Truffle trouble: what happened to the Tuberales?

Thomas LÆSSØEa,*, Karen HANSENb,†

aDepartment of Biology, Copenhagen University, DK-1353 Copenhagen K, Denmark
bHarvard University Herbaria – Farlow Herbarium of Cryptogamic Botany, Cambridge, MA 02138, USA

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, Ruhiandella, 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.

ª 2007 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

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 oreculates that have evolved ± convergently into hypogeous forms.’ Malencçon (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 ‘Plectiscineae’ 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. Burdssl (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 Geaceae, Terfeziaceae, and Tuberales) 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 Barisia 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), Geaceae (two genera), Helvellaceae (three genera), Balsamiaceae (two genera), Terfeziaceae (four genera) and Tuberales 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 Brummen (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**

Ascomycet 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 “cúp fungus”. The genus Caulocarpa was based on such hypogeous Sarcosphaera ascocoma (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: ptychotheca with persistent, recognizable hymenia and variously folded or even solid ascomata; sterotheca without hymenia and solid ascomata; and exotheca with external hymenium. None of these ascomata 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 opepulate 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 pezizalean 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/ascockey.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 astrosafricana*, was listed as a member of *Terfezia* subgen Matritrolymcyces and may require a new combination, as Matritrolymcyces 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*) *lefeburei*, 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 epigean, 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 epigean 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 (Ramaut 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
Pyronemataceae
Ascodesmidaceae
Glaziellaceae
Sarcoscyphaceae
Sarcosomataceae

Morchellaceae-Discinaceae
Helvellaceae-Tuberaceae
Rhizinaceae
Caloscyphaceae

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 Asco- bolus 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 Shimodaira–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 Pariaria–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 Pachyphloeo, 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 Mattirolymus 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
Neolecta vitellina
Byssonectria terrestris
Melastiza contorta
Otidea onotica
Otidea uniblina
Smardaeas amethystina
Greletia reticulosperma
Morchella elata
Ascobolus carbonarius
Ascobolus denudatus
Ascobolus crenulatus
Marcelleina pseudoanthracina
Marcelleina persoonii
Peziza gerardi
Iodophanus hyperboreus
Iodophanus carneus
Amylascus tasmanicus
Scabropezia flavovirens
Scabropezia scabrosa
Pachyphloeus melanoxanthus
Glischroderma sp.
Pachyphloeus citrinus
Pachyphloeus virescens
Pachyphloeus citrinus
Pachyphloeus virescens
Boudiera tracheia
Boudiera dennisii
Pachyella babingtonii
Pachyella punctispora
Pachyella violaceonigra
Pachyella habrospora
Peziza natrophila
Peziza quelepidotia
Peziza apiculata
Peziza succosa
Peziza succosella
Peziza ampelina
Peziza subbiloba
Peziza subvitellina
Peziza varia
Peziza arvenensis
Peziza amplexa
Peziza vesiculosa
Peziza succosella
Peziza amplexa
Peziza vesiculosa
Iodowynnea auriformis
Iodowynnea-Kaliharituber
Kalaharituber pfeilii
Peziza luteoloflavida
Peziza obtusapiculata
Peziza polypapulata
Peziza retrocurvata
Peziza depressa
Ruhlandiella berolinensis
Peziza whitei
Peziza limnea
Peziza badia
Peziza alaska
Peziza radiobifusca
Peziza saniola
Peziza depressa
Peziza griseorosea
Peziza atrovina
Peziza ellipospora
Terfezia boudieri
Terfezia boudieri
Terfezia claveryi
Terfezia claveryi
Caizia flexisaccus
Tirmania pinoyi
Tirmana nivea
Peziza oestrudera
t
Peziza phyllostephen
Petrican carbonaria
Petrican frachycarp
Petrican endocarpoide
Peziza vacini
Eremiomyces echinulatus
Peziza bananicola
Peziza subsapiane (2)
Peziza subsapiane (1)

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.
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 two species of Eremiomyces (with or without Ruhlandiella), Amylas–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 Amylas, 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
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 Hydnophora, 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. oregomensis, 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 Hydnophora 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
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)]; sterethecia [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 suggest that ptychothecia and sterethecia have evolved multiple times in different lineages within Pezizales.

Taxonomy

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 Shimodaira–Hasegawa test for ML (p < 0.05)

<table>
<thead>
<tr>
<th>Tree</th>
<th>MP</th>
<th>ML</th>
<th>Ln likelihood</th>
<th>Difference in LnL</th>
<th>P-value</th>
<th>Significantly worse?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineage A, unconstrained MPT</td>
<td>2078</td>
<td>Best</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lineage A, unconstrained optimal MPT</td>
<td>2082 (+4)</td>
<td>No</td>
<td>–11183.03738</td>
<td>6.37162</td>
<td>0.083</td>
<td>No</td>
</tr>
<tr>
<td>Hydnophytus monophyletic</td>
<td>2095 (+17)</td>
<td>Yes</td>
<td>11224.21753</td>
<td>0.006*</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Truffles in ‘P. depressa lineage’ monophyletic (including Ruhlandiella and Eremiomyces)</td>
<td>2093 (+15)</td>
<td>Yes</td>
<td>–11213.75681</td>
<td>–30.71942</td>
<td>0.004*</td>
<td>Yes</td>
</tr>
<tr>
<td>Truffles in ‘P. depressa lineage’ monophyletic (not including Ruhlandiella, but including Eremiomyces)</td>
<td>2085 (+7)</td>
<td>No</td>
<td>–11201.55494</td>
<td>–18.51755</td>
<td>0.020*</td>
<td>Yes</td>
</tr>
<tr>
<td>Amylascus and Pachyphlebus monophyletic</td>
<td>2098 (+16)</td>
<td>Yes</td>
<td>–11219.85875</td>
<td>–36.82137</td>
<td>0.041*</td>
<td>Yes</td>
</tr>
<tr>
<td>Pachyphlebus monophyletic</td>
<td>2090 (+12)</td>
<td>Yes</td>
<td>–11214.23004</td>
<td>–31.19266</td>
<td>0.005*</td>
<td>Yes</td>
</tr>
<tr>
<td>Mattirolomyces with Terfezia</td>
<td>2094 (+12)</td>
<td>Yes</td>
<td>–11215.54524</td>
<td>–32.50785</td>
<td>0.009*</td>
<td>Yes</td>
</tr>
<tr>
<td>Kalihartibacter with Terfezia</td>
<td>2098 (+20)</td>
<td>Best</td>
<td>–11218.85867</td>
<td>–35.82129</td>
<td>0.051</td>
<td>No</td>
</tr>
<tr>
<td>Lineage B, unconstrained MPT</td>
<td>1235</td>
<td>Best</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lineage B, unconstrained optimal MPT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fischerula–Leucangium with Hydnophytus</td>
<td>1239 (+4)</td>
<td>No</td>
<td>–6550.10480</td>
<td>–9.21752</td>
<td>0.077</td>
<td>No</td>
</tr>
<tr>
<td>Lineage C, unconstrained MPT</td>
<td>1679</td>
<td>Best</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lineage C, unconstrained MLT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stephensia with Paurocotylis</td>
<td>1694 (+15)</td>
<td>Yes</td>
<td>–8991.20102</td>
<td>–32.32599</td>
<td>0.001*</td>
<td>Yes</td>
</tr>
<tr>
<td>Stephensia monophyletic</td>
<td>1692 (+13)</td>
<td>Yes</td>
<td>–8992.05211</td>
<td>–33.17708</td>
<td>0.001*</td>
<td>Yes</td>
</tr>
</tbody>
</table>

a Difference in length between MPTs and constrained trees in parentheses.
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, b-tubulin, and RPB2 (Hansen et al. 2005). Only one truffle genus, *Mattirolyces*, 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 Ereminomyces within Pezizaceae is not resolved in our analyses, but it is likely among members of the P. depressa–Ruhlandiella, Plicaria–Hapsidomyces or P. phylogenica 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; Trape 1971, 1979). Trape (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 ascii 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 abandonned the genus and placed it in Choiromyces. In agreement with Hansen et al. (2005), Hydnotryopsis setchellii 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 monoplyetic 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. Trape (1975c, 1979). The solid ascomata have a peridium of globose cells, and asci and paraphyses in a hymenial configuration.  

**Kalaharituber** Trape & 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 terrezioides, Terfezia terrezioides).  
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. terrezioides 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. Pachyphloides, 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. Trape (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 vesciculosa.  
Several hypogeous species, with passive spore dispersal, have been accepted in the otherwise epigeous, apothecial genus Peziza. Trape (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 (sclerotiacia), 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).


Type: Ruhlandiella berolinensis.

Ruhlandiella is more or less epigeeous but with passive spore dispersal and with a somewhat convoluted ascoma, where the hymenium covers the surface (exothelial) 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 Picaria. Molecular results place it in the P. depressa–Ruhlandiella lineage (Hansen et al. 2005) (Fig 2). Muciturbo was accepted and listed in the Ascobaceae 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, Exoxone, 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 uninnucleate.

**Sphaerozone** Zobel 1854 (syn. Sphaerosoma subgen. Tulasia) 

Type: Sphaerozone ostiolum (syn. S. tulatsianum).

This is a monotypic genus with exothelial, more or less spherical, and to some extent convoluted ascoma, 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 indistinct, 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 Gymnohynotrya (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 ectomyorrhizal. 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 ascus that led early workers (e.g. Fischer 1897) to consider Terfezia outside the Tuberales. Vizzini (2003) considered the Tuberaeaceae 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). Díez 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. pinayi spores. See also the descriptions in Malençon (1973) and in Montecchi & Sarasini (2000). Díez 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 pycnothecia and stereothecia are identified in both the *Morchellaceae–Discinaceae* and *Helvellaceae–Tuberaceae* lineages (Figs 3, 6A–H). The family *Tuberella* 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 stipite-like extension, as the name indicates, which is absent on the European taxon.

**Leucangium** Quél. 1883

Type: *Leucangium ophthalmosorum* (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 neighbour-hood of the *Morchellaceae*, while the type of *Picoa* clustered with *Oidium* (*Pyronemataceae*) (data not shown in O’Donnell *et al.* 1997). L. *carthusianum* has apiculate-fusiform ascospores in saccate asci. Palfner & Agerer (1998b) described the ectomycorrhiza of this species.

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

Besides the epigeous taxa *Discina*, *Pseudohirzina* 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–Bassisia* 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, Phy- matomyces, in Barsisia, but as the type has been lost, this Japanese taxon should be re-investigated. Barsisia ascomata have a smoother surface compared to species of Balsamia. Molecular results indicate a very close relationship between Barsisia 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 aspitate, 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 Balsamicaceae 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 Pyrrolepazaceae s.l. (as tribe Otidea 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 Labyrinthomycetes. 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 Labyrinthomycetes.

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

*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 Gericulodendron-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 associated with *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 ganadìi._

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 Tuberales.

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, Astomycota), 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 _Paurocotyliis_. 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 _Paurocotyliis_, the truffles formed in _Pyronemataceae_ all still possess a hymenium; no stereothecia are found. Epigeous members have both saprotrophic and mycorrhizal representatives, but _Paurocotyliis_ 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.
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 (ptycethoclia; 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 Oitidiaceae). 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. hispida 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. Myrmeccostystis, 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, Myrmeccostystis (type: M. cerebriformis) and Genea. Trappe (1975c) synonymized Myrmeccostystis 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 singleri 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 Aeuriae 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 fulfill the small mammal niche in New Zealand) that may act as dispersal vectors in its natural setting (Castellano et al. 2004). Macro- and morphologically 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: Hydrocystis tuaitesi (syn. Petchiomyces tuaitesi).

This genus was included in Genaceae by Fischer (1938), followed by Gilkey (1954). Bursdall (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 (1959) 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 lefeburei.

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 of Phaeangium lefeburei (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 Ascomobolaceae 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 Otidae booth). 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 Sphaerzone (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. Euecker (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. colombii, 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. colombii is apparently very close to S. bombycina, but some minor macroscopic differences are noted.

Hypogeous pezizalean taxa currently not placed within clade A–C

Carbymycetaceae 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 ascomata and 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 pulvulent 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 microsorius.

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 Euotiales/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 Pseudobalsamina microsorius 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. Hauker (1959) did some developmental studies on Diehliomyces and concluded that the ascocoma were not truly folded as in a typical member of the Tuberales, and she supported a transfer to the Eurotiatales. 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 Tuberales, 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.natruftling.org/) and e.g. Buccquet-Grenet & Dubarry (2001). A very extensive bibliography on the genus Tuber can be found in Cerutti 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 Orygenales (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 Tuberales and Helvellaceae. Percudani et al. (1999) focused on hypogeous Pezizales phylogeny and species thought to belong to the Balsamiaceae, Terfeziaceae, and Tuberales. Unfortunately they included only few epigeous taxa, which resulted in Cazia, Mattirolomycis, Pachythelus, and Terfezia (Terfeziaceae) erroneously formed a monophyletic group within Pezizaceae. A study with a broader sampling of epigeous pezizaceous species followed (Norman & Eggert 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–Tuberales (Fig 3) and in three lineages within Pyronemataceae (Fig 4). The only strictly hypogeous family known is currently Tuberales. 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),
Table 2 – Different recent classification schemes of pezizalean truffles

<table>
<thead>
<tr>
<th>Tuberales (Korf 1973a)</th>
<th>Hypogeous Pezizales (Trappe 1979)</th>
<th>Suggested classification of hypogeous Pezizales</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elaphomyctaceae</td>
<td>Peziaceae</td>
<td>Peziaceae</td>
</tr>
<tr>
<td>Elaphomyces</td>
<td>Amylascus</td>
<td>Amylascus</td>
</tr>
<tr>
<td></td>
<td>Mycocelidalia (as Clelandia)</td>
<td>Cazia</td>
</tr>
<tr>
<td></td>
<td>Hydnotrzyopsis</td>
<td>Eremiomyces</td>
</tr>
<tr>
<td></td>
<td>Pezi sp.</td>
<td>Hydnobolites</td>
</tr>
<tr>
<td></td>
<td>Tirmania</td>
<td>Hydnotrzyopsis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kalahartuber</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mattirolomyces</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mycocolidalia</td>
</tr>
<tr>
<td>Terfeziaceae</td>
<td>Terfeziaceae</td>
<td>Terfeziaceae</td>
</tr>
<tr>
<td>Carbomyces</td>
<td>Choirmyces</td>
<td>Tuber</td>
</tr>
<tr>
<td>Delastria</td>
<td>Delastria</td>
<td>Tuber</td>
</tr>
<tr>
<td>Mukagomyces</td>
<td>Hydnobolites</td>
<td>Tuber</td>
</tr>
<tr>
<td>Paradoxa</td>
<td>Pachyphloeus</td>
<td>Tuber</td>
</tr>
<tr>
<td>Picoa</td>
<td>Terfezia</td>
<td>Tuber</td>
</tr>
<tr>
<td>Terfezia</td>
<td></td>
<td>Tuber</td>
</tr>
<tr>
<td>Tirmania</td>
<td></td>
<td>Tuber</td>
</tr>
<tr>
<td>Balsamiales</td>
<td>Balsamiales</td>
<td>Balsamiales</td>
</tr>
<tr>
<td>Balsamia</td>
<td>Balsamiales</td>
<td>Balsamiales</td>
</tr>
<tr>
<td>Caulocarpace</td>
<td>Balsamiales</td>
<td>Balsamiales</td>
</tr>
<tr>
<td>Choirmyces</td>
<td>Balsamiales</td>
<td>Balsamiales</td>
</tr>
<tr>
<td>Eldaria</td>
<td>Balsamiales</td>
<td>Balsamiales</td>
</tr>
<tr>
<td>Fischerula</td>
<td>Balsamiales</td>
<td>Balsamiales</td>
</tr>
<tr>
<td>Lespiulitnia</td>
<td>Balsamiales</td>
<td>Balsamiales</td>
</tr>
<tr>
<td>Labyrinthomycoses</td>
<td>Balsamiales</td>
<td>Balsamiales</td>
</tr>
<tr>
<td>Hydnobolites</td>
<td>Balsamiaceae</td>
<td>Balsamiaceae</td>
</tr>
<tr>
<td>Hydnoplicata</td>
<td>Balsamiaceae</td>
<td>Balsamiaceae</td>
</tr>
<tr>
<td>Pachyphloeus</td>
<td>Balsamiaceae</td>
<td>Balsamiacea</td>
</tr>
<tr>
<td>Phymatomyces</td>
<td>Balsamiaceae</td>
<td>Balsamiacea</td>
</tr>
<tr>
<td>Piersonia</td>
<td>Balsamiaceae</td>
<td>Balsamiacea</td>
</tr>
<tr>
<td>Protopagena</td>
<td>Balsamiaceae</td>
<td>Balsamiacea</td>
</tr>
<tr>
<td>Pseudobalsamia</td>
<td>Balsamiaceae</td>
<td>Balsamiacea</td>
</tr>
<tr>
<td>Stephensia</td>
<td>Balsamiaceae</td>
<td>Balsamiacea</td>
</tr>
<tr>
<td>Tuber</td>
<td>Balsamiaceae</td>
<td>Balsamiacea</td>
</tr>
<tr>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Geopora coperi</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Hydnocystis</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Labyrinthomycoses</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Paurocryptis</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Pachyphloeus</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Phymatomyces</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Piersonia</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Protopagena</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Pseudobalsamia</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Stephensia</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Tuber</td>
<td>Pyronemataceae</td>
<td>Pyronemataceae</td>
</tr>
<tr>
<td>Geneaceae</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Genea</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Hydnocystis</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Pachyphloeus</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Phymatomyces</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Piersonia</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Protopagena</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Pseudobalsamia</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Stephensia</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Tuber</td>
<td>Geneaceae</td>
<td>Geneaceae</td>
</tr>
<tr>
<td>Carbomyctaceae</td>
<td>Carbomyctaceae</td>
<td>Carbomyctaceae</td>
</tr>
<tr>
<td>Carbomyces</td>
<td>Carbomyctaceae</td>
<td>Carbomyctaceae</td>
</tr>
<tr>
<td>Carbomyces</td>
<td>Carbomyctaceae</td>
<td>Carbomyctaceae</td>
</tr>
</tbody>
</table>

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

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, Hydnotrzy, Pachyphloeus, Peziza, Sarcosphaera, and Tuber. They hypothesized that the ectomycorrhizal lifestyle is a pre-condition 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.
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 lineages 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.

Acknowledgements

Thanks to Jens H. Petersen for providing and editing photographs, and to Christian Lange, Jan Vesterholt, Johan Nitare, Juan Santos, and Manuel Tabarés for allowing us to use their pictures. Jim Trappe is thanked for help with references, advice on truffle matters in general and for supplying many of the specimens used by K.H. for molecular work. Rosanne Healy is thanked for providing specimens of Pachyphloeus. Donald H. Pfister is thanked for discussions and support of this work. The suggestions from two anonymous reviewers are greatly acknowledged. The research contributed by K.H. was financially supported by an NSF grant to Donald H. Pfister and K.H. (DEB-0315940). T.L. thanks David Hawksworth for initiating this review, and he and Scott LaGreca are thanked for hosting ‘The New Bottles for Old Wine: fruit body types, phylogeny and classification’ BMS annual conservation and taxonomy meeting.

References

States. IX. A new name for Peziza quericola Fogel & States (Ascomycota, Pezizales). Mycota 88: 155–156.


