Chapter 5
State of the Art of the Research on *Boletus edulis*

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5.1 Introduction

The genus *Boletus* belongs to the family Boletaceae, order Boletales, and consists of a complex of ectomycorrhizal fungal species. A large number of plants are suitable hosts: Fagales, Fagaceae (*Castanea*, *Castanopsis*, *Fagus*, *Lithocarpus*, *Quercus*) and Betulaceae (*Carpinus*, *Corylus*, *Betula*, *Ostrya*, *Populus*); Malvales, Malvaceae (*Tilia*) and Cistaceae (*Cistus*); Malpighiales, Salicaceae (*Salix*); Ericales, Ericaceae (*Arctostaphylos*); and Pinales, Pinaceae (*Abies*, *Keteleeria*, *Picea*, *Pinus*, *Tsuga*) (Olivier et al. 1997; Águeda et al. 2006; Mello et al. 2006). Among many sections, the section *Boletus* Singer (=*Edules* Fr. 1838) known as "porcini" is a conspicuous group of wild, edible mushrooms (Singer 1986; Murat et al. 2008; Sitta and Floriani 2008). Their reported distribution throughout the Northern Hemisphere was mostly limited to the sampling of European species. Nevertheless, they are popular in Europe and are becoming an economic resource North America also (Arora 2008). A global geographic sampling together with the selection of four loci—the fast-evolving in nuclear internal transcribed spacer (Fig. 5.1), the nuclear large subunit of the ribosome, the largest subunit of the nuclear gene encoding RNA polymerase II, and the mitochondrial ATPase subunit 6—has allowed Dentinger et al. (2010) to trace the molecular phylogenetics of these mushrooms. On this basis authors could recognize 18 reciprocally monophyletic species, show the monophyly of porcini, and expand their known distribution to Australia and Thailand. However, this global analysis did not consider *Boletus pinetorum*, a new species described using both morphological and molecular analysis by Korhonen et al. (2009) who focused on section *Boletus* in Fennoscandia.

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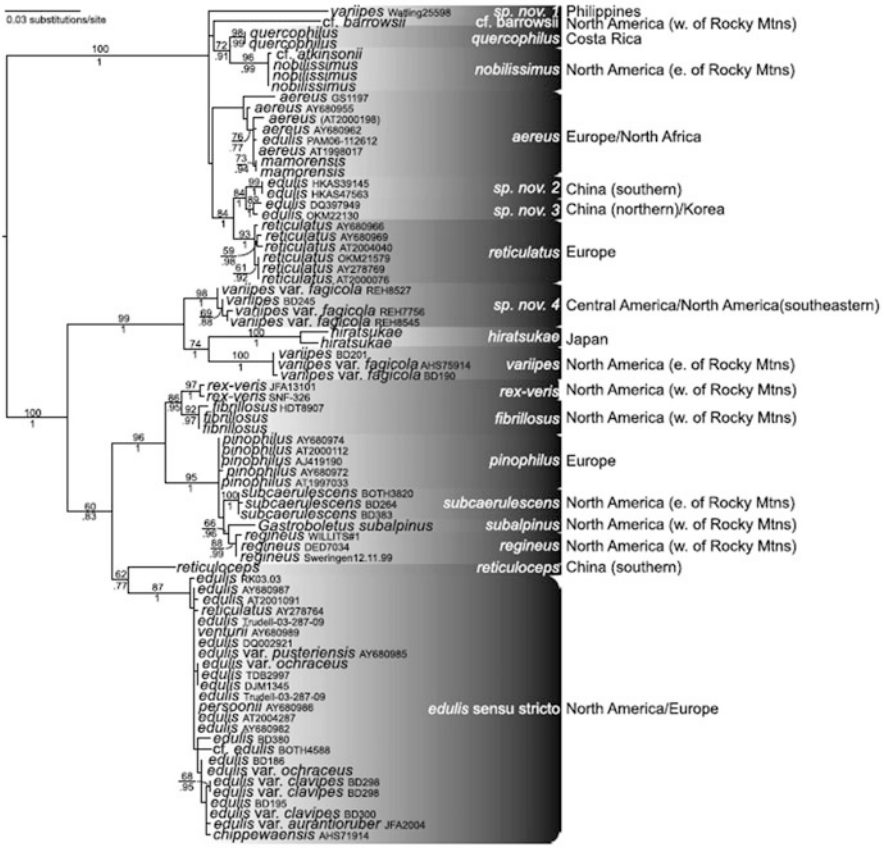

Fig. 5.1 Phylogram of the best maximum likelihood tree using *Boletus* ITS sequences. Clades that correspond to species are *shaded* and labeled with the specific epithet on the *right*. Geographic range of each species is indicated at *right*. Figure reprinted by "Molecular Phylogenetics and Evolution," 57, Dentinger et al., Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*), 2010, with permission from Elsevier

Many attempts to cultivate edible boletes have been made (Hall et al. 1998). This practice mostly concerned species belonging to *B. edulis* sensu lato (s.l.), such as *Boletus edulis* Bull.: Fr. sensu stricto (s.s.), *B. aereus* Bull.: Fr., *Boletus pinophilus* Pilát & Dermek, and *Boletus aestivalis* (Paulet) Fr. (Fig. 5.2). These fruiting bodies are in high demand; these mushrooms have a pleasant flavor and texture and are used in huge quantities for the production of dried mushroom soups. Preparations of these dried mushrooms sometimes include different species even some not belonging to section *Boletus*, such as *Boletus violaceofuscus*. Although Helbling et al. (2002) report that *B. edulis* induces allergic IgE-mediated symptoms, no taxonomic indication is given concerning *B. edulis* (sensu stricto or sensu lato), so the identity of the specific mushroom culprit remains unclear. Therefore, specific primers have been designed within the internal transcribed spacer rDNA region for the unambiguous

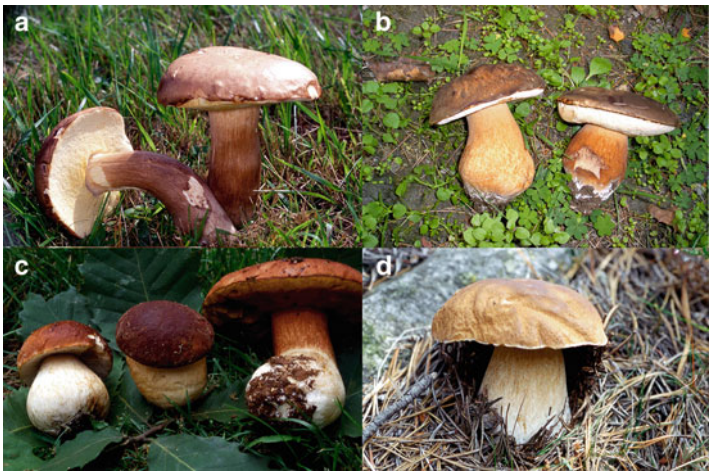

Fig. 5.2 Pictures of *Boletus aestivalis* (**a**), *Boletus aereus* (**b**), *Boletus pinophilus* (**c**), and *Boletus edulis* (**d**). Photos by Alfredo Vizzini

detection of these five species (Mello et al. 2006). The availability of these primers represents a crucial resource for detecting these fungi. Next steps are focused toward the analysis of the genetic structure of these fungi, as well as the certification of mycelia to be used as inocula in long-term reforestation programs focused on increasing bolete production. The broad host range of *B. edulis* makes it a suitable species to be introduced with various host tree species during reforestation efforts.

5.2 From *Boletus edulis* Molecular Identification to Cultivation

Many researchers have synthesized ectomycorrhizae of the *B. edulis* species complex on various hosts (Froidevaux and Amiet 1975; Tozzi et al. 1980; Molina and Trappe 1982a, b; Ceruti et al. 1983–1984, 1985; Poitou et al. 1982; Duñabeitia et al. 1996). However, the identification of the mycelia obtained by strain isolation and used as inoculum could not be confirmed by molecular analysis nor could the identification of the synthesized ectomycorrhizae. Because of this and other problems, when seedlings inoculated with *Boletus* species have been outplanted, the ectomycorrhizae of *Boletus* sp. are quickly replaced by other ectomycorrhizal fungi, and fruiting body production has never been successful (Olivier et al. 1997; Meotto et al. 1999; Wang and Hall 2004). Only more recently, Águeda et al. (2008) were able to synthesize ectomycorrhizae of *B. aereus*, *B. edulis*, and *B. reticulatus* (=*B. aestivalis*) with *Cistus* sp. and to provide molecular identification of the fungal strains isolated from fruiting bodies. Controlled mycorrhization now seems feasible opening new perspectives on the cultivation of these commercial mushrooms, which

have been collected exclusively from the wild (Cannon and Kirk 2007). In addition, *B. edulis* is regarded as a species tolerant to salinity stress, an adaptation that should be selectively advantageous in reforestation ecosystems (Liang et al. 2007). In order to understand the adaptive mechanisms of *B. edulis* to salt stress, proteomics was used to study the global expression profile of proteins in response to this stress. When the *B. edulis* was exposed to salt stress conditions (4 % NaCl, w/v), proteomic changes included proteins related to multiple cellular processes (e.g., metabolisms, energy-related processes, DNA repair, cell cycle control, and stress tolerance) (Liang et al. 2007).

5.3 In Field Characterization of *Boletus edulis* Mycelium and Mycorrhizae

Tracking the dynamics of an ectomycorrhizal fungus is a difficult task because fruiting bodies do not reflect the distribution of underground mycelial networks (Dahlberg 2001). Generally, the first analyses on the distribution of an ectomycorrhizal fungus focused on the fruiting bodies, which were followed by analyses of the mycorrhizae and extraradical mycelium in the soil. Few studies have been conducted on the ecology of the *B. edulis* complex. A first description and molecular characterization of the ectomycorrhizae of *B. edulis* on *Cistus ladanifer* L. was made by Águeda et al. (2006) by sampling field ectomycorrhizae and soil rhizomorphs under the fruiting bodies of *B. edulis*. Mycorrhizae of *B. edulis*, *B. aestivalis*, and *B. aereus* on *Castanea sativa* were detected in the field by Peintner et al. (2007). These mycorrhizae were morphologically similar to each other and showed the anatomo-morphological features previously described on *C. ladanifer*, except the color, which was darker ochre. In this investigation, ectomycorrhizae of *B. edulis* s.l. and soil fungal communities were characterized by molecular methods in a habitat with high production of *B. edulis* s.l. fruiting bodies. The first outcome of this investigation was that large amounts of basidiomes result from abundant mycorrhizae, but rare mycelia. While *Boletus* fruiting bodies dominated the aboveground fungal community, *Boletus* mycelia were rare in the soil and had a scattered distribution (Peintner et al. 2007). This finding is in agreement with a study by Zhou et al. (2001) showing that amounts of subterranean mycelia and mycorrhizae are not always correlated to the number of fruiting bodies formed by them. It is known that fructification is affected by habitat characteristics and climatic conditions (Bonet et al. 2004; Pinna et al. 2010) and depends on the presence of mycelium in the soil. Thanks to the progress of real-time PCR techniques, a quantification of *B. edulis* extraradical mycelium in a Scots pine forest soil was carried out (De la Varga et al. 2011). In this work the productivity of fruiting bodies was not correlated either with the concentration of *B. edulis* mycelia in soil samples or with the abundance of *B. edulis* mycorrhizae, whereas a statistically significant positive correlation was detected between the concentration

of mycelia of *B. edulis* in the soil samples and the presence of *B. edulis* mycorrhizae in these samples. The lack of correlation between the concentration of the mycelium in the soil and the productivity of fruiting bodies has also been shown in *T. melanosporum* orchards (Suz et al. 2008).

5.4 Applications of *B. edulis*

While ecologists are interested in deciphering the lifestyle of boletes, the common mushroom *B. edulis* is also an object of research aimed to discover important properties in edible fungi (see also Chap. 18). Since fungi are the most productive biological sources of various primary and secondary metabolites, they have long been exploited by the pharmaceutical and food industries. Heleno et al. (2011), in a targeted analysis of metabolites in wild *Boletus* species, revealed an important source of proteins, carbohydrates, fatty acids (mainly linoleic acid), sugars (mainly mannitol and trehalose), and vitamins (tocopherols and ascorbic acid) in *B. edulis*, *B. aereus*, and *B. reticulatus*, as well as phenolic acids. The finding of various macronutrients favors the consumption of these species not only for their taste and aroma but also as source of nutrients. On the other hand, the presence of phenolic acids, which has been correlated to antioxidative properties, can be exploited by food and pharmaceutical industries. In addition, polysaccharides extracted from *B. edulis* have shown important antioxidative properties and might be employed as ingredients in healthy and functional food to alleviate the oxidative stress (Zhang et al. 2011). As result of recent investigations, a lectin with antitumoral properties has been found, opening new perspectives in research aimed at developing new drugs for cancer therapy (Bovi et al. 2011). 1-Octen-3-ol is a characteristic aroma compound produced by mushrooms, including *B. edulis*, and is often added as flavoring in processed products such as dehydrated soups (probably because of huge losses of 1-octen-3-ol during the preparation of these food products) (Zawirska-Wojtasiak 2004). De Pinho et al. (2008) correlated the pattern of volatiles with the overall aroma of 11 wild edible mushrooms and discovered that only *B. edulis* presented linoleic acid that, as all a long-chain unsaturated fatty acids, shows antibacterial activity and is used as antimicrobial food additive. While metabolites are the object of interest for industrial applications, few studies have investigated their biological role in nature, such as their role in the interactions with other fungi, bacteria, and plants. In this regard, Splivallo et al. (2011) examined specific aspects of volatile ecology and biology of other ectomycorrhizal mushroom highly appreciated for their special taste and aroma, the truffles. To date, more than 200 volatile organic compounds have been described from various truffle species, and the biosynthetic pathways/genes involved in volatile biosynthesis have been traced in the recently sequenced genome of *Tuber melanosporum* Vittad. (Martin et al. 2010).

In a recent survey aimed to investigate metallic elements in wild-grown mushrooms, the use of *B. edulis* has established a baseline measure of regional mineral status and heavy metal pollution (Falandysz et al. 2011). It is known that

mushrooms are involved in the biogeochemical cycling of metallic elements and metalloids, and there is interest in understanding the mechanisms involved in bioconcentration of metallic elements by mushrooms.

5.5 Conclusions

Among the sections of the genus *Boletus*, the species belonging to the *B. edulis* complex have received more attention because of their commercial use. The application of morphological and molecular methods has allowed the production of *B. edulis* certified mycelia, and consequently inocula, and in vitro mycorrhizae, but research in field is still in its infancy. Although the detection of mycorrhizae and extraradical mycelium is feasible, no conclusions can be given on how this fungus completes its life cycle in time and space or on the population size. More long-term studies are needed to elucidate the genetic structure of *B. edulis*. Recently the ultrahigh-throughput DNA sequencing (UHTS) has dramatically changed the nature of biological research. These new sequencing technologies provide a tremendous amount of DNA sequence data with reduced costs, effort, and time as compared to Sanger sequencing (Fox et al. 2009). Among the UHTS numerous applications, genome sequencing/resequencing is opening new perspectives through understanding the biology and ecology of fungi. The sequencing of the mycorrhizal genomes has just started. The first sequenced genome is that of the basidiomycete *Laccaria bicolor* (Maire) P. D. Orton followed by the genome of the ascomycete *T. melanosporum* (Martin et al. 2008, 2010). The comparison of genomic traits in the two ectomycorrhizal fungi clearly indicated that the evolution followed along different ways in ascomycetes and basidiomycetes. Since the availability of genome sequences from ecologically and taxonomically diverse fungi would allow comparative studies to understand the evolutionary mechanisms of symbiosis, the US Department of Energy Joint Genome Institute launched the Fungal Genomics Program (FGP) (Martin et al. 2011). This program scheduled the sequencing of the genomes of 25 mycorrhizal fungi that have been chosen on the basis of their different affiliations, types of symbiosis they establish, and host specificity (http://mycor.nancy.inra.fr/blogGenomes/?p=1334). The genomes of some of them [*Amanita muscaria* (L.) Lam., *Cenococcum geophilum* Fr., *Hebeloma cylindrosporum* Romagn., *Laccaria amethystina* Cooke, *Oidiodendron maius* G. L. Barron, *Piloderma croceum* J. Erikss. & Hjortstam, *Paxillus involutus* (Batsch) Fr., *Pisolithus microcarpus* (Cooke & Massee) G. Cunn., and *Pisolithus arhizus* (Scop.) Rauschert (= *Pisolithus tinctorius*) have been just sequenced and preliminary data presented at the first Mycorrhizal Genomics Workshop held in Nancy in September 2011. *B. edulis* genome sequencing will follow soon [together with that of *Cantharellus cibarius* Fr., *Coltricia cinnamomea* (Jacq.) Murrill, *Cortinarius glaucopus* (Schaeff.) Fr., *Gymnomyces xanthosporus* (Hawker) A. H. Sm., *Lactarius quietus* (Fr.) Fr., *Meliniomyces bicolor* Hambl. & Sigler, *Paxillus rubicundulus* P. D. Orton, *Ramaria formosa* (Pers.) Quél., *Rhizoscyphus ericae* (D.

J. Read) W. Y. Zhuang & Korf, *Scleroderma citrinum* Pers., *Suillus luteus* (L.) Roussel, *Sebacina vermifera* Oberw., *Tomentella sublilacina* (Ellis & Holw.) Wakef., *Tricholoma matsutake* (S. Ito & S. Imai) Singer, *Tulasnella calospora* (Boud.) Juel, and *Terfezia boudieri* Chatin] allowing the identification of crucial genetic traits driving symbiosis and fruiting body development. This could, in the future, provide tools for the management of this precious fungus *in situ*.

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References

Águeda B, Parladé J, de Miguel AM, Martínez-Peña F (2006) Characterization and identification of field ectomycorrhizas of *Boletus edulis* and *Cistus ladanifer*. Mycologia 98:23–30. doi:10.3852/mycologia.98.1.23

Águeda B, Parladé J, Fernández-Toirán LM, Cisneros Ó, de Miguel AM, Modrego MP, Martínez-Peña F, Pera J (2008) Mycorrhizal synthesis between *Boletus edulis* species complex and rockroses (*Cistus* sp.). Mycorrhiza 18:443–449. doi:10.1007/s00572-008-0192-3

Arora D (2008) California porcini: three new taxa, observations on their harvest, and the tragedy of no commons. Econ Bot 62:356–375. doi:10.1007/s12231-008-9050-7

Bonet JA, Fischer CR, Colinas C (2004) The relationship between forest age and aspect on the production of sporocarps of ectomycorrhizal fungi in *Pinus sylvestris* forests of the central Pyrenees. For Ecol Manage 203:157–175. doi:10.1016/j.foreco.2004.07.063

Bovi M, Carrizo ME, Capaldi S, Perduca M, Chiarelli LR, Galliano M, Monaco GL (2011) Structure of a lectin with antitumoral properties in king bolete (*Boletus edulis*) mushrooms. Glycobiology 21:1000–1009. doi:10.1093/glycob/cwr012

Cannon PF, Kirk PM (2007) Fungal families of the world. CABI UK Centre (Egham), Wallingford

Ceruti A, Ceruti Scurti J, Tozzi M (1983–1984) Sintesi micorrizica tra *Boletus aereus* e *Quercus pubescens*. Allionia 26:5–17

Ceruti A, Tozzi M, Reitano G (1985) Sintesi micorrizica tra *Boletus aereus* e *Castanea sativa*. Allionia 27:5–9

Dahlberg A (2001) Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. New Phytol 150:555–562. doi:10.1046/j.1469-8137.2001.00142.x

de la Varga H, Águeda B, Martínez-Peña F, Parladé J, Pera J (2011) Quantification of extraradical soil mycelium and ectomycorrhizas of *Boletus edulis* in a Scots pine forest with variable sporocarp productivity. Mycorrhiza 22:59–68. doi:10.1007/s00572-011-0382-2

De Pinho PG, Ribeiro B, Goncalves RF, Baptista P, Valentao P, Seabra RM, Andrade PB (2008) Correlation between the pattern volatiles and the overall aroma of wild edible mushrooms. J Agric Food Chem 56:1704–1712. doi:10.1021/jf073181y

Dentinger BTM, Ammirati JF, Both EE, Desjardin DE, Halling RE, Henkel TW, Moreau PA, Nagasawa E, Soytong K, Taylor AF, Watling R, Moncalvo JM, McLaughlin DJ (2010) Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*). Mol Phylogenet Evol 57:1276–1292. doi:10.1016/j.ympev.2010.10.004

Duñabeitia MK, Hormilla S, Salcedo I, Peña JI (1996) Ectomycorrhizas synthesis between *Pinus radiata* and eight fungi associated with *Pinus* spp. Mycologia 88:897–908. doi:10.2307/3761052

Falandysz J, Frankowska A, Jarzynska G, Dryzałowska A, Kojta AK, Zhang D (2011) Survey on composition and bioconcentration potential of 12 metallic elements in King Bolete (*Boletus edulis*) mushroom that emerged at 11 spatially distant sites. J Environ Sci Health B 46:231–246. doi:10.1080/03601234.2011.540528

Fox S, Filichkin S, Mockler TC (2009) Applications of ultra-high-throughput sequencing. Methods Mol Biol 553:79–108. doi:10.1007/978-1-60327-563-7_5

Froidevaux L, Amiet R (1975) Ectendomycorrhizae of *Pinus mugo* + *Boletus edulis* subsp. *edulis* and *Pinus cembra* + *Suillus variegatus* formed in pure culture. Eur J For Pathol 5:57–61. doi:10.1111/j.1439-0329.1975.tb00935.x

Hall IR, Lyon AJE, Wang Y, Sinclair L (1998) Ectomycorrhizal fungi with edible fruiting bodies – 2. *Boletus edulis*. Econ Bot 52:44–56. doi:10.1007/BF02861294

Helbling A, Bonadies N, Brander KA, Pichler WJ (2002) *Boletus edulis*: a digestion-resistant allergen may be relevant for food allergy. Clin Exp Allergy 32:771–775. doi:10.1046/j.1365-2222.2002.01400.x

Heleno SA, Barros L, Sousa MJ, Martins A, Santos-Buelga C, Ferreira ICFR (2011) Targeted metabolites analysis in wild *Boletus* species. LWT Food Sci Technol 44:1343–1348. doi:10.1016/j.lwt.2011.01.017

Korhonen M, Liimatainen K, Niskanen T (2009) A new boletoid fungus, *Boletus pinetorum*, in the *Boletus* section *Boletus* from Fennoscandia (Basidiomycota, Boletales). Karstenia 49:41–60

Liang Y, Chen H, Tang M, Shen S (2007) Proteome analysis of an ectomycorrhizal fungus *Boletus edulis* under salt shock. Mycol Res 3:939–946. doi:10.1016/j.mycres.2007.06.005

Martin F, Aerts A, Ahrén D, Brun A, Danchin EGJ, Duchaussoy F, Gibon J, Kohler A, Lindquist E, Pereda V et al (2008) The genome sequence of the basidiomycete fungus *Laccaria bicolor* provides insights into the mycorrhizal symbiosis. Nature 452:88–92. doi:10.1038/nature06556

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R et al (2010) Perigord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464:1033–1038. doi:10.1038/nature08867

Martin F, Cullen D, Hibbett D, Pisabarro A, Spatafora JW, Baker SE, Grigoriev IV (2011) Sequencing the fungal tree of life. New Phytol 190:818–821. doi:10.1111/j.1469-8137.2011.03688.x

Mello A, Ghignone S, Vizzini A, Sechi C, Ruiu P, Bonfante P (2006) ITS primers for the identification of marketable boletes. J Biotechnol 121:318–329. doi:10.1016/j.jbiotec.2005.08.022

Meotto F, Pellegrino S, Bounous G (1999) Evolution of *Amanita caesarea* (Scop.: Fr.) Pers. and *Boletus edulis* Bull.: Fr. synthetic ectomycorrhizas on European chestnut (*Castanea sativa* Mill.) seedlings under field conditions. Acta Hortic 494:201–204

Molina R, Trappe J (1982a) Lack of mycorrhizal specificity by the ericaceous hosts *Arbutus menziesii* and *Arctostaphylos uva-ursi*. New Phytol 90:495–509. doi:10.1111/j.1469-8137.1982.tb04482.x

Molina R, Trappe J (1982b) Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. For Sci 28:423–458

Murat C, Mello A, Abba' S, Vizzini A, Bonfante P (2008) Edible mycorrhizal fungi: identification, life cycle and morphogenesis. In: Varma (ed) Mycorrhiza. Springer, Berlin, pp 703–729

Olivier JM, Guinberteau J, Rondet J, Mamoun M (1997) Vers l'inoculation contrôlée des cèpes et bolets comestibles? Rev For Fr XLIX:222–234. doi: 10.4267/2042/5671

Peintner U, Iotti M, Klotz P, Bonuso E, Zambonelli A (2007) Soil fungal communities in a *Castanea sativa* (chestnut) forest producing large quantities of *Boletus edulis* sensu lato (porcini): where is the mycelium of porcini? Environ Microbiol 9:880–889. doi:10.1111/j.1462-2920.2006.01208.x

Pinna S, Gévry MF, Côté M, Sirois L (2010) Factors influencing fructification phenology of edible mushrooms in a boreal mixed forest of Eastern Canada. For Ecol Manage 260:294–301. doi:10.1016/j.foreco.2010.04.024

Poitou N, Mamoun M, Delmas J (1982) Quelques résultats obtenus concernant la mycorhization de plantes-hôtes par les champignons mycorhiziens comestibles. Les Mycorhizes: biologie et utilization. Les Colloques de l'INRA 13:295–301

Singer R (1986) The Agaricales in modern taxonomy. Koeltz Scientific Books, Koeningstein

Sitta N, Floriani M (2008) Nationalization and globalization trends in the wild mushroom commerce of Italy with emphasis on porcini (*Boletus edulis* and allied species). Econ Bot 62:307–322. doi:10.1007/s12231-008-9037-4

Splivallo R, Ottonello S, Mello A, Karlovsky P (2011) Truffle volatiles: from chemical ecology to aroma biosynthesis. New Phytol 189:688–699. doi:10.1111/j.1469-8137.2010.03523.x

Suz LM, Martin MP, Oliach D, Fischer RC, Colinas C (2008) Mycelial abundance and other factors related to truffle productivity in *Tuber melanosporum-Quercus ilex* orchards. FEMS Microbiol Lett 285:72–78. doi:10.1111/j.1574-6968.2008.01213.x

Tozzi M, Scurti IC, Berta G (1980) Ricerche preliminari di sintesi tra *Boletus edulis* e *Quercus pubescens*. Allionia 24:5–11

Wang Y, Hall IR (2004) Edible ectomycorrhizal mushrooms: challenges and achievements. Can J Bot 82:1063–1073. doi:10.1139/b04-107

Zawirska-Wojtasiak R (2004) Optical purity of (R)-(−)-1-octen-3-ol in the aroma of various species of edible mushrooms. Food Chem 86:113–118. doi:10.1016/j.foodchem.2003.08.016

Zhang A, Xiao N, He P, Sun P (2011) Chemical analysis and antioxidant activity *in vitro* of polysaccharides extracted from *Boletus edulis*. Int J Biol Macromol 49:1092–1095. doi:10.1016/j.ijbiomac.2011.09.005

Zhou Z, Miwa M, Matsuda Y, Hogetsu T (2001) Spatial distribution of the subterranean mycelia and ectomycorrhizas of *Suillus grevillei*. J Plant Res 114:179–185. doi:10.1007/PL00013981