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ORIGINAL ARTICLE

Morphological and molecular study of *Peziza emileia* and *P. howsei*, two distinct taxa

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Abstract *Peziza emileia* Cooke and *P. howsei* Roze & Boud. are compared here; they are morphologically very similar, but the internal transcribed spacer (ITS) sequence is unique for each of the two species. Furthermore, since the holotype of *P. emileia* deposited in Kew (K) contains an unidentified fungus and the holotype of *P. howsei* in Paris (PC) no longer exists, we provide lectotypification and epitypification for both taxa.

Keywords Pezizales · ITS rDNA Sequence · RPB2 DNA Sequence · Morphology · Taxonomy

Introduction

Both *Peziza emileia* and *P. howsei* were published in 1879, and since that time their consideration as distinct species has been questioned. Some authors (Dissing 2000; Grelet 1936; Häffner 1986; Hohmeyer 1986; Hohmeyer et al. 1989; Jamoni 2004; Le Gal 1937; Moser 1963) considered *P. emileia* to be different from *P. howsei*, while others consider them to be synonyms (Dennis 1960, 1981; Le Gal 1941; Romagnesi 1978).

In these descriptions and in those by Bánhegyi 1939; Bresadola 1881; Donadini 1981; Ellis and Ellis 1988; Maas Geesteranus 1954; Schmid-Heckel 1988, and Seaver 1928, the characters used were sometimes overlapping or contrasting, especially with regard to apothecial coloration, or the descriptions are scanty, as in the case of details of spore ornamentation. In a previous phylogenetic study using large subunit sequences (LSU) (Hansen et al. 2001), *P. howsei* and *P. emileia* were not distinguishable. Through our analyses of fresh and dried samples from private and institutional European herbaria, we have obtained new morphological and molecular data to clarify the phylogenetic position of these taxa.

Background information

Cooke (1879: 226, Plate 106, Fig. 379) published *P. emileia* as growing on the ground, with hymenial surface "*laete-fusca*," outer surface "*albida*," and spores warted 16–17×8 μm. Gillet (1879) transferred it to *Aleuria*. The characters described actually are similar to those of Cooke, but he did not report spore size, and the habitat was strangely given as fimicolous. Saccardo (1889) treated the species in *Discina*, repeating Cooke's original description. Boudier (1907a, 1911) transferred it to *Aleuria*, but this binomial is illegitimate, because Gillet previously used the name. Le Gal (1937: 197–198) treated *P. emileia* in *Galactinia*, describing the spores as 17–21 (22)×8–9.5 μm with fine regular and rather dense warts, and considered *G. emileia* to be different from *G. howsei* (l.c.: 199). Le Gal (1941) revised her earlier statement after further studies. She considered *G. emileia* and *G. howsei* to be a single taxon, and wrote (l.c.: 79): "*nous avons acquis la conviction que G. Emileia n'est qu'une forme de G. Howsei*

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à hyménium fauve cannelle ne présentant aucune trace de violacé."

Peziza howsei was described by Roze and Boudier (1879: 26, Table 3, Fig. 3) with hymenial surface as violaceous with yellowish tints, outer surface white-grey and finely warted spores, 17–19×7–8 μm. Gillet (1886) transferred it to *Aleuria*, again describing the hymenium as violaceous with yellow tint, outer surface white-grey and warty spores (without specifying the dimensions). Rehm (1896) combined it in *Plicaria*, describing the hymenium as violaceous stained yellow which pales, outer surface whitish and spores of 17–19×7–8 μm, warted. Boudier (1907b) used the generic name *Galactinia* for it without other details. In the key to *Galactinia* and *Aleuria*, Le Gal (1941: 70) stated that *P. emileia* was a doubtful species, and that it is a form of *P. howsei* lacking violet pigments. Romagnesi (1978) confused the authorship of some taxa and attributed to Moser two combinations he did not actually make. Donadini (1978) incorrectly made the combination "*P. howsei (Boud.) nov. comb*.", which is illegitimate (McNeill et al. 2011, art. 52.1), probably based on the new name in *Galactinia* made by Boudier (1907b), that already was, however, a re-combination. The characters of this fungus are: hymenium violet to ochraceous, outer surface bluish to ochraceous-grey and spores of 18–20×8–9 μm, finely warted. Both names are currently in use.

Materials and methods

Morphological analyses

Macroscopic and microscopic examinations were carried out on both fresh and dried specimens. Measurements and descriptions of microscopic characters were made using material mounted in water, rehydrated when necessary with 5 % KOH. Melzer's reagent and Cotton Blue in lactic acid were also used. Specimens were studied using an Optika optical microscope (BK 1301 model) with 40× or 100× (immersion oil) objectives, and Scanning Electron Microscope (SEM) (Carl Zeiss EVO10 LS). For SEM, hymenial sections were coated with a gold layer (100 Å) using an Edwards S 150 A Sputter Coater. Spore dimensions were based on measuring 50 spores that were judged to be mature.

DNA isolation, PCR, and sequencing techniques

The Qiagen DNeasy Plant Mini Kit (Qiagen, Germany; cat. no. 69104) was used to extract genomic DNA from herbarium specimens of *P. emileia* and *P. howsei*. Specimens used are listed in Table 1 and are noted by an asterisk. A 1/10 and a 1/100 dilution of the DNA were used for PCR amplification of the ITS rDNA region (Internal Transcribed Spacer+5.8S rRNA gene) and the 6–7 region of the RPB2 gene (DNA-dependent RNA polymerase II subunit RPB2) (Hansen et al. 2005). The ITS was amplified using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). 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 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 (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 and RPB2 DNA sequences determined in this study were deposited in GenBank (Table 1).

Alignment of the DNA sequences was done using ClustalW through the Cipres Science Gateway (ML; Miller et al. 2009) and then manually adjusted with Se-Al v 2.0a11 (Rambaut 2002). The ITS and RPB2 sequences of the *P. emileia* and *P. howsei* isolates determined in this study were aligned with GenBank sequences of select *Peziza* species from clade IVb (Hansen et al. 2001), and are listed in Table 1. The outgroup taxon was *P. depressa*.

DNA sequence alignments were analyzed using Maximum Parsimony, PAUP 4.0b10 (MP; Swofford 2002) and Maximum-Likelihood with RAxML–HPC2 on Abe through the Cipres Science Gateway (ML; Miller et al. 2009). Branch support for MP and ML analyses was determined by 1,000 bootstrap replicates.

Results

Molecular results

The combined ITS and RPB2 alignment in this study, which included taxa belonging to *Peziza* group IVb (Hansen et al. 2001), yielded 1,482 bp. One region in the ITS1, consisting of 212–370 bp, was excluded in the phylogenetic analyses due to ambiguous alignment. Results of DNA sequencing from this study determined that *P. howsei* and *P. emileia* have ITS sequences that are unique for each of the species. A comparison of the pairwise differences using PAUP 4.0b10 showed that the average percent ITS sequence difference within isolates of *P. emileia* was 0.06 %, while within *P. howsei* the average percent ITS sequence difference was 3.5 %. The level of interspecific ITS sequence variation between the two

Table 1 Details of *Peziza* specimens used in phylogenetic analysis. NA indicates GenBank sequence not available. Sequences obtained in this study are designated with an asterisk (*)

Species	Herbarium and specimen number	Geographic origin	Year and collector	GenBank accession ITS	GenBank accession RPB2
*P. emileia**	**GM** 03091006	Italy	2010 G. Medardi	KJ728717	KJ728720
*P. emileia**	**L** 0833270	Netherlands	1954 R.A. Maas Geesteranus	KJ728716	KJ728719
*P. emileia**	**L** 0833260	U.K.	1992 J. van Brummelen	KJ728715	KJ728718
*P. howsei**	**PGJ** 1742	Italy	1988 P.G. Jamoni	KJ728714	NA
*P. howsei**	**GM** 13099708	Italy	1997 G. Medardi	KJ728713	NA
P. howsei	**C** KH 97-98	Denmark	1997	NA	AY500493
P. howsei	**MCVE** 6929	Italy	1991 E. Bizio	JF908528	NA
P. ampelina (1)	**MCVE** 15909	Italy	2001 G. Merdardi/C gallinaro	JF908554	NA
P. ampelina (2)	**C** KH 00.011	Denmark	1994 C. Lange	AF491629	AY500492
P. lobulata	**MCVE** 16641	Italy	2003 A. Lantieri	JF908567	NA
P. lobulata	**FH** KH 03-157	Denmark	2003	NA	AY500495
P. petersii (1)	**MCVE** 3836	Italy	1994 E. Bizio	JF908527	NA
P. petersii (2)	**MCVE** 15504	Italy	2000 G. Merdardi/C. Gallinaro	JF908550	NA
P. petersii (3)	**DAOM** 195796	Canada	——	AF133179	NA
P. proteana (1)	**MCVE** 16618	Italy	2003 P. Cugildi	JF908566	NA
P proteana (2)	**OSC** 100024 AFTOL- 71	USA	2003 M. Crockett	DQ491497	NA
P. subcitrina (1)	**C** KH 00.023	Denmark	2000 K. Hansen/S. Landvik	AF491627	NA
P. subcitrina (2)	**C** KH 97.133	Denmark	1997 C. Lange K. Hansen	AF491628	AY500520
P. depressa	**C** KH 98.28	Denmark	1995 H. Hundsen	DQ200837	AY500474

species was 15 %. The phylogenetic analyses of the ITS and RPB2 DNA sequences in the current study (Fig. 1) places these species in sister clades. This confirms that *P. howsei* and *P. emileia* are distinct but closely related species.

Taxonomy

Peziza emileia Cooke, *Mycographia* 1: 226, Pl. 106, Fig. 379. 1879.

Figure 2a, b, c, d.

≡ *Aleuria emileia* (Cooke) Gillet, *Champignons de France, Les Discomycètes. Livr.* 2: 43. 1879

≡ *Discina emileia* (Cooke) Sacc., *Syll. Fung.* 8: 100. 1889, as "*emileja*"

[≡*Aleuria emileia* (Cooke) Boud., *Icones Mycologicae*: 153, Tab. 280. 1906. comb. illeg. (later homonym)]

≡ *Galactinia emileia* (Cooke) Le Gal, Revue de Mycol. 2:197. 1937.

[≡ ? *Aleuria emileia var. splendens* Le Gal in L. Remy, *Bull. trimest. Soc. mycol. Fr.* 80 (4): 585. 1965, invalid, holotype not designated]

Typification: **Lectotype designated here**, France, "on the ground," Cooke, *Mycographia*: 226, coloured Pl. 106, Fig. 379. 1879. **Epitype designated here**, Italy, Loc. Piana di Gaver, Breno, Brescia, on the ground in a wood of conifers and *Salix*, 3 Sep 2010, leg. et det. G. Medardi [GM 03091006 – K(M) 191291].

Augmented Description—Apothecia 30–80 mm diam., irregularly and more or less deeply cup-shaped, in some cases sub-umbilicate, sessile. Hymenial surface smooth or undulating, more or less intense fawn-cinnamon, also with some slight brown tones, darker in high humidity. Outer surface delicately scurfy, whitish to pale brownish, darker in age or if soaked; margin even to lobed, sometimes shortly fissured (Fig. 2a). **Flesh** waxy, fragile, whitish or weakly brownish, layered. **Ascospores** 17–22×(−8.5) 9–11 μm, ellipsoid, with warts in form of irregular ridges, more or less longed/broadened, amoeboid in outline, 1–3 μm width×1 μm high, interconnected and covering most of the surface (Fig. 2b, c, d); hyaline to slightly pale brownish, two oil drops, uniseriate in the ascus. **Asci** 280–330×15–18 μm, subcylindrical, hyaline to weakly brownish, amyloid (amyloidity distributed more or less 15–20 μm from the apex), eight-spored. **Paraphyses** subcylindrical, 2.5−4 μm wide on the bottom, 7–8 μm at the apex, simple, septate, very pale brownish at the tip. **Subhymenium** 50–70 μm thick, *textura intricata*, hyphae more or less interwoven, septate, 5–6 μm diam. **Medullary excipulum** two-layered. Upper layer 200–300 μm thick, *textura globulosa-angularis*, cells rounded or slightly

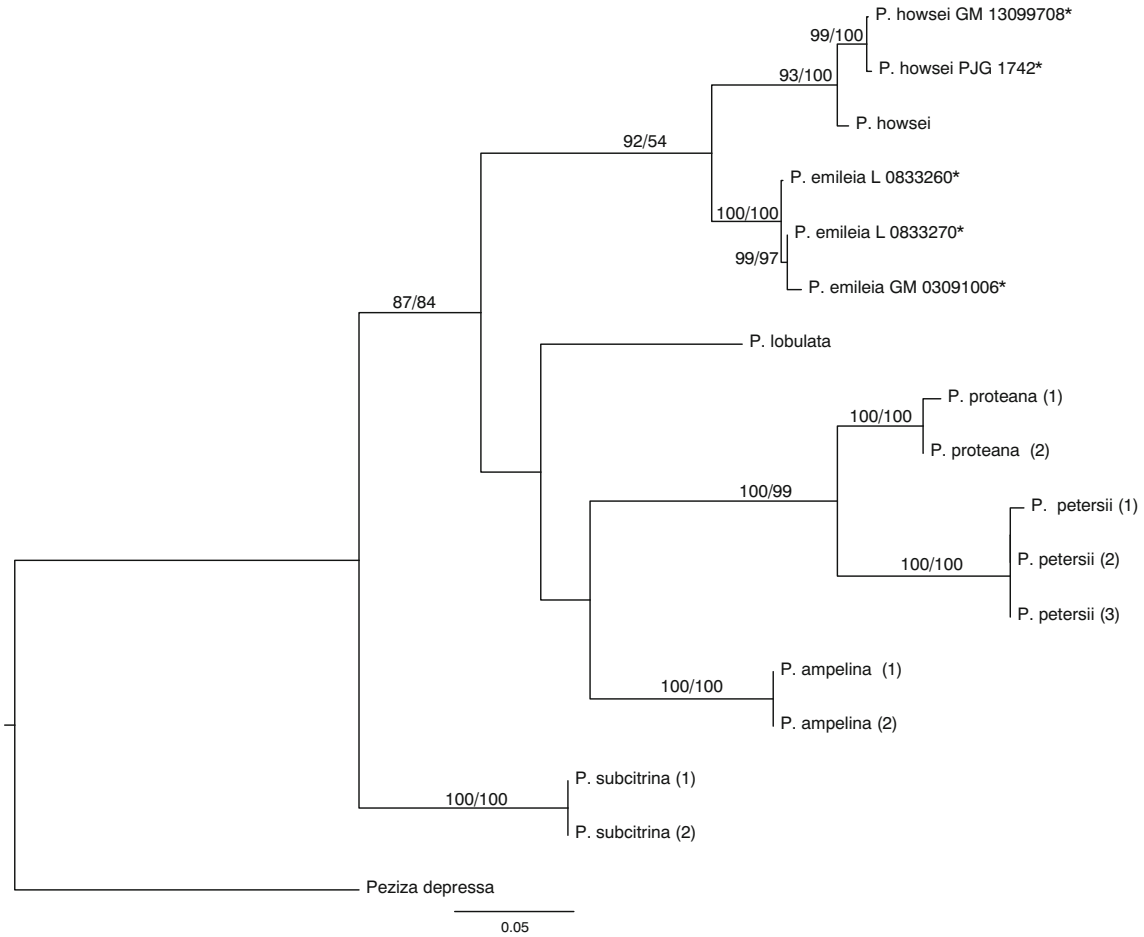

Fig. 1 Phylogenetic tree of *Peziza* species, clade IVb (Hansen et al. 2002) inferred from ITS rDNA and RPB2 DNA sequences. *P. emileia* and *P. howsei* isolates sequenced in this study have a "*" following their name and collection number. The tree presented is from Maximum Likelihood analysis using RAxML-HPC2 on Abe through the Cipres Science Gateway (Miller et al. 2009). Maximum Likelihood, and Maximum Parsimony (using PAUP 4.0b10 Swofford 2002) branch support values are indicated above the branches respectively. *Peziza depressa* was the outgroup species. Branch support for both analyses was determined by 1000 bootstrap replicates

polygonal, 25–30 μm diam. Lower layer 400–500 μm thick, of intermixed *textura intricata* and *globulosa-angularis*, hyphae interwoven, septate, 3–5 μm diam. and cells similar to those of the first layer. **Ectal excipulum** 200–300 μm thick; textura angularis, cells irregularly polygonal, 10–25 (30) μm diam.

Habitat and ecology

In small groups on the ground of the woods, scattered or fasciculate; summer–autumn.

Peziza howsei Roze & Boud., *Bull. Soc. bot. Fr.* 26 (Suppl.): LXXV, Tab. 3, Fig. 3. 1879.

Figure 2e, f, g, h.

≡ *Aleuria howsei* (Roze & Boud.) Gillet, *Champignons de France, Les Discomycetes. Livr.* 8: 206. 1886.

≡ *Plicaria howsei* (Roze & Boud.) Rehm, *Die Pilze III, Ascomyceten: Hysteriaceen und Discomyceten. Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz, Zweite Auflage, Erster Band*: 1015. 1895.

≡ *Galactinia howsei* (Roze & Boud.) Boud., *Hist. Class. Discom. Eur.*: 48. 1907.

[≡*Peziza howsei* (Boud.) Donadini, *Bull. Soc. linn. Provence* 31: 29. 1978. comb. illeg. (later homonym)]

[≡*Peziza howsei* (Boud.) Donadini, *Le genre Peziza dans le sud-est de la France*: 70. 1981. comb. illeg. (later homonym)]

Typification: **Type**: No authentic material has been located at PC; Donadini (1978: 29) specified that the lectotypus of this fungus was placed in PC under the name of *Galactinia howsei*, but not even this sample was found. **Lectotype designated here**, France, "Aurillac, bois de Condamine, en Juillet 1879, sur la terre nue," Roze & Boud. *Bull. Soc. bot. Fr.* 26 (Suppl.): LXXV, Tab. 3, Fig. 3. 1879.

Epitype designated here, Italy, Pergine Valsugana (Trento), on sandy soil in a mixed wood 13 Sep 1997, leg. et det. G. Medardi [GM 13099708 – K(M) 191412].

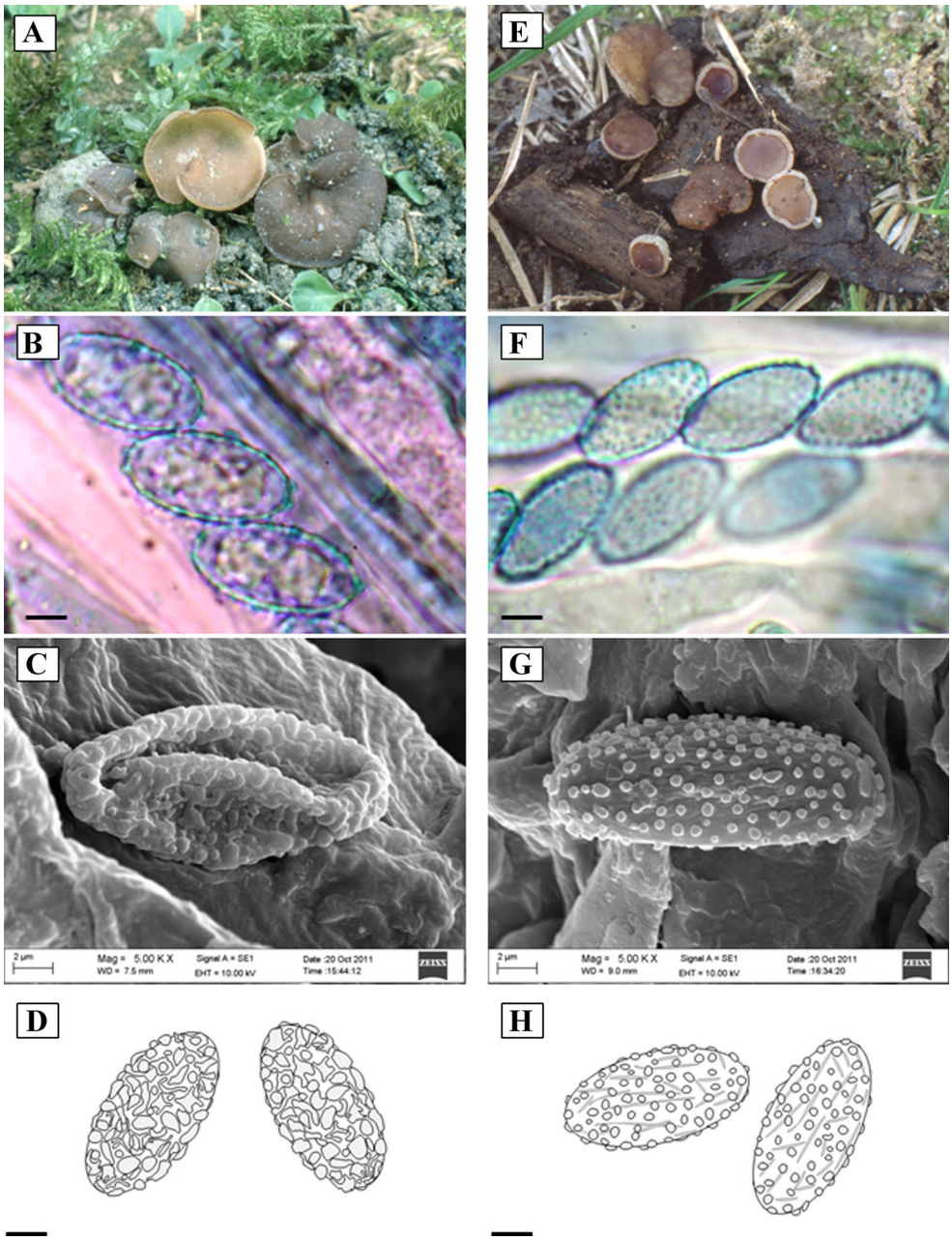

Fig. 2 Comparison of the main characters of *P. emileia* and *P. howsei*. *P. emileia*: *a*, apothecia in situ (Photo G. Medardi); *b*, micrography with LM (Bar= 5 μm), spores coloured with Cotton blue (Photo G. Medardi); *c*, micrograph with SEM, spore pattern (Photo G. Fichera); *d*, spore pattern (Bar=5 μm) (Del. G. Medardi). *P. howsei: e*, apothecia in situ (Photo G. Medardi); *f*, micrograph with LM (Bar=5 μm), spores coloured with Cotton blue (Photo G. Medardi); *g*, micrography with SEM, spore pattern (Photo G. Fichera); *h*, spore pattern (Bar=5 μm) (Del. G. Medardi)

Augmented Description—Apothecia up to 30 mm diam., flat, only concave when mature, sessile. Hymenial surface generally even, at times weakly undulate, more or less pale brownish-violaceous, sometimes with weak yellowish tones. The violaceous tint is more evident in young specimens and becomes dark brown with age or in high humidity. Outer surface smooth or delicately scurfy (especially near the margin), decisively contrasting with the hymenium, bluish-ochraceous or greyish-bluish ochraceous, paler toward the base; margin regular, pale (Fig. 2e). **Flesh** waxy, fragile, whitish to slightly brownish, layered. **Ascospores** 18−22(−23)×9−11(−12) μm, ellipsoid, with warts isolated, more or less regularly rounded, cylindrical or barely conical-truncate in profile, 0.5-1 μm diam. × 0.5 μm high, in some cases connected to form short crests; their spacing leaves some areas of the surface uncovered, hyaline or pale beige-isabelline, two oil drops, uniseriate in the ascus. **Asci** 280–330×15–18 μm, subcylindrical, hyaline to isabelline, amyloid (amyloidity diffused more or less 15–20 μm from the apex), 8-spored. **Paraphyses** subcylindrical, 2.5−4 μm wide in the lower part, up to 8 μm at the top, simple, septate, slightly pale violaceous at the apex.

Subhymenium up to 50 μm thick, *textura intricata*, hyphae more or less interwoven, septate, 5–6 μm diam. **Medullary excipulum** two-layered: upper layer up to 200 μm thick, *textura globulosa-angularis*, cells rounded or

slightly angular up to 25 μm diam. Lower layer up to 400 μm thick, more or less of *textura intricata* near the upper layer (hyphae interwoven, septate, 3–5 μm diam.), tending to *globulosa-angularis* with cells similar to the upper layer. **Ectal excipulum** up to 200 μm thick; *textura angularis*, cells irregularly polygonal, 10–25 μm diam.

Habitat and ecology

In small groups on the ground of the woods, at times also near degraded wood covered with soil; summer–autumn.

Specimens examined

Peziza emileia: **France**, Forêt de Hallate (no locality), Jun 1885, leg. F. Sarrazin, [K(M) 59526 **syntypus**] (We identified this as *P. petersii*); **Germany**, Kreis Weissefels, Leissling, Winterleike, 08 Aug 1969, leg. N. Nothnagel, (L 0833261) (the sample ref. L 0833261 contains two collections, one of *P. emileia* and one of *P. howsei*.); **Italy**, **Tuscany**, Cala Violina (Grosseto), 10 Dec 2003, leg. S. Gori, A. Laganà, C. Perini, E. Salerni, det. A. Laganà, C. Perini, E. Salerni (SIENA 6554); **Lombardia**, Schilpario (Bergamo), 20 Sep 2009, leg. et det. G. Medardi (GM 20090911); Loc. Piana di Gaver, Breno (Brescia), 03 Sep 2010, leg. et det. G. Medardi (GM 03091006); **Netherlands**, Beeds, Marienwaard, Oct 1949, leg. K. Westmyze, det. M. Le Gal (L 0833269); Bunnik, Rhÿmauven, 22 Jul 1954, leg. et det. R.A. Maas Geesteranus (L 0833270); Beeds, Gelderland, 08 Jul 1972, leg. et det. F. Tjallingii (L 0341857); **U.K.**, Isle of Wight, no date, leg. Howse, ex Herbarium Phillips [K(M) 171195 **holotypus**] (We identified this as *Aleuria* sp. or *Melastiza* sp.); Crickley Wood, Gloushershire, 16 Sep 1992, leg. et det. J. van Brummelen (L 0833260).

P. howsei: **Estonia**, Hiiu Co., Hiiesaare Lighthouse, 18 Sep 2001, leg. L. Vaher, det. B. Kullman sub *Pachyella celtica* [TAA(M) 179773]; Järvselja forest, Tartu, 27 Sep 2001, leg. et Det. B. Kullman [TAA(M) 179788]; **France**, Arbois, Jura Dep., 1901, leg. D. Metier, det. E. Boudier sub *Galactinia howsei* (PC 0084255); Bois Lebisey, Caen Caluados, 15 Jun 1995, leg. et det. T. Duchemin (THD 9506152669); Trouville sur Mer, 07 Apr 1998, leg. et det. T. Duchemin (THD 9804073326); **Germany**, Kreis Weissefels, Leissling, Winterleike, 21 Jul 1969, leg. N. Nothnagel, (L 0833261) (the sample ref. L 0833261 contains two collections, one of *P. emileia* and one of *P. howsei*); Zwoller Kerspel, Windesheim, 02 Sep 1954, leg. C. Bas, det. unidentifiable (L 0833271); **Italy**, **Piemonte**, Val d'Otro, Alagna Valsesia (Vercelli), 12 Jun 1988, leg. et Det. P.G. Jamoni (PGJ 1742); Val d'Otro, Alagna Valsesia (Vercelli), 01 Jul 1994, leg. et det. P.G. Jamoni (AMB 002819); **Veneto**, Isola della Certosa (Venice), 23 Nov 1991, leg. E. Bizio, det. D. Garofoli (EB 23119114 and MCVE 6929); Loc. Alberoni (Venice), 10 Nov 2001, leg. et det. E. Bizio (EB 10110102); **Trentino Alto-Adige**, Pergine Valsugana (Trento), 13 Sep 1997, leg. et det. G. Medardi (GM 13099708); **Lombardia**, Val d'Avio, Temù (Brescia), 03 Jul 1999, leg. et det. G. Medardi (GM 03079904); Val d'Avio, Temù (Brescia), 20 Jul 1999, leg. et det. G. Medardi (GM 20079906); Valvestino, Magasa (Brescia), 24 Jul 1999, leg. et det. G. Medardi (GM 24079901); Loc. Piana di Gaver, Breno (Brescia), 03 Oct 2011, leg. et det. G. Medardi (GM 03101102 and GM 03101103); **Sicily**, Buccheri (Siracusa), 11 Nov 2011, leg. et det. A. Lantieri (AL 11111102); **Netherlands**, Leiden (Hort. Bot.), 19 Sep 1950, leg. K. de Groot, det. M. Le Gal (L 0833280); **Portugal**, Lisbon, Jan 1908, leg. et det. M. Torrend sub *Galactinia howsei* (PC 0084254); **Serbia** – **Montenegro**, Biogradska Gora National Park, Kolašin, 26 Aug 2011, leg. et det. B. Peric (GM 260811BP); Vucji Potoj, 30 Aug/ 2011, leg. O. Peric, det. B. Peric (GM 300811BP); Biogradska Gora National Park, Mojkovac, 09 Sep 2012 leg. et det. B. Peric (GM 090912BP).

Discussion

We show, in agreement with the study of the *Peziza* core group by Hansen et al. (2002), that the ITS region is highly variable among members of the Pezizaceae. In that case, as here, there was considerable variation in the length of the ITS region even among taxa that were considered to be closely related. In this case, morphological characteristic and molecular phylogenetic differences confirm the earlier opinion that these are two distinct species.

Peziza emileia and *P. howsei* are very similar morphologically and it is difficult to distinguish them, but characters including habitat can overlap and be ambiguous. When specimens are not in an optimal state, their characterization is particularly difficult. In our studies we encountered some major difficulties. The holotype of *P. howsei* no longer exists in PC. The holotype of *P. emileia* [K(M) 171195] contains a completely different fungus, probably an *Aleuria* or *Melastiza* species with reticulated spores and inamyloid asci. We further studied a syntypus [K(M) 59526], but in this case, the sample was thought to be *P. petersii* Berk.

When we compared the original diagnoses and the related plates of *P. emileia* and *P. howsei*, we found only two differences, which are quite difficult to distinguish in the field: apothecia colour, and spore size and ornamentation. In *P. emileia*, the hymenium is fawn-cinnamon and the outer surface is whitish or whitish to weakly brownish, whereas in *P. howsei*, they are respectively brownish-violaceous with slight yellowish tints and greyish-white-pale bluish tending yellowish toward the margin (cfr. Fig. 2a, e). The spore size,

on average, is 2 μm longer in *P. howsei*. We should also note that various authors reported the spore size larger than the original diagnoses of both species, which was also our finding.

Our microscopic studies showed that the diversity of shape, density and distribution of spore warts (cfr. Fig. 2b, c, d, f, g, h) is another defining feature (not always easy to appreciate) that can help in distinguishing these two species. In outline, the warts of *P. howsei* are in the range of 0.5 μm diam. × 0.5 μm high, or slightly larger (1 μm diam. × 0.5 μm high), punctiform and more or less rounded if observed from above, and cylindrical or faintly conical-truncate, widely-spaced, in a few cases joined and organized in to short ridges. This arrangement leaves some zones of the spore surface exposed. In *P. emileia*, warts are small, irregular with more evident ridges that are more or less amoeboid or elongated/broadened in outline, variable in size (1–3 μm width×1 μm high), close and covering a great part of the surface.

The characters differentiating *P. emileia* and *P. howsei* morphologically are essentially colour of the apothecia and the different patterns of spore ornamentation.

The name *Peziza emileia* var. *splendens* is mentioned in the literature (Donadini 1978, 1981; Remy 1965). Since a holotype was not designated, the name is invalid. This fungus was described as a large, more fleshy and clustering species, with yellow-brown to bright orange hymenium and outer surface concolorous but tinged purple toward the margin, and spores 16–21×8–9 μm with very fine and dense cyanophilous warts. The lack of the holotype does not allow us to investigate the question further, and we can only assume that this taxon could be a physiological variation of the species or represents a different taxon.

Acknowledgments We wish to thank the curators of K(M), L, MCVE, SIENA, TAA(M), PC, for arranging loans of material, E. Bizio, T. Duchemin, and P.G. Jamoni for putting their collections at our disposal, and Dr. G. Fichera (Department of Biology "Marcello La Greca", University of Catania) for the valuable help in the realization of the SEM photographs. We want to express a particular gratitude to Dr. Thijsse, curator of the Leiden University Herbarium (Netherlands), for the permission to carry out the molecular analysis on their samples, and Dr. K. Hansen for information about the studied taxa.

Conflict of interest The authors declare that they have no conflict of interest.

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