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Clarification of *Peziza fimeti* with notes on *P. varia* collections on dungGIANFRANCO MEDARDI¹, ANGELA LANTIERI², DONALD H. PFISTER³,
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ABSTRACT — The smooth-spored species inhabiting dung, mainly of the *Peziza fimeti* group, were studied morphologically and through ITS sequence comparison. The results established that *Peziza varia* is also able to fruit on dung, clarifying a long-standing situation regarding two conflicting interpretations given in *P. fimeti* literature.

KEY WORDS — *Pezizales*, taxonomy

Introduction

We re-examined the concepts that have been applied to *Peziza fimeti* (Fuckel) E.C. Hansen (Hansen 1876), a species interpreted differently by various authors. In keys and descriptions *P. fimeti* is reported with two different spore sizes. Bresadola (1892, as *P. fimetaria*, as *Humaria fimetaria*), Seaver (1928), Moser (1963), Romagnesi (1978), Breitenbach (1979), Dennis (1981), Hardtke & Herrmann (1986), Ellis & Ellis (1988), Häffner & Kasperek (1989), Mornand & Péan (1994), Spooner & Butterfill (1999), Dissing (2000), Kutorga (2000), Delgado et al. (2001), Ciana (2003), García (2003), and Poumarat (2008) reported the ascospores measuring (13–)16–18(–18.6) × (6–)8–10(–12) µm. On the other hand, Svrček & Kubička (1961), Gamundí (1975), Donadini (1977, 1978, 1979, 1981), Calonge et al. (1986), Garofoli (1989), Prokhorov & Kutorga (1990), Hallgrímsson & Götzsche (1990), Cacialli et al. (1995), Wang & Wang (2000), Hansen et al. (2002), Doveri (2004), Karasch (2005), Pfister & Eyjólfsdóttir (2007), and Moyne & Petit (2008) cited larger spore sizes, (14.1–)18–22.5 × (7.2–)9–12 µm.

This confusion led Hohmeyer (1986) to define two autonomous taxa, “*fmieti* sensu Dennis, Seaver” and “*fmieti* sensu Donadini, Gamundi”, distinguished from each other only by the above mentioned spore dimensions. Because of a discrepancy between *P. fmieti* spore sizes as given by Fuckel (1871: $16 \times 8 \mu\text{m}$) in the protologue and modern measurements from the holotypus (G: $20\text{--}22.5 \times 10\text{--}12 \mu\text{m}$), Hansen et al. (2002) suggested that *P. fmieti* sensu Seaver was probably a mistake perpetuated in the literature.

Nevertheless, after we analyzed fresh and dried samples from private and institutional European herbaria, we noted another entity with ascospores similar to those described by Fuckel and to *Peziza alcis* Harmaja. Molecular phylogenetic analysis of the ITS rDNA region showed this latter species is distinct from other *Peziza* spp. on herbivore dung.

Materials & methods

Material studied

We examined both fresh and dried samples (including holotypes) from AMB, FH, G, MCVE, SIENA, TAA(M), and private herbaria. The nine herbarium specimens of *Peziza fmieti* selected for DNA extraction are listed in TABLE 1.

TABLE 1. *Peziza fmieti* specimens used in phylogenetic analysis.

LOCATION	COLLECTION DATA	HABITAT	ITS SEQUENCE NO.
Italy. Trentino-Alto Adige, Trento, Pozza di Fassa	E. Bizio, 13.08. 94 [pers. herb. EB 130894-32; FH 00301727]	Bovine dung	JQ654487
Italy. Abruzzo, L'Aquila, Civitella Alfedena	E. Bizio, 05.06. 91 [pers. Herb. EB 050691-23; FH 00301728]	Excrement	JQ654488
Italy. Sicily, Siracusa, Buccheri, Santa Maria's Wood	A. Lantieri, 29.10. 05 [pers. Herb. AL 291005-17; FH 00301725]	Bovine dung	JQ654489
Italy. Friuli-Venezia Giulia, Udine, Terzo	A. Pergolini, 15.04. 94 [AMB 002072; FH 00301726]	Equine dung	JQ654490
USA. Alaska, Anchorage, Goose Lake	L. Millman, 07.09. 11 [DHP 11-691; FH 00301724]	Moose dung	JQ654491
Russia. Chelyabinskaya Oblast, Miass	Parmasto et al., 16.07. 73 [TAAM 062922]	Excrement	JQ654492
Russia. Krasnodar, Umpyr, Caucasus Nature Reserve	M. Pallo, 10.08. 76 [TAAM 064318]	Excrement	JQ654495
Russia. Kamchatka, 47 km from Krapivnaya	B. Kullman, 06.08. 78 [TAAM 187900]	Excrement of <i>Ursus ursus</i>	JQ654493
Estonia. Jõgevamaa Co, Kursi, Puurmani Commune, Altnurga, forestry sq. 95	M. Õpik, 08.10. 97 [TAAM 171114]	Moose dung	JQ654494

SPECIMENS EXAMINED: *Peziza alcis*: ESTONIA: JÕGEVAMAA CO, KURSI, Puurmani Commune, Altnurga, Forestry sq. 95, on moose dung, 08.10.97, leg. & det. M. Õpik (TAAM 171114, as *P. fmieti*); RÄGAVERE COMM., ULJASTE, Lääne-Viru Co., on ground

and on dung, in moist *Betula-Picea* forest, date not declared, leg. K. Kalamees, det. A. Raitviir (TAAM 71260, as *P. fimeti*). FINLAND: LAPLAND, KEVO, National Park, on moose dung, 28.08.81, leg. K. Kalamees, det. A. Raitviir (TAAM 122042, as *P. fimeti*); UUSIMAA, Inkoo, Rädikila, alt. ca 15 m, grid 27° E 6665 : 339, ± mesic acid coniferous health forest, on dung of *Alces*, 09 Nov. 1977, H. Harmaja (H – holotype). USA: ALASKA, ANCHORAGE, Goose Lake, on moose dung, 07.09.11, leg. L. Millman, det. D.H. Pfister (DHP 11-691, as *P. fimeti*; FH 00301724).

Peziza fimeti: GERMANY: RHEINLAND-PFALZ, Nassau, auf Kuhmist, in einem Tannenwalde, unterhalb Mappen, Vere, Fuckel (G 00276010, as *Humaria fimeti* – holotype). ITALY: ABRUZZO, L'AQUILA, Civitella Alfedena, on excrement, 05.06.91, leg. & det. E. Bizio (pers. herb. EB 050691-23; FH 00301728); FRIULI-VENEZIA GIULIA, UDINE, Codroipo, on equine dung, 16.09.94, leg. & det. A. Pergolini (MCVE 11905); LAZIO, ROMA, Nettuno, 25.11.08, leg. & det. G. Consiglio (pers. herb. GC 08337, as *P. vesiculosa*); LOMBARDIA, BRESCIA, Botticino, S. Gallo, 27.05.00, leg. & det. G. Medardi (pers. herb. GM); Breno, Piana di Gaver, on burnt ground, 23.05.91, leg. & det. G. Medardi (pers. herb. GM, as *P. granularis*); Breno, Piana di Gaver, on bovine dung, 31.05.99, 18.06.01 and 29.06.08, leg. & det. G. Medardi (pers. herb. GM); Pisogne, Val Palot, on bovine dung, 16.05.96, leg. & det. G. Medardi (pers. herb. GM); Serle, on bovine dung, 29.06.97, leg. & det. G. Medardi (pers. herb. GM); PIEMONTE, VERCELLI, Alagna Valsesia, on excrement, 25.06.94, leg. & det. G. Ricci (MCVE 22682); VERBANIA, Druogno, Val Vigezzo, 15.05.10, leg. & det. G. Medardi (pers. herb. GM, as *P. granularis*); TRENTINO-ALTO ADIGE, TRENTO, Bersone, on equine dung, 23.06.89 and 18.05.99, leg. & det. G. Medardi, (in pers. herb. GM); Pozza di Fassa, on bovine dung, 13.08.94, leg. & det. E. Bizio (pers. herb. EB 130894-32; FH 00301727); Rabbi, Val di Rabbi, on the ground, 11.09.99, leg. G. Baiano, det. G. Medardi (MCVE 14977, as *P. granularis*); Tonadico, Passo Valles, on bovine dung, 28.06.88, leg. & det. E. Bizio (pers. herb. EB); VENETO, BELLUNO, Taibon Agordino, Valle S. Lucano, on bovine dung, 21.08.01, leg. & det. E. Bizio (MCVE 16013); VENEZIA, on burnt ground, 19.10.02, leg. & det. E. Bizio, (MCVE 1644, as *P. granularis*); Oasi Ca' Roman, on burnt ground, 03.05.97, leg. & det. D. Garofoli (MCVE 12628, as *P. granularis*); Tonezza Cimone, on bovine dung, 03.06.88, leg. & det. E. Bizio (pers. herb. EB); TOSCANA, SIENA, Ginestreto, on bovine dung, 26.01.97, leg. & det. C. Perini (SIENA 2482, as *P. vesiculosa*). RUSSIA: KAMCHATKA, 47 Km from Krapivnaya, on excrement of *Ursus ursus*, 06.08.78, leg. B. Kullman, det. not declared, (TAAM 187899); 47 Km from Krapivnaya, on excrement of *Ursus ursus*, 06.08.78, leg. B. Kullman, det. not declared (TAAM 187900); KRASNODAR, UMPYR, Caucasus Nature Reserve, on excrement, 10.08.76, leg. M. Pallo, det. A. Raitviir (TAAM 064318); Malaya Laba, Caucasus Nature Reserve, on excrement, 12.08.76, leg. M. Pallo, det. A. Raitviir (TAAM 064369); KUNASHIR ISLAND, LAGUNNOYE, on excrement, 12.08.70, leg. B. Kullman, det. not declared (TAAM 061513); RESPUBLIKA TYVA, ERZIN, on excrement, 18.07.72, leg. B. Kullman, det. not declared (TAAM 065761). TAJIKISTAN. RAMIT STATE NATURE RESERVE, Mount Hissar, on excrement, 12.04.77, leg. & det. A. Raitviir (TAAM 064569). UZBEKISTAN: Dshisaki, TURKESTAN CHAIN, Kulsai, Zaamini Nature Reserve, in *Juniperus* forest, 24.05.80, leg. K. Kalamees, det. A. Raitviir (TAAM 199165).

Peziza varia (collections provisionally assigned to *P. fimeti* sensu Seaver, before morphological and phylogenetic analysis): ITALY: FRIULI-VENEZIA GIULIA, TRIESTE, Monte Valerio, on equine dung, 19.02.02, leg. & det. F. Bersan (MCVE 23136, as *P. vesiculosa*); Muggia, Rio Storto, on bovine dung, 07.04.94, leg. & det. M. Zugna (MCVE 3637, as *P. vesiculosa*); UDINE, Terzo, on equine dung, 15.04.94, leg. & det. A. Pergolini (AMB002072, as *P. vesiculosa*; FH00301726); TRENTINO-ALTO ADIGE, TRENTO, Bersone,

on equine dung, 16.05.89, leg. & det. G. Medardi (MCVE 11322, as *P. fimeti*); VENETO, VENEZIA, Mestre, Parco Villa Franchin, on equine dung, 26.03.98, leg. L. Levorato, det. L. Levorato & A. Camoli (MCVE 13876, as *P. vesiculosa*). RUSSIA: KAMCHATKA, 47 Km from Krapivnaya, on excrement of *Ursus ursus*, 06.08.78, leg. B. Kullman, det. not declared (TAAM 116381, as *P. fimeti*); YAMALO-NENETSKIY AVTONOMNYI OKRUG, Ovgort, on horse excrement and ground near, 28.07.76, leg. Murdvee, det. not declared (TAAM 110066, as *P. fimeti*).

Morphological studies

Microscopic characters were measured and described from material mounted in water or sometimes in 5% KOH to rehydrate dried material. Other mounting media were Melzer's reagent and Cotton blue in lactic acid. Specimens were studied morphologically and photographed using an Optika optical microscope (BK 1301 model) with 40× or 100× (immersion oil) objectives. Spore dimensions were calculated measuring 50 mature spores.

DNA isolation, PCR, and sequencing techniques

Genomic DNA was extracted from the herbarium specimens (TABLE 1) using the Qiagen DNeasy Plant Mini Kit (Qiagen, Germany; cat. no. 69104). A 1/10 and 1/100 dilution of the DNA was used for PCR amplification of the ITS rDNA region using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR parameters followed LoBuglio et al. (1993), using 35 PCR amplification cycles performed in a Peltier Thermal cycler PTC-200 (MJ Research, Watertown, MA) using EconoTaq DNA Polymerase (Lucigen, Middleton, WI).

PCR amplification, purification, and sequencing techniques followed Hansen et al. (2005). DNA sequences were edited in Sequencher 4.6 (GeneCodes, Ann Arbor, Michigan). The nine DNA sequences determined in this study were deposited in GenBank (JQ654487- JQ654495).

DNA sequence analyses

DNA sequences were aligned using ClustalW through the Cipres Science Gateway (ML; Miller et al. 2009) and then manually adjusted with Se-Al v 2.0a8 (Rambaut 1996), or Mesquite v 2.75 (Maddison & Maddison 2011). The nine ITS sequences of the *Peziza fimeti* isolates were aligned with 86 ITS sequences of the *Peziza* species included by Hansen et al. (2002) (<http://treebase.org/treebase-web/search/study/summary.html?id=900>). All data was included in the analyses.

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). Due to the large number of trees obtained with parsimony analysis, a Maximum Parsimony phylogenetic search, as outlined by Hansen et al. (1999, 2002) was employed. Each maximum parsimony analysis was performed in two parts as described by Hansen et al (2002): First, 1000 heuristic searches were performed, with random taxon addition and TBR branch swapping, with MAXTREES unrestricted, keeping only up to 15 trees per replicate. Next, exhaustive swapping was performed on all of the most parsimonious trees discovered in the first part of the analysis, with MAXTREES set to 15000. Branch support for MP and ML analyses was determined by 1000 bootstrap replicates. One *Peziza ampelina* Quél. [nom.

illegit., non Pass.] and two *P. subcitrina* (Bres.) Korf sequences were included in this analysis as outgroup.

Results & discussion

Phylogenetic analyses of ITS rDNA sequences place isolate AMB 002072 (identified as *P. vesiculosa* by the collector but as *P. fimeti* sensu Seaver in our

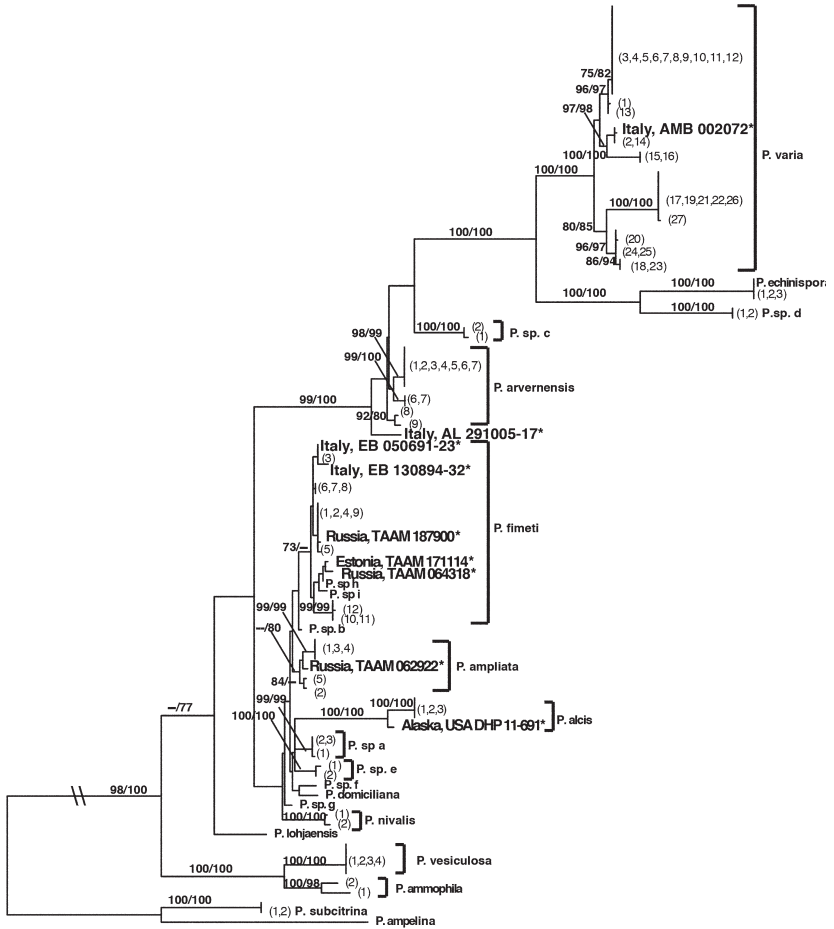


FIGURE 1. Phylogenetic tree of *Peziza* species inferred by Maximum Likelihood. Maximum Parsimony and Maximum Likelihood Bootstrap values above 70% are separated by / and shown either above the branches, or to the left of the branches when space is limited. Branch support for both analyses was determined by 1000 bootstrap replicates. Numbers in parentheses refer to isolate numbers as listed in Hansen et al. (2002). The nine ITS sequences of the *Peziza fimeti* isolates determined in this study are highlighted in bold followed by *.

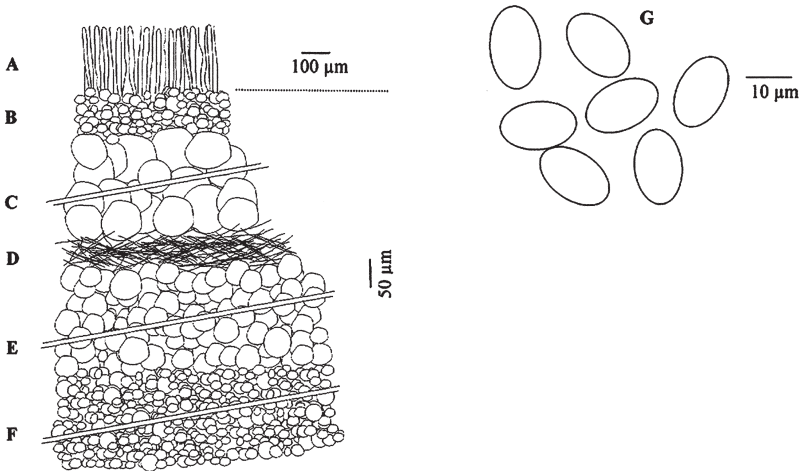


FIGURE 2. *Peziza varia*. Ascoma, vertical section: A. hymenium; B. subhymenium; C. upper medullar excipulum; D. median medullar excipulum; E. lower medullar excipulum; F. ectal excipulum; G. released ascospores.

provisional determinations) in the *P. varia* complex (FIG.1). Within this complex are several well- supported lineages (Hansen et al. 2002; this study, FIG. 1).

The *Peziza varia* complex is a heterogeneous and complicated assembly of widespread species whose diversity has not been well defined. A number of characters are highly variable, such as presence or absence of a stalk, thickness of excipular layers, cell types in the ectal excipulum, and presence or absence of moniliform paraphyses.

ITS analyses highlight the environmentally influenced variability of these morphological characters. The substratum is normally thought to be a very significant taxonomic character in *Peziza* Dill. ex Fr., but the ITS analyses indicate that samples from different substrates and from distant locations can be closely related or conspecific (Hansen et al. 2002).

Peziza varia (Hedw.) Alb. & Schwein. has 25–60 mm diam., more or less regularly cup-shaped, sessile or subsessile apothecia; a smooth, pale brown, pale hazelnut hymenial surface; hygrophanous, grey-brownish to whitish when dry and dark brown if wet, slightly scurfy receptacle surface; and a regular or somewhat wavy margin. Microscopical characters are ascospores that are 14–16(–17.5) × 9–11(–12) μm, smooth as seen in the light optical microscope but slightly warted with SEM (Hansen et al. 2002), hyaline, without oil drops;

FIGURE 3. *Peziza varia*. Fresh ascomata in situ. A. on trimmed wood; B. on trimmed and painted wood of a door; C. on sawdust mixed with soil; D. on burnt residue; E. on papery residue; F. on textile residue; G. on humous soil; H. on sandy soil; I. on gravelly soil; L. on bovine dung.



cylindrical, $\leq 280 \mu\text{m}$ long asci, clavate to moniliform paraphyses, and a multi-layered excipulum: textura globulosa-angularis subhymenium with $5\text{--}12 \mu\text{m}$ broad cells, a 3-layered medullary excipulum (internal or upper textura globulosa-angularis layer with $20\text{--}100\text{--}(110) \mu\text{m}$ broad cells; median textura intricata layer; external or lower textura angularis layer with $20\text{--}45 \mu\text{m}$ broad cells), and an ectal textura globulosa excipulum with $15\text{--}20 \mu\text{m}$ broad cells (FIG. 2).

The habitat of *P. varia* is extremely variable; it can grow on several substrates, such as wood (raw or trimmed, sometimes also painted, occasionally buried), sandy or gravelly, calcareous or acid soil, composted loam, between floor tiles, in cellars or caves, on burnt remnants, and on building, textile, or papery residues.

Our study shows that *P. varia* is also able to grow on dung of herbivores, such as equine or bovine (excluding elks), at times on *Ursus* (FIG. 3). Its occurrence on dung, supported by ITS sequence analyses and morphological comparison with the other similar fimicolous species, probably explains the smaller ascospore sizes for *P. fimeti* given by a number of authors. It is possible that some of the mistaken spore measurements are not due to erroneously transcribing data but that this particular *P. varia* ecological variant has gone unobserved.

Peziza varia shows remarkable morphologic affinities to *P. alcis*, until now recorded only from northern Europe where it is found along paths frequented by elk or on leaf-litter moistened with urine (Harmaja 1986). *Peziza alcis* differs in narrower ascospores ($15\text{--}16\text{--}(17) \times 7\text{--}9 \mu\text{m}$) and a medullary excipulum composed of a single textura globulosa layer with $20\text{--}30 \mu\text{m}$ broad cells (FIG. 4).

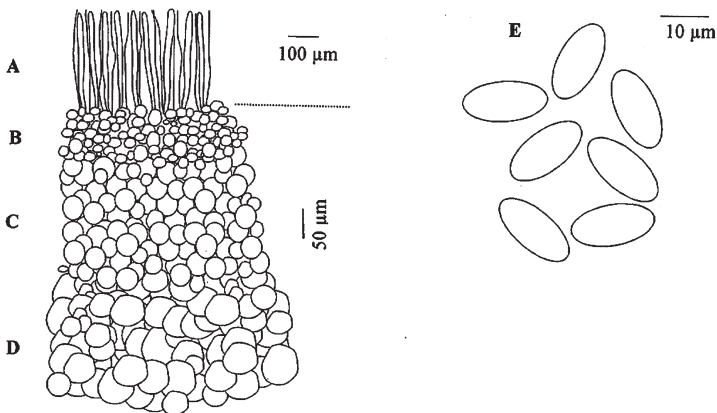


FIGURE 4. *Peziza alcis*. Ascoma, vertical section: A. hymenium; B. subhymenium; C. medullary excipulum; D. ectal excipulum; E. released ascospores.

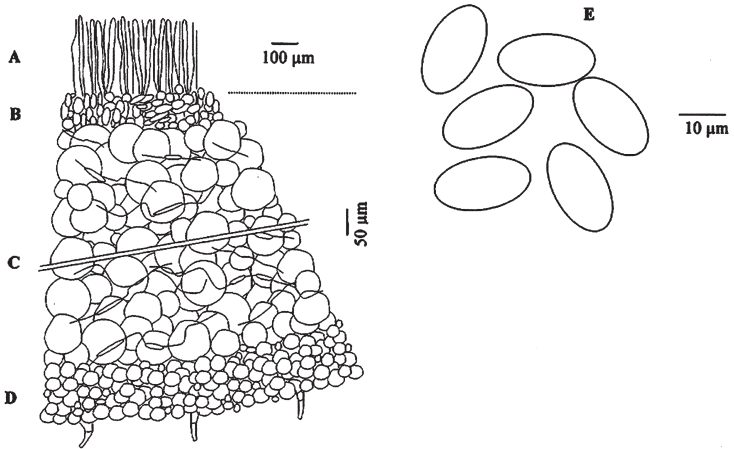


FIGURE 5. *Peziza fimeti*. Ascoma, vertical section: A. hymenium; B. subhymenium; C. medullary excipulum; D. ectal excipulum; E. released ascospores.

Our studies show that *P. alcis* is found in higher circumpolar latitudes (areas frequented by elk), while *P. varia* collected on dung generally comes from lower latitudes where it is not associated with elk.

Peziza fimeti, the most common and widespread species of its group, has been reported worldwide from temperate zones. It fruits on dung of various animals or (at times) on soil or burnt woody remnants (Hansen et al. 2002). It resembles *P. varia* in apothecial colour but has larger ascospores ((18–)20–22.5 × 10–12 µm) and a medullary excipulum composed of a single textura globulosa layer with 50–100 µm broad cells (FIG. 5). These characters, as well as apothecial size and shape, also distinguish *P. fimeti* from *P. vesiculosa* Bull., with which is often confused.

Two specimens provisionally determined as *P. fimeti* sensu Seaver were found not to belong in *P. varia*. Molecular analyses (FIG. 1) of the first sample, TAA(M) 062922 (previously studied by Hansen et al. (2002) cluster it with *P. ampliata* Pers., which normally fruits on woody residue and marsh herbaceous stems (Donadini 1981) or burnt ground (Dougoud 2001). However, dried apothecia are small, cup-shaped and thin instead of turbinate and thick, and also the structural study did not show the characteristic large cells (≤220–240 µm) usually observed in the medullary excipulum of *P. ampliata*. We have been unable to resolve the identity of this taxon. Our molecular analyses place the second divergent collection (AL 291005-17; FH 00301725) in a clade including *P. varia*, *P. arvernensis* Roze & Boud., and *P. echinospora* P. Karst.; its correct identity is also still not understood.

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