

Russulaceae associated with mycoheterotroph *Monotropa uniflora* (Ericaceae) in Tlaxcala, Mexico: a phylogenetic approach

Alejandro KONG^{a*}, Joaquín CIFUENTES^b, Arturo ESTRADA-TORRES^c,
Laura GUZMÁN-DÁVALOS^d, Roberto GARIBAY-ORIJEL^e & Bart BUYCK^f

^a Laboratorio de Biodiversidad, Centro de Investigación en Ciencias Biológicas,
Universidad Autónoma de Tlaxcala, km 10.5 carretera San Martín
Texmelucan-Tlaxcala, San Felipe Ixtacuixtla, Tlaxcala, 90120 México,
email: akongluz@hotmail.com

^b Herbario FCME, Facultad de Ciencias, Universidad Nacional Autónoma
de México. Apartado Postal 70-181, 04510 México DF, México,
email: jcifuentesblanco@hotmail.com

^c Laboratorio de Biodiversidad, Centro de Investigación en Ciencias Biológicas,
Universidad Autónoma de Tlaxcala, km 10.5 carretera San Martín
Texmelucan-Tlaxcala, San Felipe Ixtacuixtla, Tlaxcala, 90120 México,
email: arturomixo@hotmail.com

^d Departamento de Botánica y Zoología, Universidad de Guadalajara, km 15.5
carretera Guadalajara-Nogales, Las Agujas, Zapopan, 45101, Jalisco, México,
email: lguzman@cucba.udg.mx

^e Instituto de Biología, Universidad Nacional Autónoma de México,
México DF, México, email: rgaribay@ib.unam.mx

^f Muséum national d'Histoire naturelle, Département Systématique et Évolution,
CP 39, ISYEB, UMR 7205 CNRS MNHN UPMC EPHE,
12, rue Buffon, F-75005 Paris, France, email : buyck@mnhn.fr

Abstract – The aim of this study was to explore the systematic position of the fungi associated with the roots of *M. uniflora* in two localities of Tlaxcala, Mexico, using a phylogenetic approach. Thirty plants were sampled, fifteen from a coniferous forest dominated by *Abies religiosa* in La Malinche National Park, and fifteen from a mixed conifer-broadleaf forest dominated by *Pseudotsuga menziesii* in Sierra de Tlaxco. Our ITS analysis confirms the preference of *M. uniflora* to associate with Russulaceae, in particularly with *Russula* species. The roots of each plant were associated with a single fungal ITS sequence. They were analyzed phylogenetically by maximum likelihood, and were recovered in 12 moderate to

* Corresponding author, Posgrado en Ciencias Biológicas, Facultad de Ciencias, Universidad Nacional Autónoma de México

well-supported clades within the genus *Russula*. In each of these clades only a single *Russula* species was associated with *Monotropa* in Mexico, except for clade Integrae for which two Mexican species were retrieved. A total of 13 *Russula* species were retrieved from the 30 *Monotropa* plants, four from La Malinche National Park, and 11 from Sierra de Tlaxco. Two species, *R. aff. olivobrunnea* and one unidentified species in subsection *Lactarioideae*, were shared among both localities. The *Monotropa* in the *Abies* forest of La Malinche National Park were dominated by a single species, *R. aff. olivobrunnea*, present in 11 of the 15 plants. In the *Pseudotsuga* forest of Sierra de Tlaxco, *Monotropa* was associated with a higher diversity of *Russula*, eleven species in total, seven of which were associated with a single plant, while the four other *Russula* had each been retrieved from two plants. Higher diversity of ectomycorrhizal host trees in the Sierra de Tlaxco site might possibly explain the higher richness of fungal associates as suggested by host association patterns of the involved species groups. This study brings the total number of *Russula* species that associate with *Monotropa* to forty-four. In the distribution area of Monotropeoideae, nearly all terminal *Russula* clades (i.e. subsection level) that are involved in this mycoheterotroph association, equally harbor species that associate with mycoheterotroph orchids, whereas gasteromycetation appears to have exclusively evolved in clades that also harbor species developing mycoheterotroph associations.

Diversity / ITS / gasteromycetation / host association / maximum likelihood phylogeny / mycoheterotroph orchids

INTRODUCTION

Russulaceae Lotsy (Basidiomycota: Agaricomycetes) is a large cosmopolitan family of fungi, with more than 1250 species (Rinaldi *et al.*, 2008). Buyck *et al.* (2008) demonstrated the existence of four major monophyletic clades within the agaricoid members of this family, each representing a distinct genus: *Lactarius* Pers., *Lactifluus* (Pers.) Roussel, *Multifurca* Buyck & V. Hofstetter., and *Russula* Pers. Species previously placed in one of the sequestrate and pleurotoid genera, such as *Arcangeliella* Cavara, *Cystangium* Singer & A.H. Sm., *Gymnomyces* Masee & Rodway, *Macowanites* Kalchbr., *Pleurogala* Redhead & Norvell, and *Zelleromyces* Singer & A.H. Sm. are now commonly considered synonymous within either *Russula* or one of the two milkcap genera (Miller *et al.*, 2001; Lebel & Tonkin 2007, Verbeken *et al.*, 2014). Furthermore, some corticioid and resupinate species of *Boidinia* Stalpers & Hjortstam, *Gloeocystidiellum* Donk, and *Gloeopeniophorella* Rick are closely related (Larsson & Larsson, 2003) and considered to be part of the same family (Larsson 2007).

All of the agaricoid Russulaceae are presumed to be ectomycorrhizal and they represent one of the most important ectomycorrhizal groups in arctic-boreal, temperate and tropical forest ecosystems, associating mainly with Pinaceae, Fagaceae and Betulaceae in the Northern Hemisphere, with Myrtaceae and Nothofagaceae in the Southern hemisphere, and with Caesalpinioideae and Dipterocarpaceae in the tropics (Moyersoen *et al.*, 2001, Verbeken & Buyck, 2002; Haug *et al.*, 2005; Smith & Read, 2008; Tedersoo *et al.*, 2010). Also some mycoheterotrophic Orchidaceae and Ericaceae are well-known to associate with species of Russulaceae (Girlanda *et al.*, 2006; Hynson & Bruns, 2009; Ogura-Tsujita *et al.*, 2012; Okayama *et al.*, 2012; Bellino *et al.*, 2014; Merckx, 2014). In the subfamily Monotropeoideae of Ericaceae, plants lack chlorophyll and obtain their organic carbon through a fungal link connecting the ectomycorrhizas of nearby autotrophic plants with their own

mycorrhizas (Berch *et al.*, 2005; Merckx, 2014: Fig. 1.2). Three species in this subfamily, *Monotropa uniflora* L., *Monotropastrum humile* (D. Don) Hara, and *M. sciaphilum* (Andres) G.D. Wallace are associated almost exclusively with Russulaceae species (Bidartondo & Bruns, 2001, 2005; Yang & Pfister, 2006; Yamada *et al.*, 2008; Matsuda *et al.*, 2011; Min *et al.*, 2012; Lee & Eom, 2014).

Monotropa uniflora has a disjunct distribution. In America, it is found mainly from northern California to British Columbia, and then eastward across southern Canada throughout most of the eastern United States, and southward from central Mexico through Central America into Colombia. In Asia, the distribution of *M. uniflora* extends from Japan to southern China and Taiwan, to northern India, Nepal, and Bhutan (Wallace, 1975). Throughout most of its geographical distribution *Monotropa* grows in moist conifer or hardwood forests.

Historically, *Monotropa* has been reported in association with several fungi, but molecular approaches suggested that each *M. uniflora* plant is associated with a single species of Russulaceae (Bidartondo, 2005; Yang & Pfister, 2006). Currently, there are about 45 known species of Russulaceae recorded in association with *M. uniflora*, mainly *Russula* species, but also a few *Lactarius*. Bidartondo & Bruns (2001) sampled six *Russula* and one *Lactarius* from 33 *M. uniflora* plants from the United States, and only a single *Russula* species from two plants of Japan. Young *et al.* (2002) collected 15 plants of *M. uniflora* in central British Columbia, Canada, and found three species belonging to *Russula*, one in *Lactarioideae*, one in *Griseinae* and one clearly hypogeous, possibly *Gymnomyces pila* (Pat.) Trappe, T. Label & Castellano [as *Martellia pila* (Pat.) J.M. Vidal]. Bidartondo (2005: Fig. 4) presents a geographical mosaic of mycorrhizal specificity between Russulaceae and *M. uniflora*, recording four Russulaceae lineages in western North America (*R. brevipes* Peck as the dominant lineage), and ten lineages associated with *M. uniflora* in eastern North America. Yang & Pfister (2006) sampled 56 individual plants of *M. uniflora* from four sites of Massachusetts, United States, and retrieved 20 fungal phylotypes, 18 grouped in the genus *Russula* and two within *Lactarius*. From China, Min *et al.* (2012) sampled 10 individuals of *M. uniflora* and recovered four species, two *Russula*, but also one *Tricholoma* (Fr.) Staude species, as well as a saprotrophic species of *Pholiota* (Fr.) P. Kumm. In Korea, Lee & Eom (2014) collected seven individual plants of *M. uniflora* and the sequence analysis of the ITS region showed that all of the samples represented a single *Russula* species.

In this study the diversity of the fungi associated with the root system of *M. uniflora* from two localities in Tlaxcala, Central Mexico, was explored. Whereas previous studies were mainly interested in the systematic position of the fungal symbionts at the family or genus level, this study aims particularly at providing a more accurate idea of the individual subgeneric *Russula* clades that are involved in a root symbiosis with *M. uniflora* using the same molecular marker (ITS) and we examined whether there is a phylogenetic signal that underlies this symbiosis.

MATERIALS AND METHODS

Collecting sites. – Mycorrhizas of *Monotropa uniflora* were collected from two localities in the state of Tlaxcala, Mexico: La Malinche National Park (LM) and Sierra de Tlaxco (ST; Fig. 1). Both localities are separated 45.5 km and situated in the biogeographic province of the Transmexican Volcanic Belt (TVB), a largely

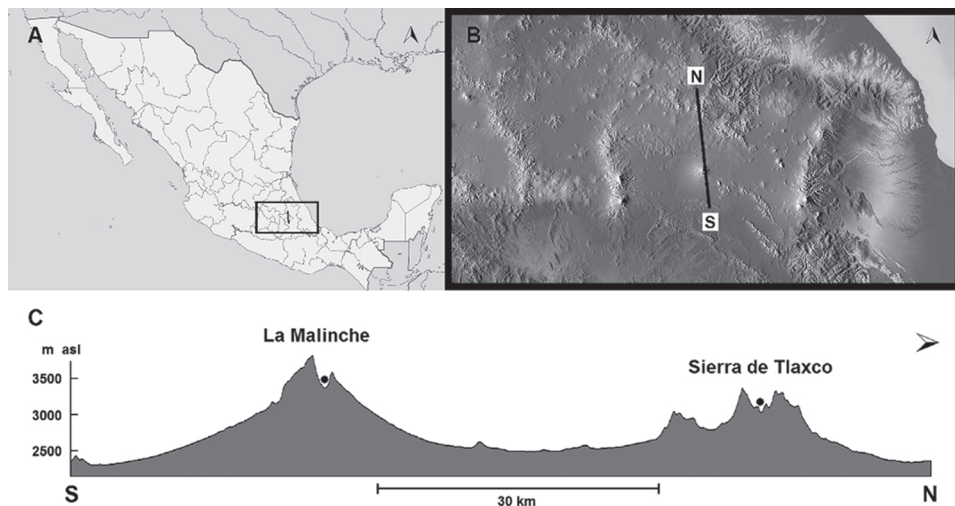


Fig. 1. Study area. **A.** Map of Mexico with a frame indicating the location of the study area. **B.** Central-eastern region of the Transmexican Volcanic Belt, showing a N-S transect along the collecting sites. **C.** Topographic profile of the N-S transect, along the La Malinche volcano and Sierra de Tlaxco, black dots mark the location of the collecting sites.

mountainous area with the highest mountains and volcanoes of Mexico, and dominated by coniferous and oak forests (Morrone, 2005). Collecting sites in LM were located 11.5 km W to the community of Ixtenco, $19^{\circ}14'15''$ - $19^{\circ}14'40''$ N, $98^{\circ}00'05''$ - $98^{\circ}00'50''$ W, between 3250-3500 m ASL, with a sub-humid temperate-semi-cold climate (rainy season mainly in summer, mean annual precipitation of 1000 mm, mean annual temperature 6-8°C), in a coniferous forest dominated by *Abies religiosa* (Kunth) Schtdl. & Cham. While ST sites were located 7.5 km NE of Tlaxco, $19^{\circ}38'25''$ - $19^{\circ}38'55''$ N, $98^{\circ}02'40''$ - $98^{\circ}03'50''$ W, between 2900-3300 m ASL, with a sub-humid temperate climate (rainy season mainly in summer, mean annual precipitation of 800 mm, mean annual temperature 10-12°C), in a mixed coniferous-oak forest, dominated by *Pseudotsuga menziesii* (Mirb.) Franco (INEGI, 2000).

Sampling and processing mycorrhizas. – Thirty plants of *M. uniflora* were collected during the summers of 2012 and 2013, fifteen in each locality, where *A. religiosa* or *P. menziesii* were dominant. Each sampled plant was separated by at least 100 m from other *M. uniflora* plants; it was removed from the soil, including its root system and wrapped in aluminum foil to be stored under refrigeration (5°C). In the laboratory, the root system was washed in tap water to remove most of the organic and mineral soil, followed by distilled water under a dissecting microscope. Ten mycorrhizal root tips from each plant were selected and placed into CTAB buffer or absolute alcohol and stored at -20°C (Fig. 2).

DNA extraction, amplification and sequencing. – Genomic DNA from mycorrhizal root tips of *M. uniflora* was extracted using the REDExtract-N-Amp Plant PCR kit (Sigma-Aldrich, St. Louis, Missouri, USA) with some modifications: individual 2-3 mm root tips were added to 20 µl of extraction solution and heated at 65°C for 10 min, followed by 95°C for 10 min. After incubation, 30 µl of dilution solution was added, and DNA extracts were stored at -20°C. The ITS region was

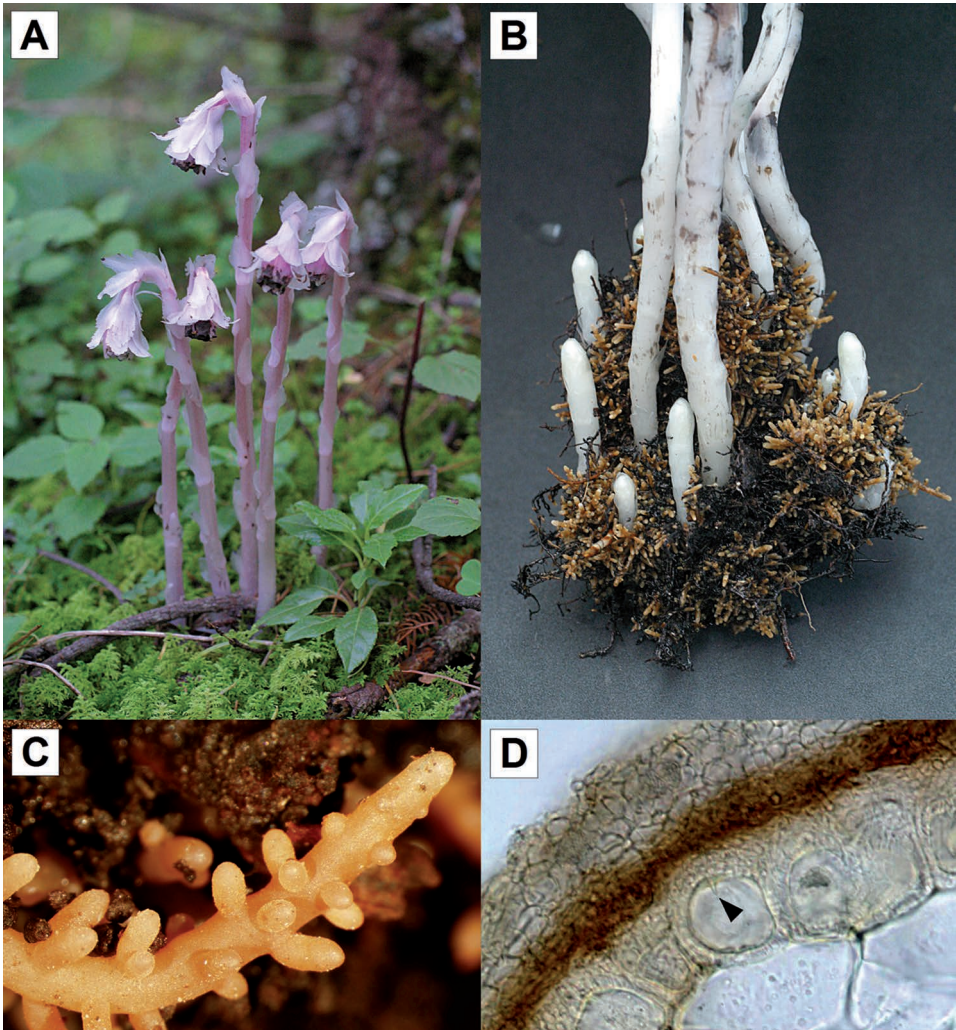


Fig. 2. *Monotropa uniflora*. **A.** Mature plants in the field from La Malinche volcano. **B.** Root system showing the typical cluster of mycorrhizas. **C.** Individual mycorrhizal root tip. **D.** Transversal section of a mycorrhiza showing a pseudoparenchymatous mantle, brown tannin layer, Hartig net surrounding epidermal cells, and the hyphal peg (arrow head) penetrating an epidermal cell.

amplified and sequenced, using primers ITS1-F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993). PCR reaction mixture contained 7.2 μl of sterile bidistilled water, 10 μl REDExtract-N-Amp Ready Mix, 0.4 μl of each primer, and 2 μl of extracted DNA. PCR were conducted following kit instructions. PCR products were purified using 1 μl of ExoSAP-IT (Affimetrix Inc. USB Products, Cleveland, Ohio, USA), 1 μl PCR-grade water, and 3.5 μl of PCR product per sample. The combination was heated to 37°C for 47 min, followed by 80°C for 15 min. Sanger sequencing was performed in an Applied Biosystems 3500XL Genetic Analyzer, with the BigDye Sequencing kit at the Laboratorio de Secuenciación Genómica de la Biodiversidad

Table 1. Species/clades and GenBank/Unite accession numbers of ITS sequences used in the phylogenetic analysis.

INGROUP: Russulaceae		
<i>Gloeocystidiellum</i>		
<i>aculeatum</i>	AY061739	China (CN)
<i>convolvens</i>	UDB016315	Estonia (EE)
<i>Gymnomyces</i>		
<i>brunnescens</i>	AY239327	United States (US)
<i>fallax</i>	AY239329	United States (US)
<i>fallax</i>	AY239349	United States (US)
<i>fallax</i>	KC152107	Mexico (MX)
<i>fragrans</i>	AY239331	United States (US)
<i>parksii</i>	AY239335	United States (US)
<i>subfulvus</i>	AY239321	United States (US)
<i>Lactarius</i>		
<i>aspideoides</i>	KJ705212	Canada (CA)
<i>atromarginatus</i>	JQ446124	Japan (JP)
<i>atroviridis</i>	KF133270	United States (US)
<i>camphoratus</i>	UDB000326	Germany (DE)
<i>luteolus</i> cf.	EU819481	United States (US)
<i>chrysorrhoeus</i>	UDB015752	Estonia (EE)
<i>decipiens</i>	KF432973	Italy (IT)
<i>deliciosus</i>	UDB002381	United Kingdom (UK)
<i>fallax</i>	JQ446103	United States (US)
<i>fuliginosus</i>	UDB000388	Germany (DE)
<i>hysginus</i>	UDB000825	Sweden (SE)
<i>illyricus</i>	JF908315	Italy (IT)
<i>imperceptus</i>	EU819485	United States (US)
<i>imperceptus</i>	JQ272401	United States (US)
<i>lignyotus</i>	UDB000390	Germany (DE)
<i>lilacinus</i>	AY606948	Germany (DE)
<i>necator</i>	UDB011671	Estonia (EE)
<i>pallidus</i>	UDB000306	Germany (DE)
<i>pubescens</i>	DQ421996	Sweden (SE)
<i>purpureus</i>	EF141541	Thailand (TH)
<i>pyrogalus</i>	UDB015966	Estonia (EE)
<i>quietus</i>	UDB011494	Estonia (EE)
<i>romagnesii</i>	DQ421989	France (FR)
<i>rufus</i>	KF241543	Norway (NO)
<i>scrobiculatus</i>	UDB011531	Estonia (EE)
<i>subdulcis</i>	UDB000042	Denmark (DK)
<i>tabidus</i>	UDB000385	Germany (DE)
<i>theiogalus</i>	AF349716	United States (US)
<i>torminosus</i>	UDB000375	Finland (FN)
<i>trivialis</i>	UDB011683	Estonia (EE)
<i>uvidus</i>	UDB015744	Estonia (EE)
<i>vietus</i>	UDB011511	Estonia (EE)
<i>zonarius</i>	EU278678	France (FR)
<i>Lactifluus</i>		
<i>annulatoangustifolius</i>	AY606981	Madagascar (MG)
<i>corrugis</i>	JN388977	United States (US)
<i>deceptivus</i>	EU598200	United States (US)
<i>edulis</i>	AY606973	Madagascar (MG)
<i>flammans</i>	HG426471	Togo (TG)
<i>gerardii</i>	EF560688	United States (US)
<i>glaucescens</i>	KF220062	France (FR)
<i>hygrophoroides</i>	HQ318285	United States (US)
<i>leae</i> [Type]	GU258241	Thailand (TH)
<i>nodosicytidiosus</i>	AY606975	Madagascar (MG)
<i>pelliculatus</i>	AY606978	Madagascar (MG)
<i>piperatus</i>	UDB011686	Estonia (EE)

Table 1. Species/clades and GenBank/Unite accession numbers of ITS sequences used in the phylogenetic analysis (*continued*)

INGROUP: Russulaceae		
<i>vellereus</i>	UDB003332	Estonia (EE)
<i>volemus</i>	UDB017983	Estonia (EE)
<i>Martellia</i>		
<i>pila</i>	AF230893	Spain (ES)
<i>pila</i>	AF230894	Spain (ES)
<i>Multifurca</i>		
<i>furcata</i>	DQ421994	
<i>ochricompacta</i>	DQ421984	United States (US)
<i>stenophylla</i> (<i>Epitype</i>)	NR120175	Australia (AU)
<i>zonaria</i>	KF432960	Thailand (TH)
<i>Russula</i>		
<i>abietina</i>	EU598179	United States (US)
<i>abietina</i>	HQ650754	United States (US)
<i>acrifolia</i>	DQ421998	Sweden (SE)
<i>acrifolia</i>	JF834363	United States (US)
<i>acrifolia</i>	KF850401	China (CN)
<i>acrifolia</i>	UDB011167	Estonia (EE)
<i>acrifolia</i>	UDB011210	Estonia (EE)
<i>acrifolia</i>	UDB015992	Estonia (EE)
<i>acrifolia</i> aff.	JF834370	United States (US)
<i>adulterina</i>	AY061651	Europe (Eu)
<i>adusta</i>	AB291768	Japan (JP)
<i>adusta</i>	AY061652	Europe (Eu)
<i>adusta</i>	JF908669	Italy (IT)
<i>adusta</i>	UDB018185	Estonia (EE)
<i>aeruginea</i>	DQ421999	Sweden (SE)
<i>albidula</i>	DQ974760	United States (US)
<i>albonigra</i>	JF834355	United States (US)
<i>albonigra</i>	KF306040	United States (US)
<i>albonigra</i>	UDB011240	Estonia (EE)
<i>albonigra</i>	UDB016040	Finland (FN)
<i>amara</i>	JF908650	Italy (IT)
<i>americana</i>	HQ604839	Canada (CA)
<i>americana</i>	KC152211	Mexico (MX)
<i>amethystina</i>	AF418640	Germany (DE)
<i>amethystina</i>	KF850402	China (CN)
<i>amethystina</i>	UDB000303	Germany (DE)
<i>amethystina</i> aff.	JF834335	United States (US)
<i>amethystina</i> aff.	JF834351	United States (US)
<i>amoenicolor</i>	AY061655	Europe (Eu)
<i>amoenipes</i>	AY061656	Europe (Eu)
<i>amoenolens</i>	AF418615	Germany (DE)
<i>amoenolens</i>	GU222264	New Zealand (NZ)
<i>amoenolens</i>	UDB000343	Germany (DE)
<i>amoenolens</i> cf.	KF245512	United States (US)
<i>anatina</i>	JF908698	Italy (IT)
<i>angustispora</i> cf.	EU598152	United States (US)
<i>anthracina</i>	JF908673	Italy (IT)
<i>anthracina</i>	UDB011194	Estonia (EE)
<i>aquosa</i>	UDB011293	Estonia (EE)
<i>archaea</i>	AY061737	Africa (Af)
<i>atroglauca</i>	UDB011109	Finland (FN)
<i>atropurpurea</i>	JF908660	Italy (IT)
<i>atrorubens</i>	UDB011173	Estonia (EE)
<i>aurantioflammans</i>	UDB011116	Finland (FN)
<i>aurantiolutea</i> cf.	MIN083-09	United States (US)
<i>aurantiolutea</i> cf.	MIN085-09	United States (US)
<i>aurata</i>	AY061659	Europe (Eu)

Table 1. Species/clades and GenBank/Unite accession numbers of ITS sequences used in the phylogenetic analysis (*continued*)

INGROUP: Russulaceae		
<i>aurea</i>	UDB011363	Estonia (EE)
<i>azurea</i>	UDB016046	Finland (FN)
<i>badia</i>	UDB016002	Estonia (EE)
<i>betularum</i>	UDB011275	Estonia (EE)
<i>bicolor</i>	KF007949	United States (US)
<i>brevipes</i>	AF349714	United States (US)
<i>brevipes</i>	EU819422	United States (US)
<i>brevipes</i>	FJ845429	Canada (CA)
<i>brevipes</i>	KF386757	United States (US)
<i>brevipes</i>	MIN117-09	United States (US)
<i>brevipes</i>	ONT118-08	Canada (CA)
<i>brevipes</i>	ONT126-08	Canada (CA)
<i>brevipes aff.</i>	JF834338	United States (US)
<i>brevipes aff.</i>	JF834353	United States (US)
<i>brevipes aff.</i>	JF834356	United States (US)
<i>brevipes aff.</i>	JF834359	United States (US)
<i>brevipes aff.</i>	JX030213	United States (US)
<i>brevipes cf.</i>	GQ166868	United States (US)
<i>brevipes cf.</i>	KC152212	Mexico (MX)
<i>brevipes cf.</i>	KF007186	Canada (CA)
<i>brevipes cf.</i>	KF007187	Canada (CA)
<i>brevipes cf.</i>	KF007188	Canada (CA)
<i>brevipes cf.</i>	KF007189	Canada (CA)
<i>brevipes cf.</i>	KF007190	Canada (CA)
<i>brunneola</i>	JF834341	United States (US)
<i>caerulea</i>	UDB000353	Germany (DE)
<i>camarophylla</i>	DQ421982	France (FR)
<i>cantharellicola</i>	KF306036	United States (US)
<i>cascadensis</i>	FJ845426	Canada (CA)
<i>cascadensis</i>	KF359616	United States (US)
<i>cerolens</i>	JN681168	United States (US)
<i>cerolens</i>	JX434672	China (CN)
<i>cerolens</i>	JX434673	Japan (JP)
<i>cerolens</i>	KF245524	Canada (CA)
<i>cessans</i>	AY061730	North America (Na)
<i>cessans</i>	FJ845437	Canada (CA)
<i>cessans</i>	UDB015971	Estonia (EE)
<i>cessans aff.</i>	JF834326	United States (US)
<i>cessans aff.</i>	JF834345	United States (US)
<i>cessans aff.</i>	JF834474	United States (US)
<i>cessans aff.</i>	JF834494	United States (US)
<i>chloroides</i>	AY061663	Europe (Eu)
<i>chloroides</i>	DQ061927	Italy (IT)
<i>chloroides</i>	DQ658888	Sweden (SE)
<i>chloroides</i>	KC581331	Canada (CA)
<i>chloroides</i>	KF432954	Ireland (IE)
<i>chloroides</i>	MRD033-08	United States (US)
<i>chloroides</i>	UDB011192	Estonia (EE)
<i>chloroides aff.</i>	JF834332	United States (US)
<i>chloroides cf.</i>	DQ422016	Sweden (SE)
<i>citrinochlora</i>	UDB016045	Norway (NO)
<i>claroflava</i>	AY061665	Europe (Eu)
<i>clavipes</i>	UDB011174	Estonia (EE)
<i>compacta</i>	EU598172	United States (US)
<i>consobrina</i>	JF908696	Italy (IT)
<i>consobrina</i>	UDB000905	Sweden (SE)
<i>crassotunicata</i>	EU597082	Canada (CA)
<i>cremeirosea</i>	EU819424	United States (US)
<i>cremeoavellanea</i>	UDB000906	Sweden (SE)
<i>cremeoavellanea</i>	UDB011366	Estonia (EE)

Table 1. Species/clades and GenBank/Unite accession numbers of ITS sequences used in the phylogenetic analysis (*continued*)

INGROUP: Russulaceae

<i>cremoricolor</i>	AJ277910	United States (US)
<i>cremoricolor</i>	KF386761	United States (US)
<i>crenulata</i>	HQ604846	Canada (CA)
<i>crustosa</i>	EU598153	United States (US)
<i>crustosa</i>	EU598193	United States (US)
<i>crustosa</i>	EU598194	United States (US)
<i>crustosa</i>	KM373243	United States (US)
<i>cuprea</i>	AY061667	Europe (Eu)
<i>cuprea</i>	KF850403	China (CN)
<i>cuprea</i>	UDB002420	United Kingdom (UK)
<i>curtipes</i>	AY061668	Europe (Eu)
<i>cyanoxantha</i>	AB451977	Thailand (TH)
<i>cyanoxantha</i>	AB458895	Thailand (TH)
<i>cyanoxantha</i>	AF345251	Thailand (TH)
<i>cyanoxantha</i>	AY606960	Germany (DE)
<i>cyanoxantha</i>	JF908664	Italy (IT)
<i>cyanoxantha</i>	JF908699	United Kingdom (UK)
<i>cyanoxantha</i>	KF002770	China (CN)
<i>cyanoxantha</i>	KF002774	China (CN)
<i>cyanoxantha</i>	KF937361	Colombia (CO)
<i>cyanoxantha</i>	KF937362	Colombia (CO)
<i>cyanoxantha</i>	KJ705232	Canada (CA)
<i>cyanoxantha</i>	UDB000329	Germany (DE)
<i>cyanoxantha</i>	UDB000330	Germany (DE)
<i>cyanoxantha</i>	UDB011230	Estonia (EE)
<i>cyanoxantha</i> cf.	EU598166	United States (US)
<i>decepiens</i>	UDB016087	Finland (FN)
<i>decolorans</i>	AF418637	Germany (DE)
<i>decolorans</i>	UDB011326	Estonia (EE)
<i>delica</i>	AF096987	Spain (ES)
<i>delica</i>	AF418605	Germany (DE)
<i>delica</i>	AY061671	Europe (Eu)
<i>delica</i>	DQ061924	Italy (IT)
<i>delica</i>	DQ061926	Italy (IT)
<i>delica</i>	GU234017	Norway (NO)
<i>delica</i>	KF432955	Belgium (BE)
<i>delica</i>	UDB001625	United Kingdom (UK)
<i>delica</i>	UDB011181	Estonia (EE)
<i>delica</i>	UDB016008	Estonia (EE)
<i>delica</i> aff.	DQ422005	Sweden (SE)
<i>delica</i> cf.	HQ445587	Norway (NO)
<i>densifolia</i>	AB291754	Japan (JP)
<i>densifolia</i>	AB291757	Japan (JP)
<i>densifolia</i>	AB291765	Japan (JP)
<i>densifolia</i>	AB291766	Japan (JP)
<i>densifolia</i>	EU526012	United States (US)
<i>densifolia</i>	FJ845430	Canada (CA)
<i>densifolia</i>	UDB000336	Germany (DE)
<i>densifolia</i>	UDB001626	United Kingdom (UK)
<i>densifolia</i>	UDB011180	Estonia (EE)
<i>densifolia</i>	UDB011237	Estonia (EE)
<i>depallens</i>	UDB015994	Estonia (EE)
<i>dissimulans</i>	EU303008	United States (US)
<i>dissimulans</i>	EU526009	United States (US)
<i>dissimulans</i>	HQ650756	Canada (CA)
<i>drimeia</i>	AY061672	Europe (Eu)
<i>dryadicola</i>	JF908710	Italy (IT)
<i>earlei</i>	DQ422025	United States (US)
<i>eccentrica</i>	EU598197	United States (US)
<i>eccentrica</i>	KC699767	South Korea (KR)

Table 1. Species/clades and GenBank/Unite accession numbers of ITS sequences used in the phylogenetic analysis (*continued*)

INGROUP: Russulaceae		
<i>emetica</i>	AY061673	Europe (Eu)
<i>emetica</i>	UDB000300	Germany (DE)
<i>exalbicans</i>	AY061674	Europe (Eu)
<i>exalbicans</i>	UDB000348	Germany (DE)
<i>farinipes</i>	UDB002462	France (FR)
<i>faustiana</i>	JF908705	Italy (IT)
<i>fellea</i>	AM113957	Denmark (DK)
<i>fellea</i>	UDB000314	Germany (DE)
<i>firmula</i>	UDB016073	Estonia (EE)
<i>fistulosa</i>	DQ421985	Africa (Af)
<i>flaviceps</i>	JF834371	United States (US)
<i>flaviscicans</i> cf.	EU598162	United States (US)
<i>foetens</i>	AY061677	Europe (Eu)
<i>foetens</i>	KF245528	United Kingdom (UK)
<i>foetens</i>	UDB000061	Denmark (DK)
<i>font-queri</i>	UDB015978	Estonia (EE)
<i>fragilis</i>	KC581327	Canada (CA)
<i>fragilis</i>	UDB018434	Sweden (SE)
<i>fuegiana</i>	ARG016-08	Argentina (AR)
<i>gilva</i>	KF002762	China (CN)
<i>globispora</i>	UDB016122	Estonia (EE)
<i>gracilis</i>	FJ845431	Canada (CA)
<i>granulata</i>	EU598192	United States (US)
<i>grata</i>	UDB000004	Denmark (DK)
<i>grisea</i>	AY061679	Europe (Eu)
<i>grisea</i>	DQ422030	Sweden (SE)
<i>griseascens</i>	UDB016038	Finland (FN)
<i>griseobrunnea</i>	GU222265	New Zealand (NZ)
<i>heterophylla</i>	DQ422006	Sweden (SE)
<i>heterophylla</i>	UDB000909	Sweden (SE)
<i>ilicis</i>	AY061682	Europe (Eu)
<i>illota</i>	DQ422024	Sweden (SE)
<i>illota</i>	KF245508	France (FR)
<i>innocua</i>	UDB015976	Estonia (EE)
<i>integra</i>	AF230896	Spain (ES)
<i>integra</i>	AY061683	Europe (Eu)
<i>integra</i>	UDB000358	Germany (DE)
<i>integra</i> aff.	JF834336	United States (US)
<i>integriformis</i>	AY061684	Europe (Eu)
<i>integriformis</i>	KF850411	China (CN)
<i>integriformis</i>	UDB016042	Finland (FN)
<i>laccata</i>	UDB016024	Finland (FN)
<i>laeta</i>	UDB016039	Finland (FN)
<i>laricina</i>	AY061685	Europe (Eu)
<i>laricina</i>	JN944008	Italy (IT)
<i>laricina</i>	KF850405	China (CN)
<i>laurocerasi</i>	AY061735	North America (Na)
<i>laurocerasi</i>	EU598184	United States (US)
<i>laurocerasi</i>	UDB000344	Germany (DE)
<i>laurocerasi</i> cf.	KF245507	United States (US)
<i>lepida</i>	DQ422013	Belgium (BE)
<i>lilacea</i>	JN944005	Slovakia (SK)
<i>littoralis</i>	AY061702	Europe (Eu)
<i>livescens</i>	UDB000894	Germany (DE)
<i>lutea</i>	HQ604848	Canada (CA)
<i>lutea</i>	UDB000912	Sweden (SE)
<i>lutea</i>	UDB011163	Estonia (EE)
<i>luteotacta</i>	UDB000913	Sweden (SE)
<i>luteotacta</i>	UDB011166	Estonia (EE)
<i>maculata</i>	AY061688	Europe (Eu)

Table 1. Species/clades and GenBank/Unite accession numbers of ITS sequences used in the phylogenetic analysis (*continued*)

INGROUP: Russulaceae		
<i>maculata</i>	UDB002498	Denmark (DK)
<i>maculata</i>	UDB002499	Belgium (BE)
<i>mairei</i>	UDB000112	Denmark (DK)
<i>mariae</i>	EU598199	United States (US)
<i>medullata</i>	UDB016041	Finland (FN)
<i>melliolens</i>	AY061690	North America (Na)
<i>melzeri</i>	AY061691	Europe (Eu)
<i>messapica</i>	AY061692	Europe (Eu)
<i>metachromatica</i>	JQ902096	Guyana (GU)
<i>metachromatica</i>	KC155393	Guyana (GU)
<i>multicolor</i>	JF908657	Italy (IT)
<i>murrillii</i>	HQ604850	Canada (CA)
<i>mustelina</i>	AY061693	Europe (Eu)
<i>mutabilis</i>	KF810137	United States (US)
<i>nana</i>	AY061694	Europe (Eu)
<i>nana</i>	UDB015077	Estonia (EE)
<i>nauseosa</i>	JF908642	Italy (IT)
<i>nauseosa</i>	UDB001716	United Kingdom (UK)
<i>nauseosa</i>	UDB011164	Estonia (EE)
<i>nauseosa</i>	UDB011329	Estonia (EE)
<i>nauseosa</i>	UDB011333	Estonia (EE)
<i>nigrescentipes</i> cf.	EU819423	United States (US)
<i>nigricans</i>	AM113962	Denmark (DK)
<i>nigricans</i>	DQ367915	Canada (CA)
<i>nigricans</i>	EU819428	United States (US)
<i>nigricans</i>	JF834331	United States (US)
<i>nigricans</i>	JQ711972	Canada (CA)
<i>nigricans</i>	UDB002421	United Kingdom (UK)
<i>nitida</i>	UDB011221	Estonia (EE)
<i>occidentalis</i>	FJ845436	Canada (CA)
<i>ochroleuca</i>	UDB016009	Estonia (EE)
<i>ochrospora</i>	DQ422012	Italy (IT)
<i>odorata</i>	AY061698	Europe (Eu)
<i>olivacea</i>	AF418634	Germany (DE)
<i>olivacea</i>	AF418635	Germany (DE)
<i>olivacea</i>	AY061699	Europe (Eu)
<i>olivacea</i>	JX425373	China (CN)
<i>olivacea</i>	UDB000322	Germany (DE)
<i>olivacea</i>	UDB011195	Estonia (EE)
<i>olivacea</i> aff.	EU284012	United States (US)
<i>olivacea</i> aff.	EU284013	United States (US)
<i>olivacea</i> aff.	EU284014	United States (US)
<i>olivina</i>	UDB016260	Finland (FN)
<i>olivina</i> (Paratype)	UDB011114	Finland (FN)
<i>olivobrunnea</i>	UDB016034	Finland (FN)
<i>olivobrunnea</i> (Holotype)	UDB011113	Finland (FN)
<i>pallescens</i>	DQ421987	Sweden (SE)
<i>pallidospora</i>	UDB016027	Finland (FN)
<i>paludosa</i>	AY061703	Europe (Eu)
<i>paludosa</i>	UDB017996	Estonia (EE)
<i>parazurea</i>	UDB000342	Germany (DE)
<i>pascua</i>	UDB017604	Norway (NO)
<i>peckii</i>	EU598174	United States (US)
<i>pectinata</i>	AY061706	Europe (Eu)
<i>pectinatoides</i>	DQ422026	Sweden (SE)
<i>pectinatoides</i>	EU819493	United States (US)
<i>pectinatoides</i>	KF245518	United States (US)
<i>pelargonia</i>	UDB011246	Estonia (EE)
<i>persicina</i>	DQ422019	Sweden (SE)
<i>persicina</i>	UDB015984	Estonia (EE)

Table 1. Species/clades and GenBank/Unite accession numbers of ITS sequences used in the phylogenetic analysis (*continued*)

INGROUP: Russulaceae		
<i>polyphylla</i>	DQ422027	United States (US)
<i>postiana</i>	AF230898	Spain (ES)
<i>postiana</i>	UDB000897	Sweden (SE)
<i>praetervisa</i>	KF245531	United Kingdom (UK)
<i>praetervisa</i>	KJ834614	KJ834614 (IT)
<i>pseudoareolata</i>	JX178488	New Zealand (NZ)
<i>pseudocarmesina</i>	JQ902076	Madagascar (MG)
<i>pseudointegra</i>	AY061708	Europe (Eu)
<i>puellaris</i>	UDB000009	Denmark (DK)
<i>puellula</i>	AY061710	Europe (Eu)
<i>puiggarii</i>	AY667425	Ecuador (EC)
<i>pulverulenta</i>	EU598186	United States (US)
<i>pumicoidea</i>	EU019931	Australia (AU)
<i>queletii</i>	UDB016001	Estonia (EE)
<i>raoultii</i>	AF418621	Germany (DE)
<i>renidens</i>	UDB011117	Finland (FN)
<i>risigallina</i>	AY061713	Europe (Eu)
<i>risigallina</i>	DQ422022	Sweden (SE)
<i>risigallina</i>	UDB015998	Estonia (EE)
<i>rivulicola (Isotype)</i>	UDB011124	Finland (FN)
<i>romellii</i>	AY061714	Europe (Eu)
<i>roseipes</i>	AY061716	Europe (Eu)
<i>roseipes</i>	JF908692	Italy (IT)
<i>roseipes</i>	KF850413	China (CN)
<i>roseipes</i>	UDB015972	Estonia (EE)
<i>roseipes</i>	UDB016003	Estonia (EE)
<i>roseoalba</i>	JQ902079	Cameroon (CM)
<i>rubellipes</i>	EU598175	United States (US)
<i>rubra</i>	AY061717	Europe (Eu)
<i>sanguinea</i>	JQ888200	United Kingdom (UK)
<i>sanguinea</i>	UDB000899	Sweden (SE)
<i>sanguinea aff.</i>	EU248591	United States (US)
<i>sardonica</i>	JQ888201	United Kingdom (UK)
<i>sichuanensis</i>	KM596859	Pakistan (PK)
<i>sichuanensis</i>	KM596866	Pakistan (PK)
<i>silvestris</i>	UDB002388	United Kingdom (UK)
<i>silvicola</i>	JF899570	Canada (CA)
<i>sinuata</i>	EU019943	Australia (AU)
<i>solaris</i>	UDB016032	Finland (FN)
<i>sororia</i>	AB211275	Japan (JP)
<i>sphagnophila</i>	AY061719	Europe (Eu)
<i>sphagnophila</i>	UDB011311	Estonia (EE)
<i>subfoetens</i>	UDB016206	Finland (FN)
<i>subfoetens cf.</i>	KF245499	United States (US)
<i>subfoetens cf.</i>	KF245500	United States (US)
<i>subfoetens cf.</i>	KF245516	United States (US)
<i>subnigricans</i>	AB291741	Japan (JP)
<i>subnigricans</i>	AB291750	Japan (JP)
<i>subnigricans</i>	EF534351	China (CN)
<i>subrubens</i>	UDB002453	Norway (NO)
<i>subrubescens</i>	KF810136	United States (US)
<i>subsulphurea</i>	KF810135	United States (US)
<i>subtilis</i>	GQ166871	United States (US)
<i>tenuiceps</i>	DQ974756	United States (US)
<i>torulosa</i>	UDB011110	Finland (FN)
<i>turci</i>	AY061720	Europe (Eu)
<i>turci</i>	EF530935	Canada (CA)
<i>turci</i>	JQ711968	Canada (CA)
<i>turci</i>	JQ711999	Canada (CA)
<i>turci</i>	KF002747	China (CN)

Table 1. Species/clades and GenBank/Unite accession numbers of ITS sequences used in the phylogenetic analysis (*continued*)

INGROUP: Russulaceae		
<i>turci</i>	KF002780	China (CN)
<i>turci</i>	KF007951	United States (US)
<i>turci</i>	KM069449	China (CN)
<i>turci</i>	UDB016082	Finland (FN)
<i>turci</i> aff.	JF834337	United States (US)
<i>variata</i>	EU819436	United States (US)
<i>variata</i>	KJ705233	Canada (CA)
<i>variata</i> cf.	EU598151	United States (US)
<i>velenovskiyi</i>	JX907817	Latvia (LV)
<i>velenovskiyi</i>	UDB011282	Estonia (EE)
<i>velutipes</i>	UDB016086	Finland (FN)
<i>ventricosipes</i> cf.	EU598187	United States (US)
<i>vesca</i>	DQ422018	Sweden (SE)
<i>vesca</i>	UDB000012	Denmark (DK)
<i>veternosa</i>	UDB000304	Germany (DE)
<i>vinosa</i>	UDB000350	Germany (DE)
<i>vinososordida</i> (type)	UDB011115	Finland (FN)
<i>violacea</i>	AY061725	Europe (Eu)
<i>violaceoincarnata</i>	UDB002549	Finland (FN)
<i>violeipes</i>	LC008521	Thailand (TH)
<i>violeipes</i>	UDB000116	Denmark (DK)
<i>virescens</i>	DQ422014	Belgium (BE)
<i>virescens</i>	UDB000117	Denmark (DK)
<i>viscida</i>	UDB002547	Denmark (DK)
<i>wernerii</i>	DQ422021	Europe (Eu)
<i>westii</i>	KF810121	United States (US)
<i>xerampelina</i>	AY061734	Europe (Eu)
<i>xerampelina</i>	UDB002526	Germany (DE)
<i>xerampelina</i> aff.	JF834361	United States (US)
<i>sp.</i>	KF002758	China (CN)
<i>sp.</i>	KF002765	China (CN)
<i>sp.</i>	KJ834628	United States (US)
<i>sp.</i>	KM219984	China (CN)
<i>sp.</i>	UDB011033	Gabon (GA)
<i>sp.</i>	UDB013973	Cameroon (CM)
<i>sp.</i> (ECM <i>Larix decidua</i>)	HM044548	Italy (IT)
<i>sp.</i> (ECM <i>Lithocarpus densiflorus</i>)	DQ273398	United States (US)
<i>sp.</i> (ECM <i>Nothofagus alpine</i>)	JX316395	Argentina (AR)
OUTGROUP		
<i>Albatrellus</i> <i>ovinus</i>	KC152062	Mexico (MX)
<i>Auriscalpium</i> <i>vulgare</i>	AY513133	Sweden (SE)
<i>Bondarzewia</i> <i>montana</i>	DQ200923	?
<i>Gloeocystidiellum</i> <i>kenyense</i>	FR878082	Portugal (PT)
<i>porosum</i>	KJ140688	United States (US)
<i>Hericium</i> <i>coralloides</i>	UDB015380	Estonia (EE)
<i>Stereum</i> <i>hirsutum</i>	AY854063	?

Table 2. GenBank numbers and origin for *Russula* samples recovered in association with *Monotropia uniflora*. Lines in bold apply to newly generated sequences for this study.

<i>GenBank</i>	<i>Sample</i>	<i>Origin</i>
AF311975	S123	Canada (CA)
AF311976	S132	Canada (CA)
AF311977	S144	Canada (CA)
AF311978	S323	Canada (CA)
AF349708	MGI5	Japan (JP)
AF349709	2371	United States (US)
AF349710	NC2172	United States (US)
AF349711	VT2364	United States (US)
AF349712	NS2087	Canada (CA)
AF349713	VT2408	United States (US)
AF349715	2344	United States (US)
AF349717	VT2407	United States (US)
AY878655	2184	United States (US)
AY878656	2052	United States (US)
AY878657	2190	United States (US)
AY878658	4152	United States (US)
AY878659	4181	United States (US)
AY878660	4939	United States (US)
AY878661	4034	United States (US)
DQ777969	A	United States (US)
DQ777970	B	United States (US)
DQ777971	C	United States (US)
DQ777972	F	United States (US)
DQ777973	G	United States (US)
DQ777974	J	United States (US)
DQ777975	K	United States (US)
DQ777976	L	United States (US)
DQ777977	M	United States (US)
DQ777978	S	United States (US)
DQ777979	U	United States (US)
DQ777980	W	United States (US)
DQ777983	O	United States (US)
DQ777984	P	United States (US)
DQ777985	R	United States (US)
DQ777986	X	United States (US)
DQ777990	JJ	United States (US)
DQ777991	KK	United States (US)
DQ777999	E	United States (US)
DQ778000	D	United States (US)
JQ396439	01-2A	China (CN)
JQ396440	02A	China (CN)
JQ396444	08-1A	China (CN)
JQ396496	63-2A	China (CN)
JQ396499	66-2A	China (CN)
JQ396502	70-2A	China (CN)
JQ396503	71A	China (CN)
JQ396510	86-2A	China (CN)
JQ396511	87-1A	China (CN)
JQ396512	88-1A	China (CN)
JQ396513	89-1A	China (CN)
JQ396514	90-1A	China (CN)
JQ396515	90-1A	China (CN)
JQ396516	92-1A	China (CN)
JQ396517	93-2A	China (CN)
JQ396518	94-1A	China (CN)
JQ396519	96-2A	China (CN)
KP780993	LM12	Mexico (MX)
KP780994	LM13	Mexico (MX)
KP780995	LM08	Mexico (MX)
KP780996	LM11	Mexico (MX)

Table 2. GenBank numbers and origin for *Russula* samples recovered in association with *Monotropia uniflora*. Lines in bold apply to newly generated sequences for this study (*continued*)

<i>GenBank</i>	<i>Sample</i>	<i>Origin</i>
KP780997	LM15	Mexico (MX)
KP780998	LM06	Mexico (MX)
KP780999	LM10	Mexico (MX)
KP781000	LM07	Mexico (MX)
KP781001	LM04	Mexico (MX)
KP781002	LM05	Mexico (MX)
KP781003	LM14	Mexico (MX)
KP781004	ST15	Mexico (MX)
KP781005	LM09	Mexico (MX)
KP781006	ST11	Mexico (MX)
KP781007	LM02	Mexico (MX)
KP781008	ST13	Mexico (MX)
KP781009	ST14	Mexico (MX)
KP781010	LM03	Mexico (MX)
KP781011	ST12	Mexico (MX)
KP781012	ST09	Mexico (MX)
KP781013	ST07	Mexico (MX)
KP781014	ST08	Mexico (MX)
KP781015	ST06	Mexico (MX)
KP781016	ST05	Mexico (MX)
KP781017	ST01	Mexico (MX)
KP781018	ST02	Mexico (MX)
KP781019	ST10	Mexico (MX)
KP781020	LM01	Mexico (MX)
KP781021	ST04	Mexico (MX)
KP781022	ST03	Mexico (MX)

y la Salud of the Instituto de Biología, Universidad Nacional Autónoma de México. Sequences were assembled and edited with the software Geneious 8.1 (<http://www.geneious.com>, Kearsse *et al.*, 2012). The 30 resulting sequences were deposited in GenBank (accession numbers: KP780993-KP781022).

Alignment and phylogenetic analysis. – Sequences were aligned using the online version of MAFFT 7.2 (Katoh & Standley, 2013), with the G-INS-i strategy, 1PAM/K=2 scoring matrix, and default values for the remaining parameters. The constructed alignments were manually edited, deleting ambiguous aligned positions and large introns (e.g., in *Russula olivacea* group). The alignment contains 540 ITS sequences, including 55 sequences (Table 2) previously reported in association with roots of *M. uniflora* (Bidartondo & Bruns, 2001, 2005; Young *et al.*, 2002; Yang & Pfister, 2006; Min *et al.*, 2012), a selection of representative sequences of the main taxonomic groups of *Lactarius* (Hesler & Smith, 1979; Heilmann-Clausen *et al.*, 1998), *Lactifluus* (Verbeken *et al.*, 2011, 2012; Stubbe *et al.*, 2012), and *Russula* (Singer, 1986; Romagnesi, 1987; Sarnari, 1998, 2005), and nine sequences of other Russulales as outgroup. Table 1 shows all sequences used in the phylogenetic analysis, with accession numbers to GenBank (Benson *et al.*, 2013), Unite (Köljalg *et al.*, 2013) or Bold Systems (Ratnasingham & Hebert, 2007) databases. The alignment is available at TreeBASE (<http://treebase.org/treebase-web/home.html>, ID: S18557). Maximum Likelihood (ML) analysis was performed in RAXML 8.1 (Stamatakis, 2014) combining a rapid bootstrapping (BS) with the ML search under the GTRGAMMA model, for 1000 replicates. Due to computational limitations a Bayesian Inference analysis was not carried out, because our alignment took more than 300 hours for 10 million generations in MrBayes (Ronquist & Huelsenbeck,

2003) and did not converge at this point (standard deviation of split frequencies > 0.05). Instead the SH-like aLRT algorithm was performed in PhyML 3 (Guidon *et al.*, 2010; Simmons & Norton, 2014). The graphical user interface SeaView 4 (Gouy *et al.*, 2010) was used, selecting the GTR model, SH-like aLRT to quantify branch support, optimizing nucleotide equilibrium frequencies, invariable sites and across site rate variation, tree searching was set as the best of NNI and SPR, and BioNJ as starting tree with optimized topology.

RESULTS

The thirty fungal sequences retrieved from mycorrhizal root tips of *M. uniflora* were all recovered within the *Russula* clade. Figs. 3 and 4 show the condensed ML trees, obtained from the ITS alignment in RAxML and PhyML analyses respectively. Both analyses differ only in the placement of some taxa as sister groups. A total of 13 species of *Russula* was detected in the two sites studied, most of them being newly reported in this association. Almost every species belonged to a separate subgeneric clade according to the performed phylogenetic analyses (Figs 3-4). From top to bottom in these phylogenies we recovered the following clades:

Clade *Integrae*. – This clade (BS=85, aLRT=0.96, Fig. 5) includes sequences of *R. integra* (L.) Fr., the type species of subg. *Polychromidia* of Romagnesi (1987) and corresponds approximately with the delimitation of subsect. *Integrinae* of Romagnesi (1967) and Bon (1988) or *Integrae* of Singer (1986) and Sarnari (2005). Other species grouped within this clade are *R. integriformis* Sarnari, *R. flaviceps* Peck and *R. laeta* Jul. Schäff. The clade contains two Mexican specimens associated with *M. uniflora* representing two different species, one from LM (LM02) as the sister species to the *R.integriformis* group, and the other from ST (ST11) recovered as the sister species to *R. integra*. They were identified as *R. aff. integriformis* and *R. aff. integra*, respectively (Table 2), but probably represent different taxa to the European species.

Clade *Olivaceinae*. – This clade (BS=99, aLRT=0.98, Fig. 6) is equivalent to the sect./subsect. *Olivaceinae* of Romagnesi (1967), Singer (1986), Bon (1988), and Sarnari (1998). It includes most of the sequences assigned as *R. olivacea* (Schaeff.) Fr. in GenBank. The specimen associated with *M. uniflora* from ST (ST10) is closely related (BS=90, aLRT=0.88) to a species from the United States identified as *R. aff. olivacea* (GenBank EU284014). Both are grouped in a well-supported subclade (BS=98, aLRT=0.98) that also includes one American, one Chinese and two European sequences, the latter three assigned to *R. olivacea*. Therefore, the Mexican specimen was identified as *R. aff. olivacea* (Table 2). However, the reference sequence for *R. olivacea* in Unite database (UDB000322, Species Hypotheses SH004639.07FU) is recovered in another clade (Fig. 6), indicating that the name *R. olivacea* is frequently applied to different species.

Clade *Laricinae*. – This well-supported clade (BS=100, aLRT=1, Fig. 7) includes sequences of *R. laricina* Velen., *R. nauseosa* (Pers.) Fr., and *R. cessans* A. Pearson. It corresponds to sect./subsect. *Laricinae* of Romagnesi (1967), Bon (1988) and Sarnari (2005). The specimen associated with *M. uniflora* in ST (ST09) is probably conspecific with two sequences assigned as *R. aff. cessans* from the United States (GenBank JF834345 and JP834494), and one as *R. abietina* Peck from Canada (GenBank HQ650754), while the sequence of the European *R. cessans*

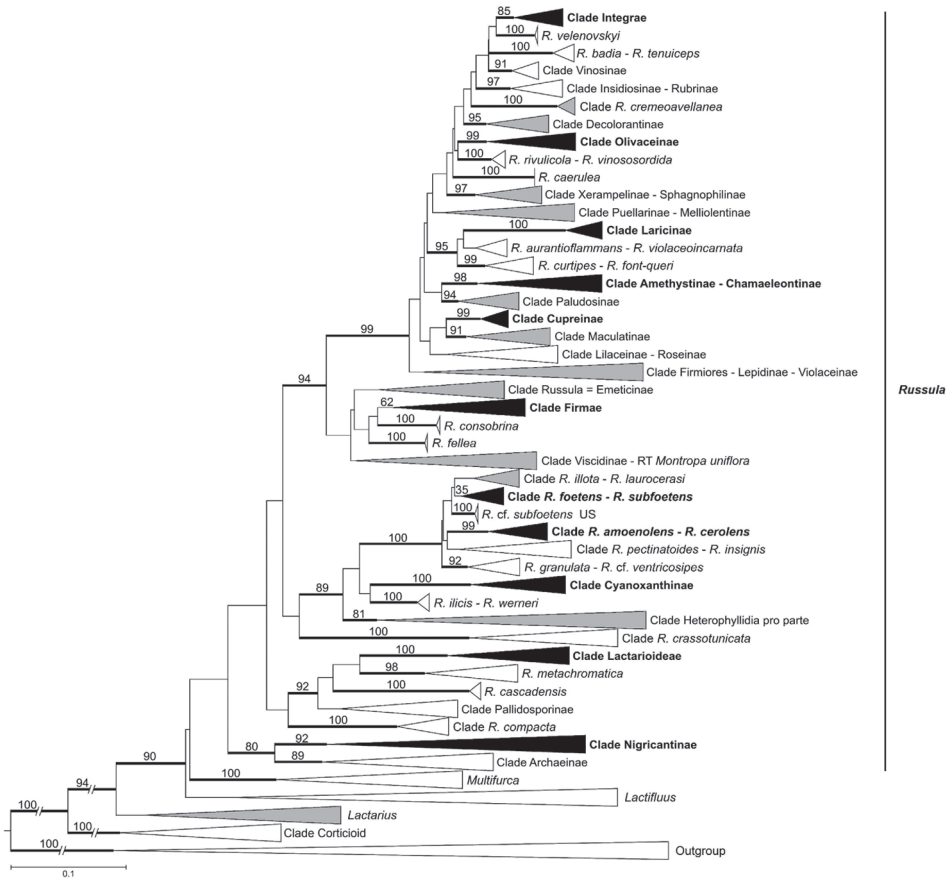


Fig. 3. Condensed RAxML tree inferred from the analysis of ITS sequences (log likelihood value = -33714.18). Branches with BS value ≥ 80 are in bold. Numbers above branches indicate BS value. Diagonal bars in branches are half of the length branch. Black truncate clades and names in bold indicate the placement of the Mexican specimens associated with *Monotropa uniflora*. Gray truncate clades indicate presence of previously recorded species in association with *M. uniflora*. Scale bar indicates nucleotide substitution per site.

(Unite UDB015971) is grouped in another subclade. The Mexican specimen was here not attributed to a particular species (Table 2) as most of the North American sequences assigned to *R. cessans* or close to this species, are clearly different from the European or Asian species concept.

Clade Cupreinae. – In this clade (BS=99, aLRT=0.98, Fig. 8) species closely related with *R. cuprea* J.E. Lange, including *R. adulterina* Secr., *R. olivina* Ruots. & Vauras, and *R. olivobrunnea* Ruots. & Vauras are grouped. In traditional classifications, *R. cuprea* and *R. adulterina* have been placed in sect./subject. *Urentinae* by Romagnesi (1967), *Urentes* by Singer (1986) and Sarnari (1998), or *Cupreinae* by Bon (1988). However, in the phylogenetic analysis, other species traditionally grouped in these sect./subject. were recovered in different clades: *R. firmula* Jul. Schäff., *R. veteriosa* Fr., and *R. badia* Quél., together with *R. rubra* (Fr.) Fr., as the clade Insidiosinae –

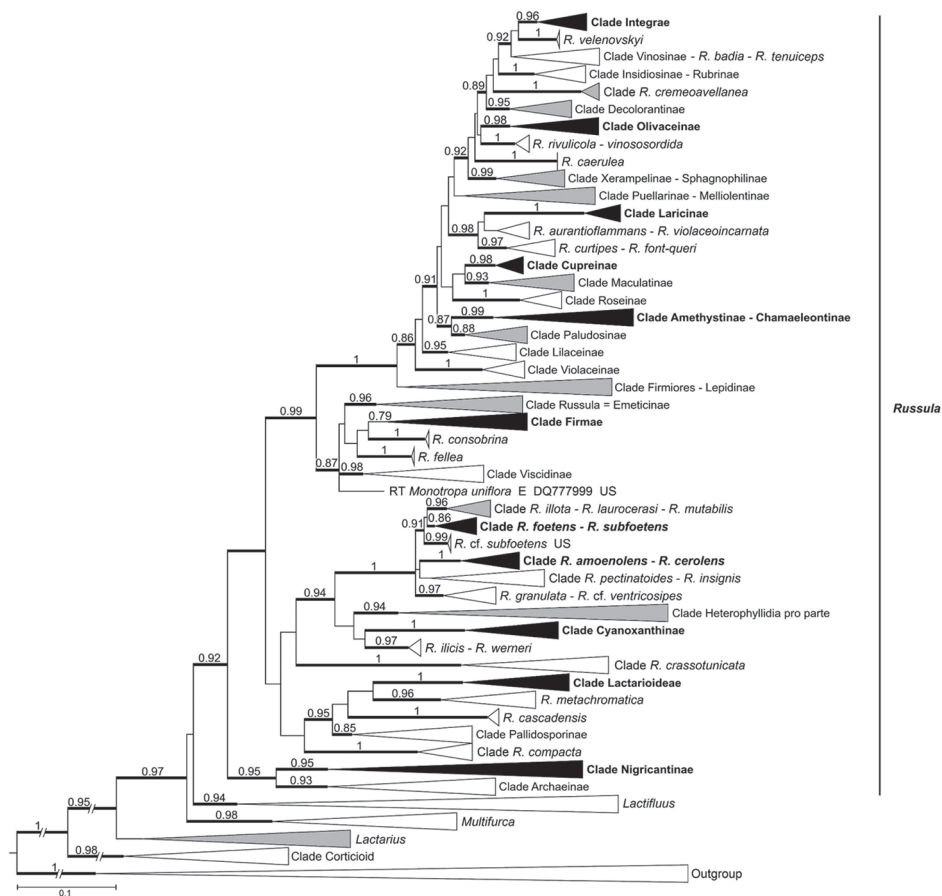


Fig. 4. Condensed PhyML tree inferred from the analysis of ITS sequences (log likelihood value = -33616.32). Branches with aLRT value ≥ 0.85 are in bold. Numbers above branches indicate aLRT value. Diagonal bars in branches are half of the length branch. Black truncate clades and names in bold indicate the placement of the Mexican specimens associated with *Monotropia uniflora*. Gray truncate clades indicate presence of previously recorded species in association with *M. uniflora*. Scale bar indicates number of nucleotide substitutions per site.

Rubrinae of Figs. 3 and 4; *R. maculata* Quél., *R. dryadicola* R. Fellner & Landa and *R. globispora* (J. Blum) Bon as the clade Maculatinae in Figs. 3, 4, and 8. *Russula olivina* was included in subsect. *Laricinae* by Ruotsalainen & Vauras (1990) and Sarnari (2005), and *R. olivobrunnea* in subsect. *Laricinae* by Ruotsalainen & Vauras (1994) but in subsect. *Integriforminae* by Sarnari (2005). Both species were recovered close to *R. cuprea* in the phylogenetic analysis. Here the name *Cupreinae* of Bon (1986, 1988) was adopted but in a different sense so as to include *R. adulterina*, *R. cuprea*, *R. olivina*, *R. olivobrunnea*, and 13 sequences closely related to *R. olivobrunnea* retrieved from Mexican *M. uniflora* roots, with 12 sequences from LM (LM04-LM15), and one from ST (ST15).

Clade Amethystinae – Chamaeleontinae. – This well-supported clade (BS=98, aLRT=0.99, Figs. 3 and 4) includes two closely related groups, corresponding

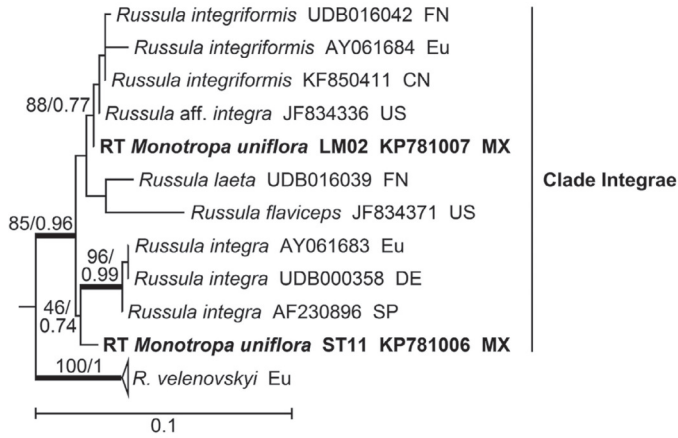


Fig. 5. Details of clade Integrae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Names of species are followed by accession number of genetic databases and its origin: CN: China, DE: Germany, ES: Spain, Eu: Europe, FN: Finland, MX: Mexico, US: United States. Names in bold are the Mexican specimens associated with *Monotropia uniflora* root tips (RT), including the sample number (prefix LM or ST). Scale bar indicates number of substitutions per site.

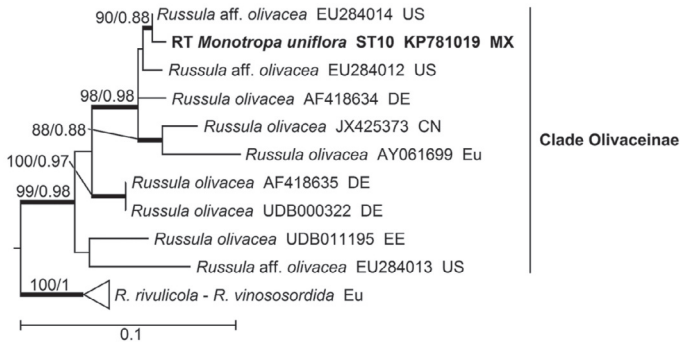


Fig. 6. Details of clade Olivaceinae in the tree obtained from the RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Names of species are followed by accession number to sequence databases and their geographical origin: CN: China, DE: Germany, EE: Estonia, Eu: Europe, MX: Mexico, US: United States. Name in bold is the Mexican specimen associated with *Monotropia uniflora* root tips (RT), including the sample number (prefix ST). Scale bar indicates number of substitutions per site.

to the sections/subsections *Amethystinae* and *Chamaeleontinae* of Romagnesi (1967), Singer (1986), Bon (1988) and Sarnari (2005). Fig. 9 shows the details of clade *Amethystinae* (BS=100, aLRT=1), where sequences of *R. amethystina* Quel., *R. murrillii* Burl., *R. roseipes* Secr. ex Bres., and *R. turci* Bres. are included. One Mexican specimen associated with *M. uniflora* in LM (LM03), was recovered as sister species of *R. roseipes*. It was identified as ‘affinis’ to this species (Table 2). On the other hand, Fig. 10 shows the clade *Chamaeleontinae* (BS=93, aLRT=0.93),

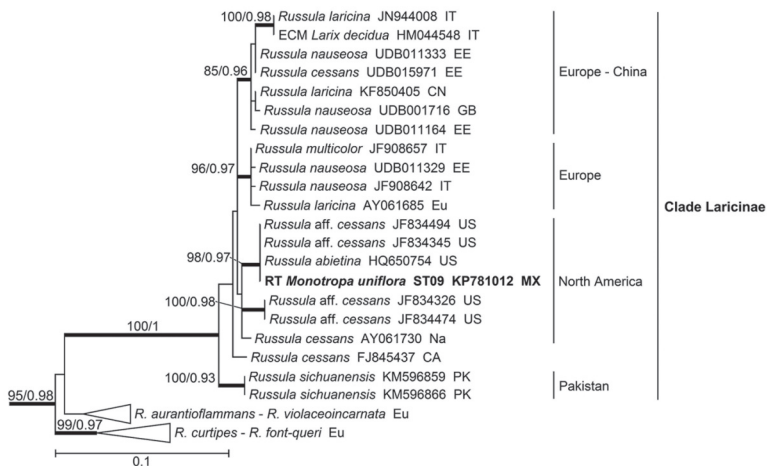


Fig. 7. Details of clade Laricinae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 85 are in bold. Numbers above branches indicate BS/aLRT values. Names of species are followed by accession number to sequence databases and their geographical origin: CA: Canada, CN: China, EE: Estonia, Eu: Europe, IT: Italy, MX: Mexico, Na: North America, PK: Pakistan, UK: United Kingdom, US: United States. Name in bold is the Mexican specimen associated with *Monotropa uniflora* root tips (RT), including the sample number (prefix ST). Scale bar indicates number of substitutions per site.

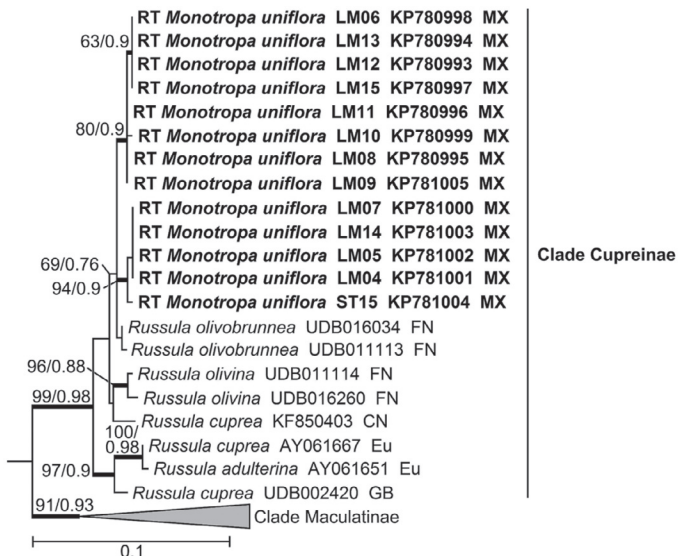


Fig. 8. Details of clade Cupreinae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 85 are in bold. Numbers above branches indicate BS/aLRT values. Gray truncate clade Maculatinae includes species previously recorded in association with *Monotropa uniflora*. Names of species are followed by accession number to sequence databases and their geographical origin: CN: China, Eu: Europe, FN: Finland, MX: Mexico, UK: United Kingdom. Names in bold are the Mexican specimens associated with *M. uniflora* root tips (RT), including the sample number (prefixes LM or ST). Scale bar indicates number of substitutions per site.

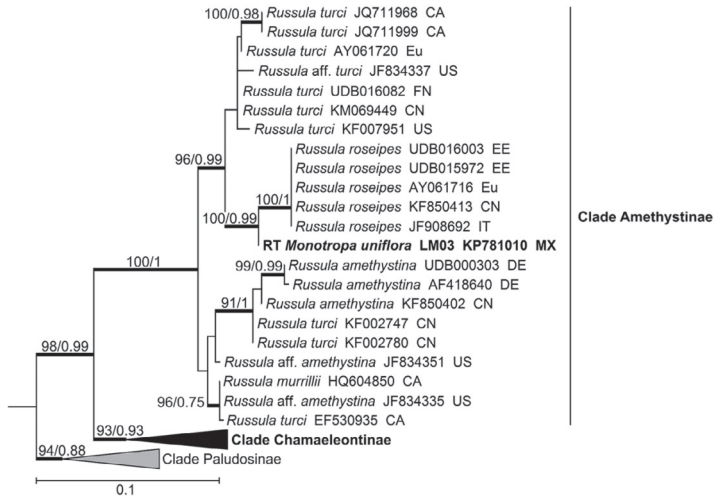


Fig. 9. Details of clade Amethystinae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 85 are in bold. Numbers above branches indicate BS/aLRT values. Black truncate clade Chamaeleontinae includes Mexican species associated with *Monotropa uniflora* and gray truncate clade Paludosinae species previously recorded in association with *M. uniflora*. Names of species are followed by accession number to sequence databases and their geographical origin: CA: Canada, CN: China, DE: Germany, EE: Estonia, Eu: Europe, FN: Finland, IT: Italy, MX: Mexico, US: United States. Name in bold is the Mexican specimen associated with *M. uniflora* root tips (RT), including the sample number (prefix LM). Scale bar indicates number of substitutions per site.

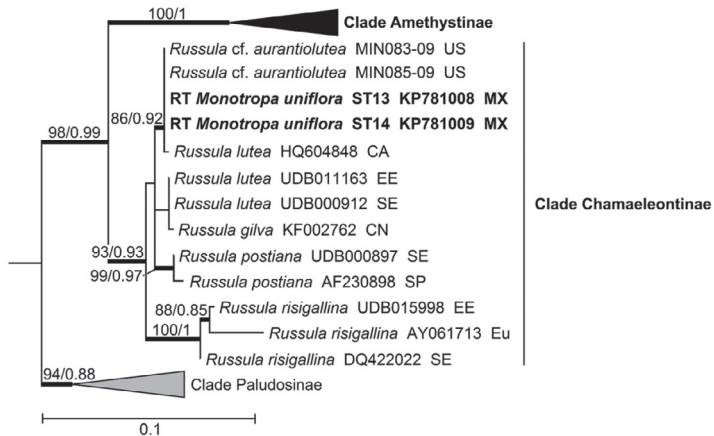


Fig. 10. Details of clade Chamaeleontinae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Black truncate clade includes Mexican species associated with *Monotropa uniflora*. Gray truncate clade includes species previously recorded in association with *M. uniflora*. Names of species are followed by accession number to sequence databases and their geographical origin: CA: Canada, CN: China, EE: Estonia, Eu: Europe, MX: Mexico, SE: Sweden, US: United States. Names in bold are the Mexican specimens associated with *M. uniflora* root tips (RT), including the sample number (prefix ST). Scale bar indicates number of substitutions per site.

where sequences of *R. cf. aurantiolutea* Kauffman, *R. gilva* Zvára, *R. lutea* (Huds.) Gray, *R. postiana* Romell, and *R. risigallina* (Batsch) Sacc. were grouped. Two Mexican sequences retrieved from *M. uniflora* roots were recovered in a subclade (BS=86, aLRT=0.92) comprising also two sequences assigned as *R. cf. aurantiolutea* from the United States (Bold Systems MIN083-09 and MIN085-09), and one as *R. utea* from Canada (GenBank HQ604848). All of them probably represent the same species. European species were recovered as independent subclades. Therefore, the Mexican species was identified as *R. aff. aurantiolutea* (Table 2).

Clade *Firmae*. – This is a not significantly supported clade (BS=62, aLRT=0.79, Fig. 11), but in previous analyses (data not shown) it was consistently recovered. It includes most of the species grouped in sect. *Firmae* by Bon (1988). The phylogenetic analysis recovered three main subclades corresponding to *Sanguineae*, *Persicinae* and *Exalbicantinae* (Fig. 11). The Mexican specimen associated with *M. uniflora* roots from ST (ST12) was included in clade *Persicinae* (BS=80, aLRT=0.95), grouping with sequences of *R. persicina* Krombh., *R. solaris* Ferd. & Winge and *R. albidula* Peck, but without being clearly more closely related to any of them. Therefore, it was identified here as '*Russula* sp.' belonging in subsect. *Persicinae* (Table 2).

Clade *Russula foetens* – *Russula subfoetens*. – Fig. 12 shows the well supported clade (BS=100, aLRT=1) that corresponds to sect. *Foetentinae* of Romagnesi (1987), or *Ingratae* of Singer (1986), Bon (1988) and Sarnari (1998). Within this clade, two Mexican sequences retrieved from *M. uniflora* roots in ST (ST07 and ST08) are closely related to a sequestrate taxon assigned to *Gymnomyces fallax* (Singer & A.H. Sm.) Trappe, T. Lebel & Castellano from the United States (GenBank AY239329) and probably represent the same species. Six other *Gymnomyces* sequences are also related including two assigned to *G. fallax*. *R. foetens* Pers. is the sister species of these sequestrate taxa. The Mexican specimens were therefore identified as *G. aff. fallax* (Table 2). On the other hand, a sequence retrieved from *M. uniflora* in Canada (GenBank AF311976) was more closely related to the European *R. subfoetens* W.G. Sm. to a sequence from the United States, as well as to the sequestrate *G. pila* (Pat.) Trappe, T. Lebel & Castellano (as *Martellia pila*, Young *et al.*, 2002).

Clade *Russula amoenolens* – *Russula cerolens*. – Within the clade (BS=100, aLRT=1) that represents section *Foetentinae* of Romagnesi (1987), or *Ingratae* of Singer (1986), Bon (1988) and Sarnari (1998), one of the best supported subclades is the group that includes sequences assigned to *R. amoenolens* Romagn., *R. pectinata* Fr., *R. cerolens* Shaffer and *R. sororia* Fr. (Fig. 13, clade *R. amoenolens* – *R. cerolens*, BS=99, aLRT=1). One Mexican sequence associated with *M. uniflora* roots from ST (ST06) is included in this subclade. Because it appears as the sister species of *R. amoenolens* – *R. pectinata* group, it was identified here as *R. aff. amoenolens* (Table 2).

Clade *Cyanoxanthinae*. – This clade (BS=100, aLRT=1, Fig. 14) corresponds to sect./subsect. *Indolentinae* of Romagnesi (1987) and Bon (1988) or *Cyanoxanthinae* of Singer (1986) and Sarnari (1998). This clade is represented by a single sequence from ST (ST05), which is closely related to a sequence from the United States assigned to *R. variata* Banning (GenBank EU819436), and another one from Canada as *R. cyanoxantha* (Schaeff.) Fr. (GenBank KJ705232). These three sequences are recovered in a well-supported subclade (BS=91, aLRT=0.94) and probably represent the same species. This subclade was recovered within a more inclusive subclade (BS=96, aLRT=0.98) containing six sequences of *R. cyanoxantha* from Europe, and two from China (Fig. 14). Therefore, the species associated with *M. uniflora* in

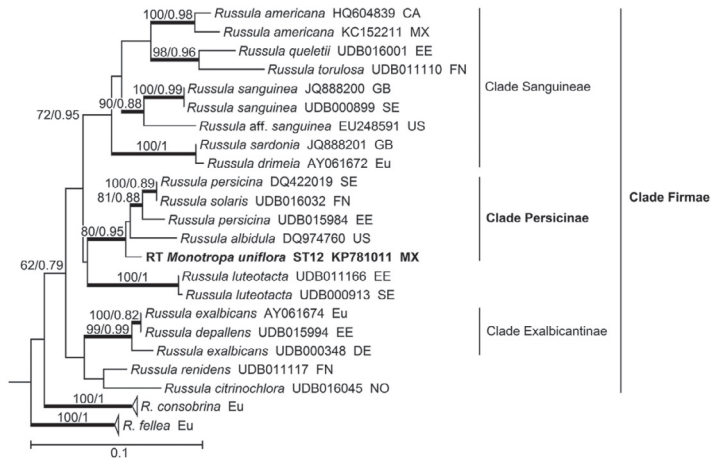


Fig. 11. Details of clade Firmae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Names of species are followed by accession number to sequence databases and their geographical origin: CA: Canada, EE: Estonia, Eu: Europe, FN: Finland, MX: Mexico, NO: Norway, SE: Sweden, UK: United Kingdom, US: United States. Name in bold is the Mexican specimens associated with *Monotropa uniflora* root tips (RT), including the sample number (prefix ST). Scale bar indicates number of substitutions per site.

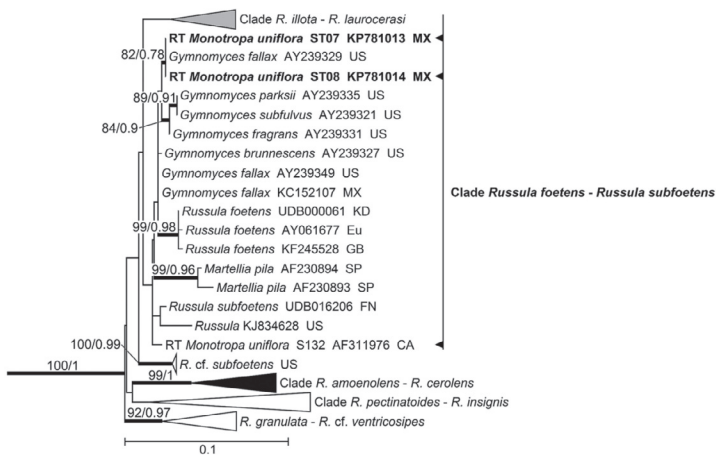


Fig. 12. Details of clade *Russula foetens* - *Russula subfoetens* in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Black truncate clade *R. amoenolens* - *R. cerolens* includes Mexican specimens to be associated with *Monotropa uniflora*. Gray truncate clade *R. illota* - *R. laurocerasi* includes species previously recorded in association with *M. uniflora*. Names of species are followed by accession number to sequence databases and their geographical origin: As: Asia, CA: Canada, CN: China, DK: Denmark, ES: Spain, Eu: Europe, FN: Finland, MX: Mexico, Na: North America, NZ: New Zealand, UK: United Kingdom, US: United States. Names in bold are the Mexican specimens associated with *M. uniflora* root tips (RT), including the sample number (prefix ST). Scale bar indicates number of substitutions per site.

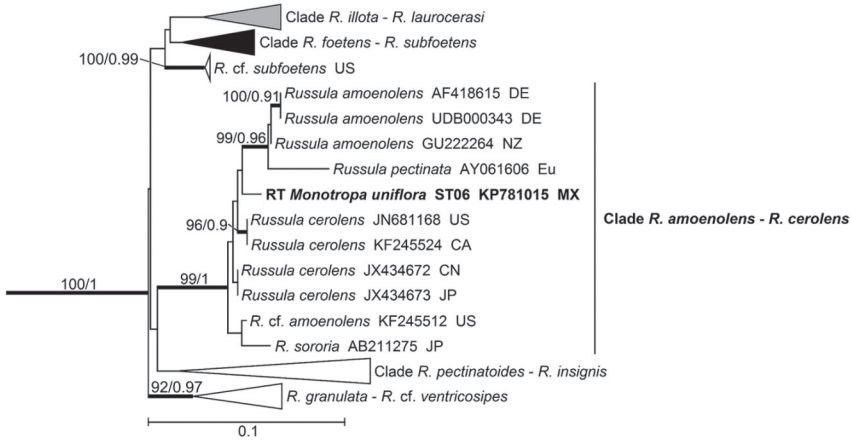


Fig. 13. Details of clade *Russula amoenolens* – *Russula cerolens* in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Black truncate clade *R. foetens* – *R. subfoetens* includes Mexican specimens associated with *Monotropa uniflora*. Gray truncate clade *R. illota* – *R. laurocerasi* includes species previously recorded in association with *M. uniflora*. Names of species are followed by accession number to sequence databases and their geographical origin: CA: Canada, CN: China, DE: Germany, Eu: Europe, JP: Japan, MX: Mexico, Na: North America, NZ: New Zealand, US: United States. Name in bold is the Mexican specimen associated with *M.uniflora* root tips (RT), including the sample number (prefix ST). Scale bar indicates number of substitutions per site.

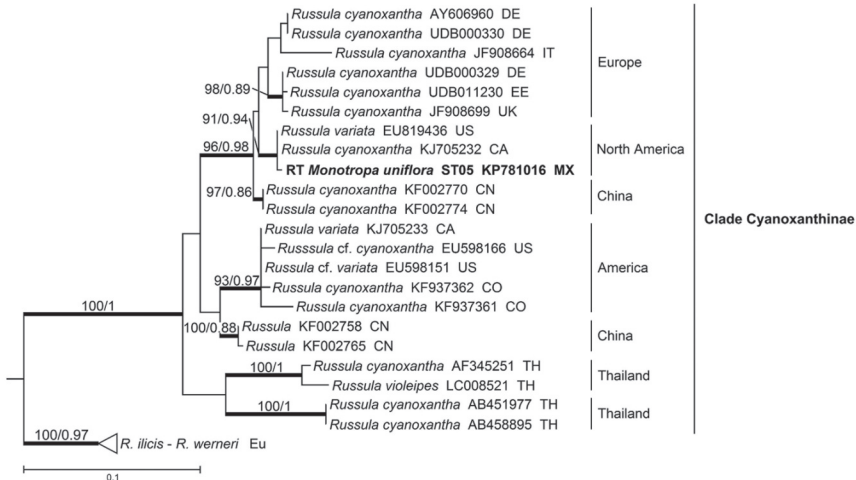


Fig. 14. Details of clade Cyanoxanthinae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Names of species are followed by accession number to sequence databases and their geographical origin: CA: Canada, CO: Colombia, CN: China, DE: Germany, EE: Estonia, Eu: Europe, IT: Italy, MX: Mexico, TH: Thailand, UK: United Kingdom, US: United States. Name in bold is the Mexican specimen associated with *Monotropa uniflora* root tips (RT), including the sample number (prefix ST). Scale bar indicates number of substitutions per site.

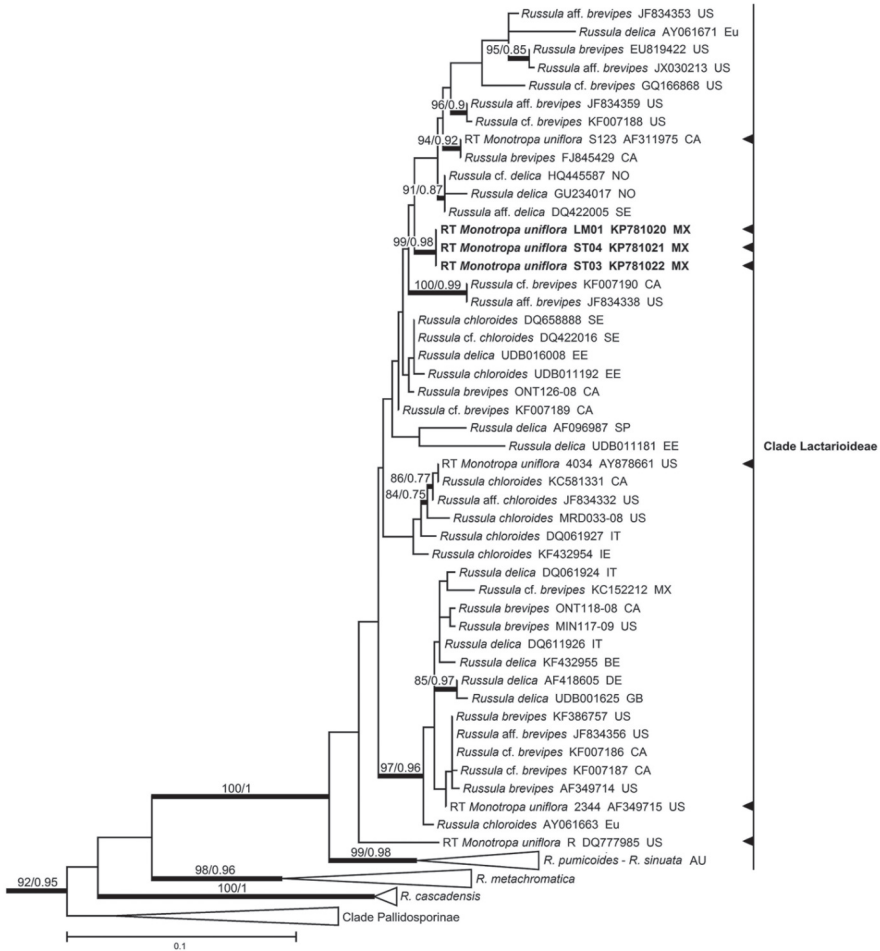


Fig. 15. Details of clade Lactarioideae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 85 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Names of species are followed by accession number to sequence databases and their geographical origin: Am: America, BE: Belgium, CA: Canada, DE: Germany, EE: Estonia, ES: Spain, Eu: Europe, GY: Guyana, IE: Ireland, IT: Italy, MX: Mexico, Na: North America, NO: Norway, SE: Sweden, UK: United Kingdom, US: United States. Names in bold are Mexican specimens associated with *Monotropa uniflora* root tips (RT), including the sample number (prefix LM or ST). Scale bar indicates number of substitutions per site.

Mexico was identified as *R. aff. cyanoxantha* (Table 2). Other sequences from North America assigned to *R. variata* or *R. cyanoxantha* grouped in a separate subclade (BS=93, aLRT=0.97), along with two Colombian sequences assigned to *R. cyanoxantha*. The latter subclade therefore appears to be exclusively American and clearly represents a different species.

Clade Lactarioideae. – This well-supported clade (BS=100, aLRT=1, Fig. 15) includes sequences assigned to *R. delica* Fr., *R. chloroides* (Krombh.) Bres. and *R. brevipes* Peck in GenBank. It corresponds to subsect. *Delicinae* of Bon

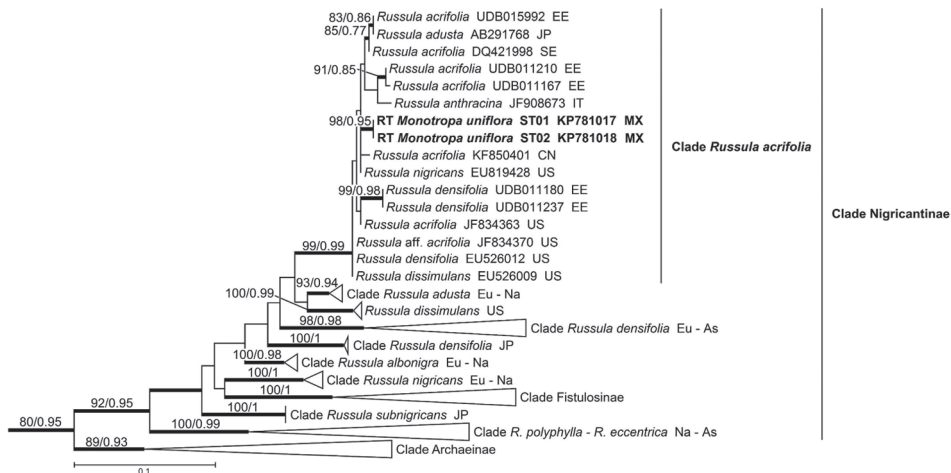


Fig. 16. Details of clade Nigricantinae in the tree obtained from RAxML and PhyML analyses. Branches with BS value ≥ 80 and aLRT value ≥ 0.85 are in bold. Numbers above branches indicate BS/aLRT values. Names of species are followed by accession number to sequence databases and their geographical origin: Af: Africa, As: Asia, CN: China, EE: Estonia, Eu: Europe, IT: Italy, JP: Japan, MX: Mexico, Na: North America, SE: Sweden, US: United States. Names in bold are Mexican specimens associated with *Monotropa uniflora* root tips (RT), including the sample number (prefix ST). Scale bar indicates number of substitutions per site.

(1988) and pale-spored species placed in sect./subsect. *Plorantinae* of Romagnesi (1967), *Plorantes* of Singer (1986) or *Lactarioides* of Sarnari (1998). This clade comprises four sequences previously reported from *M. uniflora* in North America (Bidartondo & Bruns, 2001, 2005; Young *et al.*, 2002; Yang & Pfister, 2006), as well as three new sequences from Mexico. The Mexican sequences appear to represent the same species (BS=99, aLRT=0.98), with one sequence from LM (LM01) and two from ST (ST03, ST04). The Mexican species was left unnamed because it appears to be neither closely related to any of the previously deposited sequences assigned to *R. delica*, *R. chloroides* or *R. brevipes*, nor to the four previously reported sequences associated with *M. uniflora* in the United States and Canada assigned to *R. brevipes* by Bidartondo (2005: Fig. 4). These American sequences were recovered within well-supported independent subclades in both our RAxML and PhyML analyses, and therefore likely represent different species from the Mexican samples.

Clade Nigricantinae. – This clade (BS=92, aLRT=0.95, Fig. 16) corresponds to the sect./subsect. *Nigricantinae* of Romagnesi (1967) or *Compactae* of Singer (1986), Bon (1988) and Sarnari (1998). It includes a single species associated with *M. uniflora* in Mexico and represented by two sequences from ST (ST07, ST08). These were recovered within a well-supported subclade (BS=99, aLRT=0.99) of 14 sequences, seven of which were assigned to *R. acrifolia* Romagn. (Fig. 16: clade *Russula acrifolia*). Therefore, the Mexican species was named *R. aff. acrifolia*, even though it is clear that this name appears to have been assigned to quite different species in the past.

DISCUSSION

A more precise knowledge of the Russulaceae species associated with roots of *Monotropa uniflora* is fundamental toward a better understanding of the specificity of this mycoheterotrophic symbiosis, as well as toward the importance and role of the mycorrhizal networks in temperate forest ecosystems where *Monotropa* coexists together with many ectomycorrhizal fungi and other autotrophic mycorrhizal plants. Our data join previous studies in their conclusion that the principal mycorrhizal fungi in the mycorrhizas of *Monotropa uniflora* are species of Russulaceae, but with a strong preference toward the genus *Russula* (Bidartondo & Bruns, 2001; Young *et al.*, 2002; Yang & Pfister, 2006; Min *et al.*, 2012; Lee & Eom, 2014), and that each *M. uniflora* plant is associated with a single species of Russulaceae (Bidartondo & Bruns, 2001, 2005; Young *et al.*, 2002; Yang & Pfister, 2006; Lee & Eom, 2014).

Although the purpose of this study was not to analyze the previously reported species for this association, the sequences from previous studies on *M. uniflora* [by Bidartondo and Bruns (2001, 2005), Young *et al.* (2002), Yang & Pfister (2006), and Min *et al.* (2012)] were included in our phylogenetic analysis, and 30 well-supported subclades (BS/aLRT values $\geq 95/0.95$) were recovered within the genus *Russula* (see Table 1 for details). Sequences of Lee & Eom (2014) were not included, but a performed analysis (data not shown) confirms an additional *Russula* species closely related to *R. heterophylla*, and different from the *R. heterophylla*-related species previously reported in association with *M. uniflora* by Bidartondo & Bruns (2005) or Min *et al.* (2012). Including the latter species, 44 well-supported species of *Russula* are now known to be associated with roots of *M. uniflora*.

So far, only three species (Bidartondo & Bruns, 2001; Yang & Pfister, 2006) of *Lactarius* and none of the predominantly tropical agaricoid genera *Lactifluus* and *Multifurca* have been reported to form mycorrhizas with *M. uniflora*. Min *et al.* (2012) reported on a different *Lactarius* species associated with *Monotropastrum humile*, but its ITS in GenBank (JQ396444) was mentioned as being associated with *M. uniflora* (Table 1). Also this study did not recover any *Lactarius* from the roots of the thirty *M. uniflora* plants sampled from Mexico. Beyond the clear preference of *Monotropa* for *Russula* as a mycorrhizal partner, the various *Russula* species associated with *M. uniflora* represent most of the major phylogenetic clades in the genus. Therefore, the association does not show any specificity towards a particular subgenus (Figs. 3 and 4).

Another conclusion from our study is that nearly all of the Mexican *Russula* species that associate with *Monotropa* are most closely related to North American, European or Chinese species. This is not unexpected because of the importance of coniferous hosts in the examined sites, all of which have a predominantly northern hemisphere distribution. Particularly for several of the typically conifer-associated *Russula*-groups, the closest known relatives are for the moment from Europe and China, e.g. *R. aff. roseipes* (*Amethystinae*) or *R. aff. integra* (*Integrae*). However, those species that belong to infrageneric groups that are composed of host generalists or species that are with broad-leaved hosts, have affinities that are clearly with North American species, in particular from the West Coast. The very fragmentary taxonomic knowledge of the North American *Russula* species, particularly on the West Coast (Buyck *et al.*, 2015), and the absence of type sequences for nearly all American *Russula* species from public sequence databases remain important obstacles for accurate identification of the Mexican species, although taxonomic type revisions for all of the constituent native American species of particular subsections start to

allow for a better comprehension of the native American species (e.g. Adamcik & Buyck, 2014, Buyck & Adamcik 2013). All previous studies have used the ITS region to identify which fungal species were associated with *M. uniflora*, but focused principally on the systematic position at the family or genus level. This study shows that, in the case of *Russula*, this molecular marker can already be used for a more detailed analysis at species level to provide a more accurate identification of the particular *Russula* species that are associated with *M. uniflora*, hence allowing for a better understanding of the association. Of course, we have to admit that, due to the large number of mistaken identities in public sequence databases, a correct interpretation of blast results is hardly possible without a thorough knowledge of the fungal group under investigation. The longstanding experience of the first author with the genus *Russula* was therefore an essential aspect in the identification process.

When comparing the number of samples per species (Table 2), it is clear that two patterns were observed: at the LM site few *Russula* species associated with *Monotropa* (4), one of which was clearly dominant (*R. aff. olivobrunnea*, with 11 of the 15 samples), whereas at ST more species (11) were present, all of them with nearly similar abundance (four species with two samples and seven species with one sample). Previous studies similarly found that some of the Russulaceae associated with *M. uniflora* were locally dominant, being mycorrhizal with many individual mycoheterotrophs in the same area. In British Columbia, for example, Young *et al.* (2002) retrieved a single *Russula* in 11 of the 15 plants examined (their RFLP type A; a species apparently close to *R. atroglauca* Eihell. in subsect. *Griseinae*, data not shown). In Massachusetts (United States), Yang & Pfister (2006) distinguished 20 different *Russula* phylotypes in association with *M. uniflora*, with three dominant, unique phylotypes recovered respectively from 14 plants (phylotype U; a species close to *R. cf. nigrescentipes* Peck in *Decolorantinae* of our analysis, data not shown), ten plants (phylotype J; a species in *Puellarinae-Melliolentinae* of our analysis), and eight plants (phylotype X; another species close to *R. decolorans* (Fr.) Fr. of our analysis, data not shown) from a total of 56 plants. In China, Min *et al.* (2012) recovered a close relative of *R. illota* Romagn. from 14 of the 18 sequences obtained from the mycorrhizas of *M. uniflora*. In Korea, Lee & Eom (2014) recovered a close relative of *R. heterophylla* (Fr.) Fr. from all seven plants examined. In our study, *R. aff. olivobrunnea* was present in 12 of the 15 plants sampled at LM, but in only a single plant at ST. Also, for *Monotropastrum humile* the dominance of a single *Russula* species has been recorded (Matsuda *et al.*, 2011: site C; Min *et al.*, 2012). This pattern of local dominance therefore seems to be a common phenomenon. We hypothesize that the higher diversity of *Russula* associated with *M. uniflora* in ST (Table 3) might be the consequence of the higher number of ectomycorrhizal host trees present in the collecting site. The *Pseudotsuga menziesii* forest of ST is mixed with *Abies religiosa*, *Arbutus xalapensis* Kunth, *Pinus ayacahuite* C. Ehrenb. ex Schldl., *P. hartwegii* Lindl., *P. montezumae* D. Don in Lamb., *P. patula* Schldl. & Cham., *P. teocote* Cham. & Schldl., and at least three species of *Quercus* L. (Ventura, 2009), whereas the almost pure *A. religiosa* forest in LM is occasionally mixed with widely dispersed individuals of *P. hartwegii*, *P. montezumae*, *Alnus jorullensis* Kunth and *Salix paradoxa* Kunth (López Domínguez *et al.*, 2005). Most of the previous studies did not correlate the association between *M. uniflora* and Russulaceae with the existing diversity of the autotroph ectomycorrhizal host trees in the area, an element that, in our opinion, could be primordial in the ecology and diversity of the ectomycorrhizal community. Our hypothesis seems supported by the fact that most of the *Russula* that associate with *Monotropa* in ST belong to species groups that typically never associate with conifer hosts (Table 3), but further studies

Table 3. Identification, samples, systematic position and host association of the *Russula* species associated with *Monotropa uniflora* in two localities of Tlaxcala, Mexico.

Species/Taxa	Sample		Systematic position*	Host specificity
	<i>La Malinche</i>	<i>Sierra de Tlaxco</i>		
<i>Russula</i> aff. <i>integriformis</i>	LM02		<i>Integratae</i>	<i>R. integriformis</i> s.s. is a <i>Picea</i> associate
<i>R. aff. integra</i>		ST11	<i>Integratae</i>	<i>R. integra</i> s.s. is a strict conifer associate
<i>R. aff. olivacea</i>		ST10	<i>Olivaceinae</i>	<i>R. olivacea</i> s.s. is a generalist, mainly growing with <i>Fagus</i> but more rarely also with <i>Picea</i>
<i>Russula</i> subsection <i>Laricinae</i>		ST09	<i>Laricinae</i>	All <i>Laricinae</i> are strict conifer associates
<i>R. aff. olivobrunnea</i>	LM04, LM05, LM06, LM07, LM08, LM09, LM10, LM11, LM12, LM13, LM14, LM15	ST15	<i>Cupreinae</i>	<i>Cupreinae</i> are often host generalists, but <i>R. olivobrunnea</i> s.s. is a strict <i>Picea</i> associate
<i>R. aff. roseipes</i>	LM03		<i>Amethystinae</i>	All <i>Amethystinae</i> are strict conifer associates, <i>R. roseipes</i> preferentially with <i>Pinus</i>
<i>R. aff. aurantiolutea</i>		ST13, ST14	<i>Chamaeleontinae</i>	<i>Chamaeleontinae</i> are mostly host generalists, <i>R. aurantiolutea</i> s.s. grows mainly with deciduous trees
<i>Russula</i> subsection <i>Persicinae</i>		ST12	<i>Persicinae</i>	All <i>Persicinae</i> are strict deciduous trees associates
<i>Gymnomycetes</i> aff. <i>fallax</i>		ST07, ST08	<i>Foetentinae</i>	Many <i>Foetentinae</i> are host generalists, <i>Gymnomycetes fallax</i> s.s. was originally described as a <i>Quercus</i> associate
<i>R. aff. amoenolens</i>		ST06	<i>Pectinatae</i>	Many <i>Pectinatae</i> are host generalists, <i>R. amoenolens</i> s.s. is mostly a deciduous tree associate, but grows also with conifers
<i>R. aff. cyanoxantha</i>		ST05	<i>Cyanoxanthinae</i>	All European <i>Cyanoxanthinae</i> are host generalists
<i>Russula</i> subsection <i>Lactarioideae</i>	LM01	ST04, ST04	<i>Lactarioideae</i>	<i>Lactarioideae</i> are typical host generalists
<i>R. aff. acrifolia</i>		ST01, ST02	<i>Nigrantinae</i>	Many <i>Nigrantinae</i> are host generalists, including <i>R. acrifolia</i> s.s.

* Subsection based on traditional classification of Romagnesi (1967, 1987), Singer (1986), Bon (1988), and Samari (1998, 2005).

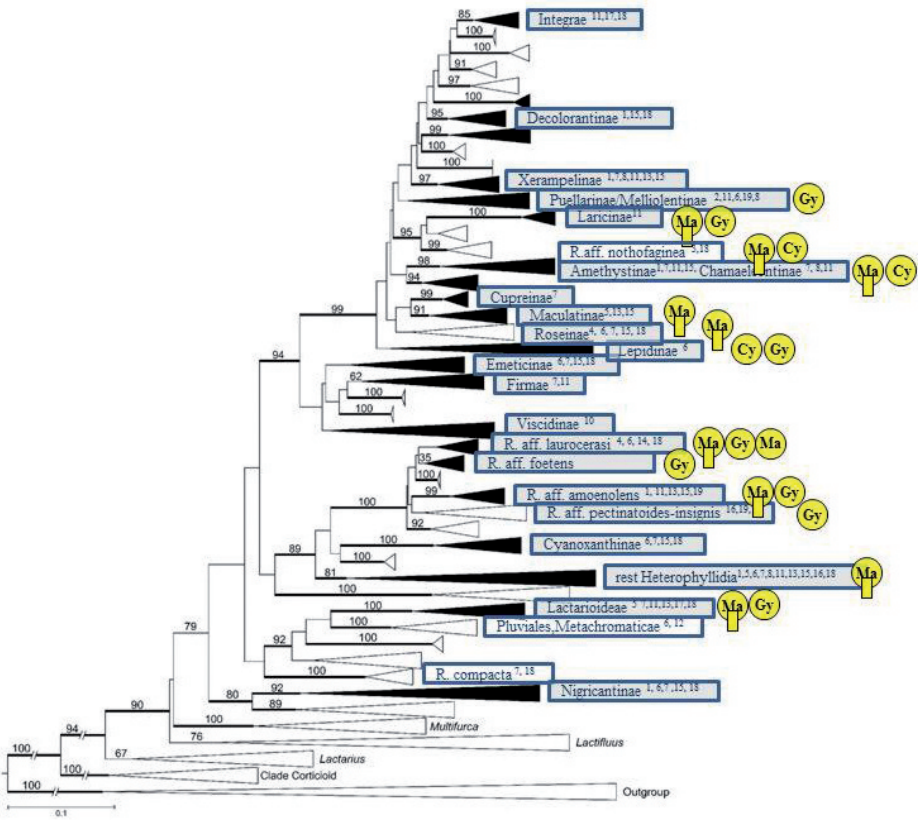


Fig. 17. Condensed RAxML tree inferred from the analysis of ITS sequences. The tree is identical to the one shown in Fig. 3, but individual names of infrageneric clades in *Russula* have been deleted, while black truncate clades now indicate the systematic placement of all presently known *Russula* that associate with *Monotropa uniflora*. Branches with BS value ≥ 80 are in bold. Numbers above branches indicate BS value. Pale gray rectangles next to phylogenetic tree clades indicate phylogenetic position of published sequences (Table 2) for *Russula* that associate with mixo- or heterotrophic orchids as deduced from the NCBI fast minimum evolution tree presentation for BLAST pairwise alignments (not shown); white rectangles indicate associations within predominantly to exclusively tropical clades. Bibliographic references for each orchid-associated clade are numbered 1 to 19: 1: Taylor & Bruns 1997; 2: Taylor & Bruns 1999 (*Corallorhiza*); 3: Bougoure & Dearnaley 2005 (*Dipodium*); 4: Dearnaley & Le Brocq 2006 (*Dipodium*); 5: Girlanda *et al.*, 2006 (*Limodorum*); 6: Roy *et al.*, 2009 (*Aphyllorchis*); 7: Okayama *et al.*, 2012 (*Lecanorchis*); 8: Motomura *et al.*, 2010 (*Cymbidium*); 9: Shefferson *et al.*, 2007 (*Cyripedium*); 10: Shefferson 2005 (is doubtful record); 11: Taylor *et al.*, 2004 (*Corallorhiza*); 12: Dearnaley 2006 (*Erythrorchis*); 13: Kennedy *et al.*, 2011 (*Hexalectris*); 14: Yamato & Iwasi 2008 (*Cephalantera*); 15: Sakamoto *et al.*, 2015 (*Cephalantera*); 16: Jacquemyn *et al.*, 2014; 17: Bidartondo *et al.*, 2008 (*Epipactys*); 18: Ogura *et al.*, 2012 (*Cymbidium*); 19: Freudenstein *et al.*, 2014 (*Corallorhiza*). Symbols on the extreme right show similarly deduced phylogenetic position for all presently deposited sequences for secotioid-gasteroid species in *Russula*, corresponding to the genera *Cystangium* (CY), *Gymnomyces* (GY), *Macowanites* (MA on a stipe) and *Martellia* (MA).

are needed to examine the influence of ectomycorrhizal host diversity on the association between mycoheterotrophs and Russulaceae.

Although we did not find any phylogenetic signal at subgenus level in the association between the major *Russula* groups and *Monotropa*, preliminary analyses of BLAST results (not shown) of species associated with mycoheterotrophic orchids show that *Monotropa* shares with these monocots nearly the exact same species groups and even some of the same *Russula* species (Fig. 17). Family Orchidaceae differs from Monotropoideae in the fact that it has a very important distribution component in tropical areas and in the southern hemisphere, where mycoheterotrophy remains largely unexplored. It can thus be expected that, compared to *Monotropa* associates, several additional, more tropical species groups in *Russula* will be involved, as suggested by some of the already identified orchid associates, e.g. the *R. compacta* clade or the *Pluviales-R. metachromatica* clade (Fig. 17).

Equally intriguing is the fact that our preliminary analysis of absolutely all of the deposited sequences of secotioid-gasteroid species in *Russula* suggests that gasteromycetation has constantly evolved in clades containing species that associate with mycoheterotrophic plants (Fig. 17). So far, *Gymnomyces* aff. *fallax* seems to be the only recovered hypogeous '*Russula*' that associates with *Monotropa*, but the phenomenon was also suggested by Okayama *et al.* (2012) for *Arcangeliella* (= *Lactarius*). Compared to mycoheterotrophy, the gasteromycetation process in *Russula* exhibits a much stronger phylogenetic signal and seems completely absent from certain of the presently recognized subgenera (Fig. 17). A more comprehensive approach of these aspects will be the subject of a forthcoming contribution.

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