Clarification of Peziza fimeti with notes on P. varia collections on dung

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Published Version</td>
<td>doi:10.5248/121.465</td>
</tr>
<tr>
<td>Accessed</td>
<td>January 31, 2017 1:06:48 PM EST</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:30168141">http://nrs.harvard.edu/urn-3:HUL.InstRepos:30168141</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
Clarification of *Peziza fimeti* with notes on *P. varia* collections on dung

**Gianfranco Medardi¹, Angela Lantieri², Donald H. Pfister³, Katherine F. LoBuglio³ & Gabriele Cacialli⁴**

¹Via Giuseppe Mazzini 21, I-25086 Rezzato (Brescia), Italy  
²Department of Biological, Geological and Environmental Sciences, Section of Plant Biology, University of Catania, Antonino Longo 19, I-95125 Catania, Italy  
³Department of Organismic and Evolutionary Biology and the Farlow Herbarium, Harvard University, 22 Divinity Ave, Cambridge, MA 02138  
⁴Via Goito 25, I-25127 Livorno, Italy  
* Correspondence to: *angelalantieri@gmail.com

**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**

This confusion led Hohmeyer (1986) to define two autonomous taxa, “fimeti sensu Dennis, Seaver” and “fimeti sensu Donadini, Gamundi”, distinguished from each other only by the above mentioned spore dimensions. Because of a discrepancy between \( P. \fimeti \) spore sizes as given by Fückel (1871: 16 × 8 µm) in the protologue and modern measurements from the holotype (G: 20–22.5 × 10–12 µm), Hansen et al. (2002) suggested that \( P. \fimeti \) 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 Fückel and to \( Peziza \alcis \) Harmaja. Molecular phylogenetic analysis of the ITS rDNA region showed this latter species is distinct from other \( Peziza \) spp. on heribvore 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 \( Peziza \fimeti \) selected for DNA extraction are listed in Table 1.

**Table 1.** \( Peziza \fimeti \) specimens used in phylogenetic analysis.

<table>
<thead>
<tr>
<th>Location</th>
<th>Collection data</th>
<th>Habitat</th>
<th>ITS Sequence no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy. Trentino-Alto Adige, Trento, Pozza di Fassa</td>
<td>E. Bizio, 13.08. 94 [pers. herb. EB 130894-32; FH 00301727]</td>
<td>Bovine dung</td>
<td>JQ654487</td>
</tr>
<tr>
<td>Italy. Abruzzo, L'Aquila, Civitella Alfedena</td>
<td>E. Bizio, 05.06. 91 [pers. Herb. EB 050691-23; FH 00301728]</td>
<td>Excrement</td>
<td>JQ654488</td>
</tr>
<tr>
<td>Italy. Sicily, Siracusa, Buccheri, Santa Maria's Wood</td>
<td>A. Lantieri, 29.10. 05 [pers. Herb. AL 291005-17; FH 00301725]</td>
<td>Bovine dung</td>
<td>JQ654489</td>
</tr>
<tr>
<td>Italy. Friuli-Venezia Giulia, Udine, Terzo</td>
<td>A. Pergolini, 15.04. 94 [AMB 02072; FH 00301726]</td>
<td>Equine dung</td>
<td>JQ654490</td>
</tr>
<tr>
<td>USA. Alaska, Anchorage, Goose Lake</td>
<td>L. Millman, 07.09. 11 [DHP 11-691; FH 00301724]</td>
<td>Moose dung</td>
<td>JQ654491</td>
</tr>
<tr>
<td>Russia. Chelyabinskaya Oblast, Miass</td>
<td>Parmasto et al., 16.07. 73 [TAAM 062922]</td>
<td>Excrement</td>
<td>JQ654492</td>
</tr>
<tr>
<td>Russia. Krasnodar, Umpyr, Caucasus Nature Reserve</td>
<td>M. Pallo, 10.08. 76 [TAAM 064318]</td>
<td>Excrement</td>
<td>JQ654495</td>
</tr>
<tr>
<td>Russia. Kamchatka, 47 km from Krapivnaya</td>
<td>B. Kullman, 06.08. 78 [TAAM 187900]</td>
<td>Excrement of ( Urus ) ursus</td>
<td>JQ654493</td>
</tr>
<tr>
<td>Estonia. Jõgevamaa Co, Kursi, Puurmani Commune, Alturnga, forestry sq. 95</td>
<td>M. Õpik, 08.10. 97 [TAAM 171114]</td>
<td>Moose dung</td>
<td>JQ654494</td>
</tr>
</tbody>
</table>

Specimens examined: \( Peziza \alcis \): ESTONIA: Jõgevamaa Co, Kursi, Puurmani Commune, Alturnga, Forestry sq. 95, on moose dung, 08.10.97, leg. & det. M. Õpik (TAAM 171114, as \( P. \fimeti \)); 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, Rädkila, 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 Tannenwald, 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); **TRENTO-ALTO ADIGE,** Trento, Bersone, on equine dung, 23.06.89 and 18.05.99, leg. & det. G. Medardi (pers. herb. GM, as *P. granularis*); **TREVISO,** Pieve di Cadè, on excrement, 21.08.01, leg. & det. E. Bizio (pers. herb. EB 002862-14; FH 00301729); **VERBANIA,** Druogno, Val Vigezzo, 15.05.10, leg. & det. G. Medardi (pers. herb. GM, as *P. granularis*); **VENETO,** Belluno, Taibon Agordino, Valle S. Lucano, on bovine dung, 21.08.01, leg. & det. E. Bizio (pers. herb. EB); **TOSCANA,** Siena, 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 12628, 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 (AMB 002072, as *P. vesiculosa*; FH 00301726); **TRENTO-ALTO ADIGE,** Trento, Bersone,
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
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 sub sessile 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 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.

**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, ≤280 µm long asci, clavate to moniliform paraphyses, and a multi-layered excipulum: textura globulosa-angularis subhymenium with 5–12 µm broad cells, a 3-layered medullary excipulum (internal or upper textura globulosa-angularis layer with 20–100(–110) µm broad cells; median textura intricata layer; external or lower textura angularis layer with 20–45 µm broad cells), and an ectal textura globulosa excipulum with 15–20 µm 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 (15–16(–17) × 7–9 µm) and a medullary excipulum composed of a single textura globulosa layer with 20–30 µm broad cells (Fig. 4).

![Figure 4. Peziza alcis. Ascoma, vertical section: A. hymenium; B. subhymenium; C. medullar excipulum; D. ectal excipulum; E. released ascospores.](image-url)
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 it 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.
Acknowledgments

The authors thank Prof. Zheng Wang (USA) and Dott. Francesco Doveri (Italy) for critically reviewing the manuscript, the curators of AMB, G, MCVE, SIENA, TAA(M), for arranging loans of material, and E. Bizio, G. Consiglio, for putting their collections at our disposal. We want to express a particular gratitude to Dr. Parmasto, curator of the Herbarium Instituti Zoologici et Botanici, University of Life Sciences (Tartu, Estonia), for the permission to carry out the molecular analysis on their samples and Prof. Shaun Pennycook for his precious advices and observations.

Literature cited

García AF. 2003. Ascomycetes de la Vall d’Albaida i comarques dels voltants (Comunitat Valenciana). Butlletí de la Sociedad Micológica Valenciana 8: 223–262


