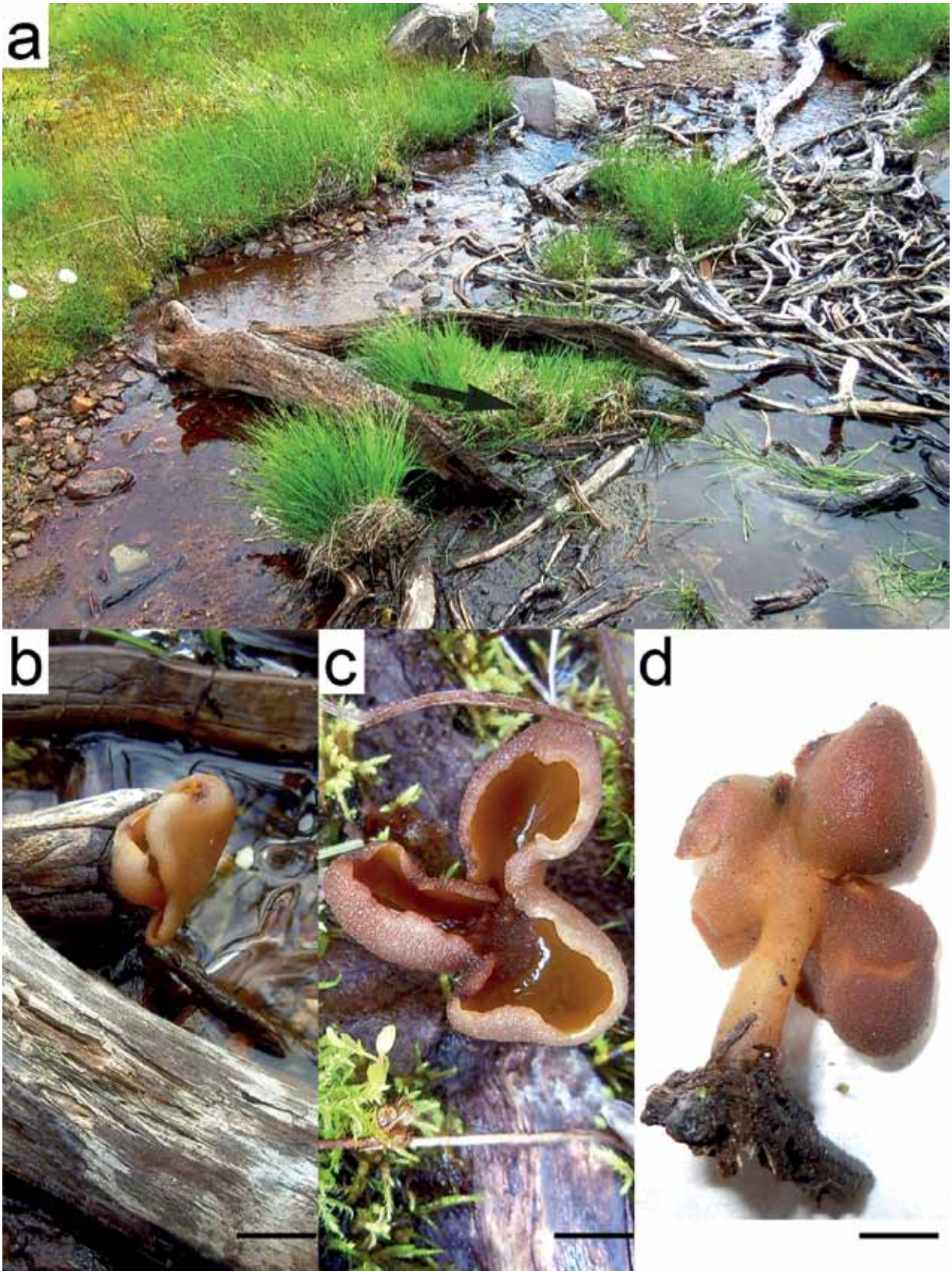

Fig. 1. *Peziza nordica*. Habitat and apothecia. Scales in b–d 1.25 cm. Photographs by Roy Kristiansen.

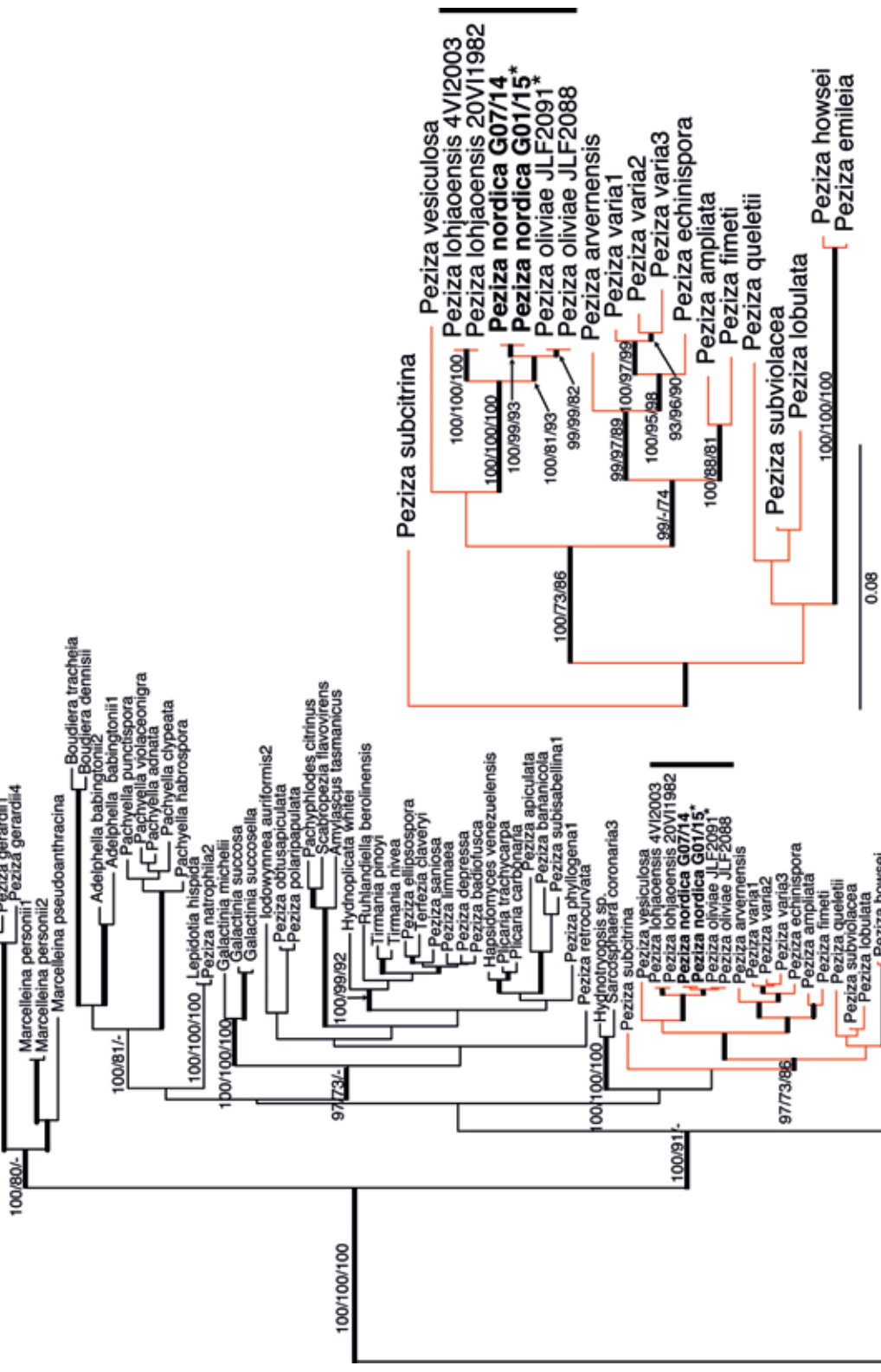

Fig. 2. Maximum Likelihood tree based on LSU rDNA and RPB2 sequence data. Branches in bold with numbers above represent clades that have Bayesian posterior probability (PP) values ≥95 %, and Bootstrap values ≥70 % for Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses. The numbers listed above the branches are PP/ML/MP support values (dashes indicate low support values). The outgroup taxa are *Ascobolus carbonarius*, *A. denudatus* and *A. crenulatus*. The vertical bar indicates the clade containing *P. nordica*. This clade is shown in the expanded tree at the right. An asterisk (*) indicates that the sequence was derived from the holotype specimen.

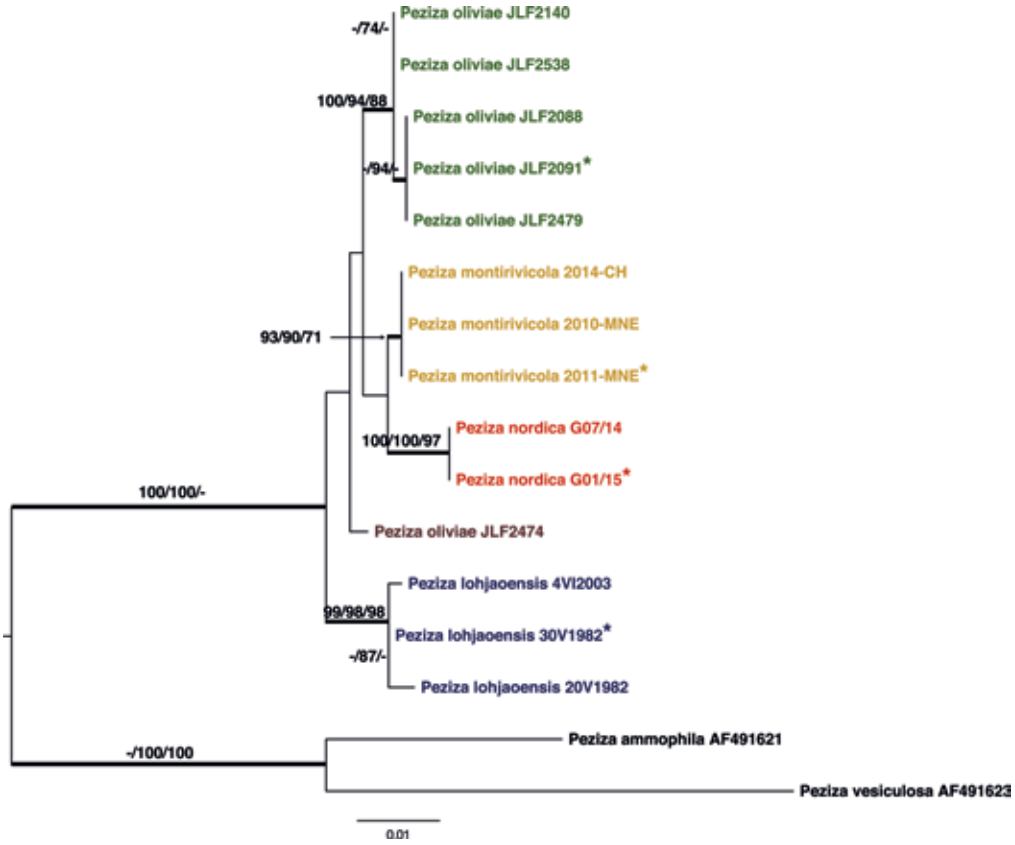

Fig. 3. Maximum Likelihood tree based on ITS rDNA sequence data. Branches in bold with numbers above represent clades that have Bayesian posterior probability (PP) values ≥95 % and Bootstrap values ≥70 % for Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses. The number listed above the branches are PP/ML/MP support values (dashes indicate low support values). The outgroup taxa are *Peziza ammophila* and *P. vesiculosa*. An asterisk (*) indicates that the sequence was derived from the holotype specimen.

rDNA sequence analyses (Fig. 3). The two methods further support the consideration of *P. nordica* as a distinct species.

Taxonomy

Peziza nordica Kristiansen, LoBuglio & Pfister, **sp. nov.** – Figs. 1, 4–6.
MycoBank no.: MB 816483

Diagnosis. – This species is similar to *P. montirivicola* but is larger, up to 80 mm broad, and has a longer stipe. The paraphyses are wider, up to 10 µm, and the asci are smaller up to 350 µm versus 457 µm in *P. montirivicola*.

Holotypus. – NORWAY, Buskerud County. Hol Community, South end of Strandavatn, Gurostølen, Hallingskarvet National Park, 1000 asl, 60° 40' 14.08" N 7° 56' 26.37" E , on wet dead water-soaked wood of birch and scrubs, 1 August 2015, leg. Roy Kristiansen (# G01/15) (FH 00304781). Isotypus (O).

Description. – Apothecia 3.5 to 8 cm diam., hymenium pale brown to brownish orange, outside smooth or slightly granular, pruinose, hygrophaneous, concolorous or yellowish, with a distinct stipe 2–4 × 0.5–1 cm, lighter than the outer surface, whitish or yellowish (Fig. 1). Flesh fragile and somewhat flexible, brown in dried material. The margin is somewhat lobed or lacerate. Asci operculate (Fig. 4 a–e), 8-spored or fewer due to abortive development (Fig. 5), 300–350 × 15–18 µm, with a well-developed amyloid ring at the tip (Fig. 4 a–e), attenuated at the base, with croziers, wall brownish or smoky, particularly noticeable in the walls of the discharged asci. Ascospores uniseriate, narrowly ellipsoid, (21–22)22.4–24.5(25–26) × (10.5) 1.4–12(13) µm, hyaline, without oil guttules but with granular contents, particularly in immature spores (Fig. 4 g), de Bary bubbles sometimes present in MLZ, first smooth and thick-walled, then developing transverse wrinkles or loosening in all mounting media, not staining in CB, thin-walled when mature. Paraphyses straight to slightly curved apically, 5–7 µm to 9–10 µm apically with

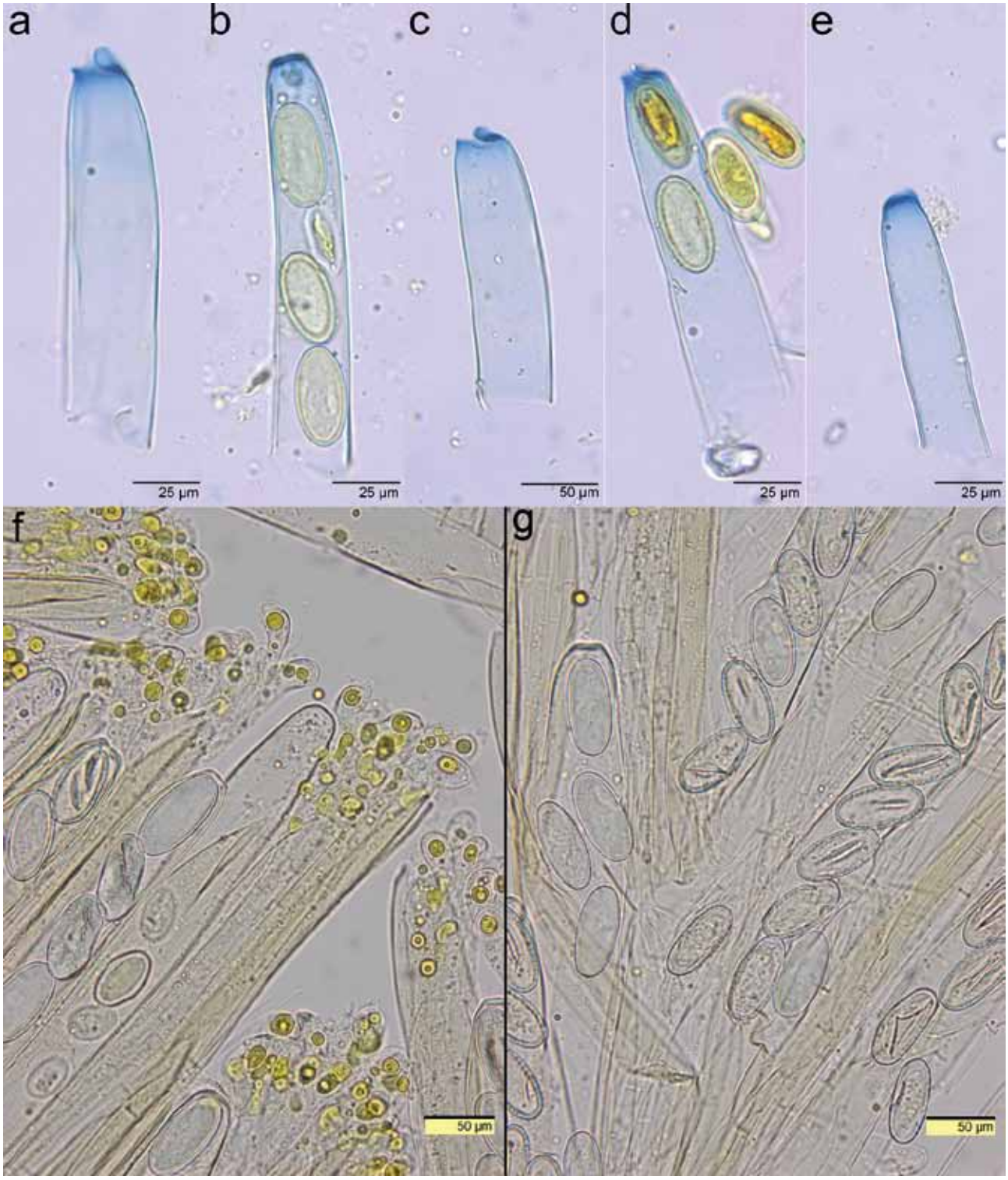

Fig. 4. *Peziza nordica*. **a–e.** Asci showing the bluing in MLZ, opercula and ascospores.
f. A portion of hymenium with ascopores and paraphyses showing yellow vacuolar material, in water. **g.** Ascospores and asci.

Tab. 2. Species and GenBank numbers of LSU and RPB2 DNA sequences included in phylogenetic analyses (Fig. 1). See Hansen et al (2005) for specimen details.

Species	LSU	RPB2
Adelphella babingtonii 1	AF335122	AY500522
Adelphella babingtonii 2	AF335123	AY500467
Amylascus tasmanicus	AF335113	AY500465
Ascobolus carbonarius	AY500526	AY500459
Ascobolus crenulatus	AY500527	AY500462
Ascobolus denudatus	AY500528	AY500460
Boudiera dennisii	AY500529	AY500508
Boudiera tracheia	AY500530	AY500507
Galactinia michelii	AY500549	AY500494
Galactinia succosa	AF335166	AY500487
Galactinia succosella	AF335167	AY500517
Hapsidomyces venezuelensis	AY500533	NA
Hydnoplicata whitei	AF335168	AY500491
Hydnotryopsis sp.	AF335116	AY500472
Iodowynnea auriformis 2	AF335118	AY500473
Iodophanus carneus	AY500534	AY500506
Iodophanus hyperboreus	AY500535	AY500458
Lepidotia hispida	U42693	AF107809
Marcelleina persoonii 1	AY500536	AY500463
Marcelleina persoonii 2	AY500537	AY500464
Marcelleina pseudoanthracina	AY500538	AY500509
Pachyella adnata	AY500541	AY500469
Pachyella clypeata	AY500542	NA
Pachyella habrospora	AY500543	NA
Pachyella punctispora	AF335145	AY500468
Pachyella violaceonigra	AF335125	AY500470
Pachyphlodes citrinus	AY500544	AY500466
Peziza ampliata	AF335128	AY500510
Peziza apiculata	AF335129	NA
Peziza arvernensis	AF335131	AY500497
Peziza badiofusca	AF335132	AY500475
Peziza bananicola	AF335133	AY500483
Peziza depressa	AF335135	AY500474
Peziza echinispora	AF335138	AY500496
Peziza ellipsospora	AF335139	AY500482
Peziza emileia	KU898062*	KJ72870
Peziza fimeti	KU898063*	KU898064*
Peziza gerardii 1	AF335142	AY500511
Peziza gerardii 2	AF335144	AY500512
Peziza gerardii 3	AY500546	AY500513
Peziza gerardii 4	AY500547	AY500471
Peziza howsei	AF335146	AY500493
Peziza limnaea	AF335147	AY500518
Peziza lobulata	AY500548	AY500495
Peziza natrophila 2	AF335153	AY500486
Peziza obtusapiculata	AY500550	AY500490
Peziza phyllogena 1	AF335155	AY500480
Peziza polaripapulata	AY500551	AY500515
Peziza queletii	AF335127	AY500492
Peziza retrocurvata	AF335159	AY500516
Peziza saniosa	NA	AY500476
Peziza subcitrina	AF335162	AY500520
Peziza subisabellina 1	AF335163	AY500484
Peziza subviolacea	AF335165	AY863000
Peziza varia 1	AF335134	AY500519
Peziza varia 2	AF335151	AY500499
Peziza varia 3	AF335150	AY500498
Peziza vesiculosa	AY500552	AY500489
Plicaria carbonaria	AY500553	AY500479
Plicaria trachycarpa	AY500554	AY500478
Ruhlandiella berolinensis	AF335175	AY500477
Sarcosphaera coronaria 3	AY500555	AY863001
Scabropezia flavovirens	AY500556	AY500461
Terfezia claveryi	AY500558	AY500503
Tirmania nivea	AF335177	AY500525
Tirmania pinoyi	AF335178	AY500502

* Sequences obtained in this study; NA = not available.

numerous yellow inclusions in the terminal cells (Fig. 4 f). These inclusions are released into water when mounted. Subhymenium composed of brown-walled hyphae, 5–6 µm diam., along with small angular cells up to about 10 µm barely differentiated of a dense textura intricata. Excipulum composed of three layers. Below the subhymenium the excipulum is constructed of textura globulosa, cells up to 40 µm, intermixed with cylindrical hyphae. Below that zone is a layer of textura intricata. Toward the outer surface cells are smaller. The outmost cells are globose and up to 80 µm, these give rise to short hyphae that are twisted or bent and sometimes unite to form low pustules (Fig. 6 a–d). The excipular cells collapse in dried specimens.

Etymology. – The epithet "nordica" referring to Scandinavia, the origin of this collection.

Habitat. – On water-soaked logs and debris of *Betula* and *Salix*.

Distribution. – Known only from the type locality.

Material examined(besides type). – Norway, Buskerud County. Hol Community, South end of Strandavatn, Gurostølen, Hallingskarvet National Park, 1000 asl, 60° 40' 14.08'' N 7°56' 26.37," on wet dead water-soaked wood of birch and scrubs, 11 August 2014, Roy Kristiansen, (# G07/14) (FH 00304780 and O).

Notes. – There are several distinctive features of this fungus. It is stipitate and in this it is unlike many of the *Peziza s. str.* species but like *P. oliviae* and *P. monterivicola*. Table 3 provides a summary comparison of the morphology and ecology of these species. *Peziza nordica* is larger than *P. oliviae*. The vacuole bodies in paraphyses in these species distinguish them from other species in the *Peziza s. str.* group. All of these species seem to occur on wood that is saturated with fresh water and occur in the spring or early in the season.

Discussion

The generic name *Peziza* has been and continues to be used across the Pezizaceae for almost all species producing large, epigeous apothecia and having asci that become blue in iodine solutions (Hansen et al. 2001, Korf 1960). This leads to a complicated and uninformative taxonomy. In the past some have argued that there were few unambiguous characters that could be used to morphologically distinguish taxa in the family. Molecular phylogenetic studies have allowed researchers to rely on characters that were in some cases dismissed, such as ascospore guttulation and ornamentation. The

Tab. 3. Overview of the morphology and ecology of the *Peziza* species included in this study.

	P. oliviae	***P. lohjaoensis***	***P. montirivicola***	***P. nordica***
Geographical range	Oregon,USA	Finland	Montenegro, Switzerland, Bosnia-Herzegovina	Norway
Size of cup	7–25 mm	10–70 mm	10–60 mm	35–80 mm
Color	olive-golden brown	brown-olive tint	brown-ochre, olive brown, chocholate brown	Brown, brownish orange
Size of stipe	1–10 × 3–7 mm	"pseudostipe"	5–10 × 5–30 mm	5–8 × 20–40 mm
Surface and color	glabrous	n/a	Granular, pale brown olive, whitish	glabrous, whitish -yellowish
Asci	250–380 × 16–22 µm amyloid with a ring	280–340 × 12–13.5 µm amyloid with a ring	307–457 × 15–23 µm amyloid, with ring	300–350 × 15–18 µm amyloid, with ring
Ascospore size, guttulation and ornamentation	15.5–24 × 9.5–12 µm ellipsoid, smooth, without guttules	17–20 × 7.5–10 µm subfusiform or narrow ellipsoid, smooth at first then verreucose with warts larger at poles	21–25 × 9.5–11.5 µm ellipsoid, smooth, without guttules, outer wall loosening or wrinkled	23.4–24.5 × 11.4–12 µm, narrowly ellipsoid, without guttules, smooth but becoming wrinkled
Paraphyses, size at tip, and contents	3–8 µm inclusions present	6–8 µm	3.5–4.5 µm with yellow inclusions	5–7/9–10 µm with yellow inclusions
Ecology	under water in small streams, dead woody debris	with *Populus tremula*, on mull, plant litter, rarely decaying wood	on immersed decaying wood of *Fagus* and *Abies* and on woody residue among mosses	on a dead water-soaked *Betula* logs.
Altitude	800–1500 m	< 50 m	1030–1662 m	1000 m
Season	June - October	May	June - September	August

Fig. 5. *Peziza nordica*. Asci showing variation in spore number, **a** mounted in water, **b** and **c** in Congo Red.

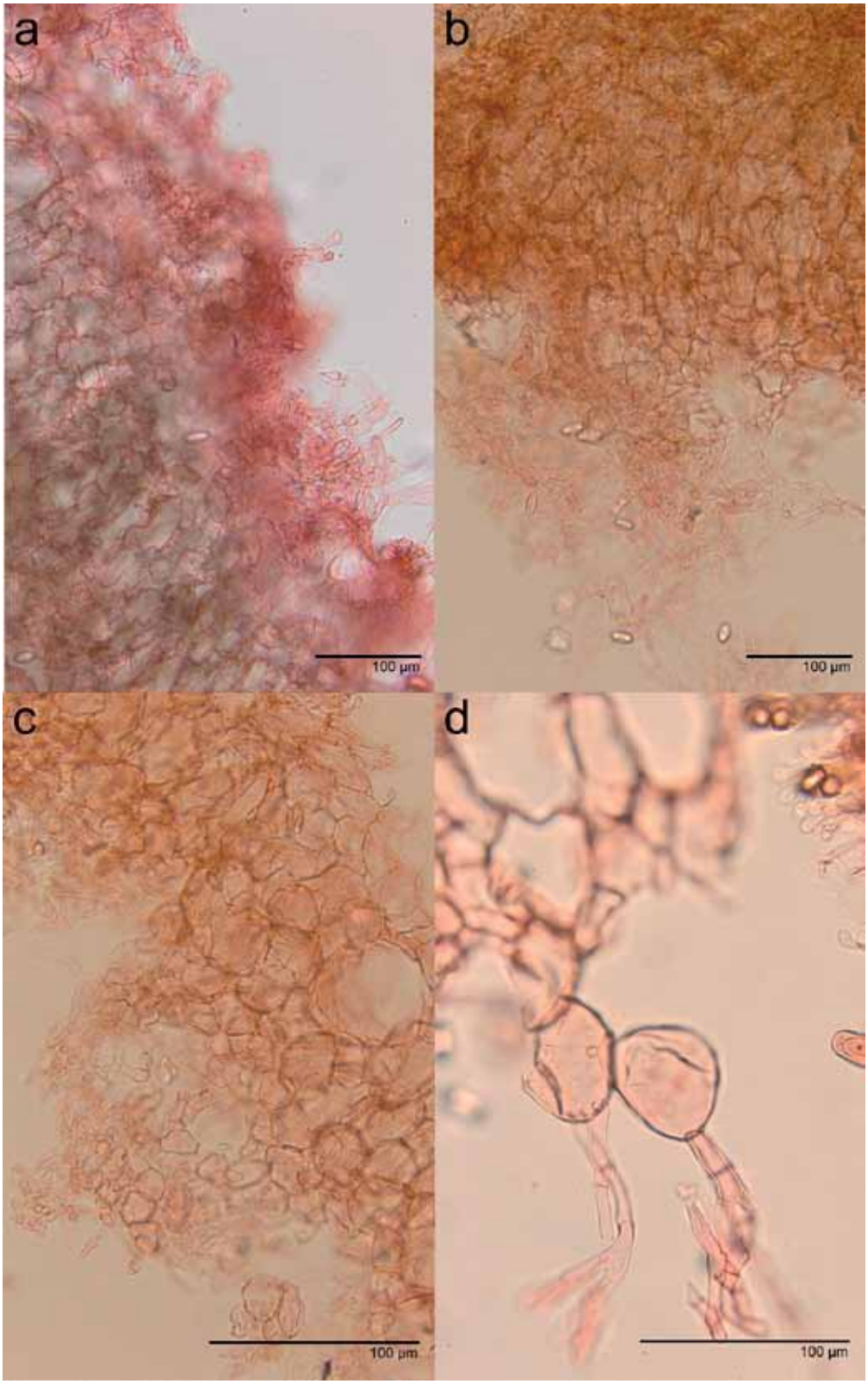

Fig. 6. *Peziza nordica*. **a–c.** Sections of the outer layer of the ascomata showing pustules. **d.** Outer cells of the excipulum with globose cells giving rise to hyphoid elements of the pustules. All sections were mounted in Congo Red in ammonia.

character of ascus bluing has also been refined with the realization that not all parts of the ascus walls become blue in the same way and that these bluing reaction differences can be reliably used to detect some groups within the Pezizaceae (Hansen et al. 2001). In this paper we have adopted several existing names for groups that show distinct phylogenetic affinities, for example, *Lepidotia* (Pfister 2015, Van Vooren et al. 2015), *Galactinia* (Pfister 2015) and *Adelphella* (Pfister et al. 2009). By taking this course we hope to begin to clarify the generic concepts in this family. Species level synonymy within the family as a whole and particularly in the *Peziza s. str.* group remains problematic.

Four taxa are involved in this study: *Peziza lohjaoensis, P. oliviae, P. montirivicola* and the newly described species, *P. nordica*. In Hansen et al. (2002) *Peziza lohjaoensis* did not group with other species and was designated as belonging to group III and its relationship with other species was considered uncertain. That study included 20 taxa in *Peziza s. str.* of which 11 were named species. *Peziza lohjaoensis* was described from Southern Finland occurring on plant litter, bare mull and occasionally on decaying deciduous wood. It occurs only in the spring and Harmaja (1986) was unable to find an existing name for the species. We have borrowed and studied all the collections of *P. lohjaoensis* from the Herbarium of the University of Helsinki. They are primarily collected in May and over a number of years. Harmaja (1986) compared this species to *P. phaeotheca* McKnight & Dublin (=*P. nivalis*), a snow bank fungus described from Western North America because of the brownish wall of the ascus. Other than the substrate preference, the brownish asci and substipitate growth habit, *P. lohjaoensis* differs from our new species in its smaller spores that are warted when fully mature. It is a distinct species in our phylogeny (Fig. 3). Apothecia in both *P. oliviae, P. montirivicola* and *P. nordica* are stipitate.

Peziza oliviae, *P. montirivicola* and *P. nordica* are particularly distinctive and interesting species because of their habit of fruiting in or near running water. They grow on wood in stream beds or on water-soaked wood near running water. Other species in the Pezizaceae that live in such habitats, for example *Pachyella* and *Adelphella* (Pfister 1973, Pfister et al. 2009), are pulvinate and lack a stipe. The bluing of the asci is also different. In those genera there is a general, diffuse bluing of the asci. This habitat is not one that is well collected and the spring occurrence may explain why these taxa have been overlooked.

Van Vooren & Moyne (2011) describe *Peziza dissingii* (now known as *Peziza sublaricina* Donadini) as a spring fruiting fungus, occurring in May. It resembles our *P. nordica* but is not stipitate. It is not a snow bank fungus. Specimens were collected at altitudes between 1250–1700 m in France. It differs from our species in that it was collected on soil and the spores are somewhat smaller and they could be ornamented with cyanophilic markings. In both cases the paraphyses have yellow vacuolar contents. Subsequently, *P. dissingii* was reported from Germany by Fellman (2014). Van Vooren & Moyne (2011) compare their species to *P. acroornata* and *P. badioides*. Both of these species have prominently ornamented ascospores, the ornamentation often being heavier at the poles of the spores. Further study and collecting may demonstrate that there are additional species in this group.

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