Truffle trouble: what happened to the *Tuberales*?

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ABSTRACT

An overview of truffles (now considered to belong in the *Pezizales*, but formerly treated in the *Tuberales*) is presented, including a discussion on morphological and biological traits characterizing this form group. Accepted genera are listed and discussed according to a system based on molecular results combined with morphological characters. Phylogenetic analyses of LSU rDNA sequences from 55 hypogeous and 139 epigeous taxa of *Pezizales* were performed to examine their relationships. Parsimony, ML, and Bayesian analyses of these sequences indicate that the truffles studied represent at least 15 independent lineages within the *Pezizales*. Sequences from hypogeous representatives referred to the following families and genera were analysed: *Discinaceae–Morchellaceae* (*Fischerula, Hydnotrya, Leucangium*), *Helvellaceae* (*Balsamia* and *Barssia*), *Pezizaceae* (*Amylascus, Cazia, Eremiomyces, Hydnotryopsis, Kaliharituber, Mattirolomyces, Pachyphloeus, Peziza, Ruhlandiella, Stephensia, Terfezia,* and *Tirmania*), *Pyronemataceae* (*Genea, Geopora, Paurocotylis,* and *Stephensia*) and *Tuberaceae* (*Choiromyces, Dingleya, Labyrinthomyces, Reddellomyces,* and *Tuber*). The different types of hypogeous ascomata were found within most major evolutionary lines often nesting close to apothecial species. Although the *Pezizaceae* traditionally have been defined mainly on the presence of amyloid reactions of the ascus wall several truffles appear to have lost this character. The value of the number of nuclei in mature ascospores as a delimiting family character is evaluated and found to be more variable than generally assumed.

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Introduction

Fungi pursuing the truffle strategy by producing underground sporocarps have long been recognized as a polyphyletic group with representatives in the former *Zygomycota* now *Glomeromycota* (*Endogone, Glomus* a.o.), *Ascomycota,* and *Basidiomycota*. Those with asci were at one time all placed in the *Tuberales* (e.g. Tulasne & Tulasne 1851; Fischer 1897; Knapp 1950; Hawker 1954; Eckblad 1968; Korf 1973a). Nannfeldt (1946) wrote: 'The question is raised whether *Tuberineae* is monophyletic or whether it is composed of different operculates that have evoluted ± convergently into hypogeous forms.'

Malençon (1938) also advanced ideas about the evolution of truffles and their transformation from epigeous apothecial species to hypogeous truffles, but, as pointed out by Burdsall (1968), his system relied too heavily on macroscopic features. Korf (1973b) discussed the evolution of convoluted pezizalean forms, both above and below ground, and although he accepted the *Tuberales*, he indicated that at least some of the taxa were derived along various evolutionary lines within the *Pezizales*. He considered *Tuberales* to be a biological unit rather than a phylogenetic one. Trappe (1971) published a similar statement, and finally Trappe (1979), proposed that the order be abandoned, with one major part being moved to the

Pezizales and just *Elaphomyces* to the *Elaphomycetales*. Fischer (1897) had earlier referred *Elaphomyces* to the 'Plectascineae' but alongside the *Terfeziaceae*. Later (Fischer 1938), *Terfeziaceae* reappeared within the *Tuberales*. Trappe (1979) kept some hypogeous lines, as families, within his *Pezizales*, but other hypogeous taxa were placed alongside epigeous species in various mixed families. Burdsall (1968) had already convincingly merged one tuberalean genus (*Geopora*) with the pezizalean genus *Sepultaria*. Eckblad (1968) gave many clear arguments for not accepting the *Tuberales* but, nevertheless, concluded the opposite. In the first *Outline of the Ascomycetes* (Eriksson 1982) *Tuberales* (with *Geneaceae*, *Terfeziaceae*, and *Tuberaceae*) were relegated to synonymy of *Pezizales*. Ainsworth & Bisby's *Dictionary of the Fungi* (Hawksworth 1983) likewise abandoned the use of *Tuberales* and listed the order under *Pezizales* (and *Elaphomycetales*). Trappe's hypothesis was tested in a long-lasting study of the ultrastructure of pezizalean taxa guided by Kimbrough and summarized in Kimbrough (1994), that for example, led to the placement of *Hydnobolites* in the *Pezizaceae*, based on both cytological and ultrastructural features of asci and ascospores. Also the placement of *Barssia* in the *Helvellaceae* followed from these studies. The most important character used was the morphology of the complicated septal pore-apparatus at the base of the asci (Kimbrough 1994). Another prominent feature, the number of nuclei in the mature spores that originated in Berthet's (1963) studies on epigeous *Pezizales*, was also taken into account when trying to delimit natural groups of truffles (e.g. Berthet 1982; Donadini 1986a, b). With the onset of the molecular taxonomy era, these early hypotheses have gradually been confirmed and expanded upon, or in some cases, corrected (e.g. O'Donnell *et al.* 1997; Norman & Egger 1999; Percudani *et al.* 1999; Hansen *et al.* 2005; Perry *et al.* 2007). In a comprehensive treatment of European (mainly Italian) truffles Montecchi & Sarasini (2000) refer former *Tuberales* taxa to *Elaphomycetales*, with just *Elaphomyces*, and *Pezizales* with seven families: *Pezizaceae* (four genera), *Pyronemataceae* (four genera), *Geneaceae* (two genera), *Helvellaceae* (three genera), *Balsamiaceae* (two genera), *Terfeziaceae* (four genera) and *Tuberaceae* with two genera. Although, they cite recent molecular results, they have chosen a conservative approach by following the systems proposed in Trappe (1979) and Pegler *et al.* (1993). One group of researchers (Parguey-Leduc *et al.* 1987b, 1990; Janex-Favre & Parguey-Leduc 2003) proposed to accept *Tuberales* based mainly on the genera *Tuber* and *Terfezia* that were considered closely related, mostly based on a perceived different development of asci and ascospores. van Brummelen (1994) gave a summary of the arguments put forward up to that time. Eriksson (2006b), influenced by data published by e.g. de Hoog *et al.* (2005), discussed what to do nomenclatorily if *Pezizales* are restricted to *Pezizaceae*. Although *Tuberales* are a possible choice, he proposed to find another name. Currently, however, there is no supported molecular phylogenetic evidence that suggests *Pezizaceae* are not part of the *Pezizales* (the *Pezizaceae* are supported as monophyletic by a BS value of 100 %, but the relationships among the included families in e.g. de Hoog *et al.* (2005) are without support).

The purpose of this paper is to review morphological and biological traits, and the systematics of the passively dispersed, more or less hypogeous *Pezizales*. Using all currently available LSU sequences from pezizalean truffles, in analyses with a broad sample of epigeous pezizalean taxa, we will further investigate the phylogenetic relationships and evolution of these truffle fungi. Ascomycetous truffles, which are now considered to be non-pezizalean (*Elaphomyces*, *Eurotiomycetes*), are not treated in detail. The taxonomic position of all accepted taxa at and above generic level are given and compared with previous classifications. The accepted classification is based on molecular phylogenetic analyses and morphological characters.

A truffle definition

Ascomycete truffles can be defined as producing sporocarps below or at ground level and with a simultaneous loss of active spore dispersal. In several genera, for example *Geopora* and *Helvella*, species with intermediate characters can be found. Also *Sarcosphaera coronaria* is an example of a fungus that has nearly become a truffle. It forms apothecia below ground and often opens by a rather small aperture, but as the spores are actively ejected it can still be classified as a "cup fungus". The genus *Caulocarpa* was based on such hypogeous *Sarcosphaera* ascomata (Trappe 1975c). Although some species tend to produce sporocarps in or on the litter, we still group them with the truffles as long as they have lost active spore dispersal. *Glaziella* and *Paurocotylis* are good examples.

Morphological features of pezizalean truffles

The ascomata are typically fleshy but can be quite hard and cartilaginous. An outer rind (peridium) is often present and can be almost woody and sculptured. Even at maturity the spores do not become powdery, except in a few genera (e.g. *Carbomyces*) that are adapted to extreme xeric conditions. There is a continuous variation from truffles with a single cavity lined with a hymenium, often with a single opening, to truffles with intricate foldings or with pockets of asci in a firm gleba. Weber *et al.* (1997) defined three different types of hypogeous ascomata within the *Pezizales*: ptychothecia with persistent, recognizable hymenia and variously folded or even solid ascomata; stereothecia without hymenia and solid ascomata; and exothecia with external hymenia. None of these ascoma types can accommodate *Paurocotylis* and *Glaziella*. These genera produce ascomata that are hollow, without paraphyses, and furthermore, are unusual in being fully exposed at maturity. Hansen *et al.* (2001) reviewed the morphological features of the truffles considered to belong to the *Pezizaceae*. Those pezizalean species that have been studied in ontogenic detail, such as *Tuber* and *Terfezia* species (Janex-Favre & Parguey-Leduc 2003), start out as apothecial before folding occurs. The asci can at one end of the variation resemble those of operculate species being cylindrical with spores in one row or at the other end be completely globose with or without a pedicel and with a variable number of often very large spores. The ascospores vary in colour from hyaline to almost black, and in surface features from smooth and thin-walled to very thick-walled with intricate ornamentation. The ascus walls can be more or less layered and amyloid or inamyloid. The *Pezizaceae* are characterized by amyloid asci, but this feature appears to have been lost in many pezizaceous truffles (Hansen *et al.* 2001, 2005).

Truffle identification and nomenclature

Castellano *et al.* (1989) have published a slightly dated key to the spores of genera found in north temperate forests. An updated key, taking further characters into use, can be found on the Internet (http://natruffling.org/ascokey.htm), and an earlier printed version was published by Trappe & Castellano (1992). Trappe's (1979) synoptical key is still useful. In Europe two main illustrated accounts with keys are current (Montecchi & Sarasini 2000; Pegler *et al.* 1993). Other important contributions include Lange (1956), Lawrynowicz (1988), and Montecchi & Lazzari (1993).

The names of pezizalean truffles are given sanctioned status if included in Fries (1821–1832) and should be used when available for a given taxon. In practice, however, another tradition has evolved, where Vittadini's (1831) much more accurate work on European truffles has been used as the *de facto* starting point for especially *Tuber* nomenclature. As Trappe (2001) has pointed out, it will be necessary to propose these Vittadini names for conservation over the sanctioned Friesian names in order not to disrupt the very long usage of these names for such economically important organisms.

Distribution, diversity, and dispersal

Although, false truffles (hypogeous *Basidiomycota*) have been collected in extreme arctic environments, the true truffles would appear to have a more limited distribution, with a clear peak in diversity in temperate–subtropical, often rather dry climates. Although a high number of publications are dedicated to truffles, a reasonable picture of the diversity and distribution of the group has still not been achieved. Castellano *et al.* (2004) from one long Australian study suggest a figure of 600 species (although mainly of false truffles), most of which remain to be described. A part of this project was described in Claridge *et al.* (2000). Only ten of these species belong to the *Ascomycota*, and two apparently to undescribed genera. [See also the extensive review of Australian and New Zealand sequestrate fungi by Bougher & Lebel (2001).] Only Europe and parts of North America can be claimed to be reasonably well covered with respect to hypogeous fungi (Castellano *et al.* 2004). Distributions of European taxa are dealt with in Lawrynowicz (1991). Parts of Asia would seem to be equally rich in truffles. Africa and South America are apparently especially poor in hypogeous ascomycetes but be aware of the likely differences in sampling efforts in various regions. Verbeken & Walleyn (2003) in a checklist of subsaharan sequestrate fungi only reported one pezizalean species, *Terfezia decaryi* from Madagascar. In addition, three species are known from the southern dry lands of continental Africa, including the Kalahari (Marasas & Trappe 1973; Ferdman *et al.* 2005). Two were separated as new genera (Ferdman *et al.* 2005). The third, *Terfezia austroafricana*, was listed as a member of *Terfezia* subgen *Mattirolomyces* and may require a new combination, as *Mattirolomyces* has been raised to generic rank. Although too little is known, it is fairly clear that many localized endemics are to be found among pezizalean truffles.

It has been hypothesized that all, or nearly all, truffles are passively dispersed with animal vectors, but there is very little experimental evidence to support this assertion. Various small mammals, including Australian marsupials (e.g. Claridge & May 1994), and voles and chipmunks in North America, collect and often hoard ascomata and by this activity are thought to play an active dispersal role (e.g. Fogel & Trappe 1978; Maser *et al.* 1978). The stomach contents of voles and chipmunks have been found to contain over 70 % truffles. So far it has not been shown that pezizalean truffle spores can germinate after gut passage but in all likelihood they can. The volatile compounds truffles exude when ripe clearly substantiate the claim that these mammals are the key dispersal vectors. Also larger mammals such as boar and deer are well known for their ability to locate and digest truffles, and presumably, also act in a beneficial way to the truffles by their dispersal abilities. The volatile compounds may resemble pheromones (Claus *et al.* 1981) and can also be used in species recognition (e.g. Marin *et al.* 1984; Pacioni *et al.* 1990). Trappe (1977) and Trappe *et al.* (2001) have speculated that the ectomycorrhizal truffle partners migrated along with the rodent dispersers and the truffles themselves, many populations later becoming isolated as a result of continental drift. Many invertebrates (*Diptera* etc) also actively seek out truffles, but although a more parasitic aspect to this relationship can be postulated, additional dispersal ability cannot be ruled out. Even birds have been claimed to actively seek out truffles and possibly act as dispersal vectors (Alsheikh & Trappe 1983b; Castellano *et al.* 2004). One example concerns the desert truffle *Phaeangium* (or *Picoa*) *lefebvrei*, which is believed to be dispersed by various species of desert-adapted larks, but also by cream-coloured courser and hoopoe. Another case deals with *Paurocotylis pila*, which at maturity has epigeous, orange–red fruit bodies coinciding with the fall of likewise bright-coloured *Podocarpus* fruits, known to be bird dispersed. Whether birds may also be involved in the dispersal of introduced British populations of *Paurocotylis* is not known.

Material and methods

Taxon sampling and alignment

To summarize and determine the phylogenetic placement of hypogeous taxa within *Pezizales*, LSU rDNA sequences from 48 hypogeous species (represented by 55 specimens) and 134 epigeous pezizalean species (represented by 141 specimens) were compiled for analyses (for sequence accession numbers, see online Supplementary Data Table 1). Sequences were selected to represent all sub-lineages within *Pezizales* based primarily on Hansen *et al.* (2001, 2005), O'Donnell *et al.* (1997), and Perry *et al.* (2007). Nucleotide sequences were aligned by hand using the software program Se-Al v. 2.0a11 (Rambaut 1996 *Se-Al: Sequence Alignment Editor*; available at http://evolve. zoo.ox.ac.uk/). The LSU rDNA contains highly divergent regions across all of the *Pezizales*. Therefore, three subset alignments were constructed, each representing one of three distinct lineages identified within the *Pezizales* (Fig 1) (Landvik *et al.* 1997; Hansen & Pfister 2007). The three alignments include representative taxa from the families *Pezizaceae* (lineage A; Fig 2); *Caloscyphaceae, Discinaceae, Helvellaceae, Morchellaceae, Rhizinaceae,* and *Tuberaceae* (lineage B; Fig 3); and
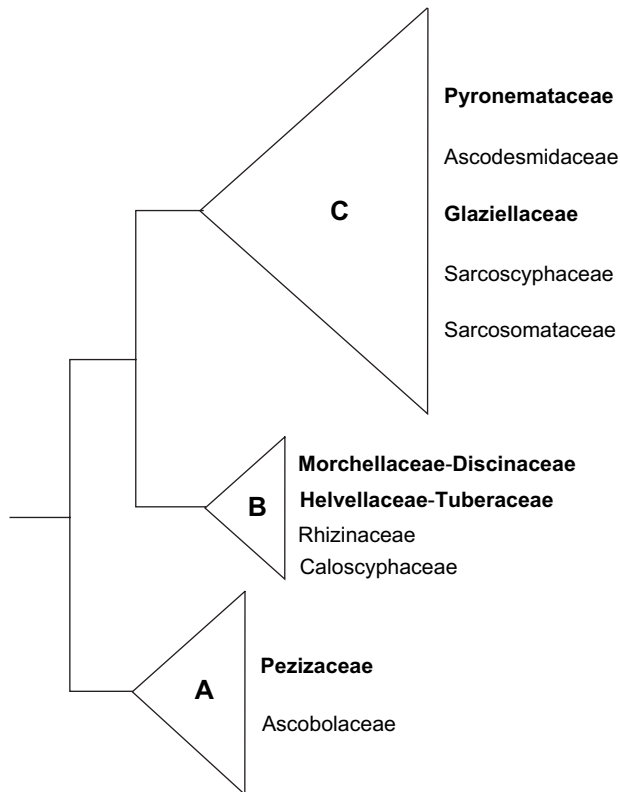
Fig 1 – Schematic tree giving an overview of the three major clades (A–C) identified within *Pezizales* using SSU rDNA sequences (after Landvik *et al.* 1997). Truffles have evolved within the families highlighted in bold. Families listed for each clade follow Eriksson (2006a). The *Morchellaceae–Discinaceae* and *Helvellaceae–Tuberaceae* lineages are according to O'Donnell *et al.* (1997).

Ascodesmidaceae and *Pyronemataceae* (lineage C; Fig 4). Members of the *Sarcoscyphaceae* and *Sarcosomataceae* were not included, because no truffle taxa were affiliated with these families. The final datasets included 68 epigeous species (from 72 specimens) and 17 hypogeous species (20 specimens) (lineage A); 22 epigeous species (one specimen each) and 22 hypogeous species (23 specimens) (lineage B); and 44 epigeous species (47 specimens) and nine hypogeous species (12 specimens) (lineage C). Based on phylogenetic analyses of higher-level relationships (e.g. Landvik 1996; Hansen & Pfister 2007; Perry *et al.* 2007), *Neolecta vitellina* was used as an outgroup for lineage A (with the ingroup also including taxa from the lineages B and C); two species of *Peziza* and *Iodophanus* for lineage B; and *Ascobolus* and *Peziza* for lineage C. Alignments are available from TreeBASE (http://www.treebase.org) as accessions M3364 (lineage A), M3363 (lineage B), and M3362 (lineage C).

Phylogenetic analyses

Analyses of the LSU were performed using PAUP version 4.0b10 for Unix (Swofford 2002) and MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001) on G5 Macintosh computers. MP, parsimony BS (PB), and Bayesian analyses were performed as in

Hansen *et al.* (2005), except Bayesian MCMC were run for 5M generations. The GTR + I + G model of sequence evolution was selected for each dataset using MrModeltest v. 2.2 (Nylander 2004). In Bayesian analyses, the first 1500 trees were deleted as the 'burn-in' period of the chain for the lineage A dataset, and Bayesian PP are based on the last 48,500 trees sampled. For the lineages B and C, the last 46,700 and 49,000 trees were used, respectively. Clades represented by PB ≥ 75 % and/or PP ≥ 95 % are considered to be significantly supported.

Based upon the results of the phylogenetic analyses, topologically constraint MP and ML analyses were used to evaluate how many times hypogeous taxa have been derived from epigeous apothecia-forming taxa, with loss of forcible spore discharge. Constraint topologies were manually specified in PAUP. The MP analyses were performed under the constraints, using the same settings as specified above (Hansen *et al.* 2005). The ML analyses consisted of heuristic searches with ten random addition sequence replicates, tree bisection–reconnection (TBR) branch swapping and starting trees obtained *via* stepwise addition. The ML GTR + I + G model parameters used, were fixed to values estimated from one of the unconstrained MP trees (from the original MP analyses). The Kishino–Hasegawa test (Kishino & Hasegawa 1989) and the Shimodiara–Hasegawa tests (Shimodaira & Hasegawa 1999) were used to compare constrained and unconstrained topologies in PAUP version 4.0b10.

Results

Phylogenetic relationships of truffles within lineage A

The LSU dataset of lineage A included 973 characters with 338 being parsimony informative. Parsimony analyses resulted in 1391 equally MPTs (1327 steps, CI = 0.333, RI = 0.678). The *Pezizaceae* are highly supported as monophyletic (PB 99 %, PP 100 %), with *Ascobolaceae* as the sister group (PB 97 %, PP 100, Fig 2). The strict consensus tree of all MPTs is highly resolved, but the deep level relationships are not well supported. Fourteen fine-scale lineages that correspond to the lineages resolved in Hansen *et al.* (2005) are recovered by all analyses. The 17 truffle species (11 genera) sampled are nested within five or six of the 14 lineages; *Eremiomyces echinulatus* is resolved separately with *Peziza vacini* in the MP analysis (Fig 2), but is placed in the *Plicaria–Hapsidomyces* lineage, along with *Peziza phyllogena* in ML and Bayesian analyses. The truffle *Amylacus tasmanicus* forms a highly supported sister taxon (PB/PP 100 %), to a highly supported clade of three species of the truffle genus *Pachyphloeus*, the anamorph *Glischroderma* sp. and the apothecial *Scabropezia* (PB 98 %, PP 100 %). The two species of the truffle genus *Hydnotryopsis* form a strongly supported group with *Sarcosphaera* (PB/PP 100 %). The three specimens of *Sarcosphaera coronaria* (from North America and Denmark) exhibit quite large sequence variation, but form a monophyletic group (PP 95 %). The placement of *Mattirolomyces* is uncertain; it is deeply nested within the *Peziza s. str.* lineage in the strict consensus tree of all MP trees, but is grouping with *Iodophanus*, as a sister group to the *Peziza s. str.* lineage in ML and Bayesian analyses (none of these positions are with
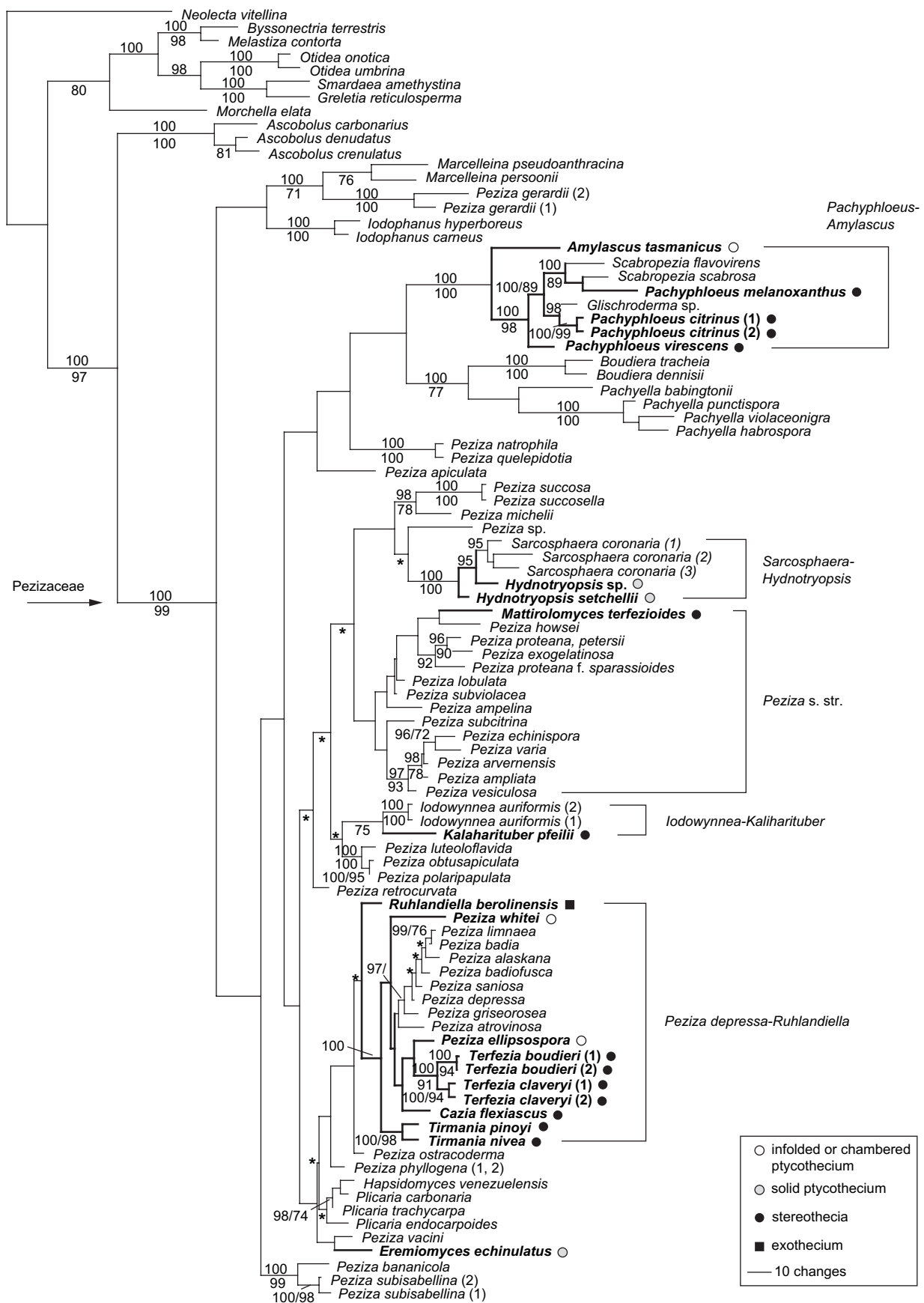
Fig 2 – Phylogenetic relationships among epigeous and hypogeous taxa in *Pezizaceae* (lineage A), derived from parsimony analyses of LSU rDNA sequences. One of 1391 most parsimonious trees. Terminal taxa represent individual specimens (from Hansen *et al.* 2001, 2005; Ferdman *et al.* 2005; Norman & Egger 1999). *Neolecta vitellina* was used to root the phylogeny. Hypogeous lineages are shown in bold. Numbers above branches represent PP (≥95 %). Numbers below branches represent PB support (≥70 %). Symbols by taxon names indicate specific fruiting body types of truffles. Fine-scale lineages, as defined in Hansen *et al.* (2005), that include truffles are indicated for discussion in the text.
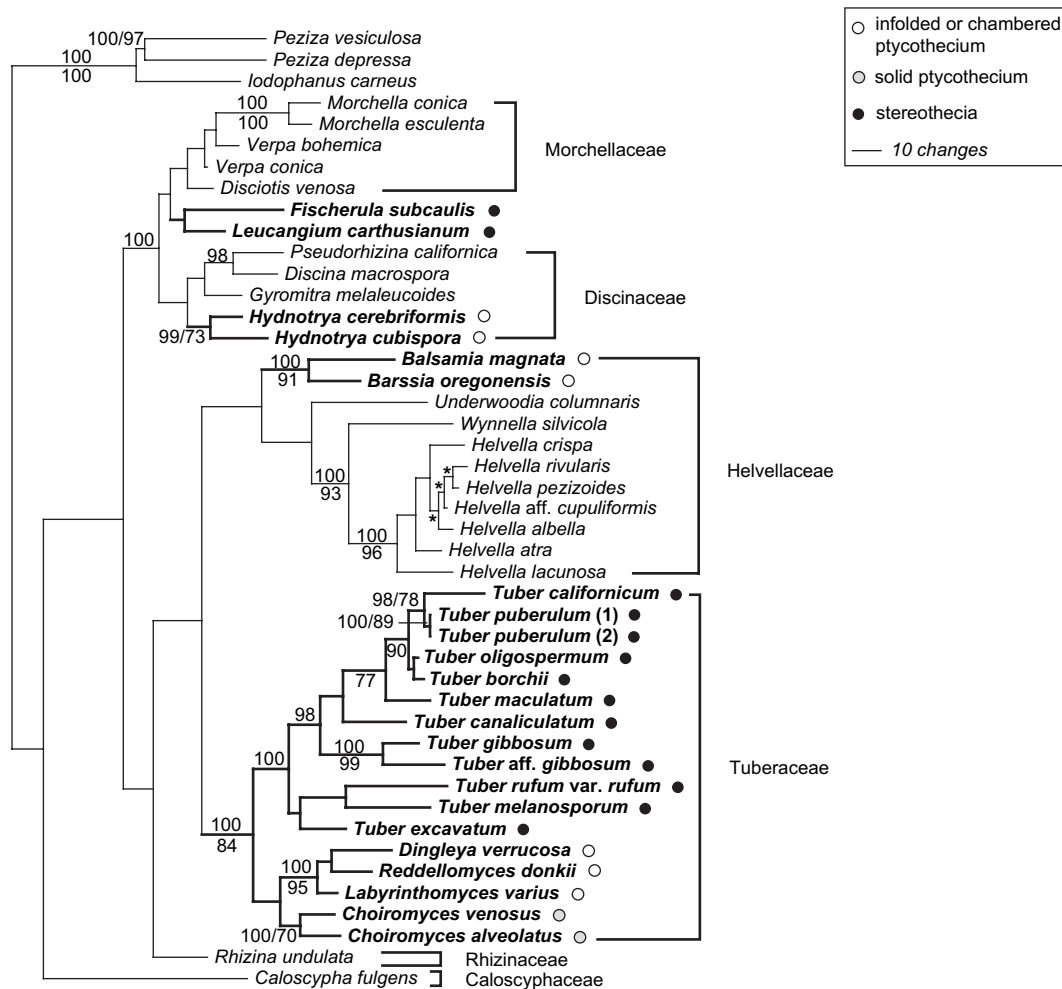
Fig 3 – Phylogenetic relationships among epigeous and hypogeous taxa within the families *Morchellaceae*, *Discinaceae*, *Helvellaceae* and *Tuberaceae* (lineage B), derived from parsimony analyses of LSU rDNA sequences. One of three most parsimonious trees. Terminal taxa represent individual specimens (primarily from O'Donnell *et al.* 1997). *Peziza vesiculosa*, *P. depressa* and *Iodophanus carneus* were used to root the phylogeny. Hypogeous lineages are shown in bold. Numbers above branches represent PP (≥95 %). Numbers below branches represent PB support (≥70 %). Symbols by taxon names indicate specific fruiting body types of truffles.

significant support). *Kaliharituber* is suggested as closely related to *Iodowynnea* (PB 75 %). The truffle genera *Cazia*, *Ruhlandiella*, *Terfezia,* and *Tirmania*, and two truffle species of *Peziza*, *P. ellipsospora* and *P. whitei*, are resolved among apothecia-forming *Peziza* species in the *P. depressa–Ruhlandiella* lineage. This lineage, excluding *Ruhlandiella*, is supported by 100 % PP, but is with only 53 % PB.

At least nine independent origins of hypogeous forms are supported by the LSU gene trees (Fig 2). Constrained MP and ML analyses forcing the two species of *Hydnotryopsis* to be monophyletic could not be rejected (Table 1). Likewise, forced monophyly of the hypogeous taxa within the *P. depressa–Ruhlandiella* lineage (not including *Eremiomyces*), did not yield trees that were significantly longer than the unconstrained MP trees. However, under this constraint the ML tree was significantly worse than the unconstrained optimal ML tree (Table 1). Trees rejected by MP and ML include the following monophyly constraints: truffles in the *P. depressa* lineage

including *Eremiomyces* (with or without *Ruhlandiella*), *Amylascus–Pachyphloeus*, *Pachyphloeus*, *Mattirolomyces* with *Terfezia*, and *Kaliharituber* with *Terfezia* (Table 1). The most conservative conclusion is thus, that forcible spore discharge has been lost only once within each of the lineages *Sarcosphaera–Hydnotryopsis* and *P. depressa–Ruhlandiella*, once in *Eremiomyces*, *Kaliharituber*, *Mattirolomyces*, and *Amylascus*, and three times in *Pachyphloeus* (assuming that active spore discharge, once lost, can not be regained).

Phylogenetic relationships of truffles within lineage B

Parsimony analyses of lineage B yielded three equally MPTs (1235 steps, CI = 0.423, RI = 0.639) produced from 699 characters, of which 233 were parsimony informative. The strict consensus tree of the three MPTs is highly resolved, but support for the families are lacking, except for *Tuberaceae* (PB 84 %, PP 100 %, Fig 3). The trees recovered by MP, Bayesian, and
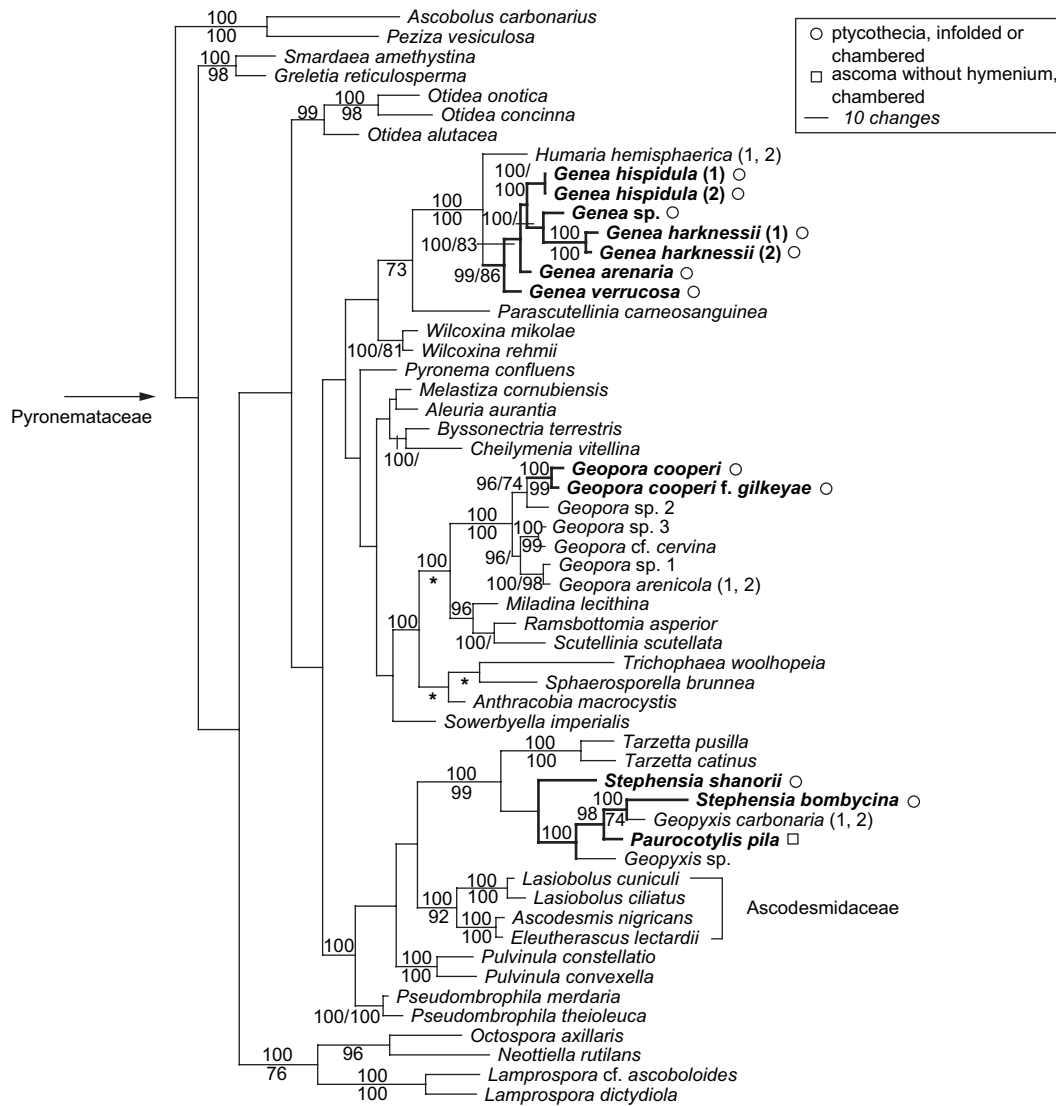
Fig 4 – Phylogenetic relationships among epigeous and hypogeous taxa in *Pyronemataceae* (lineage C), derived from parsimony analyses of LSU rDNA data consisting of 894 aligned nucleotides for 56 taxa. One of three most parsimonious trees. Terminal taxa represent individual specimens. Hypogeous lineages are shown in bold. Numbers above branches represent PP (≥95 %). Numbers below branches represent PB support (≥70 %). Symbols by taxon names indicate specific fruiting body types of truffles.

ML analyses did not possess any supported conflict. Bayesian analyses support *Helvellaceae* (PP 99 %) excluding *Underwoodia columnaris*, which is unresolved. A *Morchellaceae–Discinaceae* (PP 100 %) and a *Helvellaceae–Tuberaceae* lineage are resolved by MP and ML analyses (Fig 3), in accordance with O'Donnell *et al.* (1997), who used both SSU and LSU. The truffles *Leucangium* and *Fischerula subcaulis* are variously placed within the *Morchellaceae–Discinaceae* lineage, and their exact position is unknown. Two species of *Hydnotrya*, *H. cerebriformis* and *H. cubispora*, form a monophyletic group (PB 73 %, PP 99 %) nested within the *Discinaceae* in all analyses. *Balsamia*, *B. magnata* and *B. oregonensis*, is likewise monophyletic (PB 91 %, PP 100 %) and forms a sister group to a highly supported clade of apothecial *Helvella* species and *Wynella silvicola* in all analyses. The 11 species of *Tuber* included form a monophyletic group (PB 69 %, PP 100 %), as a sister group to a clade of four

additional truffle genera, *Dingleya*, *Reddellomyces*, *Labyrinthomyces*, and *Choiromyces s. str.*

The most parsimonious interpretation of the LSU phylogeny suggests that the truffle form originated four times within lineage B (Fig 3). Nevertheless, constraint MP and ML analyses forcing *Fischerula*, *Leucangium*, and *Hydnotrya* into a monophyletic group could not be rejected (Table 1). This suggests that forcible spore discharge has been lost at least three times within lineage B, once in the *Morchellaceae–Discinaceae* lineage, once in *Helvellaceae*, and in *Tuberaceae*.

Phylogenetic relationships of truffles within lineage C

Parsimony analyses of lineage C yielded three equally MPTs (1679 steps, CI = 0.424, RI = 0.637) from 894 total characters, of which 302 were parsimony informative. The strict

Table 1 – Evaluation of different constrained tree topologies in MP and ML analyses, compared with the MPTs and the optimal MLT, respectively, using the Kishino–Hasegawa test for MP and the Shimodiara–Hasegawa test for ML ($p < 0.05$)

Tree	MP		ML			
	Tree lenght[a]	Significantly worse?	Ln likelihood	Difference in LnL	P-value	Significantly worse?
Lineage A, unconstrained MPT	2078	Best	–	–	–	–
Lineage A, unconstrained optimal MLT	–	–	−11183.03738	–	–	Best
Hydnotryopsis monophyletic	2082 (+4)	No	−11189.40900	−6.37162	0.083	No
Truffles in 'P. depressa lineage' monophyletic (including *Ruhlandiella* and *Eremiomyces*)	2095 (+17)	Yes	−11224.21753	−41.18015	0.006*	Yes
Truffles in 'P. depressa lineage' monophyletic (not including *Ruhlandiella*, but including *Eremiomyces*)	2093 (+15)	Yes	−11213.75681	−30.71942	0.004*	Yes
Truffles in 'P. depressa lineage' monophyletic (not including *Eremiomyces*, but including *Ruhlandiella*)	2085 (+7)	No	−11201.55494	−18.51755	0.020*	Yes
Amylascus and *Pachyphloeus* monophyletic	2098 (+16)	Yes	−11219.85875	−36.82137	0.041*	Yes
Pachyphloeus monophyletic	2090 (+12)	Yes	−11214.23004	−31.19266	0.005*	Yes
Mattirolomyces with *Terfezia*	2094 (+12)	Yes	−11215.54524	−32.50785	0.009*	Yes
Kaliharituber with *Terfezia*	2098 (+20)	Yes	−11218.85867	−35.82129	0.051	No
Lineage B, unconstrained MPT	1235	Best	–	–	–	–
Lineage B, unconstrained optimal MLT	–	–	−6540.88728	–	–	Best
Fischerula–Leucangium with *Hydnotrya*	1239 (+4)	No	−6550.10480	−9.21752	0.077	No
Lineage C, unconstrained MPT	1679	Best	–	–	–	–
Lineage C, unconstrained MLT	–	–	−8958.87503	–	–	Best
Stephensia with *Paurocotylis*	1694 (+15)	Yes	−8991.20102	−32.32599	0.001*	Yes
Stephensia monophyletic	1692 (+13)	Yes	−8992.05211	−33.17708	0.001*	Yes

a Difference in length between MPTs and constrained trees in parentheses.

consensus tree of the three MPTs is nearly completely resolved, but as for the lineages A and B the deep level relationships are poorly supported. *Pyronemataceae* are suggested to be paraphyletic, because *Ascodesmidaceae* are nested within it. *Ascodesmidaceae* are highly supported as monophyletic (Fig 4). Twelve clades of pyronemataceous taxa are recovered by all analyses, which correspond to those identified by Perry et al. (2007) who used a much larger taxon sampling. The nine truffle species included are nested within three, moderate to highly supported clades with apothecial pyronemataceous taxa (Fig 4). The five species of the hypogeous genus *Genea* form a monophyletic group (PB 86 %, PP 99 %), as a sister group to the epigeous *Humaria hemisphaerica* (PB/PP 100 %). The truffle *Geopora cooperi*, forms a highly supported monophyletic group with five epigeous species of *Geopora* (PB/PP 100 %). *Geopora* is suggested to be a sister group to a clade of the apothecial *Ramsbottomia*, *Scutellinia*, and *Miladina* (PP 100 %). The truffles *Stephensia* and *Paurocotylis pila* form a highly supported group with apothecial *Tarzetta* and *Geopyxis* (PB 99 %, PP 100 %). *Stephensia* is suggested to be non-monophyletic; *Stephensia bombycina* form a well-supported group with *Geopyxis carbonaria* (PB 74 %, PP 100 %), with *Paurocotylis pila* (PP 98 %), *Geopyxis* sp. (PP 100 %), and *Stephensia shanorii* as successive sister taxa.

The most parsimonious interpretation of the LSU phylogeny suggests that forcible spore discharge has been lost at least five times within the *Pyronemataceae* (in *Genea*, *Geopora cooperi* (not completely), *Paurocotylis* and twice in *Stephensia*). The constraint analyses forcing *Stephensia* to be monophyletic, or *Stephensia* and *Paurocotylis* to be monophyletic were rejected (Table 1).

Evolution of ascomata types

At least five different forms of ascomata exist within *Pezizales*. Epigeous apothecia of various shapes with forcible spore discharge are the most common form and occur in each of the A, B, and C lineages. This is likely the ancestral form, and the molecular data suggest that the apothecia-forming *Pezizales* have given rise to at least four different types of hypogeous ascomata without forcible spore discharge (pro parte sensu Weber et al. 1997): ptychothecia [hollow to folded with internal hymenia, in *Pezizaceae, Discinaceae, Helvellaceae, Tuberaceae* and *Pyronemataceae* (Figs 2–4)]; stereothecia [solid without hymenia, in *Pezizaceae, Discinaceae–Morchellaceae,* and *Tuberaceae* (Figs 2 and 3)]; exothecia [external hymenia, *Ruhlandiella* (*Pezizaceae*, Fig 2)]; and an unnamed type found in *Glaziella* and *Paurocotylis* (*Glaziellaceae* and *Pyronemataceae*, Fig 4; recalls bladder-shaped ptychothecia, but without organized hymenia). The molecular data suggests that ptychothecia and stereothecia have evolved multiple times in different lineages within *Pezizales*.

Taxonomy

Taxonomic implications: an overview of accepted hypogeous *Pezizales* taxa

Lineage A
The *Ascobolaceae* have no confirmed hypogeous representatives but various truffle taxa have at times been placed in

the family, e.g. *Sphaerosoma* and *Ruhlandiella* (as *Muciturbo*) (e.g. Castellano *et al*. 2004). See Figs 2 and 5A-D.

Pezizaceae Dumort. 1829 (syn. *Terfeziaceae* E. Fisch. 1897)

The family *Terfeziaceae* as defined by Zhang (1992a, 1992b) are included in this family, but was accepted in the latest *Dictionary of the Fungi* (Kirk *et al*. 2001). Recent molecular results (e.g. Norman & Egger 1999; Hansen *et al*. 2005) clearly demonstrate it should be relegated to synonymy of the *Pezizaceae* (see review in Hansen & Trappe 2002). Thirteen out of 25 genera in the *Pezizaceae* (Eriksson 2006a) are exclusively truffle or truffle-like taxa, but several truffle species have also been described in *Peziza*. Hansen *et al*. (2001) gave a review of the genera. The genus *Peziza* was found to be non-monophyletic and all other pezizaceous genera nested within it (Hansen *et al*. 2001, 2005), and a revised generic arrangement is under way (Hansen & Pfister, in preparation.). Two lineages discovered comprise most of the *Peziza* species, the *Peziza s. str*. and the *P. depressa–Ruhlandiella* lineages, the latter including several truffles (*Cazia, Peziza ellipsospora, P. whitei, Ruhlandiella, Terfezia* and *Tirmania*; Fig 2). The *P. depressa–Ruhlandiella* lineage was highly supported in combined analyses of LSU, β-tubulin, and RPB2 (Hansen *et al*. 2005). Only one truffle genus, *Mattirolomyces*, clusters in *Peziza s. str*. in MP analyses, but without support (Fig 2). Three types of hypogeous ascomata exist within the family (Fig 2). The *Amylascus–Pachyphloeus* and the *P. depressa–Ruhlandiella* lineages produce both ptychothecia and stereothecia. The cardinal feature of *Pezizaceae*, the amyloid reaction of the ascus wall, has been lost in several of the hypogeous taxa (e.g. *Cazia* and *Terfezia*).

Amylascus Trappe 1971

Type: *Amylacus herbertianus*.

The type species of *Amylacus* has not been sampled for molecular phylogenetic study, but the genus is most likely monophyletic [*A. tasmanicus* has even been considered a synonym of *A. herbertianus* (Beaton & Weste 1982)] and is suggested to be closely related to *Scabropezia* and *Pachyphloeus*. *Amylascus* was originally placed in the *Terfeziaceae* or *Geneaceae* (Trappe 1971, 1975a), but later, based on the thick-walled, amyloid asci, was placed in the *Pezizaceae* (Trappe 1979). Trappe (1975a) and Beaton & Weste (1982) monographed the genus. *Amylascus* includes only the two mentioned species, both recorded only in Australia.

Cazia Trappe 1989

Type: *Cazia flexiascus*.

Originally, and at times by some subsequent authors, placed in the *Helvellaceae* (Trappe 1989), but Kirk *et al*. (2001) place it in the *Terfeziaceae*. O'Donnell *et al*. (1997) were the first to place *Cazia* in the *Pezizaceae*. As can be seen from Fig 2, it is nested within the *P. depressa–Ruhlandiella* lineage containing both epigeous and hypogeous taxa. *Cazia quercicola* Fogel & States (2002) is only the second recognized species.

Eremiomyces Trappe & Kagan-Zur 2005

Type: *Eremiomyces echinulatus* (syn. *Choiromyces echinulatus*).

Ferdman *et al*. (2005) found this species to cluster within the *Pezizaceae* (with *Terfezia* and *Tirmania* species) and not with the type of *Choiromyces*, which has affinities with the *Tuberaceae*.

Fig 5 – Fruiting body forms in lineage A (*Pezizaceae*). (A-B) *Sarcosphaera coronaria* (A) Closed apothecia, JHP-95.074 (C). (B) Open apothecia. (C) *Hydnobolites cerebriformis*, ptychothecia. (D) *Terfezia leptoderma*, stereothecia. Photos: J.H. Petersen (A), K. Hansen (B), J. Nitare (C), J. Santos (D).

Besides the molecular results the highly inflated exipular cells also suggest this fungus belongs to *Pezizaceae* rather than *Tuberaceae*. The exact placement of *Eremiomyces* within *Pezizaceae* is not resolved in our analyses, but it is likely among members of the *P. depressa*–*Ruhlandiella*, *Plicaria*–*Hapsidomyces* or *P. phyllogena* lineages (the inclusive clade A in Hansen *et al.* 2005).

Hydnobolites Tul. & C. Tul. 1843 (Fig 5C)

Type: *Hydnobolites cerebriformis*.

This genus apparently has only two accepted species, *H. cerebriformis* from Europe and *H. californicus* from North America. The type species has saccate, amyloid [when pretreated in potassium hydroxide (KOH)] asci formed in poorly defined hymenia, without well-differentiated paraphyses, in brain-like, pale ascomata. The spores are globose with a reticulate and spinulose ornament. The genus was previously placed in the *Tuberaceae* (Gilkey 1955; Korf 1973a; Castellano *et al.* 2004) or in the *Terfeziaceae* (Hawker 1954; Trappe 1971, 1979). Trappe (1979) regarded *Hydnobolites* to be close to *Pachyphloeus* and *Terfezia* (Fig 5D). Kimbrough *et al.* (1991) suggested a placement in the *Pezizaceae* based on ultrastructural observations of septal pores. They also found the asci to be weakly amyloid after treatment in 2 % KOH. No molecular data are available for *Hydnobolites*, and the placement is mainly based on the amyloid asci and the suggested close relationship to *Pachyphloeus* and *Terfezia*.

Hydnotryopsis Gilkey 1916

Type: *Hydnotryopsis setchellii*.

Gilkey (1954) later abandoned the genus and placed it in *Choiromyces*. In agreement with Hansen *et al.* (2005), *Hydnotryopsis setchelli* and an unnamed *Hydnotryopsis* are suggested as closely related to the near hypogeous *Sarcosphaera coronaria* (Figs 2 and 5A-B). The constraint analyses forcing the two *Hydnotryopsis* species to be monopyletic could not be rejected (Table 1). *Hydnotryopsis* was placed in the *Pezizaceae* by Fischer (1938), and based on the diffusely amyloid asci, followed by e.g. Trappe (1975c, 1979). The solid ascomata have a peridium of globose cells, and asci and paraphyses in a hymenial configuration.

Kalaharituber Trappe & Kagan-Zur 2005

Type: *Kalaharituber pfeilii* (syn. *Terfezia pfeilii*).

Ferdman *et al.* (2005) demonstrated (using ITS and LSU) the non-monophyletic nature of *Terfezia* and erected *Kalaharituber* for a southern African desert truffle originally described as *T. pfeilii* (basionym in error given as *Tuber pfeilii*). No epigeous representatives were included in their analyses. Our analyses indicate a relationship with the epigeous *Iodowynnea* (PB 75 %, Fig 2). Taylor *et al.* (1995) discussed the biology of *K. pfeilii* and suggested it could be mycorrhizal with species of *Acacia,* although a strong association with grasses was noted.

Mattirolomyces E. Fisch. 1938

Type: *Choiromyces terfezioides* (syn. *Mattirolomyces terfezioides*, *Terfezia terfezioides*).

This genus was reinstated by Percudani *et al.* (1999) and accepted as such by Diéz *et al.* (2002) and Ferdman *et al.* (2005), after having been sunk under *Terfezia*, where it still recides in e.g. Montecchi & Sarasini (2000). Unlike species of *Terfezia*, *M. terfezioides* occurs in woodland or in ruderal habitats rather than in deserts (e.g. Montecchi & Sarasini 2000). Kovács *et al.* (2003) reviewed the mycorrhizae studies on *Mattirolomyces* and similar taxa and concluded that there is no clear evidence for a mycorrhizal function, and an ectomycorrhizal anatomy does not develop (with *Robinia* or *Helianthemum ovatum*) but, instead, an anatomy referred to as 'terfezioid'. Kovács *et al.* (2007) maintained that the trophic strategy of this fungus remains ambiguous. It forms sclerotia in the same manner as certain species of *Morchella*. Although the position of *Mattirolomyces* is uncertain in our analyses, constraint analyses forcing *Mattirolomyces* to group with *Terfezia* were rejected (Table 1). Healy (2003) described an additional American species, but based on molecular data (R. Healy, K. Hansen and G. Kovács, unpublished results) this species is not a member of *Mattirolomyces*.

Mycoclelandia Trappe & Beaton 1984 (syn. *Clelandia*)

Type: *Clelandia arenacea* (syn. *M. arenacea*).

Beaton & Weste (1982) revised the two known species (as *Clelandia*) and Trappe & Beaton (1984) replaced the invalid homonym *Clelandia* for *Mycoclelandia*. The asci stain strongly or diffused blue in iodine solutions. There are no sequences available, but based on the known morphological features the genus clearly belongs in the *Pezizaceae*.

Pachyphloeus Tul & C. Tul. 1844 (syn. *Pachyphlodes*, *Cryptica*)

Type: *Pachyphloeus melanoxanthus*.

The ascomata typically have an apical depression or pore connecting to a few open veins. The peridium is verrucose and contains globose cells. Trappe (1979) placed the genus in the *Terfeziaceae* and gave the above synonymy (Trappe 1975c). It had mainly been treated within the *Tuberaceae* (e.g. Knapp 1951; Korf 1973a). Amyloid asci occur in some species of *Pachyphloeus* (e.g. the type species), and based on this and anatomical features the genus was placed in the *Pezizaceae* (Dissing & Korf 1980). This has been confirmed by molecular data (Norman & Egger 1999; Percudani *et al.* 1999; Hansen *et al.* 2005). Phylogenetic analyses of LSU suggest that the type species is more closely related to species of *Scabropezia* than to other species of *Pachyphloeus* (PB 89 %, PP 100 %, Fig 2). Also, constraint analyses forcing the three included *Pachyphloeus* spp. to be monophyletic were rejected (Table 1). This suggests that *Scabropezia* may be a synonym of *Pachyphloeus*.

Peziza Fr. 1822 (syn. *Hydnoplicata*)

Type (lectotype): *Peziza vesiculosa*.

Several hypogeous species, with passive spore dispersal, have been accepted in the otherwise epigeous, apothecial genus *Peziza*. Trappe (1979) noted six hypogeous species in *Peziza* and recently *Peziza infossa* (syn. *P. quercicola*) was added (Fogel & States 2002, 2003). Although this latter species is described as having operculate asci, no active spore discharge had been observed. *Peziza* has been demonstrated several times, using molecular phylogenetics, to be non-monophyletic (see above under *Pezizaceae*). The two pezizas with passive spore dispersal, *P. whitei* and *P. ellipsospora*, included in the molecular analyses, are nested within the *P. depressa*–*Ruhlandiella* lineage (Hansen *et al.* 2001, 2005) (Fig 2). In this lineage, these two taxa represent a less derived truffle form; both produce infolded ptycothecia, with a single opening, cylindrical asci with eight ascospores in a single row and paraphyses

placed in hymenia. Both species have retained the amyloid reaction of the asci. More derived truffle forms in this lineage (*Terfezia* and *Tirmania*) produce compact ascomata (stereothecia), with elongate-clavate to sub-globose asci (5-8 spored), randomly arranged in fertile areas, separated by sterile veins. *Tirmania* has amyloid asci, whereas this reaction is lost in *Terfezia*. The relationships among the taxa in the *P. depressa–Ruhlandiella* lineage are not unambiguously resolved, and a hypothesis about the evolution of these forms must await further molecular studies using more variable gene regions and a larger taxon sampling. Trappe & Claridge (2006), nevertheless, resurrected *Hydnoplicata* for *P. whitei* based on the molecular results by Hansen *et al.* (2001). However, depending on the delineation within this lineage (see also Hansen *et al.* 2005 and Fig 2), other possible and older generic names could be *Terfezia* or *Tirmania*. *Hydnoplicata* was based on *H. whitei* later transferred to *Peziza* (Trappe 1975c), and this was again confirmed by the molecular phylogenetic study by Hansen *et al.* (2001, 2005). Beaton & Weste (1982) gave an account of *P. whitei*. Also Korf (1973b) discussed this species (as *P. jactata*).

Ruhlandiella Henn. 1903 emend. Dissing & Korf 1980 (syn. *Tremellodiscus* C.G. Lloyd, ?*Muciturbo* P.H.B. Talbot 1989)
   Type: *Ruhlandiella berolinensis*.

Ruhlandiella is more or less epigeous but with passive spore dispersal and with a somewhat convoluted ascoma, where the hymenium covers the surface (exothecial) rather than being disposed internally. The paraphyses have characteristic gelatinous sheaths. Dissing & Korf (1980) placed this genus in the *Pezizaceae* with a proposed relationship to the genera *Sphaerozone*, *Boudiera*, and *Plicaria*. Molecular results place it in the *P. depressa–Ruhlandiella* lineage (Hansen *et al.* 2005) (Fig 2). *Muciturbo* was accepted and listed in the *Ascobolaceae* by Castellano *et al.* (2004), but Galán & Moreno (1998) and Hansen (2000) suggest it as a synonym of *Ruhlandiella* based on a detailed comparative study of the proposed distinguishing characters. Dissing & Korf (1980) also noted that another Hennings genus, *Exogone*, could represent an additional generic synonym and an additional species. *Ruhlandiella* (as *Muciturbo*) has been associated with a *Chromelosporium* anamorph (Warcup & Talbot 1989) in accordance with other connections in this clade (Hansen *et al.* 2005). Warcup & Talbot (1989) reported the spores of *Muciturbo* species to be uninucleate.

Sphaerozone Zobel 1854 (syn. *Sphaerosoma* subgen. *Tulasnia*)
   Type: *Sphaerozone ostiolatum* (syn. *S. tulasnei*).

This is a monotypic genus with exothecial, more or less spherical, and to some extent convoluted ascomata, and amyloid asci. These characters, on current evidence place the genus within the *Pezizaceae*. The asci are more or less as in typical members of the family but indehiscent, and the paraphyses are likewise typical. The exposed hymenium also suggests a fairly recent radiation from actively dispersed ancestors. Beaton & Weste (1978) overlooked the amyloid asci in the type species. The non-amyloid species, *Sphaerozone echinulatum* and *S. ellipsosporum*, should not be accepted in the genus, as also stated in Beaton & Weste (1982), and were duly transferred to *Gymnohydnotrya* (Zhang & Minter 1989b). Dissing & Korf (1980) first noted the amyloidity of the asci in the type species and also clarified the

nomenclatural confusion surrounding the names *Sphaerozone* and *Sphaerosoma*. There is a certain resemblance to the genus *Ruhlandiella*. All known collections are from the vicinity of ectotrophic plants, so it is most likely ectomycorrhizal. Montecchi & Sarasini (2000) illustrate and describe the genus but also cited *Sphaerosoma* as a synonym (see this).

Terfezia (Tul. & C. Tul.) Tul. & C. Tul. 1851 (Fig 5D)
   Type (lectotype): *Terfezia leonis* (syn. *Terfezia arenaria*).

Two species of *Terfezia*, *T. boudieri* and *T. claveryi*, are deeply nested within the *P. depressa–Ruhlandiella* lineage (Hansen *et al.* 2005) (Fig 2). The *Terfeziaceae* were based on the lack of structure in the arrangement of the asci that led early workers (e.g. Fischer 1897) to consider *Terfezia* outside the *Tuberales*. Vizzini (2003) considered the *Tuberaceae* and *Terfeziaceae* to exhibit extreme convergent morphology and also noted that the relationship of these families have been especially controversial. Trappe (1971) accepted the family *Terfeziaceae* within the *Tuberales* and later in the *Pezizales* (Trappe 1979). Trappe & Sandberg (1977) studied the Japanese/North American non-desert species *T. gigantea* in detail and described a rather complicated ascospore wall with minute spines, while Janex-Favre & Parguey-Leduc (1985) and Janex-Favre *et al.* (1988) studied the ascus structure and ascospores in *T. claveryi* and *T. leptoderma* and found similarities to *Tuber*. Janex-Favre & Parguey-Leduc (2003) again studied the ascomata and concluded that *Tuber* and *Terfezia* should be retained within the *Tuberales*. Norman & Egger (1999) and Percudani *et al.* (1999) found evidence for a position within the *Pezizaceae* (see also *Kalaharituber* and *Mattirolomyces*). Diéz *et al.* (2002), in a recent ITS study, hypothesized a single origin of the so-called desert truffles, *Tirmania* and *Terfezia*, but included only hypogeous taxa and no other truffle taxa from the *P. depressa–Ruhlandiella* lineage. Trappe (1971) characterized the genus *Terfezia* as the most heterogenous genus in the *Terfeziaceae*. For a review of the mycorrhizal biology of the genus see Kovács *et al.* (2003).

Tirmania Chatin 1892 [date disputed: 1890 sec Trappe; 1891 sec Hansen *et al.* 2001]
   Type: *Tirmania africana* (syn. *T. nivea*).

The amyloid reaction of the asci combined with a double ascospore wall, with the outer smooth and the inner with a reticulate-roughened wall, characterize the genus according to Alsheikh & Trappe (1983a). The two species accepted by these authors associate with species of *Helianthemum*, but the exact nature of this association is disputed (Kovács *et al.* 2003). They apparently disperse by wind after drying *in situ*, rather than relying on an animal vector. The species are prized as food items, and Rayss (1959, as cited in Alsheikh & Trappe 1983a) suggested that the manna that fed the Israelites could have been *Tirmania* truffles. Moreno *et al.* (2000) reported smooth spores in *T. nivea* and a fine net-like ornament on *T. pinoyi* spores. See also the descriptions in Malençon (1973) and in Montecchi & Sarasini (2000). Diéz *et al.* (2002) studied a number of desert truffles by molecular phylogenetic analyses, and concluded that the sampled species formed a monophyletic group. Trappe (1979) transferred the genus to the *Pezizaceae*

based on the amyloid asci. Our analyses place *Tirmania* in the *P. depressa–Ruhlandiella* lineage.

Lineage B

The monotypic, parasitic *Rhizinaceae* and *Caloscyphaceae* have no known hypogeous representatives. Truffles forming ptychothecia and stereothecia are identified in both the *Morchellaceae–Discinaceae* and *Helvellaceae–Tuberaceae* lineages (Figs 3, 6A-H). The family *Tuberaceae* is unique in its high diversity of strictly hypogeous taxa.

Morchellaceae–Discinaceae

O'Donnell *et al.* (1997) placed *Leucangium* and *Fischerula* as *incertae sedis* due to suspected long-branch attraction between these taxa. Additional sampling of hypogeous taxa in this group, including the type species of *Fischerula*, could possibly help resolve this problem.

Fischerula Mattir. 1928 (Fig 6A)

Type: *Fischerula macrospora*.

Mattirolo (1928) and Knapp (1951) separated *Fischerula* from *Tuber* based on the peculiar spore ornamentation and the more or less fusiform asci. Trappe (1975b, 1979) placed *Fischerula* in the *Helvellaceae*, a placement that can be rejected as long as the two known species are considered congeneric. The ascoma of the American taxon, *F. subcaulis*, has a stipe-like extension, as the name indicates, which is absent on the European taxon.

Leucangium Quél. 1883

Type: *Leucangium ophthalmosporum* (syn. *L. carthusianum*).

Li (1997) studied the ultrastructure of *Leucangium carthusianum*, often treated within *Picoa*, and found it to be close to species in *Morchellaceae* and *Helvellaceae*. The structure of the excipulum also indicated such a relationship. Li found the ascospores to be multi-nucleate, which would point towards the *Morchellaceae* rather than the *Helvellaceae*. Likewise, O'Donnell *et al.* (1997) found that *L. carthusianum* clustered in the neighbourhood of the *Morchellaceae*, while the type of *Picoa* clustered with *Otidea* (*Pyronemataceae*) (data not shown in O'Donnell *et al.* 1997). *L. carthusianum* has apiculate-fusiform ascospores in saccate asci. Palfner & Agerer (1998b) described the ectomycorrhizae of this species.

Discinaceae Benedix 1961 (syn. *Hydnotryaceae* M. Lange 1956)

Besides the epigeous taxa *Discina*, *Pseudorhizina* and *Gyromitra*, this family also includes the hypogeous taxon *Hydnotrya* (O'Donnell *et al.* 1997) (Figs 3, and 6C-D). The family name *Hydnotryaceae* has been used to replace *Pseudotuberaceae* (*nom. inval.*, Art. 36.1) (e.g. Burdsall 1968), but is itself invalid (no Latin nor any other kind of diagnosis, e.g. Art. 36.1).

Hydnotrya Berk. & Broome 1846 (syn. *Geoporella*, *Gyrocratera*) (Figs 6C-D)

Type: *Hydnotrya tulasnei*.

Knapp (1950, 1952) discussed the genus, including the synonym *Geoporella*, and gave a fairly detailed description, whilst a thorough key with a few misplaced taxa can be found in Gilkey (1954). Trappe (1975c) dealt with the generic names *Geoporella* and *Gyrocratera*. Trappe (1979), Donadini (1986b)

and later Abbott & Currah (1997) accepted the genus in the *Helvellaceae*, which cannot be confirmed by the molecular data. Donadini (1986a) reported 4-nucleate spores. The morphological variation within the genus spans more or less hollow ascomata with cylindrical asci to nearly solid ascomata (Figs 6C-D) with clavate-saccate asci. There is likewise a great variation in spore shape and ornamentation. Whether the variation in spore characters should be given taxonomic importance in generic assignment awaits further molecular data. Zhang (1991b) demonstrated that there is a conspicuous, but non-functional, opening in the ascus apex of *H. cerebriformis*.

Gymnohydnotrya B.C. Zhang & Minter 1989

Type: *Gymnohydnotrya australiana*.

Zhang & Minter (1989b) accepted three Australian species and placed the genus in the *Helvellaceae* based on the four nuclei in the spores. The main diagnostic feature was the lack of a peridium, an external, and in the type species also internal hymenium, and the non-pigmented spores with an unusual and intricate ornamentation (a complex reticulum) as revealed by SEM. Vizzini (2003) lists this genus in the *Discinaceae* based on the similarity to *Hydnotrya* and the 4-nucleate ascospores. There are no published LSU sequences available for phylogenetic analysis. Two of the species had previously been placed in *Sphaerozone* (Beaton & Weste 1978).

Helvellaceae Fr. 1823 (syn. *Balsamiaceae* E. Fisch. 1897)

The *Balsamiaceae*, a family accepted in an emended version by Trappe (1979) and by e.g. Pegler *et al.* (1993), were considered a synonym of the *Helvellaceae* by van Brummelen (in Dissing & Schumacher 1994) and in an emended version by O'Donnell *et al.* (1997), a conclusion that was followed by e.g. Eriksson & Winka (1998) and Hansen & Knudsen (2000). Analyses of LSU identified a *Balsamia–Barssia* lineage (PB 91 % and PP 100 %) as a poorly supported sister group to a *Helvella–Wynnella* lineage (Fig 3). However, this relationship was highly supported in combined analyses of LSU and SSU (PB 100 %, O'Donnell *et al.* 1997; Hansen & Pfister 2007).

Balsamia Vittad. 1831 (syn. *Pseudobalsamia* E. Fisch.) (Fig 6E)

Type (lecto): *Balsamia vulgaris*.

Knapp (1950) gave a detailed account of this genus, which he placed in 'section B' of his own (invalid; Art. 36.1) family *Pseudotuberaceae*, but he later (Knapp 1952) placed it in his 'Eu-tuberaceae', based on further developmental studies; a conclusion also reached by Hawker (1954). Donadini (1986b) observed four nuclei in mature spores and proposed a placement in the *Helvellaceae*. The asci can be more or less organized in a palisade-like structure. Morphologically, *Balsamia* species are typical truffles with closed fruit bodies with a veined interior and a coarse peridium (Fig 6E). The asci are sac-like with clustered spores. The spore morphology is simple as in many species of *Helvella*. Species delimitation has been a subject of discussion with Szemere (1965) taking a very broad view. Trappe (1975c) agreed that *Pseudobalsamia* should be placed in synonymy with *Balsamia*. Palfner & Agerer (1998a) described the ectomycorrhiza formed between *B. alba* and *Pseudotsuga*.

Barssia Gilkey 1925

Type: *Barssia oregonensis*.

Fig 6 – Fruiting body forms in lineage B (*Morchellaceae-Discinaceae-Helvellaceae-Tuberaceae*). (A) *Fischerula macrospora*, solid ptychothecium. (B) *Choiromyces venosus*, solid ptychothecium. (C) *Hydnotrya tulasnei*, ptychothecia, JV87-356 (C). (D) *Hydnotrya michaelis*, ptychothecia, JHP-00.018 (C). (E) *Balsamia polysperma*, ptychothecia, JV97-080 (C). (F) *Helvella astieri*, ptychothecia, (C-65663). (G) *Tuber aestivum*, stereothecia, JHP-00.395. (H) *Tuber rufum*, stereothecia, JV93-321(C). Photos: J. Santos (A), J.H. Petersen (B, D, G detail), J. Vesterholt (C, E, H), T. Læssøe (F), C. Lange (G).

Kimbrough *et al.* (1996) studied the ultrastructure of the type species. Trappe (1979) included another monotypic genus, *Phymatomyces*, in *Barssia*, but as the type has been lost, this Japanese taxon should be re-investigated. *Barssia* ascomata have a smoother surface compared to species of *Balsamia*. Molecular results indicate a very close relationship between *Barssia* and *Balsamia*, so that it may be a sound move to synonymize these genera, but more taxa, including the type of *Balsamia*, should be sampled before such a decision is made.

Helvella L. 1753: Fr.

Helvella astieri Korf & Donadini (Fig 6F) (Korf 1973b) is the only known truffle within the genus. No molecular data are available for *H. astieri*, but its placement in *Helvella* is convincing on morphological grounds. It has closed semi-hypogeous fruit bodies and apparently passive spore dispersal, but an operculum is still present. The species is very rarely recorded, but is known from France and Denmark (Hansen & Knudsen 2000). The similarity of *H. astieri* and species of *Hydnotrya* was used in placing *Hydnotrya* in the *Helvellaceae* (Trappe 1979; Pegler *et al.* 1993). Trappe (1979): 'Korf in effect emended the family (*Helvellaceae*) to include astipitate, infolded and chambered ascomata by the description of *Helvella astieri* Korf & Donadini. This species is essentially a *Hydnotrya* with operculate asci and hyaline spores'. This view was strongly opposed by Donadini (1986a), as he found spores, paraphyses and excipulum exactly as in *Helvella*.

Insufficient data [placed here in Eriksson (2006a)]:

Picoa Vittad. 1831

Type: *Picoa juniperi*.

This genus was placed in the *Balsamicaeae* by e.g. Trappe (1979) and likewise in Montecchi & Sarasini (2000). Some species have asci arranged in a clear palisade, whereas in others the asci are more dispersed. The genus can be difficult to differentiate from *Balsamia* based on the characters employed e.g. by Montecchi & Sarasini (2000). Preliminary LSU rDNA sequence data of *P. juniperi*, suggest it is more closely related to *Otidea* (unpublished data in O'Donnell *et al.* 1997) than to the taxa in clade B (as sampled by O'Donnell *et al.* 1997).

Tuberaceae Dumort. 1822

Only hypogeous taxa cluster alongside the likewise hypogeous genus *Tuber*. Ascomata produced by the *Dingleya–Choiromyces* lineage show a persistent hymenium (chambered to completely compressed ptychothecia), whereas ascomata produced by *Tuber* spp. have lost the hymenium (stereothecia). *Tuber* is the most speciose genus of ascomycetous truffles, and it is known from many areas around the world, including North America, Central America, Europe, and Asia, but apparently not from subsaharan Africa and South America. It has been introduced to Australia (Bougher & Lebel 2001). *Dingleya*, *Reddellomyces,* and *Labyrinthomyces* clearly have a centre of diversity in Australia and New Zealand.

Choiromyces Vittad. 1831 (syn. e.g. *Piersonia*) (Fig 6B)

Type: *Choiromyces meandriformis* (syn. *C. venosus*)

Although often placed in the *Helvellaceae* (e.g. in Pegler *et al.* 1993) current molecular phylogenies place the type species as

a sister to *Tuber*, making it possible to include it in the *Tuberaceae* (O'Donnell *et al.* 1997). Gilkey (1955) and also Korf (1973a) suggested this placement, whereas Hawker (1954) and others (e.g. Trappe 1979) placed the genus in the *Terfeziaceae* based on structural studies. Zhang & Minter (1989a) studied *C. gangliformis* (considered by some, e.g. Montecchi & Sarasini (2000), as a possible synonym of *C. meandriformis*) in detail and found four nuclei in the spores, which could indicate the *Helvellaceae*. However, 4-nucleate spores are also commonly found in *Tuber*. Zhang & Minter (1989a) found multi-layered ascus walls in taxa belonging to *Choiromyces* as opposed to taxa of f.ex. *Terfezia*. This complex wall system would appear to characterize taxa in the *Tuberaceae*. They also emphasized the strange, pitted spore ornamentation.

Dingleya Trappe 1979, emend. Trappe, Castellano & Malajczuk 1992

Type: *Dingleya verrucosa*.

The genus was described from New Zealand and stated to differ from *Hydnotrya* species by having a more solid, but apparently still chambered gleba and a verrucose peridium. Later, the affinities were considered to be with *Reddellomyces* and *Labyrinthomyces* (Trappe *et al.* 1992), which our analyses confirm (Fig 3). Trappe *et al.* (1992) recognized six species. It is not unlikely that in a future revision the three genera will be lumped.

Labyrinthomyces Boedijn 1939, emend. Trappe, Castellano & Malajczuk 1992

Type: *Labyrinthomyces varius*.

Trappe *et al.* (1992) accepted this genus within the *Pyronemataceae s.l.* (as tribe *Otideae* or undescribed tribe), but the type species is highly supported within *Tuberaceae* in molecular phylogenies (O'Donnell *et al.* 1997) (Fig 3). There is a strong relation to *Reddellomyces* and *Dingleya* (PB 95 %, PP 100 %). Zhang & Minter (1988), Beaton & Weste (1977), and Malençon (1973) also discussed the status of this genus, but their concept included *Dingleya* and *Reddellomyces*, whereas Trappe *et al.* (1992) restricted the genus to the type species.

Paradoxa Mattir. 1935

Type: *Paradoxa monospora*.

Knapp (1951) discussed this genus and declared 'Stellung dieses Genus ist noch unsicher'. Vizzini (2003) indicated that it nests within the genus *Tuber* (data not shown). It is normally included in the *Tuberaceae* (e.g. Montecchi & Sarasini 2000; Castellano *et al.* 2004). As the name indicates this Italian truffle has 1-spored asci, and the globose spores have a low, net-like ornament. The ascoma surface is fibrillose from closely packed hyphae. We accept it *ad interim* within the *Tuberaceae*.

Reddellomyces Trappe, Castellano & Malajczuk 1992 (syn. *Labyrinthomyces* subgen. *Simplex*)

Type: *Reddellomyces westraliensis*.

Trappe *et al.* (1992) separated this taxon from *Labyrinthomyces* and *Dingleya* based on a smooth and glabrous peridium and asci with 1–5 spores. They accepted four species. Our analyses of existing sequences indicate a close relationship between *Labyrinthomyces*, *Dingleya,* and *Reddellomyces*, a group of taxa

that Malençon (1973) treated in an expanded version of *Labyrinthomyces*. Trappe *et al.* (1992) considered these taxa to belong to the *Pyronemataceae* (but in different tribes), but as can be seen from Fig 3, they clearly are close to *Tuber*, and maybe they should be united under *Labyrinthomyces*.

Tuber F.H. Wigg. 1780: Fr. (syn. *Aschion*, *Ensaluta*, *Oogaster*, *Lespiaultinia*, *Delastreopsis*, *Terfeziopsis*, *Mukagomyces*) (Figs 6G-H)

Type: *Tuber gulosorum*. [This name is currently not understood and is open to interpretation, but most likely represents *T. aestivum* Vittad. 1831 (Fig 6G). Various other typifications are given in the literature, including *Index Fungorum*, which lists *T. aestivum*. A conservation procedure will probably be needed to solve this problem, as Trappe (2001) points out the sanctioned *T. albidum* also represents *T. aestivum*].

The apothecial nature of the primordial *Tuber* ascomata has long been known (e.g. Parguey-Leduc *et al.* 1990; Janex-Favre & Parguey-Leduc 2002, 2003) and in some species this can even be hinted at in mature specimens. Parguey-Leduc *et al.* (1987a, 1987b) studied asci and spores of *T. melanosporum* in ultrastructural detail. Li & Kimbrough (1995) studied ultrastructural characters and supported a placement within *Pezizales*. The characters found were so divergent that they suggested that *Tuber* could be polyphyletic. A *Geniculodendron*-like anamorph has been reported from *Tuber dryophilum* (Urban *et al.* 2002). It is a big genus with 63 species according to Kirk *et al.* (2001). The genus forms a rather diverse group with a well-supported separate position within the present phylogenetic analysis (Fig 3). The synonymy cited above is according to Trappe (1975c, 1979). Janex-Favre & Parguey-Leduc (2002) apparently recognized the genus *Delastreopsis*. The multinuclear condition of the mature spores is a well-known character in some species of *Tuber* (e.g. Donadini 1987). Mello *et al.* (2005) investigated the white (Piedmont) truffle (*T. magnatum*) in detail and discussed various explanations for the nuclear condition. Vizzini (2003) indicated that most species have four nuclei in the majority of the ascospores, whereas a few species have less or more nuclei in the spores. Recently, Wedén *et al.* (2005) tested whether the height of the spore ornament can be used (as has been claimed) to distinguish two disputed truffles *T. aestivum* and *T. uncinatum*. All samples formed a single fully supported group and the names should be treated as synonyms, thus confirming the conclusion reached by some early workers (e.g. Hawker 1954). Kovács & Jakucs (2006) published a detailed phylogenetic and anatomical paper on what they termed the white truffles. Papers describing *Tuber* mycorrhizae include Blaschke (1987), Rauscher *et al.* (1995) and Zambonelli *et al.* (1993, 1999). Chevalier & Frochot (1997) published a whole book on the Burgundy truffle, a name traditionally attached to *T. uncinatum*, now considered a synonym of *T. aestivum* (Wedén *et al.* 2005). The review of *Tuber* by Ceruti *et al.* (2003) should also be consulted. Roux *et al.* (1999) compared some Chinese and European truffles based on molecular studies. A suite of new species is currently being discovered and described in China (e.g. He *et al.* 2004). Trappe *et al.* (1996) provided a key to *Tuber* species with a spiny-reticulate spore ornament. There is an ongoing project to stabilize the use of *Tuber* names (e.g. Mello *et al.* 2000).

Insufficient data [In Eriksson (2006a) placed in *Pezizales incertae sedis*]:

Loculotuber Trappe, Parladé & I.F. Alvarez 1993

Type: *Loculotuber gennadii*.

The authors (Alvarez *et al.* 1993) stated this monotypic genus to differ from *Tuber* in having glebal locules and stipitate asci. The spores tend to become citriform. They speculated that the genus formed an intermediate between an unknown epigeous member of the *Pezizales* and the genus *Tuber*. Castellano *et al.* (2004) listed this genus in the *Tuberaceae*.

Lineage C

The presumably strictly saprotrophic families *Ascodesmidaceae*, *Sarcoscyphaceae* and *Sarcosomataceae* have no known hypogeous representatives. *Glaziellaceae* are suggested to belong to this clade (Hansen & Pfister 2007; Perry *et al.* 2007) (Figs 4 and 7A-H).

Glaziellaceae J.L. Gibson 1986
Glaziella Berk. 1880

Type: *Glaziella vesiculosa* Berk (syn. *G. aurantiaca*).

This genus is unusual in several respects. It fruits more or less on top of the soil and is completely hollow with a rather thin rind that contains the monosporic asci, the spore being enormous. The only species *Glaziella aurantiaca* (Fig 7A) has been interpreted in many ways, including a placement in *Xylaria* (*Sordariomycetes*, *Ascomycota*), in the *Zygomycota* and finally in the *Pezizales*. An early molecular study (Landvik & Eriksson 1994b) suggested a relationship with members of the *Pyronemataceae*. Later Landvik *et al.* (1997) expanded on this and found further evidence, but still based on a very limited taxon sampling, for a relationship (low support) with e.g. *Pulvinula* and the likewise semi-hypogeous genus *Paurocotylis*. They *ad interim* accepted *Glaziellaceae* but not *Glaziellales*. Harrington *et al.* (1999) found support for inclusion in the *Pezizales*, but did not resolve a position within, although their results could indicate a closer relationship with the *Sarcoscyphaceae* rather than with the *Pyronemataceae*. They erroneously cited the origin of the specimen as Sweden. Castellano *et al.* (2004) maintained a placement in the *Glaziellales*. Perry *et al.* (2007) had *Glaziellaceae* in a sister position to *Pyronemataceae* but with low statistical support. Eriksson (2006a) accepts the family in *Pezizales*. At least some collections of this pantropical taxon are from decidedly ectotrophic communities, but the exact nature of its biology is not known.

Pyronemataceae Schröter 1894 (syn. *Geneaceae*)

This family has been defined as having 1-nucleate ascospores and non-amyloid asci. It has relatively few hypogeous members with *Genea* as the most prominent genus. *Geopora* is represented with just one species that only *pro parte* qualifies as a truffle (active spore dispersal not completely lost). Furthermore, with the exception of *Paurocotylis*, the truffles formed in *Pyronemataceae* all still possess a hymenium; no stereothecia are found. Epigeous members have both saprotrophic and mycorrhizal representatives, but *Paurocotylis* would seem to be the only saprotrophic hypogeous member. Although *Geneaceae* have gained wide acceptance, it can be concluded both by morphological studies by e.g. Pfister (1984) and Zhang (1992a), and molecular studies (Perry *et al.* 2007) (Fig 4), that it is part of *Pyronemataceae* as currently circumscribed.

Fig 7 – Fruiting body forms in lineage C (*Glaziellaceae-Pyronemataceae*). (A) *Glaziella aurantiaca*, unnamed ascoma type, TL-6168 (C). **(B)** *Genea fragrans*, ptychothecia, JV99-373 (C). **(C)** *Humaria hemisphaerica*, apothecia, JHP-03.144. **(D)** *Genabea cerebriformis*, ptychothecia. **(E)** *Geopora cooperi*, ptychothecia. **(F)** *Geopora arenicola*, apothecia, JHP-93.114 (C). **(G)** *Hydnocystis clausa*, ptychothecia, PH00-192 (C). **(H)** *Stephensia bombycina*, ptychothecia. Photos: T. Læssøe (A), J. Vesterholt (B, G), J.H. Petersen (C, F), M. Tabarés (D), J. Nitare (E, H).**

Genea Vittad. 1831 (syn. *Hydnocaryon*)

Type (lectotype): *Genea verrucosa*.

In *Genea* the ascomata have a more or less obvious opening, and can be unfolded to strongly folded (ptychothecia; Fig 7B). The asci are arranged in hymenia, but active spore dispersal has been completely lost. The tips of the paraphyses have fused to form an epithecium that protects the hymenium (Gilkey 1954). The more or less hyaline spores have a very prominent ornamentation. Trappe (1979) accepted 29 species. Li & Kimbrough (1994) studied the ultrastructure that compared with members of the *Pyronemataceae s.l.* (as *Otideaceae*). Phylogenetic analyses of LSU rDNA support the placement in *Pyronemataceae*, and suggest that *Genea* is closely related to *Humaria hemisphaerica* (Figs 7B-C) (Perry *et al.* 2007) (PB and PP 100 %, Figs 4). Pfister (1984) proposed to place *G. hispidula* in *Humaria* based on analysis of excipular structures. Like species of *Genea*, *H. hemisphaerica* has also been shown to be ectomycorrhizal (Tedersoo *et al.* 2006).

Smith *et al.* (2006) studied the phylogeny, morphology, and taxonomy of a group of *Quercus*-associated species and listed some minor differences between *Genea* and the closely related *Genabea* (Fig 7D) and *Gilkeya*. They added a couple of new species.

Genabea Tul. & C. Tul. 1844 (syn. *Myrmecocystis, Pseudogenea*)

Type: *Genabea fragilis*.

The genus was accepted by Trappe (1975c) and again by Smith *et al.* (2006). It differs from *Genea* in having clavate to ellipsoid asci in hymenia enclosed in pockets, and in having echinulate spores rather than verrucose. *Index Fungorum* lists five binomials, two based on European material, two on North American, and one on Tasmanian. Zhang (1991a) placed *Genabea* in synonymy with *Genea*, which Korf (1973a) also had suggested. Smith *et al.* (2006) only dealt with one species, *G. cerebriformis* (Fig 7D), that clustered separately from the included *Genea* species based on LSU data. However, the type species of *Genabea* has not been sampled for molecular phylogenetic studies, which are needed in order to fully test the delimitation of *Genabea*, *Myrmecocystis* (type: *M. cerebriformis*) and *Genea*. Trappe (1975c) synonymized *Myrmecocystis* with *Genabea*.

Geopora Harkn. 1885 (syn. *Sepultaria, Pseudohydnotrya*)

Type: *Geopora cooperi*.

Burdsall (1965, 1968) studied this genus in detail and combined *Sepultaria* with *Geopora* after having found actively discharged spores in the type species of *Geopora*. Korf (1973b) gave a detailed review. Biologically *G. cooperi* (Fig 7E) behaves like an ordinary truffle but the operculum and the build up of internal pressure within mature asci have not been lost. Other species develop in the soil but open at the surface at maturity (Fig 7F). Nannfeldt (1946) also gave a rarely cited, but detailed summary (in Swedish) of the *Geopora* situation. He regarded *G. cooperi* (as *G. schackii*) as a truffle based on biological arguments, such as passive animal dispersal, smell, etc. Trappe (1975c) agreed on the above synonymy. Phylogenetic analyses of LSU confirm the placement of *G. cooperi* among epigeous *Geopora* spp. (PB and PP 100 %, Fig 4).

Gilkeya M.E. Sm., Trappe & Rizzo 2006

Type: *Hydnocystis compacta* (*Gilkeya compacta*).

This genus was erected based on a separate, although unresolved, position of *Hydnocystis compacta* in a LSU analysis of *Genea* (over six) and *Genabea* (one) species (*H. compacta* formed a trichotomy with *Genea* and *Genabea*), in combination with a deviating reddish peridium colour compared with species of *Genea* and *Genabea*. A similar molecular result was found by Perry *et al.* (2007) with *Gilkeya* and *Genabea* as (unsupported) successive sister taxa to a highly supported *Genea–Humaria hemisphaerica* clade. Further taxon sampling will hopefully resolve its position in a clearer way. *Gilkeya* and *Genabea* differ from *Genea* in having globose spores and the ascomata lack a basal tuft of mycelium.

Hydnocystis Tul. & C. Tul. 1844 (syn. *Protogenea*)

Type: *Hydnocystis piligera*.

Burdsall (1968) gave a detailed taxonomic and nomenclatural account of what he considered the only species of *Hydnocystis*, *H. piligera*. The genus is morphologically characterized by its bladder-like hypogeous ascomata with a hairy, sand-binding outer surface and an irregular opening to the outside. The spores are globose, eguttulate, and an epithecium is present. We accept its current position in the *Pyronemataceae* based on morphological characters. No sequences are available. Senn-Irlet & Aeberhard (2005) reviewed the genus in a European context, and stated that the ectomycorrhizal status of this fungus is uncertain. The placement of the species *H. clausa* (Fig 7G) is disputed. Burdsall (1968) placed it in *Geopora*; others have placed it with *Hydnocystis* (Montecchi & Sarasini 2000). Trappe (1975c) studied the type of *Protogenea* and proposed the above synonymy. *Hydnocystis singeri* from Argentina was discussed in Burdsall (1968). It was not accepted in the genus, but compared with *Labyrinthomyces* and *Phymatomyces*. It was thought to possibly represent a new genus. It is one of very few ascomycetous truffles reported from South America.

Paurocotylis Berk. 1855

Type: *Paurocotylis pila*.

Patouillard (1903) was the first to recognize that the type and only recognized species belongs to *Ascomycota*. The bright red pigmentation points to a relationship with carotenoid members of the *Pyrenomataceae*. Trappe (1979: 321) wrote 'it suggests an aleurioid fungus gone underground and fits nicely in tribe *Aleurieae sensu* Korf'. Patouillard (1903) indicated a position close to *Hydnocystis*, and noted that the remaining taxa belong elsewhere. *Paurocotylis pila* forms a monophyletic group with *Stephensia*, *Geopyxis*, and *Tarzetta* species (PB 99 % and PP 100 %, Fig 4). Originally, the microscopical similarity between *Paurocotylis* and *Stephensia* was noted. The exact nature of its ecology is far from understood. It is considered a native of New Zealand and an introduction to the UK (Dennis 1975). It is now fairly common in the northern parts of the UK, not least in Orkney (Eggerling 2004), where it fruits during the wintertime in highly disturbed soils, in vegetable plots, along roads, etc. As noted above, it has been suggested that the bright red colour may attract birds (ground-dwelling species that fulfil the small mammal niche in New Zealand) that may act as dispersal vectors in its natural setting (Castellano *et al.* 2004). Macromorphologically it resembles *Glaziella* (Fig 7A), which also has hollow ascomata, that occur more or less on top of the soil (see separate entry). Dennis (1975) noted that *Paurocotylis* spores in mature ascomata are cream coloured and found in

a powdery mass entangled with hyphae. Castellano *et al.* (2004) list *Paurocotylis* as a saprotrophic fungus, and also Dennis (1975) noted that no obvious mycorrhizal host was found in connection with the first UK find. However, the other members of the clade, e.g. *Geopyxis carbonaria* (Vrålstad *et al.* 1998) and *Tarzetta* (Tedersoo *et al.* 2006), have been shown to be ectomycorrhizal.

Petchiomyces E. Fisch. & Mattir. 1938

Type: *Hydnocystis twaitesii* (syn. *Petchiomyces twaitesii*).

This genus was included in *Geneaceae* by Fischer (1938), followed by Gilkey (1954). Burdsall (1968) studied the type of the type species and concluded that it could not be placed in *Geopora* based on the presence of an epithecium and ornamented spores. Gilkey (1939) described *Petchiomyces kraspedostoma* from California, the only additional species known besides the type from Sri Lanka. *P. kraspedostoma* has an apical opening with stiff, incurved hairs and smooth, ellipsoid spores. The genus should be revised, but we *ad interim* accept its position within the *Pyronemataceae*.

Phaeangium Pat. 1894

Type: *Phaeangium lefebvrei*.

This genus was sunk under *Picoa* by Maire (1906), but resurrected by Alsheikh & Trappe (1983b), a move not accepted by e.g. Moreno *et al.* (2000). Gutierrez *et al.* (2003) described the rather deviating mycorrhizae formed by *Phaeangium lefebvrei* (as *Picoa*) with *Helianthemum* species. We *ad interim* accept the genus (within *Pyronemataceae*).

Sphaerosoma Klotzsch 1839

Type: *Sphaerosoma fuscescens*.

Korf (1972) placed the genus in the *Ascobolaceae* following previously published characters and was *ad interim* followed by Trappe (1979). Gamundi (1976) could not find any amyloid reaction in the type material and considered it a likely member of the *Pyronemataceae* (as *Humariaceae* tribe *Otideae*). Dissing & Korf (1980) followed Gamundi but stated 'studies on fresh material are needed before the true systematic position of this genus can be evaluated'. They felt, based on circumstantial evidence, that *Sphaerosoma fuscescens* probably has forcible spore discharge. Montecchi & Sarasini (2000) cite *Sphaerosoma* as a synonym of the younger name *Sphaerozone* (*Pezizaceae*!), but it is accepted in e.g. Vizzini (2003) in the *Pyronemataceae* and *ad interim* here. Kirk *et al.* (2001) stated the number of species as three, but there are 11 names in *Index Fungorum* currently without other placement. A revision would seem to be required.

Stephensia Tul. & C. Tul. 1845 (syn. *Densocarpa*, *Elderia*)

Type: *Stephensia bombycina* (Fig 7H).

Knapp (1951) gave a description of the type species, whereas Fontana & Giovannetti (1987) described its anamorph. Uecker (1967) reported a similar anamorph for *Stephensia shanori*. Trappe *et al.* (1997) published a key to the species. Our placement (Fig 4) is based on sequences obtained by Perry *et al.* (2007). De Vito (2003) described a new species, *S. colomboi*, said to differ from previously described species in being epigeous on rotten wood. Based on the published picture the wood more or less qualifies as soil, and some of the ascomata appear to be at least partly immersed. Microscopically, *S. colomboi* is

apparently very close to *S. bombycina*, but some minor macroscopical differences are noted.

Hypogeous pezizalean taxa currently not placed within clade A–C

Carbomycetaceae Trappe 1971

Trappe (1971) erected this family as a segregate from *Terfeziaceae*. It was based on 'brown-walled asci borne in fertile pockets of large, inflated cells mixed with narrow, tubular ascogenous hyphae, and in the fertile pockets being separated by sterile veins of inflated cells only'. It never produces a hymenium in any kind of palisade. When dry the spore mass becomes pulverulent almost as in *Elaphomyces*. Eriksson (2006a) accepts the family in the *Pezizales*.

Carbomyces Gilkey 1954

Type: *Carbomyces emergens*.

This interesting taxon, only known from three species in southwestern North America, is currently under study by K. Hansen using molecular techniques. According to Trappe (1971) its mycorrhizal status has not been clarified. At maturity the ascomata are dispersed by the wind (Trappe 1979). Zak & Whitford (1986) demonstrated the hypogeous nature of immature *Carbomyces emergens*, and that rodents apparently eat the (immature?) ascomata.

Pezizalean truffles with unknown family placement (based on Eriksson (2006a)

Delastria Tul. & C. Tul. 1843

Type (mono): *Delastria rosea*.

Not much is known about this southern European/North African monotypic genus. Montecchi & Sarasini (2000) include it in the *Terfeziaceae* (here considered a synonym of the *Pezizaceae*), following Trappe (1979), and differentiate it from the other accepted genera in this family by the evanescent peridium, the pinkish colour of the gleba, 2–4-spored asci and a reticulate spore ornament. Castellano *et al.* (2004) accepted the genus in the *Tuberaceae*. DNA studies are clearly needed in order to clarify the position of this *Tuber*-like genus.

Unplaced Ascomycota *truffles (Eriksson 2006a)*

Diehliomyces Gilkey 1955

Type (mono): *Diehliomyces microsporus*.

This pest in mushroom beds (the 'compost truffle') is referred to as a 'false truffle' in Kirk *et al.* (2001), but its ascomycetous nature is not disputed, and it must be considered a genuine although rather atypical truffle. Its position is unsettled, but it could belong in *Pezizales* and parallel the case of *Orbicula*, another passively discharged, but epigeous fungus that has led a tumultuous life, but now has found its place in the *Pezizales* (Hansen *et al.* 2006). Both genera have had *Eurotiales*/*Onygenales* proposed as proper placements, mainly due to the production of small ascomata with small, globose spores. Unlike almost all other truffles this species is clearly not mycorrhizal. Diehl & Lambert (1930) introduced the species as *Pseudobalsamia microspora* after having received material from an Ohio grower where the pest was 'filling his beds

and completely stopping the production of mushrooms'. It was later found in other American sites and later also in Europe (e.g. Pegler *et al.* 1993). It resembles many typical ascomycetous truffles in having a convoluted ascoma up to 3 cm diam with an outer rind. It may have one or several openings to the exterior. The asci are evanescent, long stipitate with a sac-like, spore-containing part. Unlike typical pezizalean truffles, the spores are smooth and subglobose, 5–7 μm diam, and form an 'olivaceous sulphur-coloured dusty mass' (Diehl & Lambert 1930; Gilkey 1955). Diehl & Lambert (1930) also reported chlamydospores up to 13 μm diam, with a thick, golden-brown wall. It was grown in artificial culture, where it produced ascomata. These authors tentatively concluded that the truffle could be considered a weed in mushroom beds rather than a parasite of the mushrooms. Singer (1961) published a plate that clearly indicates the scale of a full-blown 'infection' in a mushroom bed. Hawker (1959) did some developmental studies on *Diehliomyces* and concluded that the ascomata were not truly folded as in a typical member of the *Tuberales*, and she supported a transfer to the *Eurotiales*. She found a completely irregular arrangement of the ascogenous hyphae and asci, even at very early stages of development. Currah (1985) excluded it from the *Onygenales*, where Benny & Kimbrough (1980) had accepted it.

Excluded truffle taxa

Amylocarpus Curr. 1859
   Type: *Amylocarpus encephaloides*.
   This monotypic genus has passive spore dispersal but develops on intertidal wood and, although originally included in the *Tuberaceae*, it cannot be considered a truffle in the sense of this paper. Its current position is unsettled (e.g. Landvik *et al.* 1998). It is listed as *Leotiomycetidae* with unclear position in Kirk *et al.* (2001) and as *Helotiales incertae sedis* in Eriksson (2006a).

General information
For general information on truffles refer, for example, to North American Truffling Society (www.natruffling.org/) and e.g. Bucquet-Grenet & Dubarry (2001). A very extensive bibliography on the genus *Tuber* can be found in Ceruti *et al.* (2003). Also, Trappe & Maser (1977) and Trappe *et al.* (2001) should be consulted. Recently a very illustrative guide to Andalucian truffles directed at the general public has been published (Arroyo *et al.* 2005). Dannell (1996) published a useful popular review in Swedish.

Discussion

Phylogenetic relationships of truffles within Pezizales

Within the last 13 y molecular phylogenetic studies have gradually confirmed and greatly expanded our knowledge on a repeated evolution of ascomycetous truffles across *Pezizales*. The first study to address the controversial issue of the placement of *Tuber* was that of Landvik & Eriksson (1994a), who confirmed the placement within *Pezizales*, as predicted by Trappe (1979) and others. *Elaphomyces* was erroneously indicated to be nested within *Pezizales* (Landvik & Eriksson

1994a; but see Landvik & Eriksson 1994b), but was later shown to be closely related to *Eurotiales* and *Onygenales* (Landvik *et al.* 1996). The early study by Landvik & Eriksson (1994b) showed that *Glaziella*, with the highly unusual ascomatal form, was nested within *Pezizales*. Attempts to find out the exact relationship of *Glaziella* have since been carried out (see *Glaziellaceae* above). The molecular study by O'Donnell *et al.* (1997) included a large number of truffles together with a large number of pezizalean epigeous taxa (from lineage B) and was the first to discover multiple (at least five), independently derived, hypogeous clades within *Pezizales*. It resulted in new family assignments for several truffles and revealed a relationship between *Tuberaceae* and *Helvellaceae*. Percudani *et al.* (1999) focused on hypogeous *Pezizales* phylogeny and species thought to belong to the *Balsamiaceae, Terfeziaceae,* and *Tuberaceae*. Unfortunately they included only few epigeous taxa, which resulted in *Cazia, Mattirolomyces, Pachyphloeus,* and *Terfezia* (*Terfeziaceae*) erroneously formed a monophyletic group within *Pezizaceae*. A study with a broader sampling of epigeous pezizaceous species followed (Norman & Egger 1999) that showed '*Terfeziaceae*' are not monophyletic. The study of epigeous-hypogeous relationships within *Pezizaceae* was further expanded (Hansen *et al.* 2001) and gave support for at least three independent origins of hypogeous forms within the family. Most recently, Perry *et al.* (2007) focusing on *Pyronemataceae*, with a large taxon sampling, suggested that the truffle form has arisen at least five times independently within that family. All of these studies used regions from the nuclear ribosomal genes. One multi-locus study has emerged (Hansen *et al.* 2005) substantiating the evolution of truffles within *Pezizaceae* using combined analyses of LSU rDNA and protein-coding genes, RNA polymerase II (RPB2), and β-tubulin.

Several further papers dealing with the phylogeny of truffles (e.g. Diéz *et al.* 2002; Ferdman *et al.* 2005) have unfortunately only included truffles in the analyses, which have made it difficult to pinpoint epigeous relatives and fully understand their relationships and taxonomy. Vizzini (2003) gave the most recent review of ascomycetous truffles.

The 55 species of truffles included in the current review occur in 15 separate lineages within the *Pezizales*: in nine lineages within *Pezizaceae* (Fig 2), in three lineages within *Morchellaceae–Discinaceae–Helvellaceae–Tuberaceae* (Fig 3) and in three lineages within *Pyronemataceae* (Fig 4). The only strictly hypogeous family known is currently *Tuberaceae*. Table 2 gives an overview of recent classifications of pezizalean truffles and an up-to-date classification based on both molecular and morphological characters.

Cytology

The number of nuclei in mature ascospores within the *Pezizales* has long been considered a character of major importance in defining taxa (see e.g. Berthet 1964; Korf 1973a, 1973b and Zhang 1992a,b). It has been shown that the hypogeous members of the *Pezizales* also tend to have the same number of nuclei in the spores within a certain family or genus. *Tuber* is an exception, as the spores can have from one to 18 nuclei, although most species have four nuclei in each spore (Vizzini 2003). The known numbers are summarized in Table 3. Zhang (1992a) found *Genea* (two species),

Tuberales (Korf 1973a)	Hypogeous Pezizales (Trappe 1979)	Suggested classification of hypogeous Pezizales
Elaphomycetaceae	*Pezizaceae*	*Pezizaceae*
Elaphomyces	*Amylascus*	*Amylascus*
	Mycoclelandia	*Cazia*
	(as *Clelandia*)	*Eremiomyces*
	Hydnotryopsis	*Hydnobolites*
	Peziza spp.	*Hydnotryopsis*
	Tirmania	*Kalaharituber*
		Mattirolomyces
Terfeziaceae	*Terfeziaceae*	*Mycoclelandia*
Carbomyces	*Choiromyces*	*Pachyphloeus*
Delastria	*Delastria*	*Peziza* spp.
Mukagomyces	*Hydnobolites*	*Ruhlandiella*
Paradoxa	*Pachyphloeus*	*Sphaerozone*
Picoa	*Terfezia*	*Terfezia*
Terfezia		*Tirmania*
Tirmania		
	Helvellaceae	*Helvellaceae*
	Hydnotrya	*Balsamia*
	Dingleya	*Barssia*
	Fischerula	*Helvella astieri*
Tuberaceae		
Barssia	*Balsamiaceae*	*Tuberaceae*
Balsamia	*Balsamia*	*Choiromyces*
Caulocarpa	*Barssia*	*Dingleya*
Choiromyces	*Picoa*	*Labyrinthomyces*
Elderia		*Paradoxa*
Fischerula	*Tuberaceae*	*Reddelomyces*
Lespiaultinia	*Paradoxa*	*Tuber*
Labyrinthomyces	*Tuber*	
Hydnobolites		*Morchellaceae/*
Hydnoplicata		*Discinaceae*
Hydnotrya		*Gymnohydnotrya*
Pachyphloeus		*Hydnotrya*
Phymatomyces		*Fischerula*
Piersonia		*Leucangium*
Protogenea		
Pseudobalsamia	*Pyronemataceae*	*Pyronemataceae*
Stephensia	*Geopora cooperi*	*Genabea*
Tuber	*Hydnocystis*	*Genea*
	Labyrinthomyces	*Geopora cooperi*
	Paurocotylis	*Gilkeya*
	Petchiomyces	*Hydnocystis*
	Sphaerozone	*Paurocotylis*
	Stephensia	*Petchiomyces*
Geneaceae	*Geneaceae*	*Phaeangium* = *Picoa*?
Genea	*Genea*	*Picoa*
Hydnocystis	*Genabea*	*Sphaerosoma*
Petchiomyces		*Stephensia*
		Glaziellaceae
		Glaziella
	Carbomycetaceae	*Carbomycetaceae*
	Carbomyces	*Carbomyces*

The adopted classification (right column) is based on recent molecular phylogenies combined with morphological characters.

Hydnobolites cerebriformis, *Pachyphloeus citrinus,* and *Mattirolomyces terfezioides* (as *Terfezia*), to be uni-nucleate. This led Zhang to propose the synonymy of *Geneaceae* with

Pyronemataceae, and furthermore, restricted *Terfeziaceae* to uninucleate taxa (now incorporated in the *Pezizaceae*). The *Helvellaceae* have been considered to be defined by tetra-nucleate spores, but it is now evident that this number is a plesiomorphic character (also present in *Discinaceae* and some taxa of *Tuberaceae*) and thus has very limited discriminative value. The placement of f.ex *Hydnotrya* (Trappe 1979) and *Choiromyces* (e.g. Pegler *et al.* 1993) in the *Helvellaceae* was argued along those lines. However, molecular phylogenetic analyses of SSU and LSU rDNA suggest that *Hydnotrya* belongs to *Discinaceae* and *Choiromyces* to *Tuberaceae* (O'Donnell *et al.* 1997) (Fig 3).

Ecological aspects of the truffle syndrome

Various evolutionary processes may be involved in the truffle syndrome, but the most generally accepted is the avoidance of desiccation (e.g. Thiers 1984). The high truffle diversity in arid areas favours this hypothesis. Some truffles, like *Tuber aestivum* and *T. melanosporum*, clearly have an outer layer that renders protection, to both mechanical and desiccation stresses, but many others have very delicate fruit bodies, often formed in the upper soil layers, where desiccation pressures can exist, although of a less harsh nature than above ground. Another driving force could be protection against above-ground predation of immature ascomata. At maturity the production of pungent volatile compounds attracts predators of a kind the truffles have co-evolved with, or at least adapted to, in order to facilitate spore dispersal. Pacioni *et al.* (1990) speculated on other functions of the compounds, including microbial control of the micro-rhizosphere. Spores of hypogeous fungi probably persist for longer in the soil than those of wind-dispersed relatives, which presumably is of importance in respect to life in a xeric environment and as ectomycorrhiza formers (e.g. Miller *et al.* 1994).

It is generally assumed that most hypogeous fungi, including those in the *Pezizales*, form ectomycorrhiza. Direct proof of this has not been established in all cases, but circumstantial evidence clearly indicates the validity of this assumption (e.g. Pacioni & Comandini 1999; Montecchi & Sarasini 2000). Early on some of these relationships were considered parasitic, e.g. those with *Cistaceae* (Singer 1961). Awameh & Alsheikh (1979) and Awameh *et al.* (1979) claimed that some *Terfezia* and *Tirmania* spp. form ectomycorrhiza with *Helianthemum*, but Kovács *et al.* (2003) have pointed out some important morphological discrepancies compared to typical EM structures casting doubt on these conclusions. Based on morphotyping and sequencing of ectomycorrhizal root tips, Tedersoo *et al.* (2006) identified 33 species of *Pezizales* to be ectomycorrhizal, including species of *Genea*, *Geopora*, *Helvella*, *Hydnotrya*, *Pachyphloeus*, *Peziza*, *Sarcosphaera*, and *Tuber*. They hypothesized that the ectomycorrhizal lifestyle is a precondition for the switch to hypogeous fruiting. Most well-known mycorrhizal trees would appear to be involved in associations with pezizalean truffles, including various members of the *Fagaceae*, *Betulaceae*, *Pinaceae*, and *Myrtaceae*. It is generally assumed that truffles prefer warm, fairly dry climates and calcareous soils, but this may be slightly overstated due to the emphasis of requirements for the edible *Tuber* species. Still, the overall species diversity appears to be highest in alkaline soils in warm temperate to subtropical climates. Desert areas around the world also have a special truffle

Table 3 – A compilation of the known number of nuclei in mature ascospores in hypogeous *Pezizales*		
Taxon	Nuclei/ascospore	Reference
Lineage A (*Pezizaceae*)	1 (to 4?)	
Hydnobolites cerebriformis	1	Zhang (1992a)
Mattirolomyces terfezioides, M. tiffanyae	1	Zhang (1992a; Healy 2003)
Muciturbo (= Ruhlandiella?)	1	Warcup & Talbot (1989)
Pachyphloeus citrinus	1	Zhang (1992a)
Picoa juniperi	4?	Donadini (1986b)
Lineage B (*Discinaceae-Tuberaceae*)	1–17	
Balsamia platyspora, B. vulgaris	4	Donadini (1986b)
Barssia oregonensis	4	Kimbrough *et al.* (1996)
Choiromyces gangliformis	4	Zhang & Minter (1989a)
Gymnohydnotrya australiana	4	Zhang & Minter (1989b)
Helvella astieri	4	Korf (1973b)
Hydnotrya michaelis, H. tulasnei, H. cerebriformis	4	Berthet (1982); Donadini (1986a); Zhang (1991b)
Leucangium carthusianum	4+	Li (1997)
Tuber rufum	1–2	Vizzini (2003)
Tuber aestivum, T. brumale, T. excavatum, T. indicum, T. magnatum, T. mesentericum	2–4	Vizzini (2003)
Tuber maculatum	2–8	Vizzini (2003)
Tuber borchii, T. puberulum	(2–)4–17	Donadini (1987), Vizzini (2003)
Tuber melanosporum	6–8	Parguey-Leduc *et al.* (1987a)
Lineage C (*Pyronemataceae*)	1 (to 5?)	
Genea klotzii, G. sinensis, G. variabilis, G. verrucosa	1	Donadini (1986a); Zhang (1991a, 1992a)
Geopora cooperi	1	Donadini (1987)
Hydnocystis clausa, H. piligera	1	Donadini (1986a, 1987)
Stephensia shanori	1 (to 5?)	Uecker (1967)

funga, notably including *Terfezia* and *Tirmania* species. Amongst pezizalean truffles only *Paurocotylis* is at present considered to be saprotrophic (Castellano *et al.* 2004) or suspected to be so (Dennis 1975).

Conclusion

In conclusion, the trend that started with abandoning the *Tuberales*, now robustly confirmed, has continued at the family level where 'pure' hypogeous monophyla have been reduced to a single taxon, the *Tuberaceae*. At least 15 independent origins of hypogeous forms within the *Pezizales* are supported by the LSU rDNA gene trees. Different types of hypogeous ascomata forms, infolded or chambered ptycothecia, solid ptycothecia and stereothecia, appear to have evolved multiple times independently with the linages A and B of *Pezizales*; within lineage C only infolded or chambered ptycothecia are present. No clear picture is shown by the LSU phylogenies of the hypothesis that evolution from an epigeous, actively dispersed form (apothecial) to a hypogeous, passively dispersed form (stereothecial), proceeds through an intermediate semi-immersed form. Nevertheless, several smaller clades include such forms and future studies, including additional molecular data and taxa, providing a more robust phylogeny, may likely show such a progression. Much has been learnt on truffle biology, taxonomy, and phylogeny as the *Tuberales* were abandoned as an independent order but all three fields are still very active research areas where many exciting results will be forthcoming in the near future.

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