Phylogenetic Diversity in the Core Group of Peziza Inferred from ITS Sequences and Morphology

Phylogenetic diversity in the core group of *Peziza* inferred from ITS sequences and morphology

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Species delimitation within the core group of *Peziza* is highly controversial. The group, typified by *P. vesiculosa*, is morphologically coherent and in previous analyses of LSU rDNA sequences it formed a highly supported clade. Phylogenetic diversity and species limits were investigated within the group using sequences from the complete ITS region (ITS1-5.8S-ITS2). Eighty-three specimens were selected for molecular study from a larger sample of material studied morphologically to explore the intra- and interspecific variation of each putative species. The sister group taxon, *P. ampelina* was used as the outgroup and two specimens of *P. subcitrina* were additionally included. Seven independent lineages of rDNA were identified (I–VII), each representing one to several species. These lineages form two larger clades, A (II, and I or III) and B (IV–VII), supported by macromorphology: small (generally < 2 cm), shallowly cup- to disc-shaped apothecia (A) and large (up to 15 cm), deeply cup-shaped to expanded apothecia (B). The overall exciple structure (a stratified or non-stratified medullary layer) and to some degree spore surface relief, likewise support the groupings. Clade A contains taxa with smooth or nearly smooth spores (except for *P. lohjaënsis*), while clade B contains taxa with a range of spore ornamentations, from smooth, finely warty to distinctly warty, and spiny. The position of groups I (*P. vesiculosa* and *P. ammophila*) and III (*P. lohjaënsis*) are uncertain, and these taxa also deviate morphologically from the other clade A members. The following species are recognized based on morphology and ITS rDNA analyses: *P. ammophila* and *P. vesiculosa* (I); *P. alcis*, *P. ampliata*, *P. domiciliana*, *P. fimeti*, *P. nivalis*, and a number of putative species or intraspecific entities (II); *P. lohjaënsis* (III); *P.* sp. c (IV); *P. arvernensis* (V); *P. echinispora* and *P.* sp. d (VI); and *P. varia* (VII). The nomenclature of these species is analyzed and taxa are typified as necessary. Based on ITS and morphology, we found no justification for recognizing more than one species in the 'P. varia complex', including 27 specimens that have been referred to under the names *P. cerea*, *P. micropus* and *P. repanda*, from an array of substrates and different geographical areas. Morphological characters previously used to delimit species within this complex, such as colour variation of the apothecia, presence or absence of a stipe, stratified or non-stratified medullary exciple (or thickness of the excipular layers), cell types in the outermost exciple and moniliform *vs* filiform paraphyses were not correlated with the subgroups supported by ITS analyses and appeared to be plastic. Therefore, *P. cerea* and *P. micropus* are placed in synonymy with *P. varia*. The name *P. repanda* is rejected. Levels of sequence divergence were low within group II, comprising 33 small apothecial specimens. Twelve fine-scale lineages were identified, but the analyses did not resolve relationships among these. *P. granulosa sensu* Boudier is considered a synonym of *P. fimeti*. These have previously been distinguished mainly by occurrence on various soil types, including burnt soil and soil mixed with sawdust or woodchips *vs* on dung. The substrate and habitat have been much emphasized in the taxonomy of *Peziza*, but the results obtained here indicate that populations on a diverse array of substrates may be closely related, or indeed, conspecific.

INTRODUCTION

Molecular phylogenetic studies (Norman & Egger 1996, 1999, Hansen, Læssøe & Pfister 2001) have shown that the genus *Peziza* Fr. 1822 is not monophyletic. Our previous results (Hansen *et al.* 2001), based on phylogenetic analyses of partial LSU rDNA sequences, suggest that *Peziza* is composed of at least six distinct lineages, most of which include other genera of the *Pezizaceae*. It is evident from these studies that, to reflect the phylogeny, *Peziza* must either be subdivided into several genera or a very wide generic concept must be employed. *Peziza* is estimated to comprise 80 species according to Hawksworth *et al.* (1995). No monographic treatment of the genus has ever been undertaken, although a number of subgenera and sections

have been proposed; only a few previous attempts have been made to provide a coherent system (e.g. Donadini 1977, 1978). The group treated here (group IVa, in Hansen *et al.* 2001) includes the type species of *Peziza*, *P. vesiculosa*, and thus must serve as the core for a future recircumscription of *Peziza*. The clade was strongly supported in parsimony analyses of LSU rDNA sequences (bootstrap 91%). Furthermore, the presence of a distinct amyloid ring zone at the apex of the asci is a synapomorphy (shared derived character) for the clade and its sister group, IVb (Hansen *et al.* 2001). It includes many common taxa characterized by mostly yellowish brown, disc- to cup-shaped apothecia, finely warted or smooth, eguttulate spores and simple, hyaline or pale yellowish paraphyses. Habitats comprise undisturbed and disturbed substrates, including rich soil mixed with litter, woody debris or mortar, plaster, brick walls, lime, burnt ground, highly decayed trunks, twigs, wood-chips, sawdust, manured substrate and dung. The species are primarily found in temperate and arctic-alpine areas. All investigated species produce *Oedocephalum* anamorphs (reported for *P. ammophila*, *P. ampliata*, *P. echinispora*, *P. varia* and *P. vesiculosa*) and germinate and grow easily on artificial media (e.g. Berthet 1964a, 1964b, Webster, Rifai & Samy El-Abyad 1964, Paden 1973). Based on this and the habitats, we consider this group of *Peziza* species most likely to be saprotrophic. With a few exceptions the group is equivalent to Boudier's (1885, 1907) concept of the genus *Aleuria* (Fr.) Gillet 1879 non Fuckel 1870, defined primarily by eguttulate ellipsoid spores. While the delimitation of the group seems clear-cut, species limits are highly controversial, and the number of species recognized varies. Furthermore, the nomenclatural situation is unstable. Most type specimens or original material has been lost or never kept, and where material does exists it is of limited value since distinguishing ephemeral characters are not preserved. There are many taxonomically problematic species, because of morphological uniformity. Molecular divergence based on nLSU rDNA (Hansen *et al.* 2001) is also low within the group. Many names have been referred to the core group of *Peziza*; some of that are obvious synonyms while others are difficult or impossible to interpret. The group has never been revised, but most keys to *Peziza sensu lato* include the species names discussed in this paper (e.g. Le Gal 1941, Moser 1963, Romagnesi 1978, Hohmeyer 1986, Dissing 2000, Spooner 2001). Seaver (1928), Maas Geesteranus (1967), Rifai (1968), and Dennis (1981) give descriptions of taxa as well as keys. Several apparent species complexes occur within the group. Rifai (1968) pointed out the '*P. repanda-varia-micropus-cerea* complex' (here referred to as the '*P. varia* complex'), for species with large apothecia and ellipsoid, smooth, small (*ca* 15 μm long) and eguttulate spores. Seaver (1928) only recognized one species within this complex, *P. repanda*, while others have accepted three or four species (e.g. Boudier 1905–10, Svrček 1970, Dennis 1981, Dissing 2000,

Spooner 2001). The most detailed study within this complex is by Svrček (1970), who also included species with finely warted spores and smaller apothecia. He recognized the following species: *P. ampliata*, *P. crassipes*, *P. cerea*, *P. micropus*, *P. varia* and *P. arvernensis*. His study provided a key and updated the nomenclature, although he did not resolve whether *P. repanda* was a synonym of *P. micropus* or a separate species. Another complex centres around fimicolous species and small species on soil, with the following associated names: *P. alcis*, *P. bovina*, *P. fimeti*, *P. granulosa* auct., *P. sepiatra* and *P. nivalis*. Furthermore, there has been considerable confusion over the naming of the snow bank species, *P. nivalis* and others in the same habitat (Pfister 1992), such as *P. ninguis*, *P. fortoulii*, and *P. phaeotheca* that have been treated in several papers (e.g. Donadini 1977, 1979, 1980a) and keys (e.g. Hohmeyer 1986, Spooner 2001).

The core group of *Peziza* includes some of the most difficult taxonomic and nomenclatural problems in the *Pezizales*. In this study, using molecular phylogenetic methods, we aim to evaluate some of the morphological characters and substrate specificities previously used to separate species within this group. We have examined the intra- and interspecific phylogenetic diversity of the group, using sequences of ITS1-5.8S-ITS2 rDNA (ITS) from a broad selection of samples. We have collected and used fresh specimens whenever possible. The primary goals were to delimit species within the core group of *Peziza* using sequence analyses and morphology, and to provide insight into phylogenetic relationships among the species.

MATERIALS AND METHODS

Specimens examined

Morphological studies used type and other material from C, FH, G, H, K, L, PC, PRM, TUR and TURA and from the private herbaria of Roy Kristiansen, Trond Schumacher (to be placed in O), Nancy Weber (to be placed in OSC) and Wulfard Winterhoff. The greater part, however, were collected as part of this study and are deposited in C. All studied specimens have been annotated and a complete list of specimens is available on request from K.H., the British Mycological Society Library at Kew (K), and Farlow Library of Cryptogamic Botany (FH). Morphological studies aided the selection of specimens for subsequent molecular studies. Eighty-three collections of the core group of *Peziza* were selected (Table 1) to cover the range of morphological characters and substrates. Collections from across geographical ranges were included whenever possible. *Peziza ampelina*, a representative for the suggested sister group to the ingroup (group IVb in Hansen *et al.* 2001), was used as the outgroup. In addition, two collections of *P. subcitrina* (a group IVb member in Hansen *et al.* 2001) were included.

Table 1. *Peziza* collections used in the molecular phylogenetic study.

Species[a]	Collection number	Geographic origin and substrate	Year and collector	GenBank accession no.
P. alcis (1)	s.n. (H, holotype)	Finland. Dung of elk (*Alces*)	1977, H. Harmaja	AF491612
P. alcis (2)	KH 00.019 (C)	Norway. Dung of elk (*Alces*)	2000, H. Dissing	AF491611
P. alcis (3)	TL-5529 (C)	Sweden. Dung of elk (*Alces*)	1999, T. Læssøe	AF491610
P. ammophila (1)	KH-98-88 (C)	Denmark. Near *Ammophila* in coastal sand dunes	1988, K. Hansen	AF491622[b]
P. ammophila (2)	NSW 7081 (OSC)	USA, OR. Sand dunes near vegetation	1993, F. Camacho	AF491621
P. ampelina	KH 00.011 (C)	Denmark. Bare, clayey soil	1994, C. Lange	AF491629
P. ampliata (1)	JHC 92-386 (C)	Denmark. Large rotten *Fagus* trunk	1992, J. Heilmann-Clausen	AF491590
P. ampliata (2)	KH-97-81 (C)	Denmark. Bark of indet. deciduous tree	1997, J. H. Petersen	AF491591
P. ampliata (3)	KH-97-130 (C)	Denmark. Soil, wood-chips and debris	1997, C. Lange	AF491589[b]
P. ampliata (4)	KH 00.027 (C)	Denmark. Large *Populus* trunk	2000, K. Hansen	AF491588
P. ampliata (5)	KH 00.004 (C)	Denmark. Lime	2000, C. Lange	AF491592
P. arvernensis (1)	KH-98-08 (C)	Denmark. Soil, among *Fagus* leaves	1998, A. Storgaard	AF491584
P. arvernensis (2)	KH-98-12 (C)	Denmark. Edge of pile of sawdust, wood-chips and small twigs	1998, B. Klug-Andersen	AF491582
P. arvernensis (3)	KS-95-13 (C)	Denmark. Soil, leaves of *Fagus* and sawdust	1995, K. Hansen, S. K. Sandal	AF491585
P. arvernensis (4)	KS-95-09 (C)	Denmark. Soil among leaves along road	1995, K. Hansen, S. K. Sandal	AF491583[b]
P. arvernensis (5)	L 9985 (L)	Netherlands. Wood remnants, cut *Fagus* branches along road	1986, H. van der Aa	AF491581
P. arvernensis (6)	KS-94-28A (C)	Denmark. Disturbed soil, heaps of lime and stone	1994, K. Hansen, S. K. Sandal	AF491578[b]
P. arvernensis (7)	KS-94-145 (C)	Denmark. Soil and leaves of *Fagus*, in ditch	1994, K. Hansen, S. K. Sandal	AF491577
P. arvernensis (8)	s.n. (FH)	USA, CA. Leaf duff, with *Quercus*	1998, B. Niell	AF491579
P. arvernensis (9)	NSW 6714 (OSC)	USA, OR. Edge of burned slash pile	1992, N. S. Weber	AF491580
P. domiciliana	C 52152 (C)	Denmark. Mortar on soil in forest	1989, P. M. Petersen	AF491614[b]
P. echinispora (1)	Jukka Vauras 9110F (TURA)	Finland. Fire site	1994, J. Vauras	AF491575
P. echinispora (2)	NSW 6763 (OSC)	USA, OR. Burnt ground and debris	1992, N. S. Weber, G. Coleman	AF491574
P. echinispora (3)	TL-6404 (C)	Denmark. Burnt ground	2000, T. Læssøe	AF491573
P. fimeti (1)	HK 99.483 (C)	Russia, Sakha. Dung of horse	1999, U. Peintner	AF491597
P. fimeti (2)	HK 99.113 (C)	Russia, Sakha. Dung of cow	1999, H. Knudsen	AF491596
P. fimeti (3)	TL-5449 (C)	Denmark. Dung of cow in calcareous grassland	1999, T. Laessøe	AF491598[b]
P. fimeti (4)	NSW 6266 (OSC)	USA, NM. Aged cow manure	1990, N. S. Weber	AF491595
P. fimeti (5)	HK 2000.69 (C)	Greenland. Dung of reindeer (*Rangifer*)	2000, K. Kalamees	AF491594[b]
P. fimeti (6) as *P. granulosa*	KH-98-26 (C)	Denmark. Soil/sawdust mixture under *Fagus*	1998, H. Knudsen	AF491600[b]
P. fimeti (7) as *P. granulosa*	TL-4935 (C)	Denmark. Soil mixed with wood-chips/ sawdust	1998, T. Laessøe	AF491599
P. fimeti (8) as *P. granulosa*	KS-94-12 (C)	Denmark. Fire site	1994, K. Hansen, S. K. Sandal	AF491601
P. fimeti (9) as *P. granulosa*	HK 99.362 (C)	Russia, Sakha. Sandy, clayey soil on slope from steppe to river	1999, U. Peintner	AF491593
P. fimeti (10) as *P. sepiatra*	TL-5368 (C)	England. Fire site	1999, T. Laessøe, J. H. Petersen	AF491604
P. fimeti (11) as *P. crassipes*	C 52151 (C)	Denmark. Clayey soil with hardwood sawdust	1983, S. A. Elborne, H. Knudsen	AF491606[b]
P. fimeti (12) as *P. granulosa*	KH 00.012 (C)	Denmark. Bare, very moist, rich soil	1994, K. Hansen, S. K. Sandal	AF491605
P. lohjaënsis	s.n. (H, holotype)	Finland. Bare mull, in rich wood on somewhat calcareous ground	1982, H. Harmaja	AF491576
P. nivalis (1)	KH-97-44 (C)	USA, CA. Soil and decaying grasses, at edge of snow bank, burnt ground	1997, K. Hansen	AF491620
P. nivalis (2)	NSW 6740 (OSC)	USA, OR. Soil and charred bits of vegetation near melting snow, burnt ground	1992, N. S. Weber *et al.*	AF491619
P. subcitrina (1)	KH-97-133 (C)	Denmark. Bare soil along ditch	1997, K. Hansen, C. Lange	AF491628
P. subcitrina (2)	KH 00.023 (C)	Denmark. Soil and sawdust, along forest road	2000, K. Hansen, S. Landvik	AF491627[b]
P. varia (1) as *P. cerea*	KH-97-54 (C)	Denmark. Plaster in basement	1997, J. H. Petersen	AF491559
P. varia (2) as *P. cerea*	KH-97-01 (C)	USA, MA. On wet carpet in basement and directly on plaster	1997, K. Hansen	AF491555
P. varia (3) as *P.* cf. *repanda*	Jukka Vauras 1271F (TUR)	Finland. Basement, near concrete wall of coarse sand	1982, J. Vauras	AF491570[b]

Table 1. (*cont.*)

Species[a]	Collection number	Geographic origin and substrate	Year and collector	GenBank accession no.
P. varia (4)	KH 00.003 (C)	Denmark. Hardwood sawdust and on rich soil	2000, H. Knudsen, M. Sasa	AF491569
P. varia (5)	KH 00.022 (C)	Denmark. Sawdust from *Larix* and on rich soil	1999, H. Knudsen	AF491568[b]
P. varia (6) as *P. cerea*	KH 00.033 (C)	Denmark. Gravel and sand, in driveway to a house	2000, H. H. Schierup	AF491567
P. varia (7) as *P.* cf. *micropus*	KH 00.028 (C)	Denmark. Large *Populus* trunk	2000, K. Hansen	AF491562
P. varia (8)	KH-97-02 (C)	USA, MA. Decorticated indet. hardwood	1997, D. S. Hibbett	AF491561
P. varia (9) as *P.* cf. *repanda*	NSW 6504 (OSC)	USA, OR. Wood, soil with woody debris and sunflower fruits	1991, N. S. Weber	AF491565
P. varia (10) as *P. micropus*	KH-98-104 (C)	Denmark. Dead standing *Ulmus*	1998, J. Heilmann-Clausen	AF491566
P. varia (11)	KH-98-38 (C)	Denmark. Innerside of bark of large *Salix* trunk	1998, K. Hansen	AF491564
P. varia (12)	KH-98-03 (C)	Denmark. Decayed *Salix* trunk	1998, K. Hansen	AF491563
P. varia (13) as *P.* cf. *cerea*	s.n. (TUR)	Finland. Brick wall, where excess water has penetrated the structure	1994, S. Huhtinen	AF491560
P. varia (14) as *P.* cf. *micropus*	KH-97-88 (C)	Denmark. Large *Fagus* trunk	1997, K. Hansen	AF491556
P. varia (15)	KH-99-04 (C)	USA, MA. Very decayed wood	1999, Z.-L. Yang	AF491558
P. varia (16) as *P. cerea* and *P. arvernensis*	s.n. (TUR)	Finland. Not stated	1978, Y. Mäkinen	AF491557
P. varia (17) as *P. micropus*	KH-97-107 (C)	Denmark. Large *Fagus* trunk	1997, K. Hansen	AF491549
P. varia (18) as *P. micropus*	TL-4604 (C)	Russia, Kamchatka. Decayed *Betula* trunk	1997, H. Knudsen	AF491554
P. varia (19) as *P. micropus*	KH-97-125 (C)	Denmark. Large *Fagus* trunk	1997, K. Hansen *et al.*	AF491547
P. varia (20) as *P. micropus*	TL-4691 (C)	Russia, Kamchatka. Large *Populus* trunk	1997, T. Læssøe	AF491552
P. varia (21) as *P. micropus*	JHP 00.283 (C)	Denmark. Decayed *Fagus* trunk	2000, T. Læssøe, J. H. Petersen	AF491548
P. varia (22) as *P. repanda*	TL-5396 (C)	England. Decayed *Fagus* trunk	1999, T. Læssøe, J. H. Petersen	AF491546
P. varia (23) as *P. repanda*	s.n. (TUR)	Finland. Decayed *Populus tremula* trunk	1992, L. Kosonen	AF491553
P. varia (24)	KH-99-08 (C)	USA, MA. Wood, at the base of live but old *Acer*	1999, K. Griffith	AF491550
P. varia (25)	D-400 (C)	USA, MA. Decayed *Ulmus* trunk	1999, K. Griffith *et al.*	AF491551
P. varia (26) as *P.* cf. *micropus*	KH 00.032 (C)	Denmark. Bark of decayed *Quercus* trunk	2000, M. Kemp	AF491545
P. varia (27) as *P. arvernensis*	HFG 82.167 (C)	Denmark. Decaying *Fagus* trunk	1982, H. F. Gøtzsche	AF491544
P. vesiculosa (1)	JV 95.652 (C)	Denmark. Straw	1995, S. Hansen, J. Vesterholt	AF491626
P. vesiculosa (2)	NSW 6124 (OSC)	USA, OR. Bales of hay	1989, R. Crawford, J. Weber	AF491625
P. vesiculosa (3)	C 52150 (C)	England. Mulched beds, with *Taxus* and *Cedrus*	2000, B. M. Spooner	AF491624[b]
P. vesiculosa (4)	TL-6398 (C)	Denmark. Horse manure	2000, T. Læssøe	AF491623
Peziza sp. a (1)	KH-97-99a (C)	Denmark. Almost bare calcareous soil	1997, C. Lange	AF491617[b]
Peziza sp. a (2)	KH-97-129 (C)	Denmark. Bare clayey soil in old wheel track	1997, C. Lange	AF491615
Peziza sp. a (3)	KH-97-134 (C)	Denmark. Rich soil, along forest road	1997, K. Hansen *et al.*	AF491616
Peziza sp. b	KH-98-53 (C)	Austria. Bare soil, under *Cirsium*, alt. 1800 m	1998, K. Hansen	AF491609
Peziza sp. c (1)	KH 00.002 (C)	Denmark. Hardwood sawdust and rich soil	2000, H. Knudsen, M. Sasa	AF491587
Peziza sp. c (2)	KH 00.020 (C)	Denmark. Sawdust from *Larix* and rich soil	1999, H. Knudsen	AF491586[b]
Peziza sp. d (1)	NSW 6406 (OSC)	USA, OR. Soil mixed with wood chips	1991, N. S. Weber *et al.*	AF491572
Peziza sp. d (2)	NSW 6429 (OSC)	USA, OR. Soil among grasses at edge of road	1991, N. S. Weber	AF491571
Peziza sp. e (1)	HD S85.41 (C)	Svalbard. Silt with running water	1985, H. Dissing	AF491608
Peziza sp. e (2)	HK 87-273 (C)	Greenland. Sandy clay among *Juncus* and grasses	1987, H. Knudsen	AF491607

Table 1. (*cont.*)

Species[a]	Collection number	Geographic origin and substrate	Year and collector	GenBank accession no.
Peziza sp. f	NSW 7213 (OSC)	USA, OR. Soil at campfire site	1994, L. Wilson	AF491613
Peziza sp. g	NSW 6762 (OSC)	USA, OR. Damp, disturbed mineral soil	1992, N. S. Weber, G. Coleman	AF491618
Peziza sp. h as *P. fimeti*	TAA 128087 (TAA)	Estonia. Dung of Beaver (*Castor*)	1997, A. Kollom	AF491602
Peziza sp. i	C 52153 (C)	Denmark. On dead *Phragmites*, by lake	1982, S. A. Elborne, H. Knudsen	AF491603

[a] Species determination by K. Hansen based on morphological characters and ITS rDNA.

[b] Sequences provided by Sara Landvik.

Morphological methods

All material was studied by light microscopy by K.H. The observations were made from fresh or dried collections. In addition, observations on fresh material by Nancy Weber (NSW collections) were made available for this study. Dried material was revived in water overnight, or for a minimum of 3 h. Hymenial elements were studied by teasing apart a piece of hymenium with a fine needle. Structural features of the exciple were studied using vertical, median sections made by hand or cut on a freezing microtome (15–30 μm thick) (Reichert-Jung, Frigocut E 2800). Measurements and descriptions were made on material mounted in water, or sometimes in 2–10 % KOH for rehydrated dried material. Other chemicals used were Melzer's reagent, Congo red in ammonia, and Cotton blue in lactic acid. The spore surface from several fresh collections of each putative species were viewed with a Phillips scanning electron microscope (SEM) to study the variation of the ornamentation within and between putative species; ascospores were discharged onto a cover glass, air-dried, placed on a stub and coated with gold-palladium alloy.

Molecular techniques

DNA was isolated from dried or fresh ascomata (stored in extraction buffer) and was extracted as outlined in Hansen, Pfister & Hibbett (1999), except the material was not ground in liquid nitrogen, but shaken in a Fastprep™ FP120 Cell Disruptor (BIO 101, CA). Serial dilutions of DNA (1:10, 1:100, 1:1000) were used as template for the polymerase chain reaction (PCR). The nuclear ribosomal ITS1-5.8S-ITS2 region was amplified with the primer pair ITS4 and ITS5 (White *et al*. 1990). The amplifications were done in a Peltier Thermal Cycler PTC-200 using the following program: 35–40 cycles at 94 °C for 30 s, 60 ° for 30 s, and 72 for 1.30 min, followed by a 14 ° soak. PCR fragments were purified using QIAquick spin columns (1997 Qiagen). The primers ITS1, ITS4, and internal primers ITS3 and 5.8S (White *et al*. 1990, Hibbett *et al*. 1995) were used for BigDye terminator cycle sequencing (Applied Biosystems, Foster City, CA). Cycle sequencing was done in a Peltier Thermal Cycler PTC-100 (MJ Research, Watertown, MA) using the following program: 25 cycles of 30 s at 98 ° and 4 min at 60 °,

followed by a 14 ° soak. Sequencing reactions were purified using the DyeEx™ spin kit (2000 Qiagen). Sequencing reactions were electrophoresed and data collected on an Applied Biosystems 377 automated DNA sequencer (Perkin-Elmer/ABI). Fifteen of the collections were sequenced by Sara Landvik (Novozymes, Copenhagen) using a slightly different procedure: the DNA was extracted with the FastDNA Kit S (BIO 101) and the PCR products were cleaned with the GFX PCR, DNA and Gel Band purification Kit (Amersham Pharmacia Biotech, NJ). Sequencing reactions were electrophoresed and data collected on an ABI 3700 (Perkin-Elmer).

Three groups of specimens (IV, V and VII) possessed sequences with short homopolymere regions of 8 to 12 T's or A's in different positions, which caused sequencing problems. The majority of these sequences were obtained by use of internal primers reading in both directions. To obtain complete sequence data from six of these specimens, however, and from three other herbarium specimens (*P. alcis* (1), *P. echinispora* (1) and *P. fimeti* (4)), PCR products, generated with the use of Pfu DNA polymerase, were cloned by use of the PCR-Script™ Amp cloning kit (Stratagene Cloning Systems, La Jolla, CA) following the manufacturer's instructions.

Alignment and phylogenetic analyses

Sequences were edited and contigs assembled using Sequencher 3.0 (GeneCodes, Ann Arbor, MI) and are deposited in GenBank (Table 1). Nucleotide sequences were aligned manually in the Sequence Alignment Editor Se-Al (Rambaut 1996) with alignment gaps inserted to optimize aligned sites. Alignments are available from TreeBASE (http://phylogeny.harvard.edu/treebase) as accession nos M1221 (all taxa), M1222 (Group II separately) and M1223 (Group VII separately). The ITS rDNA contained highly divergent regions, within which only certain subsets of the taxa or populations could be aligned. Therefore, parts of the final dataset were interleaved in alternating blocks of aligned complete sequences and aligned partial sequences. Two data sets, the inclusive and exclusive datasets, were developed to explore the sensitivity of the results to inclusion or exclusion of ambiguously aligned regions. In both data sets gapped positions were

included and treated as missing data (gap = missing coding). The ITS sequence of *P. lohjaënsis* was in preliminary analyses found to be very different from the ITS sequences of the rest of the ingroup. To examine the sensitivity of the results to the inclusion or exclusion of *P. lohjaënsis*, this species was omitted in a separate analysis of the inclusive dataset.

Based upon analyses including all collections, which identified two distinct larger clades (designated group II and VII), two additional alignments were constructed that included only collections from each clade. In this way the total number of gaps required for alignment were reduced and the possibility of inclusion of additional characters from regions that were too divergent to be aligned across all taxa were explored. In analyses of the two subset alignments, gaps were scored as missing or as characters (indel coding) following guidelines outlined in Hibbett *et al.* (1995). *Peziza arvernensis* (7) (numbers in parentheses refer to specimen codes in Table 1) was used as an outgroup for both clades, because it was the easiest to align from among the sister group taxa.

Phylogenetic analyses were performed in PAUP*4.0b8 (Swofford 1998) on an iMac 350 MHz. All characters were equally weighted and unordered. Each maximum parsimony analysis was performed in two parts based on strategies designed by Maddison, Ruvolo & Swofford (1992) and Olmstead *et al.* (1993). First, 1000 heuristic searches were performed, with random taxon addition and TBR branch swapping, with MAXTREES unrestricted, keeping only up to two trees per replicate. Secondly, exhaustive swapping was performed on all of the most parsimonious trees discovered in the first part of the analysis, with MAXTREES set to 15000. Relative robustness of individual branches was estimated by bootstrap, using 1000 replicates, with heuristic searches, 100 random addition sequences, TBR branch swapping and MAXTREES set at 100. For the two subset alignments, each of group II and VII, branch-and-bound searches were in addition conducted, which guarantees discovery of all most parsimonious trees. Under this search method some of the specimens with identical sequences in the group VII dataset, were excluded to reduce the search time.

RESULTS

Alignment

The datasets consisted of 86 ITS rDNA sequences determined in this study (Table 1). The inclusive data set included all characters. The exclusive data set omitted four divergent or particularly ambiguous regions: positions 173–308, 360–390, 757–780 and 807–825. The ITS region varies considerably in length (Table 2), even though these taxa are considered closely related species based on LSU rDNA sequences and morphology. ITS1 was found to vary in length from

Table 2. Nucleotide length of ITS1, ITS2 and ITS1-5.8S-ITS2 (excluding gaps).

Lineage/species	ITS1	ITS2	Total ITS
I/*P. ammophila*	218–236	182–187	557–580
I/*P. vesiculosa*	232	216	605
II	192–195	171–176	522–526
II/*P. alcis*	207	174	538
III/*P. lohjaënsis*	203	176	536
IV/*P.* sp. c	231	176	564
V/*P. arvernensis*	221	176–180	554–559
VI/*P. echinispora*	238	197	592
VI/*P.* sp. d	256	185	598
VII a	223–226	188–191	568–574
VII b/Denmark	235	210	597
VII b/Kamchatka	247	193	602

approximately 192 to 256 bp (excluding gaps) within the core group of *Peziza*, and ITS2 ranged in length from approximately 171 to 216 bp. The shortest ITS1 and ITS2 were found within the group of species with small apothecia (*P.* sp. g and *P. fimeti*), while the longest ITS1 was found in *P.* sp. d and the longest ITS2 in *P. vesiculosa*. In general the different lengths of the ITS region correspond to the groups recovered by the phylogenetic analyses (Table 2, Figs 1–3). ITS1 and ITS2 were aligned along with the intervening 5.8S rDNA (uniformly 157 bp long) and flanking partial sequences of 18S (*ca* 59 bp) and 25S rDNA (*ca* 59 bp). The aligned length of all sequences, including inserted gaps, was 899 bp.

Phylogenetic relationships

The number of included, variable and parsimony informative characters for each data set are given in Table 3, along with the number and length of the most parsimonious trees (MPTs) found in each analysis, consistency index [CI] and retention index [RI].

All taxa

The results of the different analyses, including all collections, differed slightly in bootstrap values and in degree of resolution of the strict consensus trees, but no conflicting nodes received bootstrap support above 65%. The strict consensus trees of the inclusive data set (Fig. 1) and the exclusive dataset (Fig. 2) demonstrate these differences. The basal node of the ingroup topology differs under the two sets of analyses. In the strict consensus tree of the inclusive data set the ingroup root is placed along the branch leading to group I (Fig. 1), thus grouping *P. lohjaënsis* with group II (bootstrap 63%). Whereas in the strict consensus tree of the exclusive data set the ingroup root is placed along the branch leading to the *P. lohjaënsis* lineage (Fig. 2), grouping *P. vesiculosa* and *P. ammophila* with group II (bootstrap < 50%).

The core group of *Peziza* (group IVa in Hansen *et al.* 2001) was highly supported as monophyletic (bootstrap

Table 3. Information of the various data sets analyzed and the most parsimonious trees found.

Data set	Matrix size	Characters			Trees			
		Included	Variable	Informative	Number	Length	CI	RI
Inclusive[a]	899	899	353	311	> 15 000	793	0.660	0.927
Exclusive[a]	899	689	235	197	> 15 000	532	0.635	0.922
Group II[a]	694	694	122	87	90	196	0.740	0.849
Group II[b]	709	698	134	101	108	216	0.745	0.857
Group VII[a]	766	766	97	56	156	117	0.897	0.958
Group VII[b]	780	774	109	69	156	135	0.904	0.966

[a] Gap = missing coding.
[b] Gap = indel coding.

99%). Despite the large number of trees the strict consensus trees are fairly well resolved. Seven distinct rDNA lineages of species or populations were identified (groups I–VII) that were highly supported (bootstrap 89–100%) (Figs 1–2), except for lineage V that only received 58% bootstrap support in the strict consensus tree of the exclusive data set (Fig. 2). In the strict consensus tree of the inclusive data set, however, lineage V was supported by 98% of the bootstrap replicates (Fig. 1). The single collection of *P. lohjaënsis* constitutes a separate lineage (group III). Excluding *P. lohjaënsis* from the analysis of the inclusive data set did not change the overall topology, except the strict consensus tree was more resolved within group II (topology not shown), and within and between the groups IV–VII (congruent with the tree derived from the exclusive data set, Fig. 2). The overall topology is congruent with the topology derived from LSU rDNA sequences, although not all lineages were sampled previously (Hansen *et al.* 2001).

Group I, supported by 100% of the bootstrap replicates, include four collections of *P. vesiculosa* and two collections of *P. ammophila* (Figs 1–2). The branch leading to group I is the longest in the ingroup (54 steps, inclusive dataset, Fig. 3). Furthermore, additional long branches lead to *P. vesiculosa* (25 steps) and to *P. ammophila* (34 steps). Group II includes 33 collections, all with small apothecia, that represent a range of putative species, such as *P. alcis*, *P. ampliata*, *P. fimeti* and *P. nivalis*. Relationships among these taxa are not resolved in the strict consensus tree (Fig. 1) or only with very low support (Fig. 2), but 89–98% of the bootstrap replicates support monophyly of group II taxa. In general, levels of sequence divergence (ITS rDNA) within group II are low based on the branch lengths (Fig. 3). Nevertheless, sequences of the three collections of *P. alcis* were extremely divergent within the group with a branch length of 33 bp (Fig. 3). Groups IV–VII form a clade B (bootstrap 76–86%), all of specimens with large apothecia, as a sister group to the specimens with small apothecia, groups II and I or III, (clade A) (Figs 1–3). Within clade B, group VI and VII form a highly supported clade (bootstrap 96–100%). In the strict consensus tree of the inclusive data set (Fig. 1) group IV, V and VI–VII form a trichotomy. In the

strict consensus tree of the exclusive data set (Fig. 2) group IV is a sister group to the groups VI–VII, although bootstrap support is weak (56%), with group V as a sister group to these. Group IV includes two collections of *P.* sp. c from Denmark (bootstrap 100%). Group V includes two well-supported clades of *P. arvernensis*, group Va contains seven collections of *P. arvernensis* from Denmark and the Netherlands (bootstrap 79–90%), while Vb contains two collections from western North America (bootstrap 81–99%) (Figs 1–3). Group VI, which was supported by 94–98% of the bootstrap replicates, contains two strongly supported lineages of three collections of *P. echinispora* (bootstrap 100%) and two collections of *P.* sp. d from western North America (bootstrap 100%). The next longest branch in the ingroup leads to group VI (38 steps) with the third longest leading to *P. echinispora* (36 steps) and to *P.* sp. d (36 steps) (inclusive dataset, Fig. 3). Group VII includes 27 collections from the '*P. varia* complex', including specimens that have been referred to under the names *P. cerea*, *P. micropus* and *P. repanda* (bootstrap 92–97%). In the analysis of the exclusive dataset two lineages were identified (VIIa, b), but only weakly supported (bootstrap 60% and < 50%). Whereas in analysis of the inclusive dataset four lineages were identified with moderate to high support (70–100%), but relationships between these were unresolved.

Group II and VII taxa in separate analyses

Realignment of the sequences of populations/taxa of each of the groups II and VII allowed unambiguous alignment of more of the molecular characters, with a potential for a more reliable resolution of the fine-scale relationships. The length of the alignment was reduced compared to the alignment including all 86 taxa (Table 3), since many fewer gaps were required, thus reducing the possibility of homoplasy. The topology of the strict consensus trees produced with the branch and bound searches (under indel coding) were identical to those produced with heuristic searches (Figs 4, 6). The number of informative characters increased under indel coding (Table 3) and a higher resolution was obtained for group II members, but the resolved nodes yielded
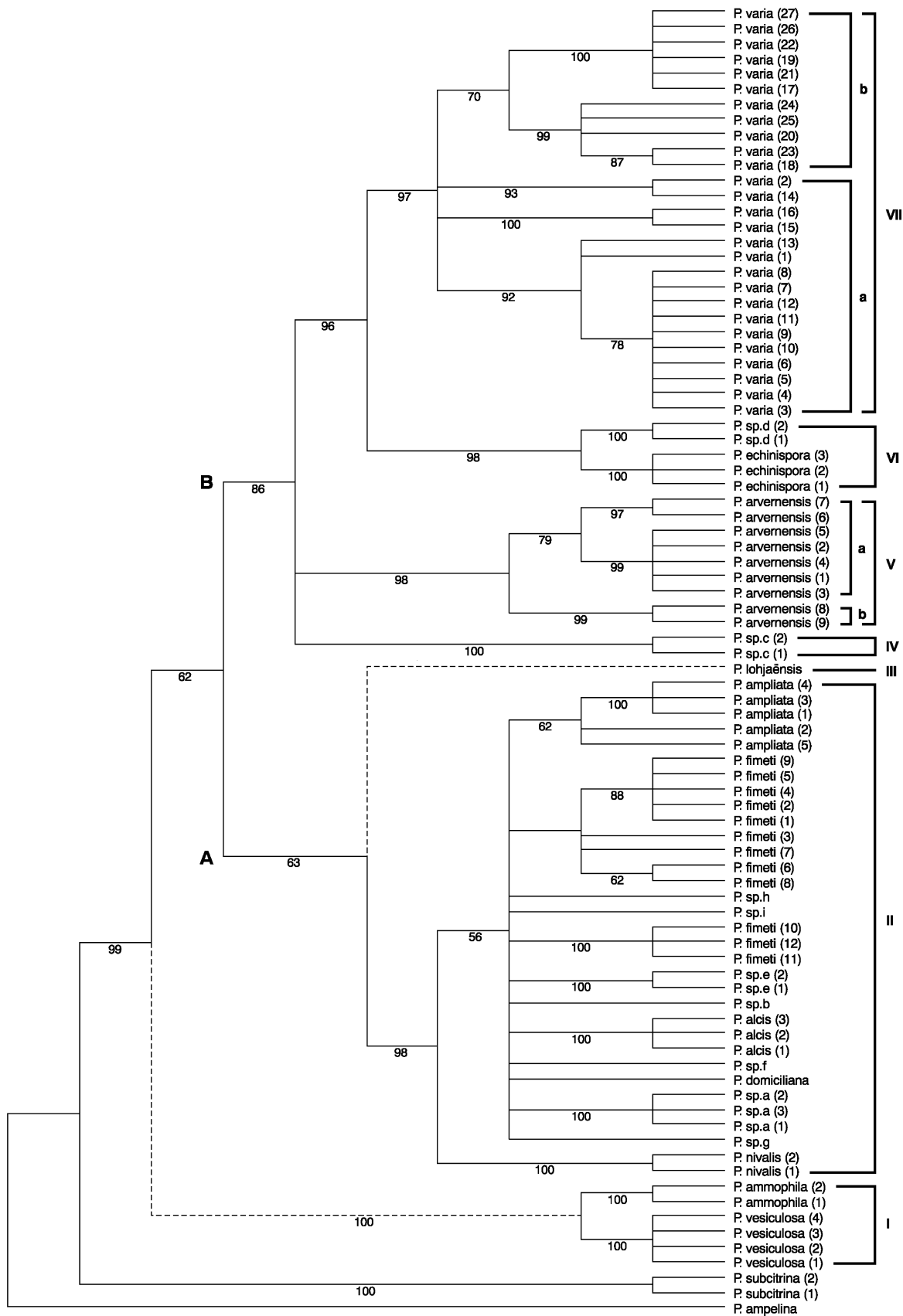
Fig. 1. Strict consensus tree of 15 000 most parsimonious trees generated from the inclusive dataset of ITS sequences. Terminal taxa are individual specimens (see Table 1). Numbers below branches are frequency of occurence in 1000 bootstrap replicates (values < 50 % not shown). Bracketed groups I–VII and the two main lineages A and B are discussed in the text. Dashed branches indicate alternate placement of *Peziza lohjaënsis* and *P. vesiculosa/P. ammophila* shown in Fig. 2.
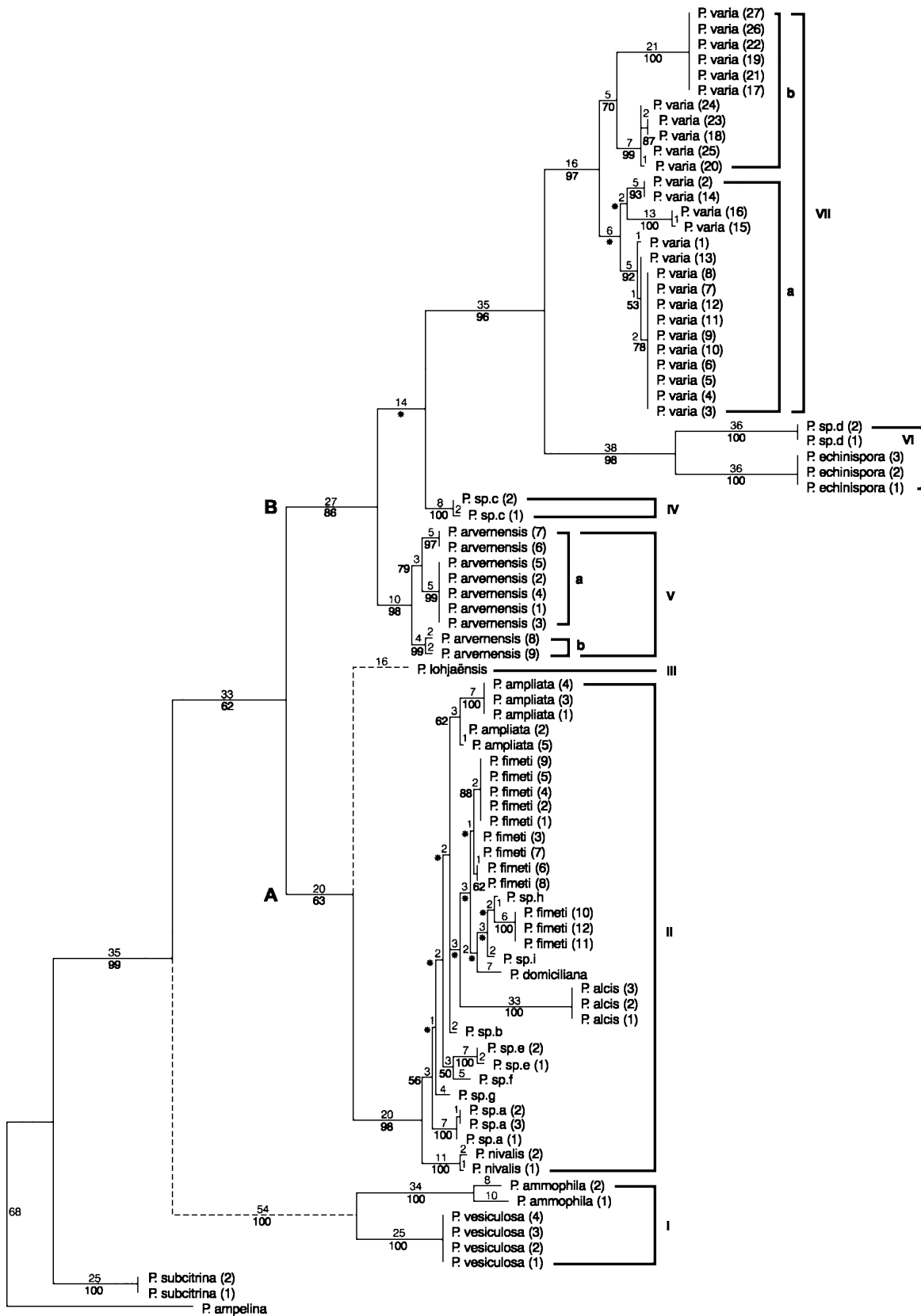
Fig. 2. Strict consensus tree of 15000 most parsimonious trees generated from the exclusive dataset of ITS sequences. Terminal taxa are individual specimens (see Table 1). Numbers below branches are frequency of occurence in 1000 bootstrap replicates. Bracketed groups I–VII and the two main lineages A and B are discussed in the text. Dashed branches indicate alternate placement of *Peziza lohjaënsis* and *P. vesiculosa*/*P. ammophila* shown in Fig. 1.

Fig. 3. Phylogram depicting one of 15 000 most parsimonious trees generated from the inclusive dataset of ITS sequences. Branch lengths are proportional to the number of steps along the branch. Branches that collapse in the strict consensus tree are indicated with an asterisk. Other symbols are as in Figs 1–2.

only weak support ($< 60\%$) (Figs 4–5). The strict consensus trees for group VII obtained under the two coding schemes were identical. Therefore, only trees produced under indel coding are presented (Figs 4–7).

Twelve lineages, which represent species or intra-specific entities (Table 4), were supported within group II (Figs 4–5). Lineages represented by more than one sequence were identical or differed only by one or two base pairs and were supported by 100% of the bootstrap replicates. To a large extent these lineages correlate with distribution, e.g. collections of *Peziza* sp. a were from Denmark, *P.* sp. e from Svalbard and Greenland, *P. nivalis* from western North America and *P. alcis* from Finland, Norway and Sweden. *P. ampliata* was supported by only 67% of the bootstrap replicates. Within the *P. ampliata* lineage, three collections from Denmark, from large trunks or wood-chips, exhibited identical sequences (bootstrap 100%). Within the *P. fimeti* lineage, five collections from Russia, Greenland and eastern North America showed almost identical sequences (bootstrap 84%). However, four collections of *P. fimeti* from Denmark that were almost identical, differed markedly from three other collections from Denmark and the UK, that were likewise almost identical (Fig. 5) (see *P. fimeti* under 'Taxonomic implications' below). ITS sequences fail to resolve relationships among the twelve lineages (Figs 4–5).

Within group VII two lineages were identified in all MPTs (VIIa, b), congruent with the analysis of the exclusive dataset that included all taxa (Figs 2, 6), but they were only weakly supported (bootstrap 55% and 76%). Two divergent and highly supported clades (100% bootstrap) are present within group VIIb (Figs 6–7). The one is composed of six collections from Denmark and the UK, the other of five collections from Finland, eastern North America and Kamchatka. There were no or few base pair differences among collections within each of these clades (Fig. 7), whereas several base pair differences have accumulated between the clades. Two clades, each of two collections, were highly supported within group VIIa (bootstrap 98 and 100%). As a sister group to these, the rest of the group VIIa members form a weakly supported clade (bootstrap 59%). In contrast, in analyses of all collections this clade received 92–94% bootstrap support (Figs 1–3). Group VIIa includes specimens from wide geographical regions (Denmark, Finland, western and eastern North America) but the specimens do not appear in any geographical pattern.

Taxonomic implications

Based on morphology and ITS rDNA analyses the following taxa are recognized in the core group of *Peziza*. Selected morphological characters partly supporting the ITS phylogeny are presented in Table 4. The species are listed as they group based on phylogenetic analyses of ITS (I–VII). For each species results are presented and discussed.

Peziza ammophila Durieu & Lév., *Expl. sci. Algérie*: pl. 28, fig. 2 and *Atlas, Expl. figs*: 10 (1868).

Type: No authentic material has been located at PC; Durieu & Lév., *Expl. sci. Algérie*: pl. 28, fig. 2 (1847) – *lectotypus hic designatus*.

Selected descriptions: Maas Geesteranus (1967: 44), Dennis (1981: 17).

Selected illustrations: Seaver (1928: pl. 64), Maas Geesteranus (1967: fig. 52), Dennis (1981: pl. V A).

Notes: Some authors (Moser 1963, Dennis 1981, Dissing 2000) have cited Durieu & Montagne as the authors of *P. ammophila*. However, although Montagne was an editor and author of some of the cryptogamic sections, Durieu & Léveillé were the authors attributed on the plate and in the explanation of Fig. 2 (Durieu & Léveillé *loc. cit.*). No description of *P. ammophila* was published when the plate was issued or in supplementary papers before 1868. Therefore, we conclude that the description of the plate, published in 1868, constitutes the diagnosis. Plate 28 was first issued in 1847, and Fig. 2 bore the name *P. ammophila*, but this was a *nomen nudum* until the caption was published in 1868. The plate shows characters in full accordance with the current concept of the taxon. It illustrates one apothecium, a mature ascus and one enlarged spore.

P. ammophila is distinguished from the rest of the ingroup by its macromorphology, development and habitat (Table 4). SEM of spores is shown in Fig. 8. ITS sequences of two collections of *P. ammophila*, from Denmark and western North America, form a highly supported clade (bootstrap 100%), but the sequences differ by several base pairs (pairwise differences: 18/601 bp). No morphological differences were detected. ITS sequences of *P. ammophila* are highly divergent from the rest of the ingroup (Fig. 3).

Peziza vesiculosa Bull., *Hist. Champ. France*: 270 (1791): Fr., *Syst. mycol.* **2**: 52 (1822).

Type: Bulliard, *Hist. Champ. France*: pl. 457, fig. 1 F & H (*loc. cit.*) – *lectotypus hic designatus*. Bulliard is not known to have preserved herbarium material (Stafleu & Cowan 1976–88). Fries (*loc. cit.*) excluded elements of Bulliard's (*loc. cit.*) original illustration, but a further deviating element (fig. 1E – too pale and expanded) is excluded here. When introduced, the name *P. vesiculosa* was superfluous, being a later synonym of Bulliard's *P. cerea*, but it is protected by sanctioning (Arts 6.4, 15.1, 52.1). An epitype should be designated when suitable material becomes available, preferably from the type locality.

Selected descriptions: Boudier (1905–10: 139), Dennis (1981: 19), Cacialli, Caroti & Doveri (1995: 545–549).

Selected illustrations: Boudier (1905–10: pl. 257), Ryman & Holmåsen (1984: 625), Breitenbach & Kränzlin (1984: pl. 54), Cacialli *et al.* (1995: pl. 77).

Notes: The four ITS rDNA sequences of *P. vesiculosa* from different geographical areas (Denmark, the UK and western North America) were completely identical,

Table 4. Selected morphological characters that characterize the recognized species of *Peziza* based on the collections used in the molecular phylogenetic study. The taxa are arranged according to the seven (groups I–VII) independent ITS rDNA lineages identified.

Species	Apothecia: diam (cm); shape	Spores: size (μm)[a]; ornamentation LM/SEM; guttulation (+/−)	Paraphyses: pigments; shape	Substrate
I				
P. ammophila	3–5; (pseudo) stipitate, deeply cup-shaped, spreading marginal rays	16–17–18 × 8–9–10; smooth/smooth; −	Hyaline to pale yellowish; straight, slightly enlarged at apex	Sand dunes, often amongst *Ammophila*
P. vesiculosa	2–10; deeply cup-shaped, margin long incurved, hymenium wrinkled to folded towards the centre	20.5–22–24 × 11–12–12.5; smooth/smooth; −	Distinct yellow guttules, turn golden in Melzers; enlarged at apex	Horse manure, straw, hay, manured soil, mulched beds
II				
P. alcis	up to 1; cup- to disc-shaped, plane at maturity, finely crenulate margin	15–16–16.5 × 7.5–8–9; smooth/smooth; −	Hyaline; bent at a sharp angle at very apex	Dung of elk
P. ampliata	1–2; cup- to disc-shaped, margin ±crenulate	18–19.5–20.5 × 9.5–10.5–11.5; smooth/smooth; −	Hyaline; straight, enlarged and slightly bent at apex	Hardwood trunks, rich soil mixed with wood-chips, chalk
P. domiciliana	1–2; shallowly cup-shaped	14.5–15–15 × 7–7.5–8; fine, regular warts (LM); 2 small	Hyaline; enlarged at apex	Mortar
P. fimeti	0.5–1.2 (−2.5); cup-shaped, later often expanding, margin crenulate	18–20–22 × 9.5–11–12.5; smooth/smooth to roughened; −	Hyaline or with distinct yellow guttules; straight or bent at a sharp angle, ±irregularly swollen apically	Dung of cow, horse, reindeer, clayey soil, soil mixed with wood-chips/sawdust, burnt ground
P. nivalis	0.6–2; disc-shaped, appressed to soil, margin crenulate, splitting 3–6 times, often recurved	18–19.5–21 × 10.5–12–13.5; smooth/ smooth or slightly granular surface; −	Hyaline; often moniliform below top cell (caused by high level of moisture)	Soil and decaying grasses, at edge of melting snow bank, burnt ground
P. sp. a (1, 2, 3)	0.8–2.5; disc- to cupshaped	20.5–22.5–23.5 × 12–13–14; smooth or finely warty (×1000)/±irregularly dispersed warts; −	Hyaline vacuoles; straight, slightly enlarged at apex	Bare clayey, calcareous or rich soil, disturbed ground
P. sp. b	up to 1.5; disc- to shallowly cup-shaped, margin raised	21–21.5–22 × 11–11.5–12; smooth/smooth or finely warty; −	Hyaline/yellowish vacuoles; straight, club-shaped at apex	Bare soil, under *Cirsium*, alt. 1800 m
P. sp. e (1, 2)	0.6–1.8; disc-shaped, margin dentate	19.5–20–21.5 × 10.5–11.5–12; smooth/smooth or roughened; −	Reddish-brown amorphous pigment on apices; club-shaped at apex	Silt with running water, sandy clay
P. sp. f	0.8–2.1; shallowly cup-shaped, becoming ± plane	21.5–22.5–23 × 11.5–12–12.5; smooth; −	Hyaline to pale yellowish; straight to curved at apex, club-shaped	Burnt ground
P. sp. g	0.5–1.3; disc-shaped with a raised margin, in age plane	22–24.5 × 12–14.5; smooth; −	Pale brown; straight, club-shaped at apex	Damp, disturbed mineral soil
P. sp. h	up to 1.0; shallowly cup-shaped	16–16.5–17 × 8–9-9.5; smooth	Hyaline or pale yellowish granules; ± bent at very apex	Dung of beaver
P. sp. i	0.4–1.0; shallowly cup-shaped to disc-shaped, plane	18–18.5–19 × 9.5–9.5–10; smooth or finely warty, with thickenings at the poles	Hyaline or pale yellowish granules; ± bent at apex	Dead *Phragmites*
III				
P. lohjaënsis	1–7; cup-shaped, often laterally compressed	18–19–19.5 × 9–9.5–9.5; low, irregularly dispersed warts, becoming larger and fused at the poles	Pale or medium brown content; club-shaped at apex	Rich, bare mull and scanty litter, or on decaying hardwood, often under leaves

Table 4. (*cont.*)

Species	Apothecia: diam (cm); shape	Spores: size (μm)[a]; ornamentation LM/SEM; guttulation (+/−)	Paraphyses: pigments; shape	Substrate
VI				
P. sp. c (1, 2)	2.8–8.0; deeply cup-shaped, outside scurfy-scaly, thick-fleshed	18.5–19.5–20.5 × 9.5–10.5–11; smooth/smooth; −	Hyaline vacuoles; slightly enlarged at apex	Sawdust and on rich soil
V				
P. arvernensis	2–11; deeply cup-shaped, often irregularly folded from the sides, margin often incurved	16–17.5–19(–20) × 7.5–9–10; distinct rounded and elongated warts, irregularly spaced, seen on surface and in outline of spores in ×400–1000/coarse, rounded to elongated warts; −	Hyaline; straight, slightly enlarged at apex	Soil in connection with leaves, small branches, or sawdust
VI				
P. echinispora	1–4.5; cup-shaped, often irregular compressed, expanding in age	15.5–16.5–17 × 7.5–8–9; high (0.5–1 μm), isolated, regular warts or spines, often denser at the poles; −	Hyaline or pale yellowish vacuoles; straight, enlarged at apex	Burnt ground
P. sp. d (1, 2)	1.1–5.5; deeply cup-shaped	19–21–22.5 × 10.5–11–12.5; smooth; −	Hyaline; straight, enlarged at apex	Soil mixed with wood chips, bare disturbed soil
VII				
P. varia	2–15; cup-shaped to expanded, ±folded, sometimes finally convex and with a depressed centre, funnel-shaped, often with a distinct, lighter coloured, crenulate margin	14.5–16–17.5 × 8–9.5–10.5; smooth or very fine, dense warts to fine warts (×1000), seen on surface, not in outline of spores or/very low, fine warts to fine, regular low warts; −	Hyaline or pale yellowish; slightly enlarged at apex	Large decorticated hardwood trunks: *Fagus, Quercus, Betula, Populus, Salix, Ulmus*, or plaster, brick walls, typically in wet basements, gravel, sawdust and rich soil

[a] smallest and largest spore size (excluding aberrant ones) in the collections studied, and the average of all.

but highly divergent from those of the other taxa in the ingroup (Fig. 3). The distinction is evident as well from morphological features displayed by *P. vesiculosa*, such as size and shape of the apothecia, distinct yellow guttules in the paraphyses (Boudier, loc. cit.: pl. 257) which turn golden in Melzer's reagent, large, smooth spores (Fig. 9) and the habitat, being restricted to ±manured straw or hay, richly manured soil and mulched beds, but apparently not or rarely directly on dung. *P. fimeti* has been confused with *P. vesiculosa*, because it occurs on dung and has smooth spores within the same size range, but *P. fimeti* differs in the small, discoid apothecia and other characters (Table 4). Separate analyses of ITS and LSU rDNA data suggest that *P. vesiculosa* and species with small apothecia on dung are highly divergent and occur in separate lineages within the core group of *Peziza*.

Peziza alcis Harmaja, *Karstenia* **26**: 44 (1986); as 'alcidis'.

Type: **Finland**: Uusimaa: Inkoo, Rådkila, alt. *ca* 15 m, grid 27° E 6665:339, ±mesic acid coniferous heath forest, on dung of *Alces*, 9 Nov. 1977, *H. Harmaja* (H – holotype!).

Selected description: Harmaja (1986).
Selected illustration: None.

Notes: The three ITS rDNA sequences of *P. alcis* from Finland (holotype), Norway and Sweden were identical, but extremely divergent from the rest of the taxa with small apothecia, which otherwise were very uniform (Figs 3, 5). Nevertheless, the ITS data does support *P. alcis* within group II (Figs 1–2). Likewise, based on morphological criteria, *P. alcis* appears to be closely related to the rest of the group II members. Within this group it is distinguished by its small spores (Fig. 10), paraphyses characteristically bent at the apex (slightly enlarged and hooked at the tips), and occurrence on elk dung.

One collection, *P.* sp. h, fruiting on dung of beaver, is morphologically indistinguishable from the collections of *P. alcis*, but differences in its ITS sequence led to its grouping with a collection on reed litter, labeled as *P.* sp. i. These collections require further study and recollecting.

Peziza ampliata Pers., *Icon. descr. fung.* **2**: 31, tab. 8, fig. 6 (1800): Fr., *Syst. mycol.* **2**: 76 (1822).

Type: No authentic material has been located in the Persoon herbarium in L; Persoon (*loc. cit.*): tab. 8, fig. 6 – **lectotypus hic designatus**.

Selected descriptions: Svrček (1970: 59), Dennis (1981: 18).

Selected illustrations: Boudier (1905–10: pl. 264), Svrček (1970: figs 1 A–B, 2).

Notes: *P. ampliata* is recognized by small apothecia, up to 2 (–3) cm diam, a rather thick flesh in comparison with the size of the apothecia (*ca* 1–3 mm thick), occurrence on large trunks or smaller pieces of hardwood, and by large, smooth spores (Fig. 11) compared to *P. varia* (Figs 20–25, Table 4). The apothecia are sessile on a broad base or broadly substipitate and are at first shallowly cup-shaped then expanded and flattened. The hymenium is yellowish brown to brown, and may have an olivaceous tint (observed for *P. ampliata* specimens 1 and 4). *P. ampliata* has been described with a non-stratified medullary exciple i.e. without a hyphal middle layer between two layers of globose cells (e.g. Svrček 1970, Dennis 1981). Nevertheless, we found apothecia both with and without stratified exciples. The type plate of *P. ampliata* depicts a single, medium brown, 24 mm wide, sessile apothecium on a decayed piece of wood.

Three of the ITS sequences, from typical *P. ampliata* specimens occurring on wood, were identical (Fig. 5). Two additional collection are provisionally referred to *P. ampliata*, based on ITS, although bootstrap support was low (67 %). One of these, *P. ampliata* (5), differs by the substrate being lime. Only specimens from Denmark were included in the molecular study.

Peziza domiciliana Cooke, *Gard. Chron.* **41**: 793 (1877).

Type: **United Kingdom**: *Scotland*: Edinburgh, on damp walls, Inverleith House, *Balfour* (K – holotype, PC – isotype).

Peziza adae Sadler, *in* Cooke, *Trans. bot. Soc. Edinb.* **13**: 45 (1877): nom. superfl. (Art. 52.1).

Selected description: Maas Geesteranus (1967: 49).

Selected illustrations: Cooke (1879: pl. 97, fig. 349), Maas Geesteranus (1967: fig. 61, spores only).

Excluded illustration: Breitenbach & Kränzlin (1984: pl. 40) (= *P. varia*).

Notes: Type material of *P. domiciliana* was studied by Le Gal (1941; as *Galactinia adae*) and Rifai (1968). They both described and illustrated the spores as minutely warted (Le Gal 1941: fig. 27, Rifai 1968: fig. 252), a character not mentioned in the original description. Like *P. varia*, *P. domiciliana* occurs on damp walls, plaster and mortar. These species have been confused, but *P. domiciliana* differs from *P. varia* by its small (Cooke: 13 × 6–7 μm; Le Gal: 12–14(15) × 6–9 μm), finely warted spores (easily seen in LM × 1000) with two small guttules. It is the only species within the core group of *Peziza* that has guttulate spores. Only a single collection with small apothecia (in C) referable to *P. domiciliana* has been seen. ITS rDNA sequences suggest *P. domiciliana* is closely related to the species with small apothecia (group II), nonetheless its obvious ability to produce apothecia up to 5 cm (Cooke 1879, Maas Geesteranus 1967).

Peziza fimeti (Fuckel) E. C. Hansen, *Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn*: 267 (1876).

Humaria fimeti Fuckel, *Jahrb. Nassauischen Ver. Naturk.* **25–26**: 338 (1871).

Type: [**Germany**: Nassau] auf Kuhmist, in einem Tannenwalde, unterhalb Mappen. Vere. Herb. Barbey-Boissier (G – holotype!; FH – isotype!).

Peziza bovina W. Phillips, *in* Stevenson, *Mycol. Scot.*: 308 (1879).

Type: [**Scotland**: *Grampian*] *Peziza* (*Humaria*) *bovina* ns. Grantown, on cow dung, Aug. 1878, *J. Keith* 80 (K(M): 59523 – holotype!).

Peziza granularis Donadini, *Bull. Soc. Linn. Prov.* **30**: 73 (1977); nom. illegit. This was a replacement name for *Aleuria granulosa* (Schum.) Gillet *sensu* Boudier. Donadini should have described this taxon as a new species, and supplied a Latin diagnosis (Spooner & Yao 1995).

Misapplication: *Peziza granulosa* Schum. *sensu* Cooke (1879) and Boudier (1905–10). Boudier's material of *Aleuria granulosa*, 'Montmorency, Junio 1879, Ipse Pegi Boudier, vit. Icones' (PC, herb. Boud.), was examined. The spores measured 19.5–20.5–21.5 × 11–11.5–12 μm, fall within the range of *P. fimeti*.

Excluded: *Peziza granulosa* Schum., *Enum. pl.* **2**: 415 (1803).

Type: Schumacher, *Flora Hafniensis Fungi Delineati* (unpubl.): fig. 131 (macroscopic colour drawing) – **lectotypus hic designatus**. Schumacher's fig. 131 illustrates a species with much larger apothecia (× 2–3) than the species which has been referred to as *P. granulosa* following Cooke (1879) and Boudier (1905–10). Schumacher did not illustrate or describe any microscopic characters. Fries (1822), who studied Schumacher's aquarelles, correctly listed *P. granulosa* under *P. repanda*.

Peziza fimetosa Fr., *Syst. mycol.* **2**: 73 [index: 621] (1822, 1823); syn. *P. fimetaria* Schum., *Enum. pl.* **2**: 426 (1803) non Pers.

Type: Schumacher, *Flora Hafniensis Fungi Delineati* **3** (unpubl.): pl. 123, fig. 3 (2 separate drawings) – **lectotypus hic designatus**. Schumacher's plate most likely shows a species of *Pseudombrophila*; the apothecia are too small for *P. fimeti* (*ca* 50 % smaller). Bresadola (1933) erroneously used the name *Humaria fimetaria* for *P. fimeti*.

Selected descriptions: Svrček & Kubicka (1961: 74), Gamundí (1975: 26), Cacialli *et al.* (1995: 539–541).

Selected illustrations: Boudier (1905–10: pl. 272; as *A. granulosa*), Bresadola (1933: tab. 1231, 2), Gamundí (1975: pl. 6), Breitenbach & Kränzlin (1984: pl. 43; as '*Aleuria*' hortensis), Cacialli *et al.* (1995: pl. 76).

Notes: We consider the material of *P. fimeti* in G (several apothecia still attached to dung in reasonable good condition) to be the holotype, since it has an original Fuckel drawing and label (with substrate and locality) attached. Material in FH has the same locality noted on the envelope. Both the original description

and the attached drawing of *P. fimeti* give the spores as 16×8 μm. In contrast, spores from the holotype measure $20–21.5–22.5 \times 10–11–12$ μm, which is in accordance with the material studied by us, on dung of cow, horse, sheep and reindeer from Denmark, Finland, Greenland, Norway, Russia (Sakha) and USA (New Mexico) (Figs 12–13). Although several keys and descriptions list *P. fimeti* with the spore size as given by Fuckel (Seaver 1928, Bresadola 1933, Moser 1963, Dennis 1981, Romagnesi 1978, Dissing 2000), others (Svrček & Kubicka 1961, Gamundí 1975, Donadini 1977, 1979) do report larger spores (in the range of $18.5–21.5 \times 9.5–12$ μm). According to Spooner & Lundqvist (pers. comm.) the only known species of *Peziza* with small apothecia and smooth spores on dung of cow and horse in the UK and Sweden has large spores (*ca* $18–22 \times 10–12$ μm). Based on our observations it seems most likely that only one smooth-spored species, *P. fimeti*, occurs on cow and horse dung and that the measurement given by Fuckel was a simple mistake, which has been perpetuated in the literature. We have studied the holotype of *P. bovina* and the spores measure $19.5–20.5–21 \times 10.5–11–12$ μm (in agreement with Gamundí 1975).

Based on ITS sequences and morphology we found no justification for separating *P. granulosa sensu* Boudier and Cooke from *P. fimeti*. These two species have been distinguished mainly on substrate, e.g. rich soil among herbs, soil mixed with sawdust and woodchips, or burnt ground *vs* cow, horse and other herbivores dung. In addition, according to Boudier (1905–10), the paraphyses in *P. granulosa* contain distinct yellow guttules and are often irregularly swollen apically and tend to be bent at a sharp angle (observed in e.g. TL-4935, KH-98-26 and KH 00.012 but not in KS-94-12). This character is difficult to observe on dried material and we have not been able to confirm it in collections from dung, but Svrček & Kubicka (1961) described it from material on cow dung. To a certain degree the guttulation/pigments of the paraphyses may be dependent on the light conditions during development of the apothecia. ITS sequences of two Danish collections, one from dung and the other from soil mixed with woodchips/sawdust, were identical (Figs 4–5). Likewise, ITS sequences of Russian and western North American collections from dung were identical to one Russian collection from clayey soil. A constraint analysis, using the group II dataset, forcing monophyly of the clades of *P. fimeti* (10–12) and *P. fimeti* (1–9) did not yield trees that were significantly worse (Kishino-Hasegawa test, $P < 0.05$) than the unconstrained trees (Figs 4–5).

In our concept *P. fimeti* is characterized by small apothecia (most often not exceeding 2 cm), with a crenulate margin, and a nearly smooth to strongly furfuraceous outer surface (pustules of globose cells) and spores $18–20–22 \times 9.5–11–12.5$ μm.

Peziza nivalis (Heim & Remy) M. M. Moser, *in* Avizohar-Hershenzon & Nemlich, *Israel J. Bot.* **23**: 162 (1974).

Galactinia nivalis Heim & Remy, *Bull. Soc. mycol. France* **48**: 63 (1932).

Type: No authentic material has been located in PC.

Selected descriptions: Breitenbach & Kränzlin (1984: 74), Pfister (1992: 171–172).

Selected illustrations: Breitenbach & Kränzlin (1984: pl. 48).

Notes: Although there has been considerable confusion over the application of the name *P. nivalis* and other names for specimens found on soil at the margin of melting snow, Pfister (1992) placed three names in the synonymy of *P. nivalis*: *P. ninguis*, *P. fortoulii*, and *P. phaeotheca*. ITS sequences of two collections of *P. nivalis* from western North America were almost identical and well-separated within the otherwise homogenous group II, with a branch length of 11 to 18 bp (Figs 3, 5). *P. nivalis* is mainly separated from other species of group II by its habitat (Table 4). It should be noted that collections of *P. nivalis* made by K.H. in the Sierra Nevada, California, always occurred next to decaying grasses, often on burnt ground. SEM of spores is shown in Fig. 14.

Putative species or intraspecific entities within group II: Peziza sp. a, b, e, f, g, h and i

A number of specimens not yet identified, referred to as *Peziza* sp. a, b, e, f, g, h and i, form separate lineages within group II, or are kept separate based on substrate or morphology (Fig. 4). These may represent distinct species, but the lack of well-supported resolution between the lineages within group II (Figs 4–5), provides no basis for taxonomic differentiation (see 'Group II and VII taxa in separate analyses' above), and indicates that the morphological characters used and differences in habitat are plastic and not informative for species distinctions in this complex. *Peziza* sp. a, f, g have slightly larger spores (Fig. 15) than the rest of group II, *P.* sp. e has an amorphous pigment, that covers the apices of the paraphyses, and others occur on distinctive substrates (Table 4).

Peziza lohjaënsis Harmaja, *Karstenia* **26**: 46 (1986).

Type: **Finland**: *Varsinais-Suomi*: Lohja rural commune, Jalassaari, Heimo, alt. 45 m, grid 27° E 6682:329, slightly moist, predominantly deciduous (e.g. *Populus tremula*), rich woods on somewhat calcareous ground, on bare mull, 30 May 1982, *H. Harmaja* (H – holotype!).

Selected description: Harmaja (1986: 46–47).

Selected illustration: Harmaja (1986: figs 4–5).

Notes: *P. lohjaënsis* has a unique ITS sequence, and its relationship within the ingroup is uncertain. Unfortunately only one collection was available for molecular study, so intraspecific diversity could not be explored. *P. lohjaënsis* is so far known only from
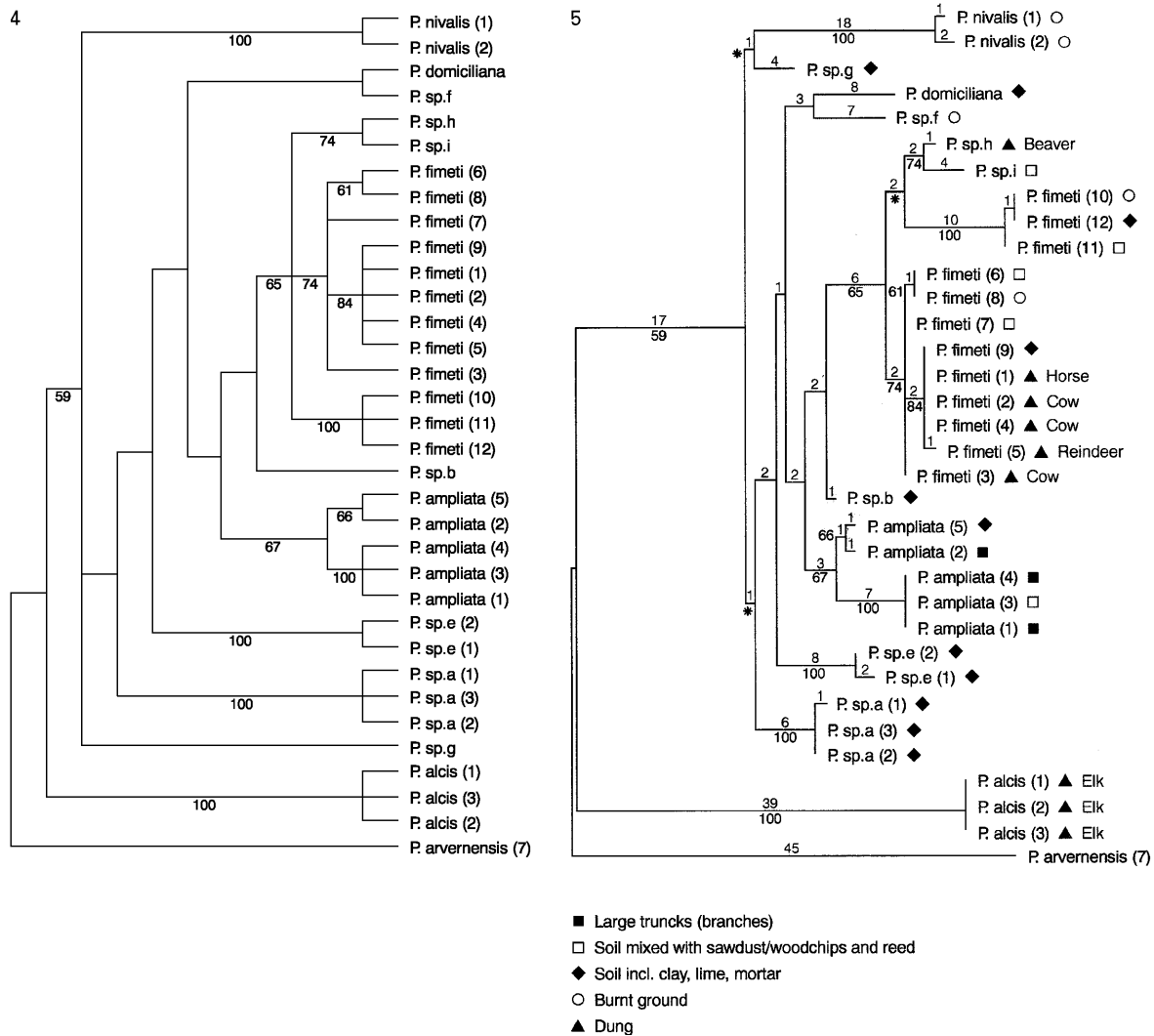
Figs 4–5. Phylogenetic trees generated under indel coding for realigned data of group II. Terminal taxa are individual specimens. Numbers by branches in black are frequency of occurence in 1000 bootstrap replicates (values < 50 % not shown). **Fig. 4.** Strict consensus tree of 108 most parsimonious trees. **Fig. 5.** Phylogram depicting one of 108 most parsimonious trees. Branch lengths are proportional to the number of steps along the branch. Symbols by specimen names indicate the substrate according to the legend.

southern Finland (Harmaja 1986). It is recognized by the spore ornamentation in combination with the large apothecia, asci with brownish walls and paraphyses with a pale or medium brown content (Table 4). Spore measurements from the holotype (Table 4) are in the range given by Harmaja (1986).

Peziza sp. c

Completely smooth spores in LM and SEM characterize this species. In addition, *Peziza* sp. c is distinguished from *P. varia* by the larger spores (Table 4). The exciple structure is of four layers (not including the sub-hymenium) as is typical for *P. varia* and *P. arvernensis*. Only the two collections of *P.* sp. c sampled for molecular studies have been seen. These showed rather thick-fleshed apothecia (1.5–3 mm thick), with a scurfy-scaly outside, and with the hymenium constricted in the centre forming ribs. Colours of the hymenium are yellow to grey brown. The two ITS sequences of *P.* sp.

c from Denmark were almost identical (group IV), and well-separated from the rest of the ingroup (Figs 1–3). *Peziza* sp. c should be described when more material becomes available, or a name may be traced when further revisions are undertaken.

Peziza arvernensis Roze & Boud., *Bull. Soc. bot. France* **26**: 76. pl. 3, fig. 4 (1879).

Type: No authentic material has been located at PC; Roze & Boud. (*loc. cit.*): pl. 3, fig. 4 (a, b, c) – **lectotypus hic designatus**.

? *Peziza sylvestris* (Boud.) Sacc. & Traverso, *Syll. fung.* **22**: 612 (1913).

Aleuria sylvestris Boud., *Icon. mycol. livr.* **10**: n°. 136, pl. 261 (1906) [preliminary text of *Icon. mycol.* with 'circulaires'].

Type: No material from the type locality, Fontaine-bleau, has been located at PC. However, material from the Boudier herbarium (PC) from 'Montmorency

(Seine-et-Oise) – France – 10 juin 1891', 'Lyon misit Dr. Riel (Julio)' and 'Pierrefonds Oct. 1889, ad terram humosam in fagetis', has been examined. These specimens previously were studied by Le Gal (1941), who found, as we have, that the spores are finely warted. On the contrary, Boudier described and illustrated the spores as smooth, but with very small vacuoles. The spores measure 16–16.5–17.5 × 9–9.5 μm (Boudier 17–18 × 9–10 μm). The status of this name will remain unclear until the type material is traced or a neotype is selected. Most authors have followed Le Gal (1941) in using the name *P. sylvestris* for a warty-spored species (Svrček 1970, Donadini 1977, Hohmeyer 1986, Dissing 2000) and often placed it in synonymy with Boudier's *P. arvernensis*. Seaver (1928) used *P. sylvestris* for a smooth-spored species on rubbish piles and soil in woods, but noted that it could possibly be *P. cerea*.

Selected descriptions: Svrček (1970: 75–76).

Selected illustrations: Boudier (1905–10: pl. 261), Breitenbach & Kränzlin (1984: pl. 36).

Notes: The type plate of *P. arvernensis* depicts a single, deeply cupshaped, apothecium, in black and white, 56 mm wide, punctate-warty spores and asci.

The nine included collections of *P. arvernensis* form a monophyletic group in all MPT's (bootstrap 58% and 98%, Figs 1–3). Two geographical lineages were identified, one consisting of collections from Denmark and the Netherlands (Va), and the other with material from western North America (Vb). Four ITS sequences from Denmark and one from the Netherlands were identical, but differed by several nucleotides from two additional sequences from Denmark (Fig. 3). The spore ornamentation in the two latter collections was much finer, but otherwise these specimens did not seem to differ. The two specimens from western North America showed almost identical ITS sequences.

P. arvernensis can easily be confused with *P. varia*, but is distinguished from other members of lineage B by the spore ornamentation, of both rounded and elongated warts, which are irregularly and widely spaced and can be seen both on the surface and in the outline of the spores by LM (× 400/1000) and by SEM. The warts are *ca* 0.3–0.5 μm high (Figs 17–18). In addition, *P. arvernensis* occurs on soil with leaves, small branches or sawdust, typically in beech forest.

Peziza echinispora P. Karst., *Fungi fenn. exs.* no. 541 (1866).

Type: *Fungi fenniae Exsiccati* no. 541 (1866) (H!) – **lectotypus hic designatus**, FH!, K *fide* Rifai (1968) – isolectotypes.
Aleuria umbrina Boud., *Icon. mycol.* pl. 279 (1905–10).
Peziza anthracophila Dennis, *Bri. Cup Fungi*: 13 (1960); nom. nov. for *A. umbrina* Boud. non Pers. 1822.

Type: Not studied.

Selected descriptions: Karsten (1871: 54–55), Rifai (1968: 228), Dennis (1981: 20).

Selected illustrations: Boudier (1905–10: pl. 279; as

A. umbrina), Le Gal (1941: fig. 6; as *A. umbrina*, spores only), Maas Geesteranus (1967: fig. 62), Dennis (1981: pl. V F), Breitenbach & Kränzlin (1984: pl. 41).

Notes: Spores of the lectotype measure 15.5–16.5–17 × 7.5–8–9 μm, which is in the range given by Karsten (1869, 1871). The taxonomy and nomenclature of *P. echinispora* poses no problems. Both molecular and morphological characters support *P. echinispora* as a well-separated species/lineage. The spore ornamentation composed of isolated, regular spines, up to 1 μm high (Fig. 19), is unique within the ingroup. Nevertheless, characters such as eguttulate spores, an *Oedocephalum* anamorph and the amyloid ascus ring zone, justify its placement in the core group of *Peziza*. To our knowledge, *P. echinispora* is only found fruiting on burnt ground. The three ITS sequences of *P. echinispora* from Denmark, Finland and eastern North America were identical (Fig. 3).

Peziza sp. d

Peziza sp. d, from Denmark, appears morphologically identical to *P.* sp. c, from Oregon, except for the slightly larger spores. However, phylogenetic analyses do suggest that these are not conspecific. ITS sequences from the two collections of *P.* sp. d were identical and form a monophyletic group with *P. echinispora* (group VI) (Figs 1–3).

Peziza varia (Hedw.: Fr.) Fr., *Syst. mycol.* **2**: 61 (1822).

Octospora varia Hedw., *Descr. micr.-anal. musc. frond.* **2**: 22, tab. 6 D (1789): Fr., *Syst. mycol.* **2**: 61 (1822).

Type: No authentic material has been located in G; Hedwig (*loc. cit.*): tab. 6 D (figs 1–5) – **lectotypus hic designatus**. Epitype: **Denmark**: Aarhus, Fuglesangsallé 88, on concrete wall damaged by water in a basement, 4 Aug. 1997, *J. H. Petersen*, KH-97-54 (C). Additional collections from the locality are deposited in C and FH.

Peziza micropus Pers., *Icon. descr. fung.* **2**: 30, tab. 8. fig. 3 (1800): Fr., *Syst. mycol.* **2**: 54 (1822).

Type: No authentic material has been located in the Persoon herbarium in L; Persoon (*loc. cit.*): tab. 6, fig. 3 – **lectotypus hic designatus**.

Peziza cerea Fr., *Syst. mycol.* **2**: 52 (1822): Fr., *loc. cit.*; non *P. cerea* Bull., *Herb. Fr.*: tab. 44 (1780).

Type: Sowerby, *Engl. Fungi* **1**: tab. 3 (1797) – **lectotypus hic designatus**. Since Fries (*loc. cit.*) excluded the Bulliard type of *P. cerea* by not referring to tab. 44, the name should be treated as authored by Fries alone (Art. 48). Among the descriptions cited by Fries was that of Sowerby (*loc. cit.*) which represents a fungus that we consider conspecific with *P. varia*. A case could also be made for regarding *P. cerea* as a *nomen ambiguum* but, since modern use of the epithet is consistent with our sense of *P. varia*, the proposed synonym is the clearest solution. Bulliard's fungus most likely is *P. vesiculosa*.

Excluded: *Peziza repanda* Pers., *Icon. pict. sp. fung.* **4**: 49 (1808): Fr. *Syst. mycol.* **2**: 51–52 (1822).

Type: No authentic material has been located in the Persoon herbarium in L. A case could be made for selecting the original Persoon plate (*loc. cit.*: pl. 20, fig. 2 [*bis*]) but this would not clarify the status of this name. Persoon described the habitat of *P. repanda* as 'in sylvaticis ad terram'. Fries (*loc. cit.*) used the name for a species on old trunks, primarily of *Fagus*, but also included blackened soil [burnt ground] as the substrate. Some authors have followed Persoon in respect to substrate (Dennis 1981, Dissing 2000, Spooner 2001), others Fries (e.g. Seaver 1928), and still others have used the name for a species occurring on soil and only exceptionally on wood (Rifai 1968, Hohmeyer 1986). In all cases the name has been used for a species with smooth spores in the size range given for *P. varia*. We have not seen material of *P. varia* on bare soil (without mortar, lime, sawdust, etc.). Since the name lacks a type specimen and has been used inconsistently it is best treated as of uncertain application.

Selected descriptions: Rifai (1968: 233–234, 237–240; as *P. varia*, *P. cerea*), Svrček (1970: 64–74; as *P. cerea*, *P. micropus*, *P. varia*), Dennis (1981: 18, 21; as *P. miropus*, *P. varia*, *P. cerea*).

Selected illustrations: Boudier (1905–10: pl. 259; as *Aleuria cerea*, pl. 267; as *A. varia*, pl. 268; as *A. micropus*), Svrček (1970: figs 3 AB, 5 A–G, 8 A–C), Breitenbach & Kränzlin (1984: pl. 40; as *P. domiciliana*), Ryman & Holmåsen (1984: 624–625; as *P. cerea* and *P. repanda*).

Notes: Hedwig's *P. varia* plate depicts two, brown, stipitate apothecia up to 48 mm wide, and some immature specimens on the substrate, and remarkably, a section showing the hymenium and exciple structure, including the stratified medullary exciple. Persoon's plate of *P. micropus* illustrates a single, brownish, stipitate apothecium, 18 mm wide, with a strongly furfuraceous, paler outside, on a piece of wood. Sowerby's *P. cerea* plate contains a range of pale yellowish brown, stipitate, more or less funnel-shaped apothecia in various stages of maturity, up to 61 mm wide, some on the substrate, others cut off.

Phylogenetic analyses of ITS sequences supports the 27 collections of the '*P. varia* complex' as one monophyletic group (group VII, bootstrap 92–97%) (Figs 1–3). Two lineages were identified (VIIa, b) (Figs 2, 6–7), which could possible represent two species. Group VIIa members are morphologically united by slightly finer spore ornamentation than group VIIb members. Spores within group VIIa appear smooth or nearly smooth in LM ($\times$1000) and have very low, dense warts in SEM, *ca* 0.1 μm high (Figs 20–22), whereas spores within group VIIb appear finely warted on the spore surface in LM ($\times$1000), and have fine, dense warts in SEM, up to *ca* 0.4–0.5 μm high (Figs 23–25). In addition, group VIIb specimens were found exclusively on large hardwood trunks, with the group of collections from Denmark and the UK typically on

Fagus (*P. varia* (26) was on *Quercus*) (100% bootstrap), and with the group from Finland, eastern North America and Kamchatka on *Acer*, *Betula*, *Populus* and *Ulmus* (100% bootstrap) (Figs 6–7, Table 4). Several specimens within group VIIa, however, were likewise found on large hardwood trunks (Table 4), of both *Fagus*, *Populus*, *Salix* and *Ulmus*, besides being found on sawdust and on rich soil with sawdust, on plaster in basements, brick walls, on gravel etc. (Fig. 7). The name *P. cerea* primarily has been used for specimens occurring on plaster covering timber or straw, brick walls or on coarse sand in wet basements and garages, on limestone or on soil mixed with mortar (Maas Geesteranus 1967, Svrček 1970, Dissing 2000, Spooner 2001), but also on woody debris, rotting sandbags etc. (Rifai 1968, Dennis 1981) whereas the names *P. micropus* and *P. repanda sensu* Fries have been used for specimens on decayed hardwood trunks. ITS sequences from specimens on the floor and walls in basements, were identical or nearly identical to ITS sequences from some of the specimens on large hardwood trunks (Figs 6–7).

Apart from the subtle difference in the spore markings, we found no morphological features that support the division of group VII in two (VIIa, b) or five species, equivalent to the well-supported clades identified in analyses of the inclusive dataset (Figs 1, 3). Since analyses of ITS only supported the two lineages VIIa and VIIb with 55–60% and <50–76% of the bootstrap replicates respectively, we found no justification for recognizing more than one species, *P. varia*, within group VII. It should be noted that the '*P. varia* complex' in general is described as smooth spored, although it previously has been shown by SEM (Berthet 1970, Donadini 1977, 1979) that the spores are finely ornamented. The warts can often be observed in LM, but sometimes the spores appear smooth.

P. varia has been distinguished from *P. cerea* and *P. micropus* by the presence of moniliform paraphyses (e.g. Rifai 1968, Dennis 1981, Ginns 1980, Dissing 2000, Spooner 2001). However, as already observed by Donadini (1980b) and Kullman (1995), we found the development of moniliform paraphyses in specimens within this group (and in group I and II) to be a result of high levels of moisture in the micro-environment, which make the cells in the paraphyses swell. Svrček (1970) also noted that this character was without any taxonomic value.

According to Svrček (1970), *P. cerea* most often develops apothecia with a medullary exciple of globose cells, lacking a middle layer of interwoven hyphae, running parallel with the outer surface (a stratified exciple) characteristic of *P. micropus* and *P. varia*. Dennis (1981) used the name *P. micropus* for a species with a non-stratified exciple. We observed a stratified exciple in all specimens, but of very variable thickness ((30–)60–280 μm thick) and often difficult to discern in dried material. We found no correlation between the ITS lineages and the thickness of the hyphal layers or
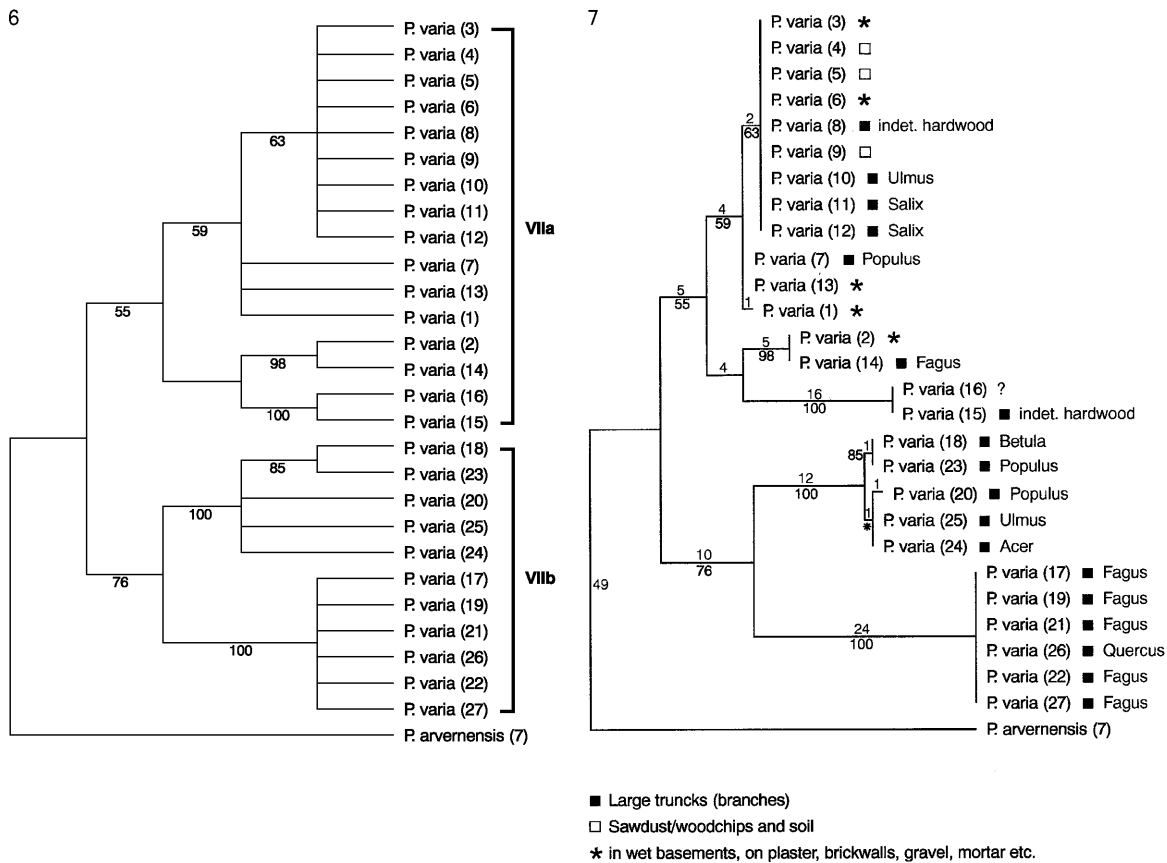
Figs 6–7. Phylogenetic trees generated under indel coding for realigned data of group VII. Terminal taxa are individual specimens. Numbers by branches in black are frequency of occurence in 1000 bootstrap replicates (values < 50% not shown). **Fig. 6.** Strict consensus tree of 156 most parsimonious trees. **Fig. 7.** Phylogram depicting one of 156 most parsimonious trees. Branch lengths are proportional to the number of steps along the branch. Symbols by specimen names indicate the substrate according to the legend.

the thickness of the total exciple. Most probably the thickness of the middle hyphal layer and the exciple overall is affected by edaphic factors. Likewise, the intensity of colour, size and shape of the apothecia may, to a certain degree, be influenced by the substrate and the light conditions, e.g. development of lighter coloured apothecia in dark basements compared to open, outdoor areas, and larger, luxuriant forms on sawdust and highly decayed trunks of *Fagus*. In agreement with Häffner (1992, 1993) and Kullman (1995), we did not find the presence or absence of a stipe to be taxonomically informative. Both sessile and substipitate specimens (stipe up to 1.5 cm long × 1.3 cm broad) were represented within the five lineages of group VII. *P. cerea*, *P. micropus*, *P. repanda* and *P. varia* have all been characterized by apothecia covered by an outer hyphal layer, with free hyphal tips, appearing whitish, felty upon drying. We found this layer to be very variable in thickness and in some specimens completely absent (in *P. varia* (10) and *P. varia* (26)).

Svrček (1970) noted that *P. varia* differs from *P. micropus* by having a brownish pigmented hyphal layer

in the medullary exciple, opposed to *P. micropus* with a relatively light coloured hyphal layer. Whether this colour variation was a stable character, however, remained unclear to Svrček and has not been confirmed in our study.

Kullman (1995) studied apothecia from one population on plaster over a period of a month and a half. She observed that some of the features considered to be characteristic of the three species *P. cerea*, *P. micropus* and *P. varia* developed over this period, e.g. presence or absence of a stem and the colour of the exciple, and therefore she considered these names to refer to one and the same species.

DISCUSSION

There are weaknesses in the dataset but these do not appear to be a result of inclusion of ambiguously aligned regions, which could have added noise or been misleading. All phylogenetic resolution receiving even moderate bootstrap support from either of the datasets tested, is compatible with results from all datasets and did not effect conclusions in this study.
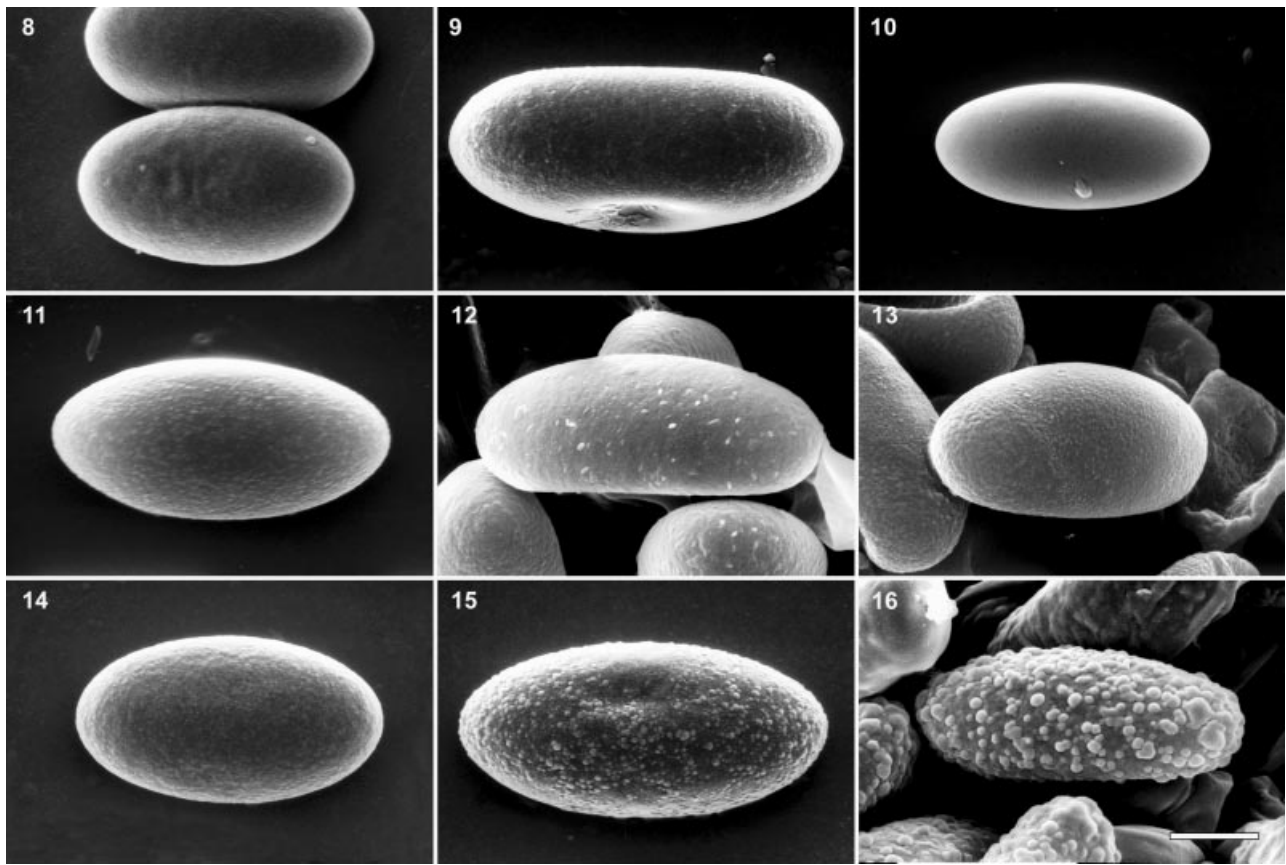
Figs 8–16. Spore ornamentation as seen by SEM for specimens of groups I–III (A). **Figs 8–15.** Group I and II. Smooth or nearly smooth spores. **Fig. 8.** *Peziza ammophila* (1). **Fig. 9.** *P. vesiculosa* (4). **Fig. 10.** *P. alcis* (2). **Fig. 11.** *P. ampliata* (2). **Fig. 12.** *P. fimeti* (2). **Fig. 13.** *P. fimeti* (7). **Fig. 14.** *P. nivalis* (1) **Fig. 15.** *P.* sp. a (1). **Fig. 16.** Group III. *P. lohjaënsis* (holotype). Irregular, rounded to elongated warts, becoming ±fused at the poles. Bar = 5 µm.

Intra- and interspecific ITS variation

The seven lineages (groups I–VII), distinguished based on ITS sequences, each represent one to several species. Our results suggest that there may be ITS rate heterogeneity within the core group of *Peziza*. *P. varia* (group VII) exhibits a high degree of intraspecific variation in the ITS-region compared to, for example, *P. vesiculosa* and *P. echinispora*, which show no variation (Fig. 3). This could be a result of a higher evolutionary rate of ITS within lineage VII and/or partly a result of geographical separation. It appears that the two divergent and highly supported geographical clades within group VIIb have not exchanged genes for a long time (Fig. 7). Some variation in anatomical characters is present within group VII (macromorphology and exciple structure), but these character differences showed no correlation with the groupings obtained by ITS. In general, levels of sequence divergence within group II are low, based on the branch lengths (Fig. 3), which may suggest a lower evolutionary rate of ITS within this lineage or a very recent radiation. In addition, the variation in morphology within group II is low, being primarily in spore size ranges, and in the pigmentation and shape of the paraphyses. The ITS sequence divergence among groups I–VII is overall quite large, which also can be seen in terms of length variation of the ITS region (Table 2).

Phylogeny within the core group

Higher level relationships

The two prominent lineages, A and B (groups II ±I/III and IV–VII respectively), identified in the phylogenetic analyses, correspond to macromorphologically discrete groups: taxa with small, shallowly cup- to disc-shaped apothecia, generally <2 cm (A) and taxa with large, deeply cup-shaped to expanded apothecia (B) (Table 4, Figs 1–3). Exceptions are *Peziza lohjaënsis* (group III), *P. ammophila* and *P. vesiculosa* (group I), which all produce large apothecia. The position of these taxa is uncertain, resulting in low bootstrap support for lineage A (<50–63 %), which may include either *P. lohjaënsis* or *P. ammophila* and *P. vesiculosa* (Figs 1–3). Lineage A contains taxa with smooth or nearly smooth spores (Figs 8–15), often with a unilateral, gelatinous coating (not seen in *P. ammophila*, *P. domiciliana*, *P. lohjaënsis*, *P. nivalis*, *P.* sp. a and *P.* sp. f). A unilateral gelatinous spore coating has not been seen within lineage B. A similar type of mucilaginous substance is present within some coprophilous species of *Ascobolus* (*Ascobolaceae*),
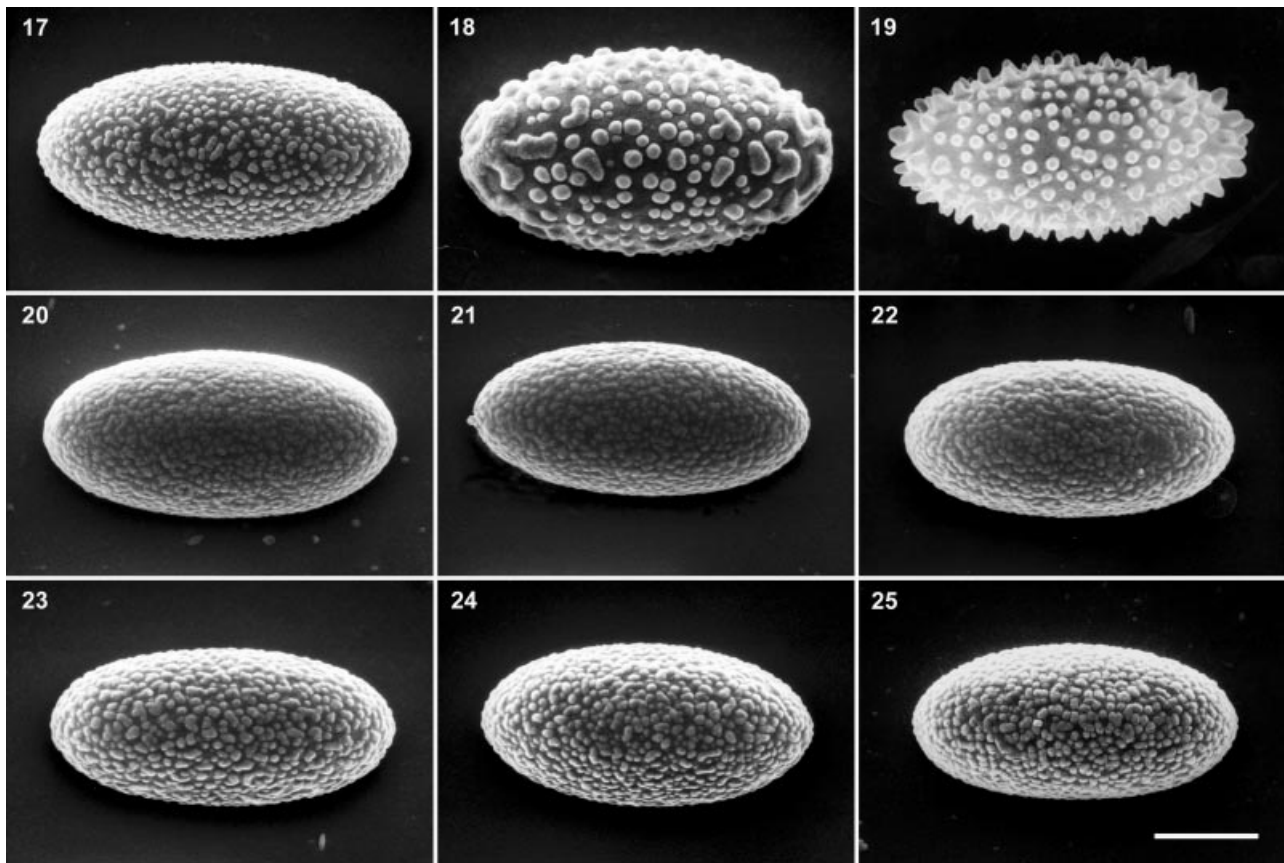
Figs 17–25. Spore ornamentation as seen by SEM for specimens of groups V, VI and VII (B). **Figs 17–18.** Group V. Relatively coarse, rounded and elongated warts, irregularly and widely spaced, seen both on the surface and in the outline of the spores. **Fig. 17.** *Peziza arvernensis* (7). **Fig. 18.** *P. arvernensis* (2). **Fig. 19.** Group VI. Isolated, regular spines. *P. echinispora* (3). **Figs 20–22.** Group VIIa. Extremely low, dense warts. **Fig. 20.** *P. varia* (1). **Fig. 21.** *P. varia* (14). **Fig. 22.** *P. varia* (12). **Figs 23–25.** Group VIIb. Fine, dense warts. **Fig. 23.** *P. varia* (20). **Fig. 24.** *P. varia* (21). **Fig. 25.** *P. varia* (22). Bar = 5 µm.

the sister group of the *Pezizaceae*. It is a likely symplesiomorphy for lineage A. *P. lohjaënsis* differs by having spores with a distinct ornamentation of irregular, rounded to elongated warts, which become ±fused at the poles (Fig. 16). Species in lineage B have a range of spore surfaces from smooth, *P.* sp. c and *P.* sp. d, to nearly smooth or finely ornamented, *P. varia* (Figs 20–25), to distinctly warted, *P. arvernensis* (Figs 17–18) or spiny, *P. echinispora* (Fig. 19).

The overall apothecial excipular structure within lineage B is uniform, with a stratified medullary exciple being composed of 3 layers: (1) of large globose cells with few interwoven hyphae; (2) a dense layer of hyphae running parallel with the outer surface; and (3) a layer identical to the first, of predominantly large globose to angular cells. The outer exciple is not clearly differentiated, but is composed of small globose to angular cells, which typically terminate in short, ±branching hyphae, forming an often thick tomentose layer. This outer layer turns whitish on drying and sometimes the hyphae aggregate irregularly giving the surface a scurfy appearance. The thickness of the different layers may vary according to substrate and microclimatic conditions. Likewise, the excipular structure is fairly uniform within members of lineage A. In

this case the medullary layer is non-stratified being composed of globose cells with few interwoven hyphae; often smaller, globose, ±brownish pigmented cells form pustules on the outside. Stratified medullary excipules may be present and have been observed in fresh material of *P. alcis* (2) and *P. ampliata* (4). The subhymenium and outer exciple are generally of small, globose to angular cells. The placement of *P. vesiculosa* within lineage A (Fig. 2) is not supported by the structure of the exciple. In this taxon there is a stratified medullary exciple as in lineage B. *P. ammophila* should be studied with respect to this character.

Inter- and intraspecific relationships (I–VII)

The grouping of *Peziza vesiculosa* and *P. ammophila* could be a result of 'long branch attraction' (Felsenstein 1978). Nevertheless, morphological features do not contradict a close relationship between these taxa. Both produce large, deeply cup-shaped apothecia, which initially are almost closed, hollow spheres, with long incurved margins, and the spores are completely smooth and eguttulate. The typical habitats also show similarities. *P. ammophila* is found with (decaying) grasses (*Ammophila*) in dunes, and *P. vesiculosa* is often found

on manured straw or hay. *P. ammophila* has been placed in *Sarcosphaera* (e.g. Seaver 1928, Kotlaba & Pouzar 1963) based on its initial, partly hypogeous development and the deeply cup-shaped apothecia with margins that split into irregular rays. Likewise, it and another species, *P. austrogaster*, has been considered to be 'unique' within *Peziza*, and a subgenus *Asterosphaera* was established with *P. ammophila* as the type species by Pouzar (1972). Most authors have considered the similarities in macromorphological development between *Sarcosphaera* and *P. ammophila* to be a result of convergent or parallel evolution. This is congruent with our results based on LSU sequences (Hansen *et al.* 2001) and ITS rDNA sequences, which support *P. ammophila* in *Peziza s. str.* Furthermore, the presence of a distinctly amyloid ascus ring zone and an *Oedocephalum* anamorph (Paden 1973) justify its placement in the group. *Sarcosphaera* represents a separate lineage (Hansen *et al.* 2001).

All taxa with small apothecia (generally < 2 cm) sampled within the core group of *Peziza* form a monophyletic group (group II). This includes *P. fimeti* (including *P. granulosa sensu* Boudier; see above), *P. alcis*, *P. nivalis*, *P. domiciliana*, *P. ampliata* and a number of putative, undetermined species. The named species figure in most keys and other literature on *Peziza* (e.g. Le Gal 1941, Donadini 1977, 1979, Hohmeyer 1986, Dissing 2000, Spooner 2001). Based on analyses of ITS, group II members appear to be very closely related, assuming that the ITS rDNA phylogeny is congruent with the population phylogeny, and they may not all represent separate evolving lineages. The uniformity within this group is reflected in the structure of the apothecia, where only very subtle differences are seen. Species within group II have been distinguished primarily by hymenial colours; presence or absence of a crenulate margin; spore sizes and shape; content of the paraphyses; and, to a large extent, the substrate. They produce apothecia in a wide range of habitats but, based on the ITS phylogeny, most are not confined to specific habitats (Fig. 5). *P. fimeti* fruits not only on cow and horse dung but also on other types of dung, sawdust-mixed soil, bare soil and burnt ground. Likewise, analyses of ITS did not identify other lineages of specimens occurring on bare ground (clay, rich soil and chalk) *vs* wood, or on burnt *vs* unburnt ground (Fig. 5). Nevertheless, *P. alcis* appears to be a well-defined lineage confined to elk dung and *P. nivalis* to dead grasses in snow beds (Figs 4–5).

Group III contains *P. lohjaënsis*, which forms either a separate lineage as a sister group to the rest of the core group of *Peziza* (Fig. 2) or a weakly supported monophyletic group with group II (Figs 1, 3). Harmaja (1986) suggested that *P. lohjaënsis* was related to *P. phaeotheca*, a snowbank fungus (treated as a synonym of *P. nivalis* by Pfister (1992), a group II member), which like *P. lohjaënsis* possesses brownish ascus walls and occurs in spring. On the other hand, other features, such as the size of the apothecia and spore orna-

mentation (Figs 16–18), point to a closer relationship with *P. arvernensis* (group V). Further morphological and molecular studies of additional specimens of *P. lohjaënsis* are needed.

Group IV represents an as yet unidentified species, *P.* sp. c, which constitutes a well-separated lineage. The position of *P.* sp. c within lineage B is uncertain, being unresolved in the strict consensus tree of the inclusive dataset (Fig. 1). In the strict consensus tree of the exclusive dataset it forms a separate lineage as a sister group to *P. echinispora*, *P.* sp. d, and *P. varia* (groups VI–VII) (Fig. 2). The two specimens of *P.* sp. c were found in the same habitat as some specimens of *P. varia*, that is, sawdust and soil. Morphologically *P.* sp. c is primarily distinguished within lineage B by the larger, completely smooth spores (Table 4).

Group V contain nine collections of *P. arvernensis*, which are highly supported as monophyletic in the inclusive dataset analysis (98 % bootstrap, Fig. 1) but only weakly supported in the exclusive dataset analysis (58 % bootstrap, Fig. 2). Two geographical subclades, Va and Vb, were highly supported in both analyses; no morphological differences were observed between the subclades. The position of *P. arvernensis* within lineage B is unresolved in the inclusive dataset (Fig. 1). In the exclusive dataset it is a sister group to the rest of lineage B (Fig. 2). Based on ITS sequences, spore ornamentation, and habitat (Figs 17–18), *P. arvernensis* appears to be a distinct species.

Group VI includes two distinct species, *P. echinispora* and *P.* sp. d. No obvious morphological characters support this particular grouping within lineage B, which could, like group I, be explained by 'long branch attraction' (Felsenstein 1978). Rather, based on the spore characters (size and lack of ornamentation) and the substrate, a close relationship between *P.* sp. d and *P.* sp. c would be expected. The spore ornamentation in *P. echinispora*, isolated, regular spines, up to 1 μm high, is unique within the ingroup. The excipular structure of *P. echinispora* is similar to that found in other lineage B members.

Within group VII we recognize a single lineage, *P. varia*, based on ITS, morphological criteria studied, and substrate. It could be argued that the two lineages, VIIa and VIIb, recovered in all MPT's of the exclusive dataset and the restricted group VII dataset (Figs 2, 6–7) and supported by small differences in spore markings, could represent two species (see notes on *P. varia* under 'Taxonomic implications' above). Nevertheless, the strongly supported subclades within group VII seem only to represent different populations of *P. varia*. Characters such as moniliform *vs* filiform paraphyses, presence or absence of a stipe, colour variation in the apothecia, stratified or non-stratified medullary exciples (or thickness of the exciple layers) and cell types in the outermost layer of the exciple, previously used to delimit species within the '*P. varia* complex', were not correlated with the subgroups identified by analyses of ITS sequences and appeared to

be plastic. In addition, substrate has been emphasized in the taxonomy of the 'P. varia complex' but our results indicate that populations on a diverse array of substrates, may be closely related (Fig. 7). On the other hand, we may not understand the substrate preferences and the biology of these fungi well enough for meaningful comparisons.

CONCLUSIONS

Overall this study implies that morphological features previously used to distinguish many of the taxa within the core group of *Peziza* are plastic. The use of substrate as a primary character for species recognition has, in addition, been misleading within some lineages. Spore ornamentation and size, and paraphyses pigmentation and shape proved, for the most part, to be useful at the specific level. Apothecia size and shape, overall exciple structure, and to some degree spore surface relief, seem to be phylogenetically informative for higher level relationships within the group. Two main clades, including seven independent lineages were identified. Within these we currently recognize thirteen species, along with a number of entities of yet uncertain status. Our critical evaluation of the taxa involved, their delimitation and the designation of types as required, provides a stable framework for future investigations of these fungi.

To understand phylogenetic relationships and define species boundaries within group II, analyses of other genes may prove useful (Taylor *et al*. 2000). Candidate genes would include the intergenic spacer IGS rDNA (Anderson & Stasovski 1992), part of RPB2 (Liu, Whelen & Hall 1999) or translation elongation factor (EF-1a) gene (O'Donnell 2000). Likewise, phylogenetic studies of multiple genes within group VII, would be valuable in a further exploration of the species limits and the ITS subclades identified in the present study. Morphological features of anamorphs rather than of teleomorphs have proven phylogenetically informative at the species level in some groups of fungi (Takamatsu, Hirata & Sato 1998, Réblová & Winka 2000). Although only *Oedocephalum* anamorphs have been reported within the core group of *Peziza*, these exhibit considerable variation. Culture characteristics and anamorph features could possibly contribute new and phylogenetically valuable information in this morphologically conserved group.

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