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A phylogenetic study of *Boletus* section *Boletus* in Europe

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Key words

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Abstract A phylogenetic study of the species in *Boletus* sect. *Boletus* was undertaken using the molecular markers ITS1-5.8S-ITS2 and GAPDH. Four well-supported lineages, one comprising *Boletus edulis* s.l., the others referring to *B. aereus*, *B. reticulatus* and *B. pinophilus* have been distinguished. The ML and MP trees of ITS showed remarkably low resolution within the *B. edulis* clade, and confirmed earlier published results, despite the use of samples from a wider geographical area and different hosts. The results of GAPDH demonstrate clearly that this low resolution must be ascribed to a low genetic variability with the *B. edulis* clade, and make clear that morphological and ecological characters have been overestimated within this species complex. *Boletus edulis* is therefore defined as a variable species with a wide morphological, ecological and geographic range, and includes several specific and subspecific taxa described in the literature (e.g. *B. betulicola, B. persoonii, B. quercicola* and *B. venturii*). Three other European species (*B. aereus*, *B. pinophilus* and *B. reticulatus*) are well delimited species based on morphology and our genetic data.

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INTRODUCTION

Boletus sect. *Boletus* is well characterised morphologically by the white, unchanging context, white pores becoming yellow to olive with age and a reticulate surface of the stipe (Singer 1986). Molecular data suggest that sect. *Boletus* is well delimited from the rest of the genus (Binder 1999).

Within sect. *Boletus* the species concept and species delimitation has however been a matter of dispute, resulting in a fairly large number of specific and infraspecific names and a complex nomenclatural history. Because most species of sect. *Boletus* are much sought for because of their culinary value, they have received attention not only from taxonomists, but also gastronomists. As a result many taxa have been described, often based on single ecological characters, such as mycorrhizal partner, or on highly variable morphological characters, such as the colour of the pileus. Particularly with regard to taxa similar to the type species of the section, *B. edulis*, a number of species and infraspecific taxa have been described, for example in relation to the mycorrhizal partner or on account of deviating colours of the basidiocarp (Table 1).

Van der Linde (2002) made an extensive literature study followed by a morphological revision (Van der Linde 2004) of sect. *Boletus* in northwestern Europe. Morphological and morphometric studies on material from various geographic regions revealed that *B. edulis* has a wide ecological range, being associated with numerous different partners, both deciduous and coniferous trees (e.g. *Betula*, *Fagus*, *Picea*, *Pinus*, *Quercus* and *Tilia*). Therefore it includes several taxa that are supposed to be exclusively associated to certain hosts (*B. betulicola* and *B. quercicola*). Morphological characters, such as the colour of the pileus, development of a reticulum on the stipe, size and shape of the spores and the terminal elements of the pileipellis, varied a great deal, and could not be used to distinguish discrete morphological entities (Van der Linde 2004). As a result it was proposed to reduce the number of taxa to four (Table 2). Beside *B. edulis* and a white variety *B. edulis* var. *albus* (= *B. persoonii*), three other species could be distinguished in Europe on morphological criteria, *B. aereus, B. pinophilus* and *B. reticulatus* (= *B. aestivalis*). Van der Linde (2004) distinguished furthermore three morphotaxa within the wide concept of *B. edulis*, one associated with *Fagus*, with a very dark pileus and strongly developed net on the stipe (morphotaxon A in the present paper), and one associated with *Tilia*, with a reddish pileus, strongly developed net and rather wide terminal elements of the pileipellis (= morphotaxon B). Morphotaxon C initially was thought to be *B. pinophilus* on account of its association with *Pinus* on nutrient-poor sandy soil in the Netherlands, but its colour and microscopical characters revealed that it should be considered a form of *B. edulis*.

Molecular data have been very useful in understanding phylogenetic relationships in Basidiomycota at all taxonomic levels (Binder 1999, Moncalvo et al. 2002, Den Bakker et al. 2004a, b). Using ITS2 and the glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH) sequences, Den Bakker et al. (2004b) confirmed that host specificity can be an important character in distinguishing species in the genus *Leccinum*. Den Bakker & Noordeloos (2005) compared other morphological and ecological data with molecular data to revise the species of the genus *Leccinum* in Europe. Leonardi et al. (2005) studied the *B. edulis*-complex with molecular data obtained from the internal transcribed spacer 1-5.8S rRNA-internal transcribed spacer 2 (= ITS) using a total of 39 samples from Italy and France. Their results support roughly those of our morphological studies.

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Table 1 Species and infraspecific taxa described in section *Boletus* in Europe.

Name and authors	Host
B. aereus Bull.	*Quercus*
B. betulicola (Vasilkov) Pilat & Dermek	*Betula*
B. carpinaceus Velen.	*Carpinus*
B. edulis Bull. var. *edulis*	Various hosts (coniferous and deciduous)
B. edulis var. *albus* Persoon = *B. persoonii* M.Bon	*Pinus sylvestris* and deciduous trees
B. edulis var. *arcticus* Vasilkov	*Betula nana*
B. edulis var. *arenarius* Engel & al.	*Pinus sylvestris*
B. edulis forma *aurantioruber* (Dick & Snell) Vassilkov	*Abies*, *Picea*
B. edulis var. *citrinus* Pelt.	Unknown
B. edulis subsp. *clavipes* (Peck) Singer	*Picea*, *Abies*
B. edulis var. *pusturiensis* Ferrarese & Simonini	*Picea* and *Pinus*
B. edulis forma *roseipes* Vassilkov	Unknown
B. edulis var. *subhepaticus* Fayod	*Fagus*
B. edulis forma *viridicaerulescens* Estades & Lannoy	*Picea*, *Abies*, and *Pinus uncinata*, *P. cembra*
B. edulis subsp. *trisporus* Watling	Unknown
B. fulvomaculatus Estades & Lannoy	Deciduous trees (*Quercus*)
B. pinophilus Pilat & Dermek	*Pinus* (*Picea*)
B. pinophilus var. *fuscoruber* (Forq.) Estades & Lannoy	*Picea*, *Abies*
B. quercicola (Vassilkov) Singer	*Quercus*
B. reticulatus Schaeff. (= *B. aestivalis*) Paulet	*Quercus*
B. venturii M. Bon	Deciduous and coniferous trees

Table 2 Species in sect. *Boletus* as accepted by Van der Linde (2004).

Species	Morphological characteristics	Host and ecology
Boletus aereus Bull.	Pileus dry, finely tomentose, often also finely pruinose-pulverulent in marginal zone; very dark brown; stipe reticulate over whole length; dark brown; stipe reticulate over whole length; pileipellis elements up to 11 µm wide with fine intracellular grains	With *Quercus*, on heavy loamy, often somewhat calcareous soil; in road-sides and parks; thermophilic
Boletus edulis Bull. var. *edulis*	Pileus surface glabrous, greasy to touch, pale to dark brown, yellow brown, reddish brown (white in var. *albus*); stipe with fine reticulation in upper half, rarely over entire length. Pileipellis with terminal elements 4.0–19 µm wide in intracellular pigment	With a broad spectre of both deciduous and coniferous trees (*Picea*, *Quercus*, *Betula*, *Fagus*, *Tilia*, rarely *Pinus*). On various soil types, ranging from moist to fairly dry
Boletus edulis var. *albus* Persoon	As in the type-variety, but fruitbodies purely white	With *Pinus* or *Quercus*, on humose soil
Boletus pinophilus Pilát & Dermek	Pileus with thick, gelatinous pellicle, dark red brown, glabrous; stipe with coarse reticulation over the top, rarely over entire length; pileipellis with terminal elements up to 27 µm wide with reddish brown parietal and incrusting pigment, which dissolves in KOH	Almost exclusively with *Pinus*, rarely with *Picea*, on poor, acid, sandy soil
Boletus reticulatus Schaeff.	Pileus moderately dark to dark yellow-brown or reddish brown, dry, tomentose, with age often somewhat fissurate or craqued; stipe with rather pronounced reticulation all over; pileipellis elements up to 20 µm wide with fine intracellular grains	With *Quercus*, on clay or sand mixed with loam, on fairly dry places, such as road-sides and parks
Morphotype A ‘Fagus’.	Pileus very dark brown, dry; pruinose-tomentose; stipe strongly swollen and reticulate all over; pores long covered with a white layer	With *Fagus* on rich, humose soil
Morphotaxon B ‘Tilia’	Pileus surface glabrous, greasy to touch, vivid red-brown; stipe with a strong white net all over on reddish brown background; terminal elements of pileipellis tapering towards apex; 12–50 × 4.5–15 µm	With *Tilia* in road-sides on peaty soil
Morphotaxon C ‘Pinus’	Pileus grey-brown to reddish brown; glabrous, but not viscid; terminal elements of pileipellis slightly swollen, up to 15 µm wide	With *Pinus* on poor, acid, sandy soil

Bolletus aereus, *B. aestivalis*, *B. edulis* and *B. pinophilus* formed four distinct groups. *Bolletus edulis* var. *pusteriensis*, *B. persoonii* and *B. venturii* could molecularly not be distinguished from *B. edulis*.

We wish to extend this analysis using two nuclear encoded regions: one the ITS regions as used by Leonardi et al. (2005), and the GAPDH region which has been shown to give good interspecific resolution in Basidiomycota (Den Bakker et al. 2004b).

We will also use the specimens studied morphologically by Van der Linde (2004) from western and northern Europe to address the following questions:

- Are taxa (species, subspecies, morphotypes) within the *B. edulis* complex genetically distinct?
- What further insights can be gained into the evolutionary history of *Boletus* with the addition of a second genetic marker (GAPDH)?
- How does our ITS data compare to previous results by Leonardi et al. (2005)?

MATERIALS AND METHODS

Selection of specimens

A selection was made from the material used by Van der Linde (2004) in his morphological studies. These were collected mainly in the Netherlands in 2001 and 2002 and during collecting trips to Borgjö (Sweden) in August 2001, Vuokatti (Finland) in September 2001 and central Austria in 2002. A list of collections used is given in Table 3.

DNA extraction

DNA was extracted from herbarium specimens using the method described in Den Bakker et al. (2004a). The presence of DNA was checked on a 1 % agarose gel in 0.5× TBE, stained with ethidium bromide.

Amplification

For amplification of ITS, the forward primer ITS5 and the reverse ITS4 (White et al. 1990) were used. Total reaction volume was 25 µl containing 2 µl of diluted template DNA. The PCR reaction was performed using the following protocol: an initial denaturation step of 94 °C for 4 min, followed by 37 cycles of: 1 min denaturation at 94 °C, annealing at 52 °C for

Table 3 Collections used in this research project, showing collection number, GenBank accession number (http://www.ncbi.nlm.nih.gov), country of origin and presumed host. Country names are abbreviated; A = Austria, B = Belgium, FIN = Finland, NL = The Netherlands, S = Sweden.

Sample No.	Collection No.	Species	GenBank accession No. (ITS/GAPDH)	Country	Host genus
1	SvdL49	*B. aereus*	EU417844/EU417876	NL	*Quercus*
2	SvdL94	*B. aereus*	EU417845/EU417877	NL	*Quercus*
3	SvdL24	*B. betulicola*	EU417846/EU417896	FIN	*Betula* or *Picea*
4	SvdL58	*B. betulicola*	EU417847/EU417895	NL	*Betula*
5	SvdL74	*B. betulicola*	EU417848/EU417894	NL	*Quercus* or *Betula*
6	SvdL36	*B. betulicola*	EU417849/EU417893	NL	*Betula*
7	SvdL41	*Morphotaxon* A	EU417850/EU417879	NL	*Fagus*
8	SvdL32	*Morphotaxon* A	EU417851/EU417880	NL	*Fagus*
9	SvdL21	*B. edulis*	EU417852/EU417881	FIN	*Picea*
10	SvdL14	*B. edulis*	EU417853/EU417882	S	*Picea* or *Pinus*
11	SvdL16	*B. edulis*	EU417854/EU417883	S	*Picea*
12	SvdL9i	*B. edulis*	EU417855/EU417884	S	*Picea* or *Betula*
13	SvdL69	*B. edulis*	EU417856/EU417885	NL	*Quercus*
14	SvdL61	*B. edulis*	EU417857/EU417886	NL	*Quercus*
15	SvdL43	*B. edulis*	EU417858/EU417888	NL	*Quercus*
16	SvdL45	*B. edulis*	EU417859/EU417889	NL	*Quercus*
17	SvdL35	*Morphotaxon* B	EU417860/EU417890	NL	*Tilia*
18	SvdL67	*Morphotaxon* B	EU417861/EU417891	NL	*Tilia*
19	O1101601	*B. edulis* var. *albus*	EU417862/EU417892	B	*Picea*
20	SvdL50	*Morphotaxon* C	EU417863/EU417905	NL	*Pinus*
21	SvdL93	*Morphotaxon* C	EU417864/EU417904	NL	*Pinus*
22	SvdL12	*B. pinophilus*	EU417865/EU417903	S	*Pinus*
23	SvdL18	*B. pinophilus*	EU417866/EU417902	S	*Pinus*
24	O1097	*B. quercicola*	EU417867/EU417901	A	*Quercus*
25	O1095	*B. quercicola*	EU417868/EU417900	A	*Quercus*
26	O1085	*B. quercicola*	EU417869/EU417899	A	*Quercus* or *Castanea*
27	SvdL47	*B. edulis*	EU417870/EU417898	NL	*Quercus*
28	SvdL79	*B. reticulates*	EU417871/EU417878	NL	*Quercus*
29	MEN 9336	*B. fechtneri*	EU417872/EU417875	NL	*Quercus*
30	SvdL15	*B. edulis*	EU417873/EU417887	S	*Betula*
31	SvdL63	*B. betulicola*	EU417874/EU417897	NL	*Quercus* or *Betula*

1 min and extension at 72 ˚C for 1 min. This was followed by a final extension of 5 min at 72 ˚C. The amplified DNA samples were checked on a 1 % agarose gel stained with ethidium bromide. PCR products were cleaned using the QIAquick PCR Purification (Qiagen, Hilden, Germany). In cases where multiple bands were found, the PCR products with the correct length (± 850 bp) were cut out and cleaned following the QIAquick Gel Extraction Kit Protocol (Qiagen, Hilden, Germany).

From the single copy nuclear gene GAPDH only the last half of the gene was amplified. Forward primer GPD0623F (5'-TTGCCAAGGTCGTCAACG-3') and reverse primer GPD reverse (5'-GAGTAWCCSCATTCGTTATCGTACC-3') were used from Den Bakker et al. (2004b). The PCR reactions for amplification of GAPDH followed Den Bakker et al. (2004b). The products were checked and cleaned as for ITS products.

Sequencing

The cleaned PCR products of ITS were sequenced using the ITS2, ITS3, ITS4 and ITS5 primers (White et al. 1990). The primers GPD0623F and GPD reverse, plus the PCR primers, were used for GAPDH sequencing. The samples were sequenced on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA, USA) using standard dye-terminator chemistry following the manufacturer's protocols.

Phylogenetic analysis

The raw data were processed using Sequencher v. 4.0 (Gene Codes Corporation Ann Arbor, Michigan, USA). ITS sequences (with and without the samples from Leonardi et al. (2005)) were aligned in ClustalX (Thompson et al. 1997) and refined by eye. The ClustalX parameters were gap opening penalty of 5 and a gap extension penalty of 3. Alignment of the GAPDH sequences was done by eye in Se-Al (v. 2.0-11) (A. Rambaut, University of Oxford, available from http://evolve.zoo.ox.ac.uk/software.html).

To increase phylogenetic resolution the ITS and GAPDH data sets were combined in MacClade 4.0.5 (Maddison & Maddison 2002) for 31 taxa in which both genes were sequenced. To test a priori whether the ITS and GAPDH datasets contained a congruent phylogenetic signal a partition homogeneity test was performed using 100 replicates, 10 random sequence additions, maxtrees set to 100, TBR branch swapping, unordered and unweighted characters and gaps treated as missing. Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed in PAUP v. 4.0 (Swofford 2002) for the combined ITS-GAPDH sequences and all the ITS sequences (this study and Leonardi et al. 2005).

For the MP analysis the heuristic search option, 100 random sequence additions, tree bisection reconnection (TBR) branch swapping, gaps treated as missing data and unordered and unweighted characters were used. For measuring relative support of the clades, a bootstrap analysis (Felsenstein 1985) was performed using 1 000 bootstrap replicates, 10 random sequence additions and TBR branch swapping. To limit the length of the bootstrap analysis no more than 10 trees per (random sequence addition) replicate were saved.

Modeltest v. 3.06 (Posada & Crandall 1998) was used to find the model of sequence evolution that best fitted the data set using the hierarchical likelihood ratio test (hLRT). These parameters of the model were used in a ML analysis and a distance bootstrap in PAUP.

For the combined ITS-GAPDH sequences the HKY85+G substitution model (with Ti/Tv ratio = 2.1349, gamma shape parameter = 0.2526, and base frequencies set to: A = 0.2164, C = 0.2682, G = 0.2423 and T = 0.2731) was used (Hasegawa et al. 1985).

The ML analysis was performed with 10 random sequence additions and TBR branch swapping. No more than 100 trees were saved for each bootstrap replicate. For the combined ITS-GAPDH dataset, the single ML tree had a log-likelihood score of -3326.71559. A distance bootstrap analysis was conducted with 1 000 bootstrap replicates, neighbour-joining, TBR branch swapping and not more than 5 trees were saved per bootstrap replicate.

The ML analysis of all the ITS sequences (Leonardi et al. 2005, and this study) was performed using the substitution model HKY+G (with Ti/Tv ratio = 1.7679, gamma shape parameter = 0.3354, and base frequencies set to: A = 0.2154, C = 0.2542, G = 0.2416 and T = 0.2888) (Hasegawa et al. 1985). The other parameters were 10 random sequence additions, TBR branch swapping and maxtrees set to 100. The ML tree had a log likelihood score of 2492.05247.

For both the ITS-GAPDH and the ITS dataset (sequences of this study and Leonardi et al. 2005), a Bayesian analysis was performed in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). The combined ITS-GAPDH dataset was partitioned to apply a different model of sequence evolution to each gene. These models were determined using MrModeltest (J.J.A. Nylander, available from: http://www.ebc.uu.se/systzoo/staff/nylander.html). The K80+G substition model with a Ti/Tv of 1.9919, equal base frequencies and gamma shape parameter of 0.2569 was applied to the ITS part of the alignment (Kimura 1980). For the GAPDH partition a HKY85+G subsitution model was used (with Ti/Tv = 2.0506, gamma shape parameter = 0.2956 and base frequencies set to: A = 0.2092, C = 0.3047, G = 0.2320 and T = 0.2540) (Hasegawa et al. 1985). The number of generations was set to 3 million and one tree was saved per 100 generations. A 50 % majority rule consensus tree was made in PAUP from the outcomes of the Bayesian analysis. A 'burn-in' of 1 million generations was used, which was well after stationarity was reached.

RESULTS

The ITS and GAPDH alignment consisted of 860 and 496 characters respectively of which 57 and 39 were parsimony informative. The partition homogeneity test indicated that the two molecular data sets (ITS and GAPDH) could be combined (P = 0.560). This alignment consisted of 1356 basepairs (bp) and contained 96 parsimony-informative sites. The MP analysis produced 6 600 most parsimonious trees of 305 steps. The strict consensus tree of the MP trees is congruent to the ML tree (Fig. 1).

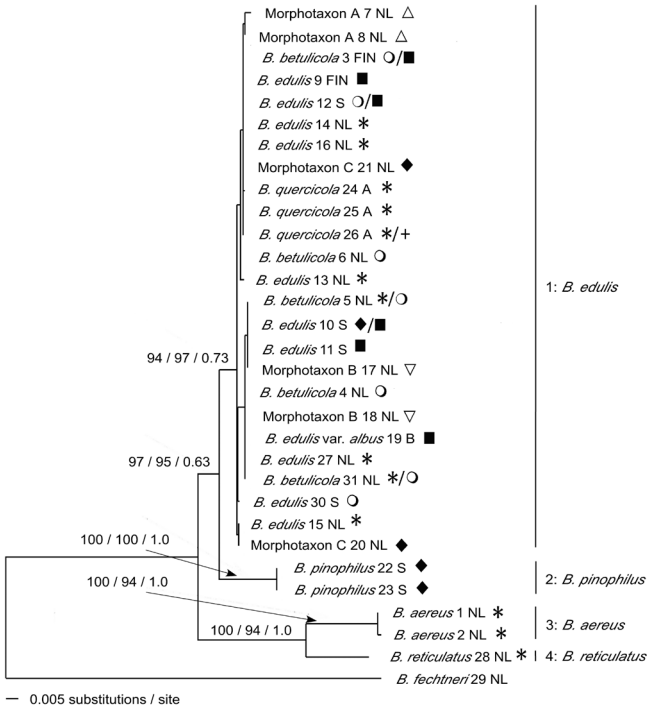

Fig. 1 Maximum likelihood tree from the combined ITS and GAPDH data. *Bolletus fechtneri* is selected as the outgroup. Above the branches bootstrap support values are displayed based on maximum parsimony (percentage), distance (percentage) and posterior probability (frequency) respectively. Behind the name and number of the samples the collection location and host genus are shown. NL = The Netherlands, FIN = Finland, S = Sweden, B = Belgium, A = Austria. ■ = *Picea*, ◆ = *Pinus*, ✻ = *Quercus*, ❍ = *Betula*, △= *Fagus*, ▽= *Tilia*, + = *Castanea*.

In Fig. 1 four well-supported clades are seen. Well-supported is used here for a MP and ML bootstrap (BP) above 70 % and/or posterior probability (PP) above 0.95. The first, *B. edulis* clade, includes the species *B. betulicola*, *B. edulis*, *B. persoonii*, *B. quercicola* and the three unnamed morphotaxa. There was no phylogenetic resolution within this group and very little genetic variation. The second clade, containing the *B. pinophilus* from Sweden (samples 22 and 23), is a sistergroup of the first clade. The third clade, containing *B. aereus* (samples 1 and 2) and *B. reticulatus* (sample 28) formed a sister group to the *B. edulis*/*B. pinophilus* clades. This group was further divided with the two *B. aereus* samples (group 3) and their sister species *B. reticulatus* (sample 28, group 4).

The total ITS alignment consisted of 964 base pairs of which 157 bases were parsimony informative. The MP analysis produced > 10 000 most parsimonious trees of 214 steps. A strict consensus topology is shown in Fig. 2. The ML tree and MP tree did not differ significantly in topology. Most ingroup relationships were not resolved, only four clades were supported with good to moderate BP or PP support. Moderate support is defined here as high support for BP (> 70 %) and low support for PP (< 0.95). The first, moderately supported clade is *B. edulis,* which includes the species *B. betulicola*, *B. edulis*, *B. edulis* var. *pusteriensis*, *B. persoonii*, *B. quercicola*, *B. venturii* and the

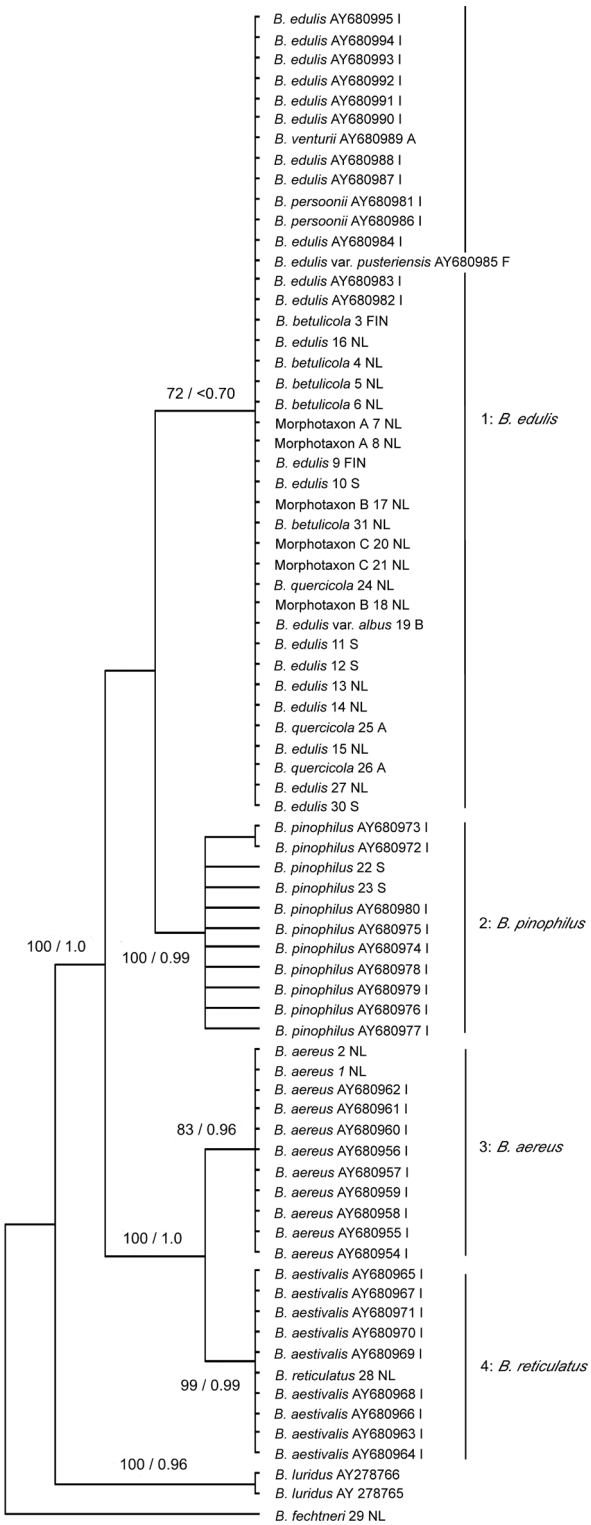

Fig. 2 A strict consesus MP topology of over >10 000 trees. *B. fechtneri* and *B. luridus* form the outgroup. Numerals associated with branches show BP support (percentage) followed by posterior probability (frequency). Leonardi et al. (2005) sequences preceded by their GenBank Accession numbers and country of origin. Samples from this study followed by sample number (Table 1) and country of origin. A = Austria, B = Belgium, F = France, FIN = Finland, NL = The Netherlands, I = Italy, S = Sweden.

three morphotaxa from various hosts and locations in Europe. There was no supported resolution within any of these clades. A second clade contains *B. pinophilus*. Finally, a *B. aereus* clade (group 3) and a *B. reticulatus* clade (group 4) (*B. reticulatus* = *B. aestivalis*), which also grouped into a well-supported clade.

DISCUSSION

Phylogenetic analysis of the ITS and GAPDH and the ITS sequences including the samples of Leonaridi et al. (2005) consistently produced the same four clades. These clades are:

1. A clade containing a large number of samples identified as being *B. betulicola*, *B. edulis*, *B. edulis* var. *albus, B. edulis* var. *pusteriensis, B. quercicola, B. venturii* and morphotaxon A, B, C;
2. *B. pinophilus*;
3. *B. aereus*; and
4. *B. reticulatus*.

There is no resolution within the *B. edulis* group. All the clades are well to moderately supported using bootstrap and posterior probabilities.

Within the *B. edulis* clade, almost no genetic variation was observed, though many distinguishable morphotypes were included plus a wide geographic sampling. Within each group no differences has been found between Northern Europe and Southern Europe *B. edulis*, *B. reticulatus* and *B. pinophilus*.

Our molecular results and those of Leonardi et al. (2005) indicate that within the *B. edulis* clade there is little genetic variation, even for a marker often used in species level distinction in the fungi. The ITS is commonly used for many organisms and is useful for separating species in Basidiomycetes. For example, Hughes et al. (1999) used ITS sequences to distinguish species of the genus *Flammulina* and Kretzer et al. (1996) used ITS for recognizing different species in the genus *Suillus* s.l. But the ITS region also has limitations. According to Bruns (2001), there is often little genetic variation among very closely related species. To corroborate the results of the ITS data, we used an additional section of DNA (GAPDH) as this region was useful in distinguishing species of the genus *Leccinum* (Den Bakker 2004b). Very little genetic differences were also found using the GAPDH dataset. The assignment of different putative species, for specimens that show slight morphological variation or different host plants, cannot be supported by our genetic data suggesting that they are all one species.

A collection with a purely white fruit body, often referred to as *B. persoonii* in the literature, appeared to be similar to *B. edulis* in morphology, and must therefore be considered a mere white form of that species, for which the name *B. edulis* var. *albus* is available. Three morphotaxa, which could be distinguished from *B. edulis* in the morphological study of Van der Linde (2004), did not get support from the molecular data and must be considered to fall within the genetic variability of *B. edulis* (see Table 2).

The lack of resolution in the *B. edulis* clade due to low variability of ITS and GAPDH, suggests that there is an overestimation of the significance of morphological and ecological characters, as was already made apparent by the study of Van der Linde (2004). We therefore consider all taxa in the *B. edulis* clade as belonging to one morphologically variable species.

GAPDH data was able to improve the support of the recognised clades from the ITS data but not resolve relationships, especially within the *B. edulis* clade.

In conclusion, host plant specificity has a very restricted value for the recognition of species in sect. *Boletus*. *Boletus edulis* appears to be associated with a large number of host trees, both deciduous and coniferous. *Boletus pinophilus* seems to be the only species within the section which has a rather strict association with *Pinus*, although it sometimes also is found in pure *Picea* or *Abies* stands. The latter is sometimes referred to as *B. pinophilus* var. *fuscoruber*. *Boletus reticulatus* and *B. aereus* are restricted to a number of *Quercus* species.

We therefore recognise four species throughout Europe: *B. aereus, B. edulis, B. pinophilus* and *B. reticulatus*.

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