Chapter 5
Taxonomy, Biology and Ecology of *Tuber macrosporum* Vittad. and *Tuber mesentericum* Vittad.

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5.1 Introduction

Nowadays, some truffle species such as *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad., *Tuber borchii* Vittad. and *Tuber brumale* Vittad. have a flourishing market and are routinely and successfully cultivated. Plantations are established with mycorrhized seedlings produced by commercial nurseries giving important extra income to farmers, especially in marginal lands or disadvantaged areas (Chevalier 1998; Olivier 2000; Hall and Yun 2002; Bencivenga and Baciarelli Falini 2012). In addition to those previously mentioned species, for some other less famous truffles, locally collected by truffle hunters, national and international markets are struggling but recently starting to develop.

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In particular, the black smooth truffle *Tuber macrosporum* Vittad. (also known as "garlic truffle" for its aromatic resemblance of garlic) has an aroma that is vaguely similar to the esteemed white truffle *Tuber magnatum* Pico (Vittadini 1831; Hollós 1911; Ceruti 1968; Montecchi and Lazzari 1993; Iotti et al. 2002; Riousset et al. 2012).

Despite its attractive aromatic traits, *T. macrosporum* is only accidentally collected in Italy by truffle hunters who are trying to find *T. magnatum*, growing in the same environment; hence, it has no commercial interest and does not enjoy privileged hunting. Small ascoma size, insecure, strongly weather-dependent yields in natural habitats, the common practice for traders to mix it with *T. aestivum* or immature *T. melanosporum*, are all the reasons of its limited reputation. Notwithstanding the above facts, *T. macrosporum* is certainly an attractive species and can merit more attention because of its enticing organoleptic features and its wide distribution across Europe. Moreover, its successful cultivation on experimental orchards (Vezzola 2005, 2010) has resulted in the production of seedlings inoculated with *T. macrosporum* spores by commercial nurseries with the aim of expanding cultivation.

Another species of a certain market which deserves specific attention is *Tuber mesentericum* Vittad., also called "black truffle of Bagnoli Irpino", from the name of a city in the Province of Avellino (Italy), where this truffle has a long history and tradition of harvest and gastronomic use (Garofoli 1906). It is also famous in northeast of France where it is more expensive than *T. aestivum* [often about 450 € per kg in detail markets, e.g. Pulnoy township (Claude Murat, pers comm)]. This species often emits a strong and very distinct phenolic-like aroma that makes it not so appreciated outside the traditional area (Vittadini 1831; Granetti et al. 2005; Riousset et al. 2012). Opinions on the gastronomic and consequently economic value of this truffle are anyway controversial, subjective and often linked to local traditions or customs. Immature fruiting bodies of *T. mesentericum* can be easily confused with the morphologically very similar *T. aestivum* and *T. aestivum* var. *uncinatum* (Chatin) I. R. Hall, P. K. Buchanan, Y. Wang and Cole, which now are considered conspecific (Paolocci et al. 2004; Wedén et al. 2005; Molinier et al. 2013) even if the identity of "uncinatum" survives as a commercial type of *T. aestivum* mainly in Italy and in France (see Chap. 3).

In this chapter, morphological characteristics of *T. macrosporum* and *T. mesentericum* fruiting bodies and ectomycorrhizas (ECMs) are described; taxonomic controversies about macro- and microscopic traits necessary for specific identification are disclosed; phylogenetic findings are highlighted in order to clarify the position of *T. macrosporum* and *T. mesentericum* in the *Tuber* phylogenetic tree, and a summary of their ecological requirements (including soil, climate and host trees) is finally provided.

5.2   Methodology

From fresh/dried *T. macrosporum* ascomata collected in Italy but also in some locations in Europe, genomic DNA was directly amplified with ITS1 and ITS4 universal primers (White et al. 1990) for fungal barcoding and then sequenced on both strands following the methodology reported by Bonito (2009). Forward and reverse sequences were then edited into contigs in BioEdit (Hall 1999). Corrected sequences were submitted to GenBank (Benson et al. 2013) with the following accession numbers: KP738345 to KP738396. Additional available ITS sequences of *T. macrosporum* were also downloaded from GenBank (www.ncbi.nlm.nih.gov) to improve the phylogenetic resolution of major clades. For *T. mesentericum*, only public sequences were used for the molecular analyses. Sequence accessions and details are reported in the phylogenetic trees (Figs. 5.2 and 5.5).

Before building phylogenetic trees, all the obtained sequences labelled as *Tuber macrosporum* and *Tuber mesentericum* downloaded from GenBank were aligned in MEGA6 to detect mislabelled sequences or misidentifications. Sequences that did not align correctly were then compared to others in GenBank using the BLAST algorithm to verify their identity (Altschul et al. 1990).

Phylogenetic tree reconstructions were performed using the maximum likelihood method (Tamura et al. 2011). The trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree, and bootstrap values (999 replicates) are shown next to the branches (Felsenstein 1985). Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

5.3   Characteristics of *Tuber macrosporum*

5.3.1   *Morphology of* Tuber macrosporum *Ascomata*

Ascoma maturation usually ranges from August to December, in the same period of *T. magnatum*, with the peak of mature specimens during the autumn. Typical ascomata of *T. macrosporum* have generally an irregular shape, lobed, but also regular and/or subglobose, with a diameter of 2–5 cm, exceptionally bigger (Fig. 5.1a). The blackish peridium is irregularly stained by reddish-brown, very short and flat warts of variable shape and size. Gleba varies from grey brown to brown lilac and purple brown when mature, with thick, branching and winding white veins. The asci of $90 - 120 \times 60 - 80 \, \mu m$ size contain 1–3(4), generally three yellowish-brown spores. The ellipsoid spores are considered definitely the biggest between the main truffle species. These spores are $40 - 70(-80) \times 30 - 55(-60) \, \mu m$ (Fig. 5.1b, c), covered with reticulate-alveolate, polygonal, 2–4 μm high, dense, closed and small meshes.

Fig. 5.1 *Tuber macrosporum* characteristics: (**a**) mature ascomata; (**b**) gleba with spores; (**c**) a two spores ascus (25 μm); (**d**) natural productive site with *Q. cerris*, *Q. pubescens* and *O. carpinifolia*; (**e** and **f**) ECMs with cystidia (0.4 mm); (**g**) ramification of cystidia (25 μm); (**h**) outer mantle layer (25 μm)

5.3.2 Tuber macrosporum *Ectomycorrhizal Synthesis and Morphology*

The first mycorrhizal synthesis of *T. macrosporum* with hornbeam seedlings was published by Giovannetti and Fontana (1980–1981) with the description of the ECMs and their distinctive traits. Some subsequent works expanded the topics: oaks and hazel seedlings were inoculated by *T. macrosporum* spore slurry and the obtained ECMs were photographed and described (Zambonelli et al. 1993; Granetti 1995; Vezzola 2005; Agerer and Rambold 2004–2008). Nevertheless, those descriptions are controversial and do not really focus on simple and valuable morphological traits that are fundamental for a correct species identification. Moreover, no molecular confirmation for ECMs belonging to *T. macrosporum* was reported in literature before 2012 when Benucci and colleagues (2012) described morphologically *T. macrosporum* ECMs on *Quercus robur* L., *Quercus cerris* L. and *Corylus avellana* L. and identified its DNA through the use of species-specific primers (Benucci et al. 2011). The same authors also described and characterized the ECM communities of cultivated and natural *T. macrosporum* sites (Benucci et al. 2014).

Tuber macrosporum ECMs on *Q. robur* and *C. avellana* are simple or ramified in a monopodial-pinnate or monopodial-pyramidal pattern (Fig. 5.1e, f). Simple ECM tips are almost straight, cylindrical or club shaped with rounded ends. The colour of the ECMs varies considerably: the youngest are light yellow with pale grey shades and cystidia are sinuous and septate, with very thick walls and branched at various angles (frequently with sharp angles). The colour of the cystidia varies from light yellow when young (sometimes with a greyish shade) to ochre at maturity. They are ramified (Fig. 5.1g) and tend to merge, creating anastomoses that form an abundant web of mycelium around the ECM that is typically orange in colour (Fig. 5.1e, f). Formation of needle-shaped cystidia reported by Granetti et al. (2005) is never found in any of the *T. macrosporum* ECMs examined by Benucci et al. (2012).

The mantle is pseudoparenchymatous and composed of four to six cell layers. The Hartig net penetrates into the first two to three cell layers of the root parenchyma (Benucci et al. 2012). The outer mantle surface is either covered densely by mycelium (cottony) or it is smooth to loosely grainy. In both cases, it is composed of angular (type L according to Agerer and Rambold 2004–2008) and epidermoid (type M) cells that form an uneven, regular puzzle-like pattern (Fig. 5.1h). Benucci et al. (2012) showed that ECM mantle might differ among the apex, middle part and base of the ECM with the middle part being the most variable.

5.3.3 Tuber macrosporum *Taxonomy and Phylogeny*

According to Index Fungorum, the global nomenclator of fungal taxonomic names (www.indexfungorum.org), the correct name of this species is *Tuber macrosporum* Vittad.

Recent phylogenetic studies on the *Tuber* genus, based on the ITS (internal transcribed spacer) region and LSU (large subunit) of the nuclear rDNA (ribosomal DNA), show that the Macrosporum clade is one of the ancestral lineages and includes two species: *T. macrosporum* and *Tuber canaliculatum* Gilkey (Jeandroz et al. 2008; Bonito et al. 2013). In addition, molecular evidence of truffles belonging to the Macrosporum group has been reported also for Japan (Kinoshita et al. 2011).

It is worth noting that some sequences downloaded from GenBank have been misidentified or mislabelled and do not belong to *T. macrosporum*. In particular, FJ809838, FJ809839, JN392325 and HE601929 show the highest similarity with *T. canaliculatum*; JQ288921 shows the highest similarity with *Tuber malenconii* Donadini, Riousset, G. Riousset and G. Chev; and HE602584 shows the highest similarity with *Tuber pseudoexcavatum* Y. Wang, G. Moreno, Riousset, Manjón and G. Riousset.

The maximum likelihood phylogenetic reconstruction based on the ITS region shows *T. macrosporum* position in the Macrosporum clade that includes the North American species *T. canaliculatum* (Fig. 5.2) (Bonito et al. 2010). Two bootstrap-supported distinct clades are present in the tree: most of the Italian sequences cluster in the clade II, while clade I comprises many samples from Central and Eastern Europe. Interesting to note that in the clade I two sequences from ECM tips (JX474822 and JX474809) clustered together and with sequences obtained from fruiting bodies (e.g. KP738346) which were collected in Sigillo (Italy) and previously analysed by Benucci et al. (2014) in a fungal community analysis study. Even if with high divergence, the sample AB553344 from a truffle fruiting body collected in Japan showed to be close to *T. canaliculatum*, suggesting the possible presence of a new Asiatic species belonging to the Macrosporum clade.

5.3.4   Tuber macrosporum *Geographic Distribution and Ecological Demand*

The truffle *T. macrosporum* has a wide distribution in Europe, being considered common in Serbia, Hungary and Romania, less frequent in Italy and rare in France and Great Britain, but also occurs in Switzerland, Germany, Ukraine, Croatia and Slovenia and has been recently reported from Slovakia, Poland and Turkey (Ceruti et al. 2003; Miko et al. 2006; Hall et al. 2007; Marjanović et al. 2010; Piltaver and Ratoŝa 2010; Benucci et al. 2012; Stobbe et al. 2012; Hilszczańska et al. 2013). Mature ascomata can be found as early as June (Vezzola 2010), but more often from September to December. *Tuber macrosporum* is generally collected from plain sites or from foothills to low mountains, often found on north-oriented slopes, lowlands or floodplains of watercourses (Vittadini 1831; Milenkovic and Marjanović 2001). Although annual rainfall in *T. macrosporum* sites was reported variable
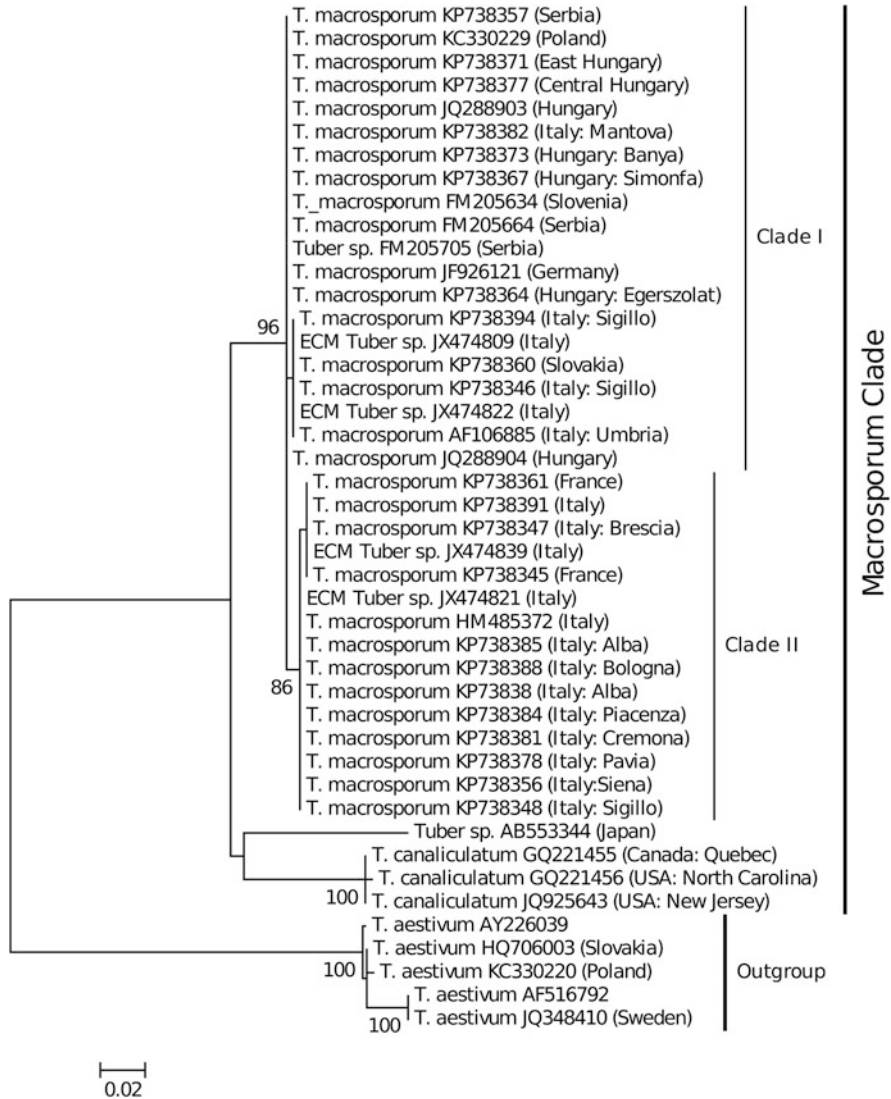
Fig. 5.2 *Tuber macrosporum* maximum likelihood phylogenetic tree based on the Jukes–Cantor model (Jukes and Cantor 1969): bootstrap values >65 % are shown next to branching nodes. A discrete gamma distribution [+*I*] was used to model evolutionary rate differences among sites. The analysis involved 44 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 337 positions in the final dataset. Sequences produced in this study have accessions starting with KP
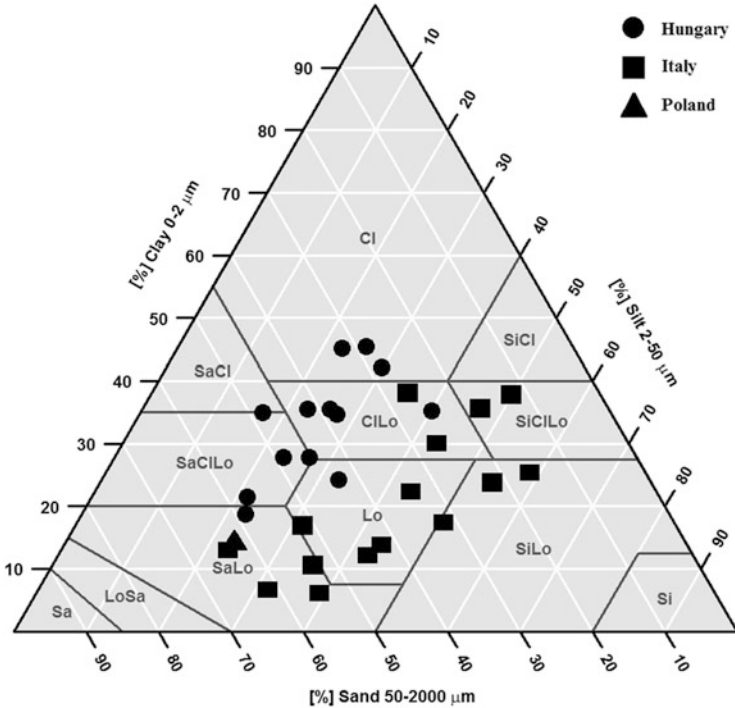
Fig. 5.3 Soil textures of different *T. macrosporum* habitats (Benucci 2011 modified; Gógán Csorbai 2011; Hilszczańska et al. 2014)

(520–850 mm), water dependence of the species is undoubted and soil moisture is very often complemented by arriving waters (subsurface water, flooding, etc.).

Soil genetic types include chernozems, luvisols, and planosols but also rendzic leptosols (Gógán Csorbai 2011; Hilszczańska et al. 2014). The species regularly shares habitats with *T. magnatum*, resulting in similar characteristics of lime-rich, neutral or slightly alkaline soil with both good aeration and humid environment. However, recent findings revealed that soil compaction in *T. macrosporum*-inhabited soils is very common. Compacted layers are typical in 30–60 cm depth, but in some cases, they occur close to the surface (5–10 cm). Due to compacted layers and the presence of water, gleys and ferric precipitations are frequent (Gógán Csorbai 2011). Soil granulometry of samples coming from different geographical origins represents slight variability in soil textures but without extreme patterns (Fig. 5.3). The most common soil types are clay loam, loam and sandy loam.

Some findings cite *T. macrosporum* from neutral or alkaline soils of pH around 7.5 with various lime contents (Djurdjevic et al. 1999; Miko et al. 2006; Hilszczańska et al. 2014). Researches focusing on the ecological demands of *T. macrosporum* affirm its preference to the above-mentioned characteristics; however, lime-free, slightly acidic environment cannot be considered as limiting factor for the species (Gógán Csorbai et al. 2010). Results also reveal high organic

Table 5.1 Main chemical characteristics of soil samples from 88 *T. macrosporum* habitats (Benucci et al. 2014, modified; Gógán Csorbai et al. 2010; Gógán Csorbai 2011)

	Range	Median	Average	SD
pH H_2O	5.7–8.2	6.87	6.89	0.59
pH KCl	4.6–7.3	6.54	6.48	0.59
$CaCO_3$ (%)	0–59	1.95	6.55	9.74
Organic matter (%)	3.2–13.4	7.32	6.95	2.16
AL-P (ppm)	5–512	49	103.26	123.59
AL-K (ppm)	85–1790	330.5	441.96	295.59

Range, median, average and standard deviation (SD) value are reported

matter and variable content of phosphorus and potassium in natural habitats (Table 5.1).

The developing environment of *T. macrosporum* is also characterized of different symbiotic partners involved in the life cycle of this truffle. Mixed deciduous, closed-canopy forests are considered as very suitable habitats for *T. macrosporum*. The most common host trees of the species are oaks (*Quercus pubescens* Willd., *Q. robur*, *Quercus petraea* Liebl., *Q. cerris*), hazelnut (*C. avellana*), hornbeams (*Ostrya carpinifolia* Scop., *Carpinus betulus* L.), willows (*Salix viminalis* L., *Salix alba* L., *Salix vitellina* L., *Salix caprea* L.), lindens (*Tilia cordata* Miller, *Tilia platyphyllos* Scop.), beeches (*Fagus sylvatica* L.) and poplars (*Populus nigra* L., *Populus tremula* L., *Populus alba* L.) (Ceruti et al. 2003; Miko et al. 2006; Marjanović et al. 2010; Gógán Csorbai 2011).

5.4 Characteristics of *Tuber mesentericum*

5.4.1 Morphology of **Tuber mesentericum** *Ascomata*

The morphology of *T. mesentericum* ascomata is very similar to that of *T. aestivum*; the overlapping features of the two species make some authors designate them as "*Tuber aestivum-mesentericum* complex" (Pacioni and Pomponi 1991). Ascomata of *T. mesentericum* are rounded or subglobose, can reach the size of 10 cm in diameter and are covered with brown-black pyramidal warts (Fig. 5.4a, on the right). Transverse streaks (Fig. 5.4e) are present in the peridium warts in *T. aestivum* and in *T. mesentericum* as well, even if less evident and frequent in the latter. Even if basal depression or cavity has been considered a distinguishing feature for differentiating ascomata of *T. mesentericum* from ones of *T. aestivum* (Montecchi and Sarasini 2000; Ceruti et al. 2003), in our experience, this characteristic is not a valid taxonomic trait, as it is possible to find ascomata of *T. aestivum* with the basal cavity as well as *T. mesentericum* ascomata without it (Fig. 5.4a). Gleba colour is typically dark grey brown, often with violet shades (Fig. 5.4a, on the right) with numerous white intensively winding veins at full maturity, in contrast to the gleba
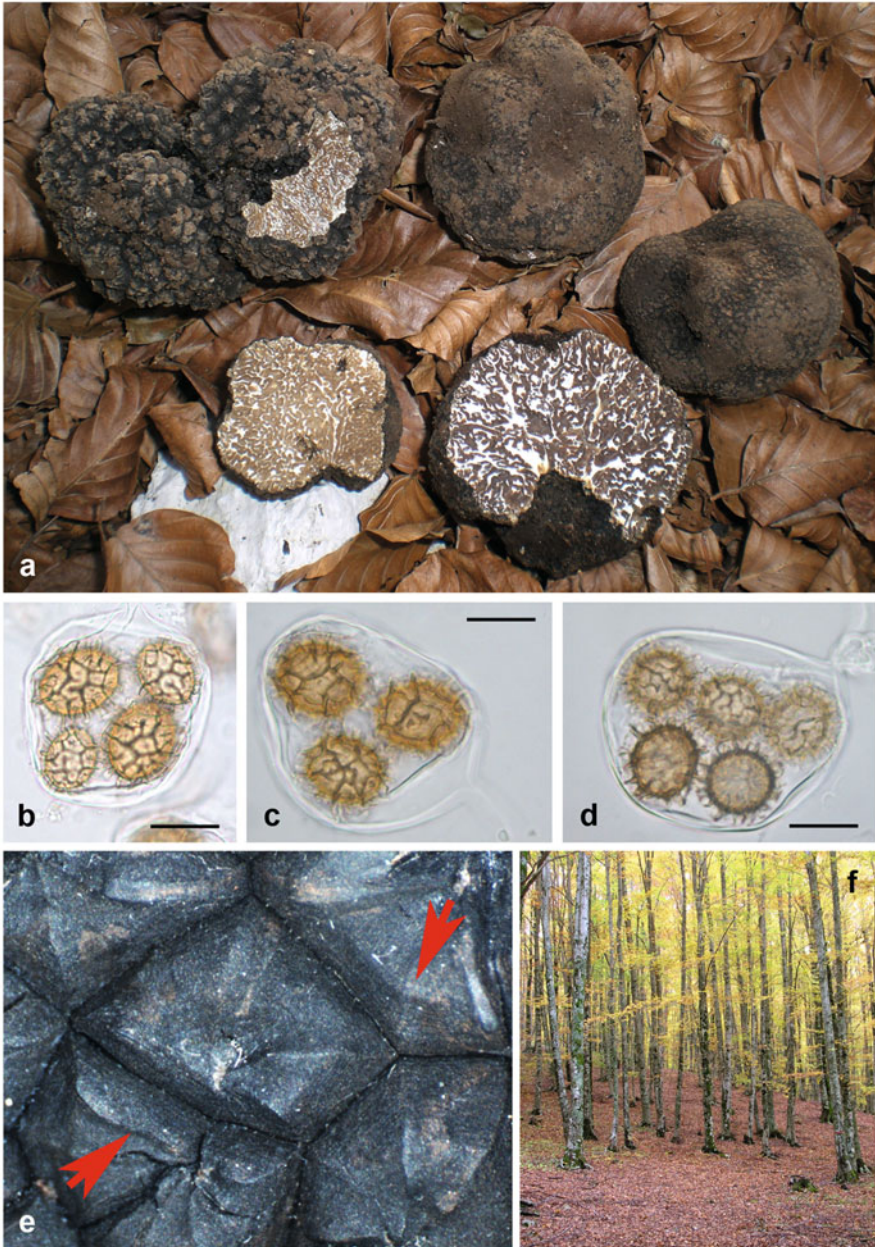
Fig. 5.4 *Tuber mesentericum* characteristics: (**a**) mature *T. aestivum* var. *uncinatum* (on the *left*) and *T. mesentericum* (on the *right*) ascomata; (**b–d**) different *T. mesentericum* ascospores (25 μm); (**e**) transverse streaks in *T. mesentericum* peridium warts (*red arrows*); (**f**) *T. mesentericum* natural productive site with *F. sylvatica*

of *T. aestivum* (Fig. 5.4a, on the left) that ranges from yellowish or light brown to ochre, but never dark brown with violet shades.

Globose or subglobose and pedunculate asci contain (1)2–4(6), yellowish-brown, ellipsoid spores (Figs. 5.4b–f) of $28 - 33 \times 20 - 23$ μm size according to Montecchi and Sarasini (2000) and Ceruti et al. (2003). It is worth noting that in our experience spore shape can vary from ellipsoid to perfectly globose (Figs. 5.4b–d), and this variability (more or less important) can be detected even in the same ascoma. Spore surface is reticulate-alveolate with irregular polygonal meshes of 3–5 μm height. Meshes are typically incomplete and often with a crest in the inside, but it can also happen, even in the same ascoma, to find complete meshes similar to those of *T. aestivum* spores (Figs. 5.4b–d).

Tuber mesentericum scent is generally strong, with frequent unpleasant note reminding of phenol, tar and/or iodine. This note, highly variable, can be immediately perceivable in the specimens when freshly harvested or can reveal itself only some days after, especially if the truffles are conserved at low temperatures in the fridge. It can be absent in mature specimens, as when freshly harvested, as some days after; on the contrary, sometimes it can be present even in immature ascomata. It has been showed for *T. aestivum* that the variability in the truffle aroma caused by volatile organic compounds (VOCs) can have a genotype basis (Splivallo et al. 2012; see Chap 3). Besides it can be also influenced by soil and ascoma-associated microbes (Buzzini et al. 2005; Splivallo et al. 2014). Anyway, the phenolic unpleasant note of *T. mesentericum* can be present or absent even in the same area of harvesting, and this variation has not been studied in details yet. In our experience, it is possible to find freshly harvested truffles with *T. mesentericum* morphological characteristics, but with a pleasant aroma, complex and deep, similar to those of *T. aestivum* or even *T. melanosporum*. A collection of mature *T. mesentericum* specimens (in the order of some tens of kilos) absolutely free of phenolic aromatic component were observed in Mediterranean habitats of Salento (Southern Italy) and in the province of Rome, under *Q. ilex* and in flat areas with reforestations of *Quercus* spp., in late spring and early summer. These particular *T. mesentericum* ascoma collections are at present under study.

5.4.2 Tuber mesentericum *Ectomycorrhizal Synthesis and Morphology*

The ECMs of *T. mesentericum* have been mentioned as early as 1988 (Giraud 1988), collected in a natural truffle field. Detailed description of the ECMs on nursery plants revealed monopodial-pyramidal ramification type on *C. avellana* (Rauscher et al. 1995), dichotomous on *Pinus pinea* L. (Zambonelli et al. 1995) and monopodial-pinnate and monopodial-pyramidal on *Q. pubescens* seedlings (Zambonelli et al. 1993). Ectomycorrhizas are reported as densely wolly, their color can vary from ochre, to yellowish brown and to red. The surface of the mantle

layer is plectenchymatous and pseudoparenchymatous with angular (type L, according to Agerer and Rambold 2004–2008) mantle cells of $3 - 11 \times 6 - 20$ μm size. Cystidia are awl shaped, bristle-like (type A) with proximal ramification, although no ramification was also reported (Zambonelli et al. 1995). Brownish cystidia were measured of 1.9–5 μm of diameter and 130–1520 μm long. Despite the morphological descriptions of the ECM, no molecular evidence is present in literature regarding isolation of DNA from *T. mesentericum* ECMs so far.

5.4.3   Tuber mesentericum *Taxonomy and Phylogeny*

In Index Fungorum, the names *Tuber mesentericum* Vittad., with its variety (var. *mesentericum* Vittad.), and *Tuber mesentericum* var. *tesserulatum* Zobel are reported. *Tuber bituminatum* Berk. and Broome is considered a synonym of *T. mesentericum* by several authors (Montecchi and Sarasini 2000; Granetti et al. 2005), but in Index Fungorum, this name refers to a holotype of *T. aestivum* (Kew Royal Botanic Gardens—Accession n. 30594). The species *Tuber bellonae* Quél. (synonym of *Tuber bituminatum* var. *sphaerosporum* Ferry de la Bellone) is reported to be close to *T. mesentericum* with some distinctive morphological features, in particular the globose spores and the higher spore volume (Pacioni and Fantini 1997). In our opinion, also *T. bellonae*, from the morphological point of view, can be included into the variability of *T. mesentericum*, even if some authors, without any molecular evidence, continue to consider it a separate species (Ławrynowicz et al. 2008). A study in progress will add molecular to morphological data to investigate the intraspecific diversity of *T. mesentericum*.

   According to Bonito and colleagues (2013), *T. mesentericum* belongs to the Aestivum clade of the *Tuber* genus phylogeny, together with *T. aestivum*, *T. panniferum* Tul. and C. Tul. and *T. magnatum*. The maximum likelihood phylogenetic tree based on *T. mesentericum* ITS sequences downloaded from GenBank shows the presence of three distinct clades (Fig. 5.5). The clade I includes mainly sequences coming from Central-North Europe, comprising sequences from Sweden, and Gotland Island (Wedén et al. 2005) which likely went through a reproductive isolation. In the clades II and III, only sequences from Italian *T. mesentericum* ascomata are present, with the exception of two sequences from Spain (FM205536 and FM205535) and one from France (JQ348414). The phylogenetic reconstruction includes also *T. aestivum* sequences, which are close in the basal lineage with the clades of *T. mesentericum* and together are separated from the out-group (Fig. 5.5). The data reported here are consistent with the finding of Sica et al. (2007) showing a strong genetic structuring of the samples in different geographical areas, with the Italian clade very well distinguishable. In this instance, it may therefore be assumed that *T. mesentericum* is a species complex, but wider sampling campaign and higher genetic support (e.g. multiple gene phylogenies, population studies) are needed to confirm this hypothesis.
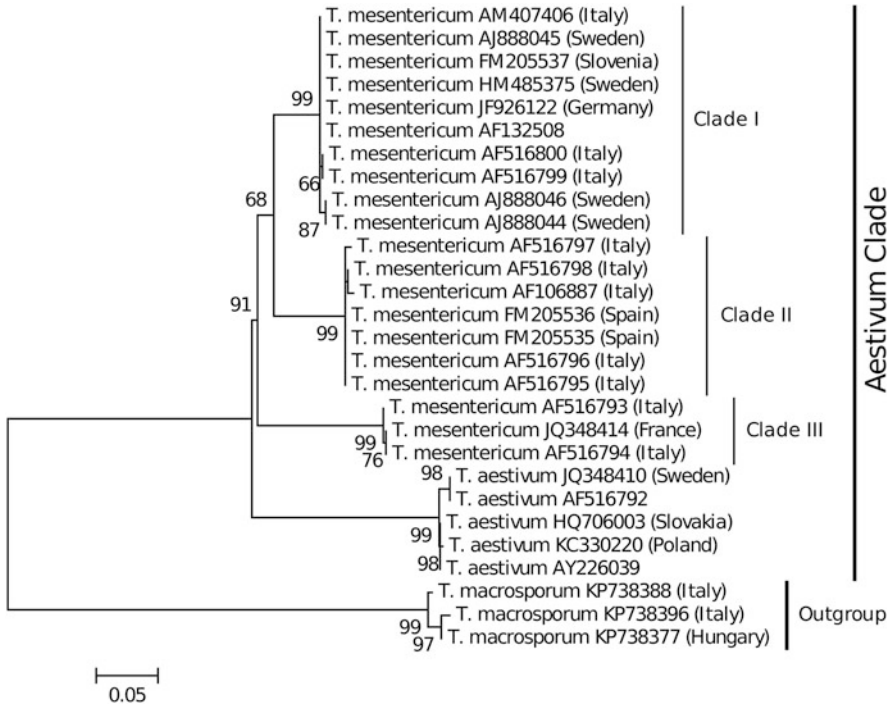
Fig. 5.5 *Tuber mesentericum* maximum likelihood phylogenetic tree based on the Tamura 3-parameter model (Tamura 1992). Bootstrap values >65 % are shown next to branching nodes. A discrete gamma distribution [+*I*] was used to model evolutionary rate differences among sites. The analysis involved 28 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 440 positions in the final dataset

5.4.4 Tuber mesentericum *Geographic Distribution and Ecology*

Tuber mesentericum is a well-distributed species in Europe, occurring from Spain to Turkey and from Sweden to Italy (Pegler et al. 1993; Montecchi and Sarasini 2000; Wedén et al. 2001; Ceruti et al. 2003; Granetti et al. 2005; Sica et al. 2007; Castellano and Türkoğlu 2012).

Tuber mesentericum can grow from hilly to mountainous areas, in the former frequently associated with *C. avellana* and *O. carpinifolia*, whereas in the latter principally in relationship with *F. sylvatica*; the truffle can be common up to the altitudinal limit of the beech, that means 1800 m AMSL and more. *Tuber mesentericum* prefers north-oriented slopes and humid environment, with well-shaded, closed forests, but orientation seems to be less determinant on lower elevations (Granetti et al. 2005; Miko et al. 2006). Soil parameters proved to be variable citing mostly high lime content with a pH of 7–7.7 but also as low as 6–6.5, agreeing in well-aerated, wet soils rich in organic matter and also in volcanic

subacid soils (Granetti et al. 2005). Able to share the habitat with all the black truffle species, but only occasionally in the growth areas of *T. aestivum* and *T. melanosporum*, *T. mesentericum* becomes predominant species in the mountain belt, especially in the calcareous beechwoods, where only *T. aestivum* var. *uncinatum*, and rarely *T. brumale*, can be also found.

The ripening season of *T. mesentericum* can begin in the summer, with a peak between the end of the autumn and the beginning of the winter. In the mountain belt, the harvest is often interrupted by the snowfalls; nevertheless in Italy, fresh and mature *T. mesentericum* ascomata can be found (sometimes along with *T. aestivum* var. *uncinatum*) after snow melting during the spring, even in April or May at higher altitudes (approx.1500–1800 m AMSL). These specimens are often in a perfect state of preservation, but this condition can last only for few days.

A more complete list of host plants comprises oaks (*Q. robur*, *Q. cerris*, *Q. petraea* and *Q. pubescens*), beeches (*F. sylvatica*), pines (*Pinus nigra* Arnold and *Pinus sylvestris* L.), hornbeams (*C. betulus* and *O. carpinifolia*), hazelnuts (*C. avellana*) and lindens (*Tilia* spp.) (Ceruti et al. 2003; Granetti et al. 2005; Miko et al. 2006; Marjanović et al. 2010; Bencivenga and Baciarelli-Falini 2012).

5.5 Conclusions

Tuber macrosporum and *T. mesentericum* are two minor truffles with a promising market and this perspective fosters researchers to disclose their taxonomy, biology and ecology. In this chapter, we showed that *T. macrosporum* is an adaptable species growing in soils with variable pH and lime content, and at the same time, it prefers fresh environment characterized by the constant presence of water. The unreliable, strongly water-dependent natural yields could be one of the main reasons for low quantities of *T. macrosporum* in the international market, which hinder its success. The establishment of plantations could resolve the problem. In recent years, due to its outstanding organoleptic characteristics, some Italian nurseries started producing mycorrhized plants with *T. macrosporum* and some orchards were realized. In spite of some successful initiatives of already productive *T. macrosporum* plantations, the species is still considered as marginal; therefore, further research is necessary to enhance *T. macrosporum* cultivation. The species has low intraspecific variability, and according to the available data, the relation between its genetic diversity and geographic distribution remains unclear. Concerning *T. mesentericum*, its ecology overlaps the one of *T. aestivum*, both prefer calcareous soils with sub-alkaline or neutral pH, but the former can in some cases be found in volcanic subacid soils. Unlike *T. macrosporum*, *T. mesentericum* shows evident patterns of genetic diversity in relation to geographic origin. The identification of five clusters of sequences, well-distinguished one from the other, suggested the existence of cryptic species within *T. mesentericum*. A study in progress aims to investigate morphological and genetic

intraspecific diversity of *T. mesentericum* species complex and its relationships with its sister species *T. aestivum*.

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