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Tuber foetidum found in Finland

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Abstract – *Tuber foetidum*, a white truffle belonging to the *T. macrosporum* group, is confirmed from Finland based on morphological and DNA analyses. The Finnish specimen was found in soil with relatively high pH in coniferous forest. The phylogenetic tree based on nuclear ribosomal ITS sequences indicated that the Finnish material is most similar to, but not identical with, *Tuber foetidum* samples from Hungary and Estonia.

Key words – *Ascomycota, Tuberales,* ectomycorrhiza, nrITS sequence

Introduction

Truffles, as strictly defined, are hypogeous fungi of the genus *Tuber,* which grow in symbiosis with certain trees. Due to rather controversial taxonomic treatments of large numbers of synonyms and varying species definitions, the real number of species is still unknown. The genus is mainly distributed in the Northern Hemisphere (Jeandroz et al. 2008). Truffles in Fenno-Scandinavia are less well documented compared with the Mediterranean region. Fries (1909), who gave the first modern account of *Tuber* species in Scandinavia, listed three species: *T. aestivum* Vittad., *T. maculatum* Vittad., and *T. rufum* Picco. Up to now, Denmark has the most records in this region, with 6 white and 3 black truffle species (Lange 1956). Five *Tuber* species are known from Sweden, including two black truffles, *T. aestivum* and *T. mesentericum* Vittad. (Danell 1996, Wedén et al. 2001). Recently the Burgundy truffle (*T. aestivum* f. *uncinatum* (Chatin) Montecchi & Borelli) has been produced on a small commercial scale in Gotland (Wedén et al. 2009). In Finland, where truffles are

not part of the culinary tradition, the first records of *Tuber* are *T. borchii* Vittad. and *T. maculatum* (Kosonen 2002). *Tuber borchii* is the only truffle species with gastronomic value found in Finland so far.

On 26 November 2006 a truffle ascocarp was found in a natural spruce forest dominated by *Picea abies* trees located in Lahti, Finland (100 km north to Helsinki) with the help of Ciro, a trained truffle dog. The truffle was morphologically and molecularly confirmed as *T. foetidum* Vittad., which represents the third *Tuber* species in Finland and the northernmost record for the species.

Materials and methods

Morphology

Morphological examinations of the ascocarp followed methods set forth in Pegler et al. (1993). Macroscopical descriptions are based on the field notes of the fresh ascocarp. The collection was air-dried with an electrical drier at 50–60°C. Ascospores were observed and measured in KOH. Sections through the peridium were cut anticlinally. All pictures were taken on an Olympus Optiphot-2 microscope. A voucher specimen is deposited in the institutional herbarium of Zoltán Bratek (ZB-3454).

Soil analysis

One kg of soil was collected by removing the litter and covering vegetation from the spruce forest near Lahti. Soil analyses were performed according to Wedén et al. (2004).

Sequence analysis

DNA extraction and PCR amplification of ITS-rDNA region was performed according to Kårén et al. (1997) with minor modifications. ITS1 and ITS4 primers (White et al. 1990) were used for PCR and sequencing reactions. For cycle sequencing ABI Prism BigDye™ Terminator Cycle Sequencing Ready Reaction Kit 3.1 (Applied Biosystems) was used. Capillary electrophoresis was carried out on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. The BlastN 2.2.2. program (Altschul et al. 1997) was used to search for published data similar to the monitored sequences in the international database (GenBank-EMBL-DDBJ-PDB). Phylogenetic analysis was performed with MEGA version 3.1 (Kumar et al. 2004). For tree reconstruction Neighbor-Joining analysis was used by default parameters of MEGA programs with one thousand replicates in bootstrap test. *Tuber melanosporum* Vittad. ITS sequence (AF132501) was selected as outgroup based on preliminary phylogenetic analysis. Phylogenetic trees were built using the Neighbor-Joining (NJ) methods (Kumar et al. 2004). The GenBank accession number of the new ITS sequence obtained by this work is FN568055.

Results

Ecology

The *T. foetidum* sample was found at a depth of 15 cm in a forest dominated by Norway spruce (*Picea abies*), with scattered birch (*Betula* sp.) and pine (*Pinus sylvestris*). Norway spruce, which comprised 80% of the canopy, averaged 25 m

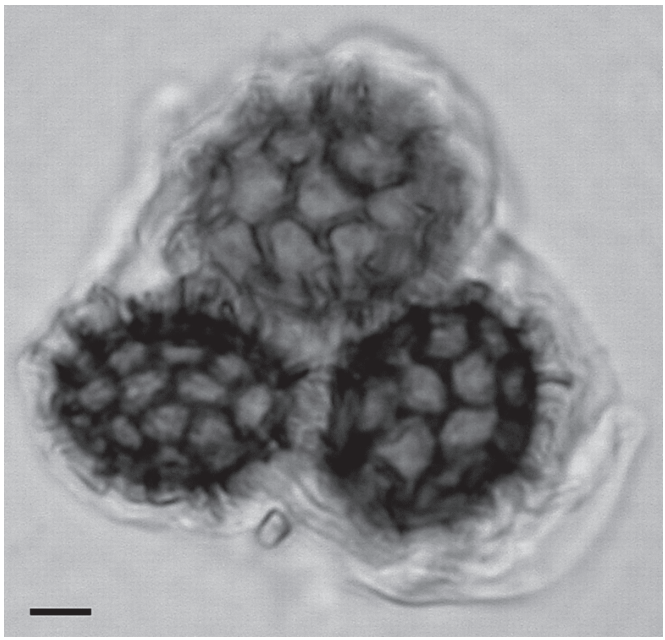

Figure 1. Spores of the Finnish *Tuber foetidum* sample.
Magnification is 100-fold. Scale bar = 10 μm.

in height, 25 cm in diameter, and 40–50 years of age. The soil is cambisol-type silt with a litter layer, lacking stone and organic humus layer. The pH at a ~40 cm measured 6.5; Finnish forest soil pH values generally average 3.5–4.5. The high pH implies that the site may have been used as a farm field or lime fertilizers have been applied earlier.

Morphology

Ascocarp 9 mm in diam.; surface pale ochraceous brownish, minutely warted to verrucose, not hairy; gleba paler than the surface, rarely marbled; with unpleasant odor. Peridium 330–380 μm thick, pseudoparenchymatous with polygonal or roundish cells 15–19 μm in diam.; cell wall yellow, 0.5–1 μm thick; cystidia lacking. Asci ellipsoid, hyaline, thin-walled, 1–5 spored, lacking a stem; 1-spored asci counting for 20.2%, 2-spored 35.8%, 3-spored 33.9%, 4-spored 9.2% and 5-spored 0.9%. Ascospores ellipsoid, in 1-spored asci: 43.7–36.5 × 38.9–25.5 μm, on average: 40.9 × 31.1 μm (n=10), in 1–2 spored asci 43.3–21.7 × 31.7–21.7 μm, in 4-spored asci 28.7 × 21.3 μm; spore wall 2–4 μm thick, light golden brown, ornamented with a regular reticulum, formed by mostly hexagonal meshes 3.6–12.2 μm along the spore length and 2.4–8.5 μm across the spore width. A 3-spored ascus is shown in Figure 1.

Sequence analysis

The ITS sequence obtained from the truffle sample covered the entire 560 bp long ITS region; lengths of ITS-1, ITS-2, and 5.8S rRNA gene are 207, 196, and 157 bp, respectively. BLAST searches indicate that the sample sequence matches most closely three identical ITS sequences (Figure 2), two from *Tuber foetidum* (AJ557543, AJ557544 in Halász et al. 2005) found in Hungary and one from a *Tuber* sp. (AJ534706) sample found in Estonia (Tedersoo et al. 2003). The 5.8S rRNA gene sequence is identical in our sample and the Hungarian and Estonian *Tuber* materials. Five and two base differences between the Finnish sample and the three above mentioned sequences were found in the ITS-1 and ITS-2 regions, respectively.

Discussion

In the *Tuber macrosporum* group, *T. foetidum* is known by its stinking odor and verrucose ascocarp surface (Lange 1956, Pegler et al. 1993, Halász et al. 2005). The peridial surface with minute brownish warts, the ellipsoid reticulate spores, and the pseudoparenchymatic peridium of the Finnish specimen correspond to the morphological criteria of *T. foetidum* (Riousset et al. 2001, Montecchi & Sarasini 2000).

The N-J tree clustered the Finnish sample into the clade that harbors two Hungarian *T. foetidum* ascocarp samples and the Estonian ectomycorrhizal sample. Inside this clade, the Hungarian and Estonian samples form a well-supported branch with 100% bootstrap support apart from the Finnish sample sequence (Figure 2). *Tuber maculatum, T. puberulum,* and *T. borchii* (Hungarian samples now deposited in the Zoltán Bratek herbarium; see Halász et al. 2005) clearly belong to a different branch. Despite intraspecific sequence variability of *T. puberulum* sequences from samples originating from different habitats, the *T. puberulum* sequences cluster together with high bootstrap support, just like *T. borchii* sequences. The *T. foetidum* clade (including ZB3454), which shows less variation than the *T. puberulum* clade, is clearly separated from the *T. maculatum*–*T. borchii*–*T. puberulum* groups. For these reasons we classify the Finnish specimen as *T. foetidum*.

ITS sequence differences indicate, however, that the Finnish genotype has begun to evolve apart from the other *T. foetidum* specimens. Further research is needed to explore the origin and status of Finnish *T. foetidum* population. This raises the possibility that *T. foetidum* sequences from other regions might also differ, as suggested by the separation of the two French *T. macrosporum* (FM205664, FM205663) sequences from the other clades. *Tuber foetidum,* which is found in western Europe between 39°N and 62°N (Jeandroz et al. 2008) and has been recorded in the Scandinavian region from Denmark (Lange 1956)

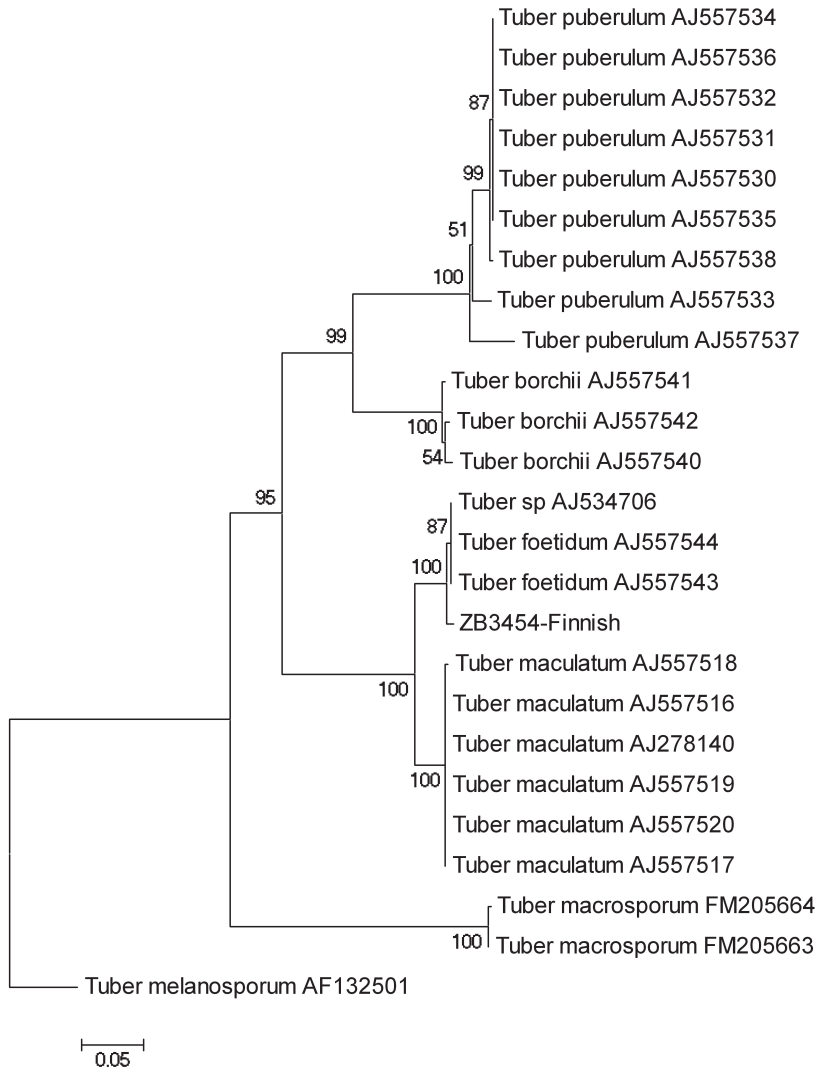

FIGURE 2. Phylogenetic tree based on ITS sequences (ITS-1 and ITS-2). Bootstrap consensus Neighbor-Joining tree based on K-2-p distance matrix (1000 replicates) is shown. Outgroup is *Tuber melanosporum* (AF132501). Scale bar indicates number of nucleotide changes per site.

and Uppland, Sweden (Anderberg & Anderberg 2001) is regarded as a rare species both inside and outside Scandinavia (Lange 1956, Pegler et al. 1993).

Tuber foetidum seems to have a broad range of host trees. In southern Europe, it grows in association with fagaceous trees (*Quercus* and *Fagus*) but in the British Isles it has been found in association with *Larix* (Pegler et al. 1993). The truffle was found in deciduous forests with unknown host associations in Denmark (Lange 1956) and under hazel in Sweden (Anderberg & Anderberg 2001). Sequence analyses by Tedersoo et al. (2003) confirm that *T. foetidum* (as "*Tuber* aff. *maculatum*") formed ectomycorrhizae with birch (*Betula pendula*). Spruce is not a commonly reported host tree for *Tuber* spp. We were unable to trace the ectomycorrhizae, but Norway spruce was the dominant tree in the Finnish forest, we feel that either spruce or Scots pine may serve as hosts of *T. foetidum*.

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