Environmental Microbiology (2005) **7**(10), 1535–1546 doi:10.1111/j.1462-2920.2005.00837.x

Species recognition in the truffle genus *Tuber* – the synonyms *Tuber aestivum* and *Tuber uncinatum*

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Summary

The two morphologically similar truffles *Tuber aestivum* and *T. uncinatum* have caused confusion because *T. uncinatum* is regarded by different authors, as either a distinct species, variety, subspecies, or synonym of *T. aestivum*. A clarification of the relationship between the two truffles would help both conservation biology and cultivation. We aimed both to test the reliability of the only quantitative morphological character used to distinguish the two taxa, i.e. the height of the spore reticulum, and to compare sequences of the ribosomal DNA (rDNA) internal transcribed spacer (ITS) region. Our study included 117 fruit bodies of *T. aestivum* and *T. uncinatum*, originating from eight European countries. The results showed that the spore reticulum height is not diagnostic. The phylogenetic analysis of ITS sequences from 81 fruit bodies and an additional 32 sequences from GenBank showed that *T. aestivum* and *T. uncinatum* were intermingled in one highly supported (100% bootstrap) monophyletic clade, separate from its sister species *Tuber mesentericum*. We conclude that *T. aestivum* and *T. uncinatum* are synonyms and the species should be named *T. aestivum*, as the oldest name has priority. For traders, *T. aestivum* syn. *T. uncinatum* should be used until conformity has been reached.

Introduction

Black truffles of the ascomycete genus *Tuber* have a long history of economic importance because they are appreciated as delicacies. They grow in ectomycorrhizal symbiosis with trees and shrubs such as *Quercus*, *Tilia* and *Corylus* species (Chevalier and Frochot, 1997; Olivier *et al*., 2002). The molecular phylogeny of *Tuber* has been studied repeatedly, leading to convincing morphological characterization of all species studied, except for *Tuber aestivum* Vittad. and *T. uncinatum* Chat. (Guillemaud *et al*., 1996; Gandeboeuf *et al*., 1997; Amicucci *et al*., 1998; Mello *et al*., 2000; Mello *et al*., 2002a; Paolocci *et al*., 2004). *Tuber aestivum* and *T. uncinatum* are sometimes treated as different species, different varieties or as synonyms (Chevalier and Frochot, 1997). It is of crucial importance for conservation biology efforts, as well as for the truffle growers and traders, to know their taxonomic status.

Vittadini described *T. aestivum* in 1831 (Vittadini, 1831). In 1887 Chatin described a new species, *T. uncinatum*, and thereby also amended the circumscription of *T. aestivum* (Chatin, 1887). The new species was named *T. uncinatum* (lat. *uncinatus* = 'hook'), due to the presence of hooks in the spore reticulum, a key character for the species. But the hooks seen by Chatin were in fact only the flexible walls of the spore reticulum bending slightly at the top (Chatin, 1887; Fischer, 1897; Chevalier *et al*., 1979; Chevalier and Frochot, 1997). The gleba of *T. uncinatum* was, when compared with *T. aestivum*, claimed to possess a deeper colour; the peridium having smaller, not transversally striated facets, and it was further claimed to having a more pleasant scent and to mature later during the year.

The spores of *T. aestivum* and *T. uncinatum* have a reticulate-alveolate spore ornamentation with irregular, polygonal meshes numbering 3–4 (5) along the longer axis (Montecchi and Sarasini, 2000). As for the height of the spore reticulum, Vittadini did not comment on this at all. Some authors have suggested that fruit bodies with a spore reticulum height around 2 μm should be classified as *T. aestivum* and fruit bodies with a spore reticulum height around 4 μm as *T. uncinatum* (Chevalier and Frochot, 1997; Riousset *et al*., 2001). Only Mello *et al*. (2002b) used the limits <4 μm for *T. aestivum* and >4 μm for *T. uncinatum*. Both methods leave a rather large group of fruit bodies, which have an intermediate spore reticulum height unaccounted for (Mello *et al*., 2002b; Paolocci *et al*., 2004). Fruit bodies marketed as *T. aestivum* or *T. uncinatum* are, however, seldom classified microscopically, but rather by their scent and colour of the gleba, two

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qualities that are naturally linked, as the darker gleba indicates pronounced spore maturity, and only mature truffles develop a scent.

Since Chatin (1887), authors have considered *T. uncinatum* either as a subspecies of *T. aestivum* (Fischer, 1897; Lange, 1956); as a variety of *T. aestivum* (Hollós, 1911; Knapp, 1950; Chevalier *et al*., 1979; 1994; Gandeboeuf *et al*., 1994a,b; Chevalier and Frochot, 1997; Riousset *et al*., 2001), as a form of *T. aestivum* (Montecchi and Sarasini, 2000) or as the same species (Hawker, 1954; Ceruti, 1960; 2003; Szemere, 1965). Mello *et al*. (2002b) and Paolocci *et al*. (2004) recently obtained opposing results from molecular data using *T. aestivum*/*T. uncinatum*. Paolocci *et al*. (2004) could not distinguish *T. aestivum* from *T. uncinatum*, but instead suggested them to be morphotypes, while Mello *et al*. (2002b) assigned them to different taxa based on their analysis. In both studies *T. aestivum* and *T. uncinatum* from a restricted number of geographical sites were used.

No collections are listed in the protologues of *T. aestivum* (Vittadini, 1831) or *T. uncinatum* (Chatin, 1887) and no neotypification has been undertaken. Consequently, there are no types for *T. aestivum* and *T. uncinatum*. According to Ceruti *et al*. (2003), a *T. aestivum* specimen seen by Vittadini is present in the Mattiroliano herbarium in Turin, Italy. This specimen may possibly be used for typification. Consequently, types have not been included in studies trying to resolve this question (Chevalier *et al*., 1994; Mello *et al*., 2002b; Paolocci *et al*., 2004; A. Vizzini, pers. comm.). A thorough nomenclatural study of this case, including typification, is needed.

The aims of this study were to (i) investigate the reliability of the spore reticulum height as a character to distinguish *T. aestivum* and *T. uncinatum* (Chevalier and Frochot, 1997; Mello *et al*., 2002b; Paolocci *et al*., 2004) and (ii) make a phylogenetic analysis of ribosomal DNA sequences to clarify if these taxa were conspecific or not. For the Swedish truffle project (Wedén *et al*., 2001; 2004a,b), which includes cultivation and conservation aspects, we needed to identify the Swedish taxon/taxa.

Results

Microscopic analysis

Spore reticulum heights were measured in 100 fruit bodies of *T. aestivum/T. uncinatum* (Table 1). Fruit bodies from Italy, France, Spain, Hungary, Denmark and Germany were initially identified as either '*T. aestivum*' or '*T. uncinatum*' before we received them for this study. We could not distinguish '*T. aestivum*' from '*T. uncinatum*' based on our spore reticulum measurements (Table 1), thus '*T. aestivum*' and '*T. uncinatum*' refer to the initial identification. The Swedish fruit bodies have therefore not been separated into '*T. aestivum*' or '*T. uncinatum*', but are only called *T. aestivum* ('Swedish taxon' in Fig. 1). The mean height of the spore reticulum did not always correspond to the initial identification and even varied within fruit bodies, making it impossible to use for separation between the two taxa (Table 1; Fig. 1). The differences in spore reticulum heights were clearly not diagnostic due to the very large overlaps (Fig. 1; Table 1; Ekman and Nordin, 1993).

The number of spores per ascus ranged from one to eight and varied between different fruit bodies and even between different parts of the gleba of the same fruit body. In the fruit body TaeW065S, for example, the first sample had a majority of asci containing two spores, while the second sample from another part of the gleba had mostly four to five and sometimes six spores per ascus. Two asci containing eight spores were observed in sample TaeW032I. The two peridium types assigned to the two taxa (Chatin, 1887) occasionally occurred on the same fruit body, having one side with small facets and another side with large, transversally striated facets.

Internal transcribed spacer (ITS) analysis

One hundred and twenty rDNA ITS sequences were analysed. Of these, 34 were from fruit bodies previously determined as '*T. aestivum*' (Italy, France, Spain, Germany and Denmark), 41 as '*T. uncinatum*' (Italy, France, Spain and Hungary), 38 as *T. aestivum* syn. *T. uncinatum* (Sweden and England), here labelled only as *T. aestivum*, six as *T. mesentericum* and one as *T. magnatum* Pico (Table 1). Of the 120 sequences, 32 were obtained from GenBank (Table 2).

Many sequences were identical and hence excluded from the phylogenetic analysis (Table 3). The mean intraspecific variation in the clade of the 113 *T. aestivum*/*T. uncinatum* sequences was 0.8%, ranging from 0% to 3.5%. Within the Swedish *T. aestivum*, the difference range was 0–0.3%, equivalent to 0–2 base pair substitutions.

The phylogram resulting from the parsimony analysis showed that '*T. aestivum'* and '*T. uncinatum'* formed a well-supported (100% bootstrap) monophyletic clade, different from their sister species *T. mesentericum* and the outgroup *T. magnatum* (Fig. 2; Table 3). Sequences of '*T. aestivum*' and '*T. uncinatum*' did not form monophyletic subclades, but were highly mixed (Fig. 2; Table 3).

Discussion

The ITS region of the rDNA was chosen for this study as it has shown to be well suited for discriminating species within *Tuber* (Roux *et al*., 1999; Mello *et al*., 2002b; Paolocci *et al*., 2004) and many other fungi (Gardes and

Table 1. One hundred and four specimens of *Tuber aestivum*/*T. uncinatum* were analysed morphologically (spore reticulum height) and 81 specimens genetically (ITS sequencing) in this study.

Specimen	Initial identification	Country of origin, region	UPS No.	Collection date	Spore reticulum height (μm)	EMBL accession No.
TaeW001S	*Tuber aestivum*	Sweden, Gotland	F-118708	1999-09-16	4.15 ± 0.34; 3.5–4.5	–
TaeW002S	*Tuber aestivum*	Sweden, Gotland	F-118709	2000-10-06	4.45 ± 0.37; 4.0–5.0	AJ888075
TaeW003D	*Tuber aestivum*	Germany, Baden-Württemberg	F-118821	2003-01-04	4.60 ± 0.88; 3.5–6.5	AJ888129
TunW004F	*Tuber uncinatum*[a]	France, Yonne	F-118777	1999-09	3.95 ± 0.50; 3.5–5.0	AJ888080
TaeW011I	*Tuber aestivum*[b]	Italy, Liguria	F-118778	1999-11-19	3.60 ± 0.66; 2.5–5.0	AJ888089
TaeW012I	*Tuber aestivum*[b]	Italy, Liguria	F-118779	1999-11-19	3.35 ± 0.34; 3.0–4.0	AJ888115
TaeW013I	*Tuber aestivum*[b]	Italy, Liguria	F-118780	1999-11-19	3.30 ± 0.48; 2.5–4.0	–
TaeW014I	*Tuber aestivum*[b]	Italy, Liguria	F-118781	1999-11-19	3.40 ± 0.66; 2.5–5.0	–
TaeW015I	*Tuber aestivum*[b]	Italy, Liguria	F-118782	1999-11-19	3.05 ± 0.28; 2.5–3.5	AJ888116
TaeW016I	*Tuber aestivum*[b]	Italy, Lombardia	F-118783	1999-11-22	3.85 ± 0.53; 3.0–4.5	AJ888090
TaeW017I	*Tuber aestivum*[b]	Italy, Lombardia	F-118784	1999-11-22	3.40 ± 0.52; 2.5–4.0	AJ888094
TaeW018I	*Tuber aestivum*[b]	Italy, Lombardia	F-118785	1999-11-22	3.90 ± 0.34; 3.0–4.5	AJ888106
TaeW019I	*Tuber aestivum*[b]	Italy, Lombardia	F-118786	1999-11-22	3.85 ± 0.47; 3.0–4.5	AJ888107
TaeW020I	*Tuber aestivum*[b]	Italy, Lombardia	F-118787	1999-11-22	4.25 ± 0.49; 3.5–5.0	AJ888095
TaeW021I	*Tuber aestivum*[b]	Italy, Lombardia	F-118788	1999-11-22	3.90 ± 0.57; 3.5–5.0	AJ888091
TunW022I	*Tuber uncinatum*[b]	Italy, Lombardia	F-118789	1999-11-22	3.75 ± 0.43; 3.0–4.5	AJ888096
TunW023I	*Tuber uncinatum*[b]	Italy, Lombardia	F-118790	1999-11-22	4.45 ± 0.83; 3.5–6.0	AJ888092
TaeW024I	*Tuber aestivum*[b]	Italy, Marche	F-118791	2002-08-02	3.40 ± 0.46; 2.5–4.0	–
TaeW025I	*Tuber aestivum*[b]	Italy, Marche	F-118792	2002-08-02	2.90 ± 0.57; 2.0–4.0	AJ888093
TaeW026I	*Tuber aestivum*[b]	Italy, Umbria	F-118793	2001-07-03	3.15 ± 0.63; 2.5–4.0	–
TaeW027I	*Tuber aestivum*[b]	Italy, Umbria	F-118794	2001-07-03	4.20 ± 0.59; 3.5–5.5	AJ888117
TaeW028I	*Tuber aestivum*[b]	Italy, Umbria	F-118795	2001-07-03	2.75 ± 0.43; 2.0–3.5	AJ888118
TaeW029I	*Tuber aestivum*[b]	Italy, Umbria	F-118796	2001-07-18	3.55 ± 0.69; 2.5–4.5	AJ888058
TaeW030I	*Tuber aestivum*[b]	Italy, Umbria	F-118797	2001-07-24	3.10 ± 0.52; 2.0–3.5	–
TaeW031I	*Tuber aestivum*[b]	Italy, Umbria	F-118798	2001-07-24	3.45 ± 0.44; 2.5–4.0	–
TaeW032I	*Tuber aestivum*[b]	Italy, Umbria	F-118799	2001-07-18	3.40 ± 0.57; 2.5–4.5	AJ888119
TaeW033I	*Tuber aestivum*[b]	Italy, Umbria	F-118800	2001-07-24	4.00 ± 0.41; 3.5–4.5	–
TunW034I	*Tuber uncinatum*[b]	Italy, Umbria	F-118801	2001-07-18	3.65 ± 0.67; 3.0–5.0	AJ888097
TunW035H	*Tuber uncinatum*[a]	Hungary, Baranya	F-118814	2003-07-20	3.60 ± 0.78; 2.5–5.0	AJ888098
TunW036H	*Tuber uncinatum*[a]	Hungary, Borsod-Abaúj-Zemplén	F-118815	2003-07-20	4.25 ± 0.68; 3.0–5.5	AJ888113
TunW037H	*Tuber uncinatum*[a]	Hungary, Pest-Jász-Nagykun-Szolnok	F-118816	2003-07-20	3.25 ± 0.49; 2.5–4.0	–
TunW038H	*Tuber uncinatum*[a]	Hungary, Pest-Jász-Nagykun-Szolnok	F-118817	2003-07-20	4.15 ± 0.71; 3.5–5.5	AJ888114
TunW039H	*Tuber uncinatum*[a]	Hungary, Jász-Nagykun-Szolnok	F-118818	2003-07-20	3.85 ± 0.82; 2.5–5.0	–
TunW040F	*Tuber uncinatum*[a]	France, Yonne	F-118776	1997-09	4.65 ± 0.67; 3.5–6.0	AJ888056
TaeW041U	*Tuber aestivum*	England, Oxford	F-118822	1999	No data	AJ888120
TaeW042K	*Tuber aestivum*	Denmark, Møn	F-118819	1999-09-18	3.75 ± 0.54; 3.0–5.0	–
TaeW043K	*Tuber aestivum*	Denmark, Aarhus	F-118820	2000-11-12	4.25 ± 0.79; 3.0–5.5	AJ888121
TaeW044S	*Tuber aestivum*	Sweden, Gotland	F-118710	2000-10-08	4.30 ± 0.59; 3.5–5.5	–
TaeW045S	*Tuber aestivum*	Sweden, Gotland	F-118711	2000-10	3.85 ± 0.47; 3.5–5.0	–
TaeW046S	*Tuber aestivum*	Sweden, Gotland	F-118712	2003-10-10	3.90 ± 0.57; 3.0–5.0	–
TaeW047S	*Tuber aestivum*	Sweden, Gotland	F-118713	2003-10-10	4.05 ± 0.60; 3.5–5.0	AJ888049
TaeW048S	*Tuber aestivum*	Sweden, Gotland	F-118714	2000-10-08	3.85 ± 0.34; 3.5–4.5	AJ888061
TaeW049S	*Tuber aestivum*	Sweden, Gotland	F-118715	2003-09-19	3.95 ± 0.50; 3.0–4.5	AJ888083
TaeW050S	*Tuber aestivum*	Sweden, Gotland	F-118716	2003-09-19	3.80 ± 0.48; 3.0–4.5	–
TaeW051S	*Tuber aestivum*	Sweden, Gotland	F-118717	2000-10-05	3.15 ± 0.34; 2.5–3.5	AJ888082

Table 1. *cont.*

Specimen	Initial identification	Country of origin, region	UPS No.	Collection date	Spore reticulum height (μm)	EMBL accession No.
TaeW052S	*Tuber aestivum*	Sweden, Gotland	F-118718	2000-10-04	3.70 ± 0.48; 3.0–4.5	AJ888084
TaeW053S	*Tuber aestivum*	Sweden, Gotland	F-118719	2000-06-27	4.25 ± 0.35; 4.0–5.0	AJ888063
TaeW054S	*Tuber aestivum*	Sweden, Gotland	F-118720	2000-09-25	4.25 ± 0.56; 3.5–5.0	–
TaeW055S	*Tuber aestivum*	Sweden, Gotland	F-118721	1999-10-11	3.85 ± 0.41; 3.0–4.5	AJ888078
TaeW056S	*Tuber aestivum*	Sweden, Gotland	F-118722	1999-09-18	3.90 ± 0.46; 3.5–4.5	AJ888050
TaeW057S	*Tuber aestivum*	Sweden, Gotland	F-118723	2000-10-04	4.25 ± 0.59; 3.0–5.0	AJ888051
TaeW058S	*Tuber aestivum*	Sweden, Gotland	F-118724	1999-09-16	3.60 ± 0.46; 3.0–4.5	AJ888085
TaeW059S	*Tuber aestivum*	Sweden, Gotland	F-118725	1999-09-21	3.95 ± 0.60; 3.0–5.0	–
TaeW060S	*Tuber aestivum*	Sweden, Gotland	F-118726	1999-09-16	3.40 ± 0.46; 3.0–4.0	AJ888052
TaeW061S	*Tuber aestivum*	Sweden, Gotland	F-118727	2002-08	4.35 ± 0.94; 3.0–6.0	–
TaeW062S	*Tuber aestivum*	Sweden, Gotland	F-118728	2003-10-10	3.95 ± 0.55; 3.0–4.5	–
TaeW063S	*Tuber aestivum*	Sweden, Gotland	F-118729	1999-09-20	3.45 ± 0.55; 3.0–4.5	–
TaeW064S	*Tuber aestivum*	Sweden, Gotland	F-118730	2003-09-20	3.60 ± 0.39; 3.0–4.0	AJ888062
TaeW065S	*Tuber aestivum*	Sweden, Gotland	F-118731	1999-09-20	4.25 ± 0.49; 3.5–5.0	AJ888060
TaeW066S	*Tuber aestivum*	Sweden, Gotland	F-118732	2000-10-08	3.70 ± 0.48; 3.0–4.5	AJ888070
TaeW067S	*Tuber aestivum*	Sweden, Gotland	F-118733	2000-10-06	4.10 ± 0.62; 3.0–5.0	AJ888086
TaeW068S	*Tuber aestivum*	Sweden, Gotland	F-118734	1999-09-19	3.90 ± 0.39; 3.5–4.5	AJ888071
TaeW069S	*Tuber aestivum*	Sweden, Gotland	F-118735	1999-09-19	3.60 ± 0,52; 2.5–4.0	AJ888122
TaeW070S	*Tuber aestivum*	Sweden, Gotland	F-118736	2000-10-10	3.75 ± 0.76; 3.0–5.0	AJ888072
TaeW071S	*Tuber aestivum*	Sweden, Gotland	F-118737	1999-09-21	3.35 ± 0.41; 2.5–4.0	–
TaeW072S	*Tuber aestivum*	Sweden, Gotland	F-118738	1999-09-21	3.75 ± 0.64; 3.0–4.5	AJ888064
TaeW073S	*Tuber aestivum*	Sweden, Gotland	F-118739	1999-09-21	4.05 ± 0.73; 3.0–5.5	AJ888087
TaeW074S	*Tuber aestivum*	Sweden, Gotland	F-118740	1999-09-21	3.50 ± 0.41; 3.0–4.0	AJ888088
TaeW075S	*Tuber aestivum*	Sweden, Gotland	F-118741	2000-10-06	3.80 ± 0.35; 3.0–4.0	AJ888073
TaeW076S	*Tuber aestivum*	Sweden, Gotland	F-118742	2000-10-06	3.70 ± 0.42; 3.0–4.5	AJ888059
TaeW077S	*Tuber aestivum*	Sweden, Gotland	F-118743	1999-09-19	4.40 ± 0.74; 3.0–5.5	AJ888074
TaeW078S	*Tuber aestivum*	Sweden, Gotland	F-118744	1999-07-27	(4.5) – no data	AJ888079
TaeW106S	*Tuber aestivum*	Sweden, Öland	F-118861	2000	3.85 ± 0.53; 3.0–5.0	AJ888109
TaeW107S	*Tuber aestivum*	Sweden, Öland	F-118862	2000	3.38 ± 0.25; 3.0–3.5[c]	AJ888110
TaeW115S	*Tuber aestivum*	Sweden, Gotland	F-118745	2003-11-10	4.20 ± 0.42; 3.5–5.0	AJ888065
TaeW116S	*Tuber aestivum*	Sweden, Gotland	F-118746	2003-11-09	4.40 ± 0.78; 3.0–5.5	AJ888053
TaeW117S	*Tuber aestivum*	Sweden, Gotland	F-118747	2003-11-09	3.75 ± 0.76; 2.5–5.0	–
TaeW118S	*Tuber aestivum*	Sweden, Gotland	F-118748	2003-11-06	4.40 ± 0.57; 3.5–5.0	AJ888066
TaeW119S	*Tuber aestivum*	Sweden, Gotland	F-118749	2003-11-06	No data	AJ888054
TaeW120S	*Tuber aestivum*	Sweden, Gotland	F-118750	2003-11-07	No data	AJ888067
TaeW121S	*Tuber aestivum*	Sweden, Gotland	F-118751	2003-11-07	3.75 ± 0.35; 3.5–4.5	AJ888123
TaeW122S	*Tuber aestivum*	Sweden, Gotland	F-118752	2003-11-10	3.80 ± 0.63; 3.0–4.5	AJ888055
TaeW123S	*Tuber aestivum*	Sweden, Gotland	F-118753	2003-11-10	4.45 ± 1.12; 3.5–7.0	AJ888068
TaeW124S	*Tuber aestivum*	Sweden, Gotland	F-118754	2003-09	3.75 ± 0.64; 3.0–4.5	AJ888069
TunW125F	*Tuber uncinatum*[a]	France, Haute-Marne	F-118755	No data	4.85 ± 0.71; 4.0–6.0	AJ888125
TunW126F	*Tuber uncinatum*[a]	France, Meurthe-et-Moselle	F-118756	2000-12-05	4.45 ± 0.55; 3.5–5.0	AJ888126
TunW127F	*Tuber uncinatum*[a]	France, Meurthe-et-Moselle	F-118757	2000-12-22	4.30 ± 0.56; 3.5–5.5	–
TunW128F	*Tuber uncinatum*[a]	France, Meuse	F-118758	2000-11-27	3.70 ± 0.42; 3.0–4.5	–
TunW129F	*Tuber uncinatum*[a]	France, Meuse	F-118759	2000-12-05	(5.5, 5.5) – no data	AJ888081
TunW130F	*Tuber uncinatum*[a]	France, Yonne	F-118760	No data	4.65 ± 0.94; 3.5–6.5	AJ888057
TunW131F	*Tuber uncinatum*[a]	France, Meuse	F-118761	200-12-05	4.00 ± 0.75; 3.0–5.0	–
TunW132F	*Tuber uncinatum*[a]	France, Meuse	F-118762	2000-11-27	3.40 ± 0.74; 2.0–4.5	–
TunW133I	*Tuber uncinatum*[a]	Italy, Marche	F-118802	1991-06-27	3.35 ± 0.63; 2.5–4.5	AJ888099

TunW134I	*Tuber uncinatum*[a]	Italy, Marche	F-118803	1991-06-23	3.67 ± 0.29; 3.5–4.0[d]	–
TunW135I	*Tuber uncinatum*[a]	Italy, Emilia-Romagna	F-118804	1998-10-01	3.50 ± 0.53; 3.0–4.5	AJ888077
TunW136I	*Tuber uncinatum*[a]	Italy, Piemonte	F-118805	1999-07-14	No data	AJ888100
TunW137I	*Tuber uncinatum*[a]	Italy, Marche	F-118806	1992-10-10	4.85 ± 0.78; 4.0–6.0	–
TunW138I	*Tuber uncinatum*[a]	Italy, Piemonte	F-118807	1999-12-25	4.05 ± 0.73; 3.0–5.5	AJ888101
TaeW139F	*Tuber aestivum*[a]	France, Alpes-Maritimes	F-118763	2003-03-05	No data	–
TaeW140F	*Tuber aestivum*[a]	France, Bouches-du-Rhône	F-118764	No data	(4.0) – no data	AJ888102
TaeW141F	*Tuber aestivum*[a]	France, Dordogne	F-118765	1990-06-10	3.70 ± 0.63; 3.0–4.5	AJ888108
TunW142F	*Tuber uncinatum*[a]	France, Drôme	F-118766	2003-06	3.80 ± 0.75; 2.5–5.0	AJ888127
TunW143F	*Tuber uncinatum*[a]	France, Côte d'Or	F-118767	1997-10-25	(3.0) – no data	AJ888076
TaeW144F	*Tuber aestivum*[a]	France, Hérault	F-118768	1992-06	3.80 ± 0.75; 3.0–5.0	AJ888103
TaeW145F	*Tuber aestivum*[a]	France, Lot	F-118769	1991-07-05	4.00 ± 0.67; 3.0–5.5	AJ888124
TaeW146F	*Tuber aestivum*[a]	France, Var	F-118770	2003-06-15	(3.5) – no data	–
TunW147F	*Tuber uncinatum*[a]	France, Yonne	F-118771	2002-02	4.25 ± 0.72; 3.5–5.0	AJ888104
TunW148F	*Tuber uncinatum*[a]	France, Yonne	F-118772	2001-08-23	4.20 ± 0.54; 3.5–5.0	–
TaeW149F	*Tuber aestivum*	France, Vaucluse	F-118773	1987-07-01	3.15 ± 0.47; 2.5–4.0	AJ888111
TaeW150F	*Tuber aestivum*[a]	France, Vaucluse	F-118774	1991-06-25	3.10 ± 0.57; 2.0–4.0	–
TaeW151F	*Tuber aestivum*[a]	France, Var	F-118775	2001-06-15	2.65 ± 0.58; 1.5–3.5	–
TaeW152E	*Tuber aestivum*[a]	Spain, Castilla la Vieia – León	F-118808	1993-07-05	No data	–
TunW153E	*Tuber uncinatum*[a]	Spain, Catalunya	F-118809	1993-07-05	4.07 ± 0.45; 3.5–4.5[e]	–
TunW154E	*Tuber uncinatum*[a]	Spain, Catalunya	F-118810	1993-07-05	(3.5) – no data	AJ888128
TaeW155E	*Tuber aestivum*[a]	Spain, Castilla la Vieia	F-118811	1993-07-05	No data	–
TunW156E	*Tuber uncinatum*[a]	Spain, Castilla la Vieia	F-118812	1993-07-05	3.80 ± 0.54; 3.0–5.0	AJ888105
TaeW157E	*Tuber aestivum*[a]	Spain, Castilla la Nueva	F-118813	1993-07-05	2.20 ± 0.27; 2.0–2.5[f]	AJ888112
TmsW005	*Tuber mesentericum*	Sweden, Gotland	F-118824	2000-10-07	Not determined	AJ888043
TmsW088	*Tuber mesentericum*	Sweden, Gotland	F-118825	2000-10-06	Not determined	AJ888044
TmsW091	*Tuber mesentericum*	Sweden, Gotland	F-118826	1999-09-16	Not determined	AJ888045
TmsW094	*Tuber mesentericum*	Sweden, Gotland	F-118827	2003-08-26	Not determined	AJ888046
TmsW095	*Tuber mesentericum*	Sweden, Gotland	F-118828	2000-10-06	Not determined	AJ888047
TmsW096	*Tuber mesentericum*	Sweden, Gotland	F-118829	1999-09-19	Not determined	AJ888048
TmgW085	*Tuber magnatum*	Unknown	F-118823	1997-11	Not determined	AJ888042

a. Initial identification of fruit bodies by Dr Sergio Arcioni, Istituto di Genetica Vegetale Sez. Perugia, CNR, Italy.
b. Initial identification of fruit bodies by Dr Gérard Chevalier, INRA Clermont-Ferrand, France.
c. $n = 4$.
d. $n = 3$.
e. $n = 7$.
f. $n = 5$.

Six fruit bodies of *T. mesentericum* and one fruit body of *T. magnatum* were also sequenced. We could not distinguish '*T. aestivum*' and '*T. uncinatum*' on the basis of our spore reticulum measurements above, hence the 49 Swedish (S) and one English (U) fruit bodies are all listed only as *T. aestivum*. D, German; E, Spanish; F, French; H, Hungarian, I, Italian; K, Danish specimens. Spore reticulum height measurements from spores ($n = 10$) of 10 different four-spored asci are presented as mean ± SD; range. Tae, *T. aestivum*; Tmg, *T. magnatum*; Tms, *T. mesentericum*; Tun, *T. uncinatum*. In samples TaeW041U, TaeW078S, TaeW107S, TunW134I, TaeW139F, TunW143F, TaeW152E, TunW153E, TunW154E, TaeW155E and TaeW157E most or all of the spores were immature, preventing full measurement. In samples TunW129F, TunW136I, TaeW140F and TaeW146F spores had been released from asci thereby preventing full measurement. Fruit body vouchers are deposited at the Uppsala herbarium (UPS). EMBL (GenBank) accession numbers are listed for the 88 sequenced specimens.

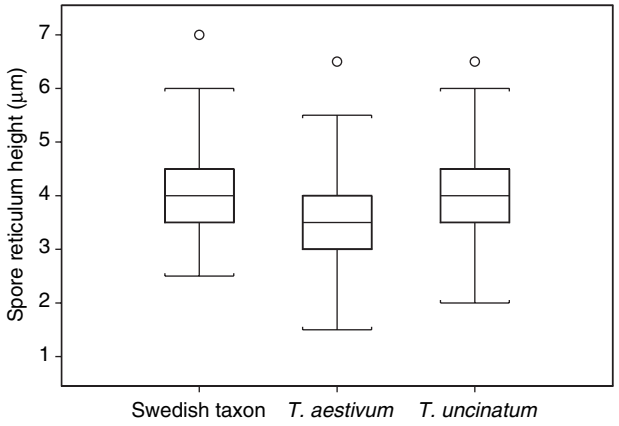

Fig. 1. Box plot of spore reticulum heights (µm) illustrating medians, first and third quartiles, range and extreme values (○). Forty-five fruit bodies of *Tuber aestivum* syn. *T. uncinatum* from Sweden: 'Swedish taxon' (450 measurements). Thirty fruit bodies of '*T. aestivum*' (300 measurements) and 25 fruit bodies of '*T. uncinatum*' (250 measurements) *sensu* Gérard Chevalier (INRA Clermont-Ferrand, France) and Sergio Arcioni (Istituto di Genetica Vegetale Sez. Perugia, CNR, Italy).

Bruns, 1993), and even for species pairs (Johannesson *et al*., 1999; Ryman *et al*., 2003). The ITS region, however, is not phylogenetically informative enough for population studies within a species (Fig. 2; Table 3; Wedén *et al*., 2004b). Problems related to use of ITS for species recognition have been discussed by, e.g. Álvarez and Wendel (2003), but could not be suspected in this case, due to the absence of support for a separation between '*T. aestivum*' and '*T. uncinatum*'. The small variation in the ITS sequence among *'T. aestivum'* and *'T. uncinatum'* specimens neither corresponded to spore reticulum height, nor to any other known morphological, chemical or ecological character. Both Johannesson and colleagues (1999) and Ryman and colleagues (2003) separated closely related species, formerly considered as one species, by analyses of the ITS region. They also found morphological key characters corresponding to the distinct clades, representing different mycorrhizal host preferences. In our analysis *T. aestivum*/*T. uncinatum* form one clade and we found no morphological differences. This is in contrast to the conclusion of Mello and colleagues (2002b), suggesting a difference between the two taxa, based on comparison of sequenced ITS. When comparing more specimens using RFLP of ITS, they could not separate '*T. aestivum*' and '*T. uncinatum*' (Mello *et al*., 2002b). Possibly, their findings from the ITS sequencing analysis resulted from the small number of selected specimens, mainly from one site in Italy, and the exclusion of all morphologically intermediate specimens (Mello *et al*., 2002b).

The two fruit bodies from the island of Öland, Sweden, shared a single base substitution not present in any other fruit body, but more fruit bodies and other methods are needed to study the Öland population. Sequences of Swedish, Danish and German fruit bodies were found in the same subclade, although mixed with sequences from other parts of Europe, hence making it impossible to point at the origin of the Swedish population. As truffle spores are known to be spread mainly by animal vectors (Trappe *et al*., 2001), the number of central and southern European fruit bodies with ITS sequences identical to those of the Swedish fruit bodies, suggest random parallel evolution of a small number of base pairs, rather than recent long distance distribution from a common origin (Table 3; Fig. 2). The ITS variation may also be a remnant that evolved before colonization of northern Europe by *T. aestivum* (Pamilo and Nei, 1988).

Our morphological analysis clearly illustrate that spore reticulum height is not diagnostic. It displays a continuum, neither suited for the discrimination between species nor suited for varieties or morphotypes (Table 1; Fig. 1). Paolocci and colleagues (2004) argued that the height of

Table 2. Thirty-two GenBank sequences were included in this study.

Number	Taxon	GenBank accession No.
TunA779I	*Tuber uncinatum*	AF516779
TaeA781I	*Tuber aestivum*	AF516781
TaeA782I	*Tuber aestivum*	AF516782
TaeA783I	*Tuber aestivum*	AF516783
TaeA784I	*Tuber aestivum*	AF516784
TaeA785I	*Tuber aestivum*	AF516785
TaeA786I	*Tuber aestivum*	AF516786
TaeA787I	*Tuber aestivum*	AF516787
TaeA788I	*Tuber aestivum*	AF516788
TaeA789I	*Tuber aestivum*	AF516789
TunA790I	*Tuber uncinatum*	AF516790
TunA791I	*Tuber uncinatum*	AF516791
TunA792I	*Tuber uncinatum*	AF516792
TaeA036I	*Tuber aestivum*	AF226036
TunA037I	*Tuber uncinatum*	AY226037
TunA038I	*Tuber uncinatum*	AY226038
TunA039F	*Tuber uncinatum*	AY226039
TunA041I	*Tuber uncinatum*	AY226041
TunA042I	*Tuber uncinatum*	AY226042
TunB509X	*Tuber uncinatum*	AF132509
TunV199I	*Tuber uncinatum*	AJ492199
TunV201I	*Tuber uncinatum*	AJ492201
TunV202I	*Tuber uncinatum*	AJ492202
TunV203I	*Tuber uncinatum*	AJ492203
TunV205I	*Tuber uncinatum*	AJ492205
TunV206I	*Tuber uncinatum*	AJ492206
TunV207I	*Tuber uncinatum*	AJ492207
TunV208I	*Tuber uncinatum*	AJ492208
TunV209I	*Tuber uncinatum*	AJ492209
TunV210I	*Tuber uncinatum*	AJ492210
TaeV215F	*Tuber aestivum*	AJ492215
TaeV216E	*Tuber aestivum*	AJ492216

Numbers in the first column refer to the number used in the phylogenetic analyses (Fig. 2): Tae, *T. aestivum*; Tun, *T. uncinatum*; A, sequence published by Paolocci *et al*. (2004); B, sequence published by Roux *et al*. (1999); V, sequence published by Mello *et al*. (2002b); the three numbers correspond to the last three numbers of the GenBank accession number; the last letter indicate country of origin: France (F), Italy (I), Spain (E) and unknown (X).

Table 3. Fruit bodies with identical ITS sequences.

Sequence in Fig. 2:	**TaeW011I**	**TaeW029I**	**TunW036H**	**TunW038H**	**TunA039F**	**TunA042I**	**TaeW047S**	**TaeW049S**	**TaeW106S**	**TaeW149F**
Identical sequences:	TaeW016I	TunW004F	TunV203I	TaeA781I	TaeW018I	TaeA788I	TaeW002S	TaeW052S	TaeW107S	TaeW157E
	TaeW017I	TunW040F			TaeW019I		TaeW048S	TaeW058S		
	TaeW020I	TunW129F			TunA041I		TaeW051S	TaeW067S		
	TaeW021I	TunW130F			TaeW141F		TaeW053S	TaeW073S		
	TunW022I	TunW135I			TunV201I		TaeW055S	TaeW074S		
	TunW023I	TunW143F					TaeW056S			
	TaeW025I	TunA790I					TaeW057S			
	TunW034I	TunA791I					TaeW060S			
	TunW035H	TunA792I					TaeW064S			
	TunA037I						TaeW065S			
	TunA038I						TaeW066S			
	TunW133I						TaeW068S			
	TunW136I						TaeW070S			
	TunW138I						TaeW072S			
	TaeW140F						TaeW075S			
	TaeW144F						TaeW076S			
	TunW147F						TaeW077S			
	TunW156E						TaeW078S			
	TunV205I						TaeW115S			
	TunA779I						TaeW116S			
	TaeA782I						TaeW118S			
	TaeA783I						TaeW119S			
	TaeA784I						TaeW120S			
	TaeA785I						TaeW122S			
	TaeA786I						TaeW123S			
							TaeW124S			

Specimens in bold face are included in the parsimony analysis (Fig. 2) and are 100% identical in their ITS sequences to the specimens listed below them. Fruit bodies sequenced in this study (W) are listed in Table 1 and sequences obtained from GenBank (A or V) are listed in Table 2. Internal transcribed spacer (ITS) sequences of specimens in column 3 (TaeW029I) and column 8 (TaeW047S) are identical. Tae, *Tuber aestivum*; Tun, *T. uncinatum*. French (F), Italian (I), Hungarian (H) and Spanish (E) specimens are classified as either '*T. aestivum*' or '*T. uncinatum*'. Swedish (S) specimens are recorded only as *T. aestivum*.

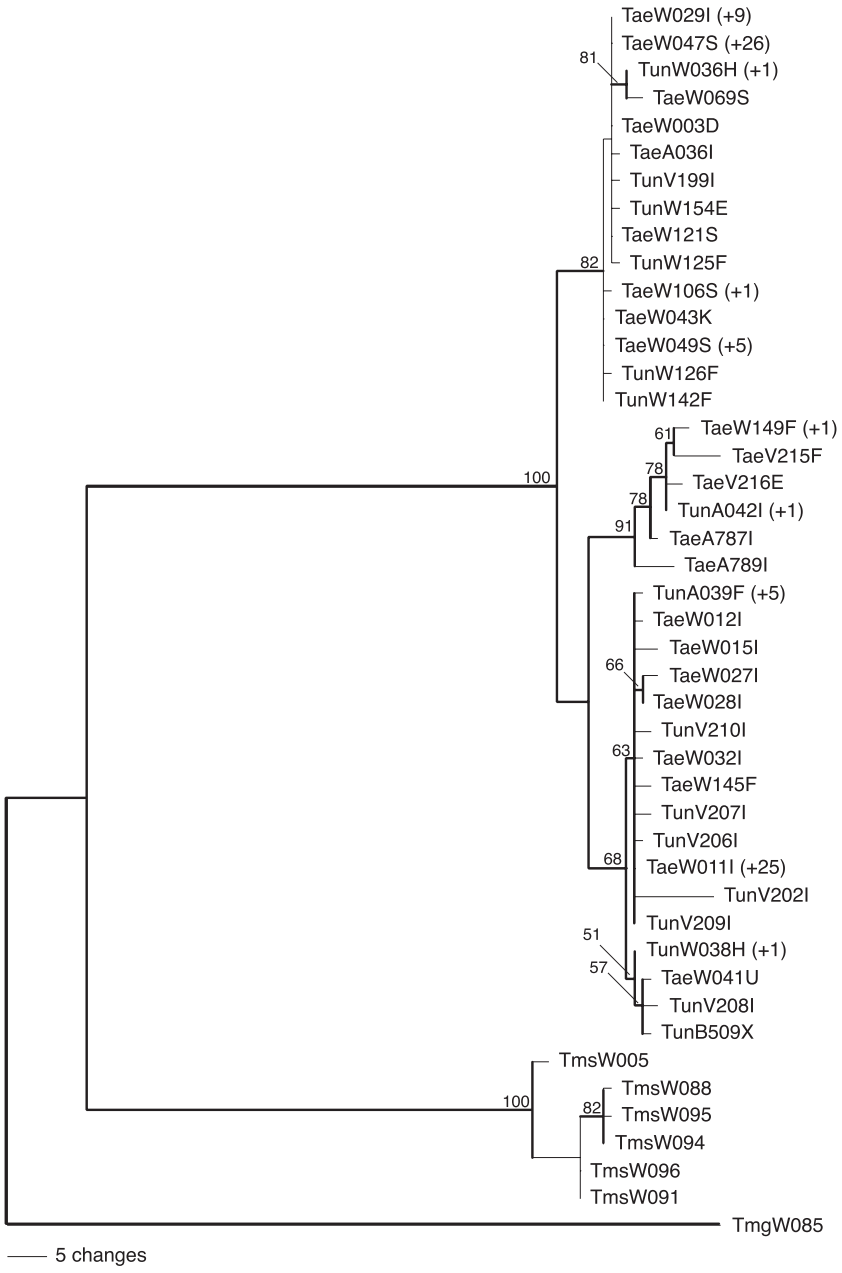

Fig. 2. One of the most parsimonious trees [317 steps; consistency index (CI) = 0.9338; retention index (RI) = 0.9707] found in the analysis of the ITS sequences representing *Tuber aestivum* (Tae), *T. uncinatum* (Tun) and the their sister species *T. mesentericum* (Tms). *Tuber magnatum* (Tmg) is included as an outgroup. The German (D), Spanish (E), French (F), Hungarian (H), Italian (I), Danish (K) and unknown (X) specimens were determined previous to our study to either '*T. aestivum*' or '*T. uncinatum*'. The Swedish (S) and the English (U) specimens are all named *T. aestivum*, as we found it impossible to distinguish between *T. aestivum* and *T. uncinatum* based on our spore reticulum measurements (Table 1). The capital letter in the middle of the specimen number indicate the origin of the sequence: W = sequences obtained in this study (Table 1); A, B and V = GenBank sequences (Table 2). Identical sequences are excluded and listed in Table 3. Numbers in brackets indicate the number of identical sequences (Table 3). *Tuber aestivum* and *T. uncinatum*, sensu Gérard Chevalier (INRA Clermont-Ferrand, France), Sergio Arcioni (Istituto di Genetica Vegetale, Sez. Perugia, CNR, Italy) (Mello *et al.*, 2002b; Paolocci *et al.*, 2004) have identical sequences and the same identical sequence may also origin from several different countries. Bootstrap percentages > 50 are indicated above branches. Branches present in the strict consensus tree are indicated with bold lines. Branch lengths are proportional to number of changes. *Tuber aestivum* and *T. uncinatum* are mixed in a well-supported monophyletic clade (bootstrap = 100).

the spore ornamentation is phenotypic, tending to be higher in more humid, semi-shaded conditions favourable for fruit body development.

Due to its wide habitat range (Chevalier and Frochot, 1997; Wedén *et al.*, 2004a), *T. aestivum* may also grow in conditions less favourable for the development of scent. *Tuber aestivum* in southern Europe, occurring in the same regions as the xerophilic *T. melanosporum* Vittad., often exhibits a relatively weak scent (Chevalier, 1979). Weakly scented *T. aestivum* fruit bodies also occur during dry years or during the summer months. Our preliminary field observations in Sweden suggest that fruit bodies close to the soil surface during hot and dry summers may mature early, but incompletely and without developing the characteristic taste and scent. When hunting for truffles today in Vittadini's old truffle grounds, the truffles found have been identified as '*T. uncinatum*' (Montecchi and Borelli, 1990), which indicate that Vittadini's concept of *T. aestivum* included Chatin's '*T. uncinatum*'.

Our microscopical analysis showed the importance of describing a reproducible method for recording measurements, and sufficient sampling for statistical analysis when describing morphological traits, enabling comparisons between studies. For molecular data it is important to indicate the mode of sequence alignment for accurate reproduction.

Internal transcribed spacer sequences did not discriminate between the two alleged taxa (Fig. 2). The height of the spore reticulum was not diagnostic (Fig. 1), neither were the closely linked characters of colour of the gleba, season of maturity, scent and taste (Table 1; Paolocci *et al*., 2004). The morphology and size of the peridium facets may vary even on the same fruit body. Because no stable characters differentiate the alleged two taxa, there is no basis for suspecting that the two names represent two varieties. It is possible that organoleptic properties are a constant character and a taxon exists with mature spores devoid of scent (Chevalier *et al*., 1994). If that taxon would be genetically distinct from *T. aestivum* it would deserve recognition, but it is not congruent with Chatin's description of *T. uncinatum*. We thus conclude that as traditionally used, '*T. aestivum'* and '*T. uncinatum'* are the same species. Therefore, the distinction between *T. aestivum* and *T. uncinatum* on the French truffle market is really a matter of product quality and not a difference between species. It is important that truffle consumers and traders know the correct definitions to avoid misunderstandings and fraud. The marketing of immature (scentless) truffles should be banned as it would otherwise encourage digging to find immature truffles. Mature truffles are found by their pronounced scent, leading the truffle hunter to the exact location of the truffle by, e.g. a trained truffle dog. Hence, digging 'blindly' for immature truffles could damage natural truffières and adjacent flora.

Because *T. aestivum* is an older, valid name than is *T. uncinatum* for the same species, the former name has priority according to the International Code of Botanical Nomenclature (Greuter, 2000). However, either the names *Tuber gulosorum* Wiggers:Fr. or *T. albidum* Fr. might have priority over *T. aestivum* Vittad. (Trappe, 2001). No types have been designated for *T. gulosorum* or *T. albidum*, however, and their identity is uncertain. Neither name has been used for over a century, whereas the name *T. aestivum* has been in common, international use in both science and commerce since Vittadini coined it in 1831. A proposal to the International Botanical Congress to conserve *T. aestivum* over *T. gulosorum* and *T. albidum* would be in order.

Experimental procedures

Selection of fruit body material

The 117 dried '*T. aestivum'* and '*T. uncinatum'* fruit bodies from eight European countries analysed in this study (Table 1) are deposited at the Uppsala herbarium (UPS). Of these, 49 originated from Sweden (representing 28 different sites on the island of Gotland and one site on the island of Öland), 23 from France (representing 20 sites in 13 counties), 30 from Italy (representing 10 sites from six regions), six from Spain (representing six sites from four regions), five from Hungary (representing five sites in four counties), two from Denmark (from two different islands), one from England and one from Germany. For the Swedish material, two fruit bodies from each site were selected, choosing the two specimens collected as early and as late as possible during the season (Table 1). This was done in order to investigate seasonal differences in mature fruit bodies, because *T. aestivum* is said to mature earlier in the season. French, Spanish and Hungarian fruit bodies were kindly supplied and identified as either '*T. aestivum'* or '*T. uncinatum'* by Dr Gérard Chevalier, INRA Clermont-Ferrand, France. Italian fruit bodies were kindly supplied and determined to either '*T. aestivum'* or '*T. uncinatum'* by Dr Sergio Arcioni, Istituto di Genetica Vegetale Sez. Perugia, CNR, Italy and Dr Gérard Chevalier. Danish, English and German fruit body samples were kindly supplied by Dr Christian Lange, The Copenhagen Botanical Museum, Dr Andy Taylor, Swedish University of Agricultural Sciences, and Dr Michael Pfaff, University of Lund respectively.

Morphological analysis

The only quantifiable morphological character suggested to separate *T. aestivum* from *T. uncinatum* is height of the spore reticulum (Chevalier *et al*., 1979; Chevalier and Frochot, 1997; Riousset *et al*., 2001; Mello *et al*., 2002b; Paolocci *et al*., 2004). The spore reticulum can be measured in different ways, but this is rarely described. Chevalier and colleagues (1979) described a method where the length of the spore is measured including and excluding the spore reticulum. The spore reticulum height is then calculated by dividing the difference between these two measurements by two. It is not always possible to measure the spore reticulum at the two ends of the oval spore due to the orientation of the spore reticulum or the spore itself in the plane of the microscope slide, i.e. to avoid optical measurement errors. The literature does not indicate how many measurements are needed per fruit body for species determination. Reported spore measurements might vary due to maturity, the fruit body being fresh or dried, microscopic slide solvent (e.g. water, ethanol, lactic acid), number of spores per ascus, and number of spores measured. We used this method: dried fruit body material (gleba) was transferred to a microscope slide and rehydrated by adding a drop of 70% ethanol and after 5 min a drop of distilled water. After 30 min the rehydrated gleba was squashed with the side of a razor blade to make the preparation as thin as possible to enable correct measurement of the reticuli. Finally, a microscope glass coverslip was applied tightly over the preparation. The spores were viewed in a Leitz Laborlux K microscope with a ×100 objective. A *camera lucida* (drawing tube) was used, giving the on-paper magnification of ×2000. By this means the spore reticulum height could be measured with an ordinary ruler, where 1 mm was equivalent to 0.5 μm in the slide preparation. To avoid optical errors, the focus was readjusted for each measurement to ensure that the spore reticulum being measured was in the same plane as the microscope slide.

The following four fruit bodies were first examined: TaeW026I (three tissue samples), TaeW032I (two samples), TaeW065S (three samples) and TaeW115S (four samples). Each sample was taken from different parts of the gleba. Two to five spore reticulum heights were measured for each spore

in a four-spored ascus. Such spores were measured until a stable mean for a tissue sample was achieved. The means from different tissue samples were compared. This resulted in up to 233 spore reticulum height measurements from 78 measured spores in 20 asci in each tissue sample. The focus was adjusted so that the reticuli could be measured in the plane of the microscope slide, thereby having a readily visible base and top and not being subject to any three-dimensional optic length errors or be mistaken with the length of the alveolar wall. The reticulum was measured in a 90° angle from the outer spore wall. Measurements were taken adjacent to an alveolar wall node (where three alveolar walls meet), as the node structure is sturdier and less prone to fold over. Only fully mature spores (i.e. not colourless spores) in intact asci were measured.

The extensive measurements of the four fruit bodies above showed a variation of the height of the spore reticulum between different tissue samples within one fruit body, between different asci in the same sample, between spores in the same ascus and within the same spore. The following compromise was chosen. One tissue sample was taken from the gleba of each of 117 fruit bodies. The spore reticulum was measured once on one spore in an ascus containing four spores. This was repeated in 10 asci, resulting in a total of 10 measurements of the reticulum height for each fruit body (Table 1).

Statistical analysis of morphological data

Mean values, standard deviation with an n-1 weighting and range were calculated for each fruit body (Table 1). Computing was performed in S-PLUS 2000 (Mathsoft, 1999). Each taxon was represented by 25 fruit body specimens. Only fruit bodies with 10 measurements were included.

DNA extraction, amplification and sequencing

Total DNA was extracted from dried fruit body material (gleba) of *T. aestivum*, *T. uncinatum*, *T. mesentericum* and *T. magnatum* using the DNeasy Plant Mini Kit (Qiagen, Basel) according to the manufacturer's manual. DNA was precipitated in 30 μl of supplied buffer. Polymerase chain reaction (PCR) amplification was conducted with the primers ITS1f (Gardes and Bruns, 1993) and LR1new (AGGAAAAGAAACC AAC) to specifically amplify the fungal ITS1, 5.8S and ITS2 regions of the ribosomal nuclear DNA. Each 45 μl of PCR reaction contained 0.8 mM dNTP, 5.5% glycerol, 1.5 mM $MgCl_2$, 0.5 mM of each of the primers ITS1f and LR1new, 0.2 μ TAQ polymerase (ABGene, Epsom) and 22.5 μl of DNA diluted 1:10 in distilled water. After an initial denaturation at 95°C for 4 min, the PCR (Eppendorf Mastercycler personal, Hamburg) was run for 35 cycles (1 min 94°C, 1 min 54°C, 45 s 72°C with a 0.4 s $cycle^{-1}$ extension at 72°C) followed by a final synthesis at 72° for 5 min. Some samples which did not yield visible PCR products, or only very faint PCR products after electrophoresis, were successfully amplified in a subsequent nested PCR, using the primers ITS5 (Vilgalys, 2001) and ITS4 (White *et al.*, 1990) and parameters as described above. Polymerase chain reaction products were diluted 1:200 before being used in the nested PCR. Amplified DNA was purified with Multiscreen PCR (Millipore, Billerica) and eluted in 35 μl of distilled water. Sequencing was carried out with the primers ITS5 and ITS4. After an initial denaturation of 1 min at 96°C, the sequencing reaction ran for 30 cycles (10 s at 96°C, 5 s at 50°C, 4 min at 60°C) using the ABI PRISM BigDye v. 3.1 labelling method (Perkin-Elmer). Contigs were assembled in Bioedit (http://www.mbio.ncsu.edu/RNaseP/home.html).

Multiple sequence alignments, gap coding and parsimony analysis

Another 32 GenBank sequences from fruit bodies morphologically determined as *'T. aestivum'* or *'T. uncinatum'* were included in the study (Table 2). Multiple sequences were aligned manually in Se-Al (Rambaut, 1996) placing indels in positions that minimized the number of substitutions within an aligned region, under the premises that indels and substitutions had equal weight (Oxelman *et al.*, 1997; Andreasen *et al.*, 1999; Långström and Oxelman, 2003). The aligned data matrix is available from the corresponding author on request. Gap coding was performed in the program GAPCODER (J. Healy and N.D. Young, 2004; http://www.tufts.edu/vet/richlab/young/GapCoder/), implementing the simple gap coding method described by Simmons and Ochoterena (2000). Maximum parsimony analyses of the ITS region matrices were performed in PAUP* version 4.0b10 for Macintosh™ (Swofford, 2002), with a heuristic search strategy having four random addition sequence replicates and TBR branch swapping. The analysis was run with the MULTREES option off, because the high similarity between the sequences otherwise generated so many trees that it was impossible to compute, the only difference between the trees being rearrangements within the end nodes. Maximum parsimony bootstrap analyses for the identification of well-supported monophyletic groups was performed with full heuristics in PAUP* with random addition of 1000 replicates, TBR branch swapping, MULTREES option off and random addition of sequences with four replicates. A tree was constructed both to show whether or not morphologically determined *'T. aestivum'* and *'T. uncinatum'* specimens from central and southern Europe would separate in distinct clades and to study where the Swedish specimens would be distributed among the foreign specimens.

Acknowledgements

Gérard Chevalier and Sergio Arcioni are greatly acknowledged for providing determined fruit body material for this study. Ovidiu Constantinescu, Gérard Chevalier, Svengunnar Ryman, Stefan Ekman, Sunniva Aagaard and Mikael Thollesson have contributed their expertise to the spore measurement study, as have Nahid Heidari, Elisabeth Långström, Magnus Popp, Bengt Oxelman and Cajsa Anderson with molecular analyses. We also thank Bertil Widbom at Gotland University for valuable support. This study was financed by the Municipality of Gotland, the European Agriculture Guidance and Guarantee Fund under the Objective 5b Gotland Programme, the European Regional Development Fund under the Objective 2 Islands Programme, the Gotland

County Administration, and further supported by Konsul Faxes Fund, the KK Foundation (Kunskaps-och kompetensutvecklingsstiftelsen), Carl the XVI Gustafs 50th Anniversary Fund and Carl Tryggers Foundation.

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