

# ECTOMYCORRHIZAL SYMBIONTS OF SILVER FIR (*ABIES ALBA* MILL.) IN SLOVENIA

## EKTOMIKORIZNI SIMBIONTI NAVADNE JELKE (*ABIES ALBA* MILL.) V SLOVENIJI

Tina UNUK NAHBERGER<sup>1</sup>, Hojka KRAIGHER<sup>1</sup> & Tine GREBENC<sup>1</sup>

<http://dx.doi.org/10.3986/fbg0107>

### ABSTRACT

#### Ectomycorrhizal symbionts of silver fir in Slovenia

Ectomycorrhizal symbionts of silver fir have been in Slovenia rarely analyzed and identified, thereby little is known about their diversity and distribution across Slovenia. The aim of this study was to identify ectomycorrhizal fungal symbionts of silver fir in silver fir natural distribution area in Slovenia. Ectomycorrhizal fungi were identified based on morphological and anatomical descriptions and by ITS DNA barcoding. Altogether we identified 86 different ectomycorrhizal symbionts of silver fir, many of them were in symbiosis with silver fir, in our study identified for the first-time. The most abundant ECM fungal taxa were assigned to *Russula*, *Tomentella* and *Lactarius* genus.

*Keywords:* silver fir, ectomycorrhizal symbionts, ITS barcoding, Slovenia

### IZVLEČEK

#### Ektomikorizni simbionti navadne jelke v Sloveniji

Ektomikorizni simbionti navadne jelke v Sloveniji, so do sedaj bili le redko predmet raziskav, zato je poznavanje njihove pestrosti in razširjenosti v Sloveniji pomanjkljivo. Namen raziskave je bil identificirati glivne vrste, ki tvorijo ektomikorizno simbiozo z navadno jelko na naravnem območju razširjenosti navadne jelke v Sloveniji. Ektomikorizne simbionte smo identificirali s pomočjo morfološko-anatomskih značilnosti ter ITS DNK barkod. Skupaj smo identificirali 86 različnih ektomikoriznih glivnih vrst, pri čemer smo veliko vrst v ektomikorizni simbiozi z navadno jelko v naši študiji identificirali prvič. Med številčnejše ektomikorizne glive so se uvrstile glive iz rodov *Russula*, *Tomentella* in *Lactarius*.

*Ključne besede:* navadna jelka, ektomikorizni simbionti, ITS barkoda, Slovenija

<sup>1</sup> Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, tina.unuk@gozdis.si; hojka.kraigher@gozdis.si, tine.grebenc@gozdis.si

## 1 INTRODUCTION

Mountainous silver fir (*Abies alba* Mill.) has a central European distribution, with a continuous population in Alps and central Europe, where its distribution limits on mountainous regions of eastern, western, southern Europe (WOLF 2003). Silver fir distribution in Slovenia represents a central area of the species total areal, where it similarly grows natively in the mountainous areas. It represents 14 % of the total forest cover in Slovenia (BONČINA et al. 2002). It rarely forms pure stands, as populations usually grow mixed with European beech (*Fagus sylvatica* L.) and/or Norway spruce (*Picea abies* (L.) H. Karst.). In central European forests, silver fir has been successful tree species as it has an ability to grow in low-light conditions, thereby silver fir is favored by the selection thinning forest management system, widely applied in Slovenia. Combining this management system with a silver fir characteristics commonly results in silver fir dominated forests (KLOPČIČ & BONČINA 2011).

Silver fir is an ectomycorrhizal (ECM) tree species, forming symbiosis with ECM fungi from Ascomycota and Basidiomycota. In the past, silver fir received only little attention as ECM host tree. In most studies ECM symbionts of silver fir have been identified only based on morphological and anatomical descriptions of ECM or based on fruit bodies occurrence (AGERER 1987; BERNDT et al. 1990; COMANDINI et al. 1998; PACIONI et al. 2001; DE ROMÁN & DE MIGUEL 2005). This approach is in most cases deficient, especially at a spe-

cies-level. Only few studies were published in which authors identified ECM fungal symbionts using molecular methods (EBERHARDT et al. 2000; SCHIRKONYER et al. 2013; WAŽNY 2014; RUDAWSKA et al. 2016; WAŽNY & KOWALSKI 2017; UNUK et al. 2019; MRAK et al. 2020). ECM fungal symbionts of silver fir are also poorly represented in an online database DEEMY (<http://www.deemy.de>), where after latest update in 2019 only twelve were described for silver fir, among all 554 description available therein (AGERER & RAMBOLD 2004). In Slovenia only recently some papers emerged publishing ECM fungal symbionts diversity with silver fir (UNUK et al. 2019) impacting plant growth and vigor by influencing the quality, direction, and flow of nutrients and water between plants and fungi. Linkages of plant phenological characteristics with below-ground root-associated fungal communities have rarely been investigated, and thus our aim was to search for an interplay between contrasting phenology of host ectomycorrhizal trees from the same location and root-associated fungal communities (ectomycorrhizal, endophytic, saprotrophic and pathogenic root-associated fungi while other common tree species, such as beech and spruce were frequently studied (KRAIGHER et al., 1995, KRAIGHER 1996; AGERER et al., 1996, PUČKO et al. 2005; KRAIGHER et al. 2007; GREBENC & KRAIGHER 2007; DE GROOT et al. 2016).

The aim of this study was to identify ectomycorrhizal symbionts of silver fir in Slovenia.

## 2 METHODS

Samplings were performed in three silver fir (*Abies alba* Mill.) dominated stands at sites Jelovski boršt (45.05 N, 15.05 E), Lehen na Pohorju (46.33 N, 15.20 E) and Ljubelj (46.24 N, 14.15 E). At each site one silver fir dominating sampling plot (20 m × 20 m) was established. Inside individual site 5 adult silver fir trees, which showed no signs of illness or damages were selected for sampling. Samplings were performed once per month from March-October in year 2016 and 2017. One soil core sample (10 cm in diameter and 20 cm deep) per month was sampled for each selected silver fir tree at individual site (1 soil core × 5 silver fir trees × 3 sites), according to protocol described in KRAIGHER (1996). Roots from samples were soaked in tap water, before being washed to remove all the soil particles attached to the roots. Further, roots were separated into coarse and fine roots, according to ŽELE-

ZNİK et al. (2007, 2016). Fine roots were characterized according to the method of AGERER (AGERER 1987) until 250 root tips per individual soil core sample were characterized. Three to five root tips of each individual characterized morphotype were freeze dried and used for ITS DNA barcoding.

Total DNA from each morphotype was extracted with a DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following manufacturer's instructions. The ITS region of nuclear ribosomal DNA was amplified from isolated DNA using the fungus specific primer pair ITS1F and ITS4 (GARDES & BRUNS, 1993; WHITE et al., 1990), following the modified procedure described in Sulzbacher et al. (SULZBACHER et al. 2016) Lawrence, KS 66044-8897. *Restingomyces reticulatus* gen. et sp. nov. is a recently discovered false truffle species from Atlantic "restinga" rainforest in northeastern Brazil.

Molecular and morphological characters separate this new sequestrate species from other described taxa in the order Phallales (Phallomycetidae, Basidiomycota). PCR products were run on 1.5 % agarose gels in 0.5 x TBE buffer. Amplified DNA fragments were cut out of agarose gels and purified with innuPREP DOUBLE-pure Kit (Analytik Jena AG, Jena, Germany) following manufacturer's instructions. After the DNA fragments' purification, sequencing was performed at a commercial sequencing laboratory (Macrogen Inc., Seoul, South Korea). All morphotypes were sequenced in both directions with the primers ITS1F and ITS4 (GARDES & BRUNS, 1993; WHITE et al., 1990). The obtained sequences were processed in Geneious version 11.1.4

(<https://www.geneious.com>, (KEARSE et al. 2012). BLASTN algorithm from NCBI website (National Center for Biotechnology Information; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and from UNITE website (<https://unite.ut.ee/>, (NILSSON et al. 2019)) was used to assess the similarity of obtained ITS sequences to sequences in GenBank and UNITE public sequence databases. Sequences that remain unclassified at a kingdom or family level were discarded. Final criteria for a database match were as followed: query cover  $\geq 80$  % and sequence similarity  $> 92$  % (representing approximate cut-off value at genus level) or sequence similarity  $\geq 97$  % - 100 % (representing approximate cut-off at species level) (PORRAS-ALFARO et al. 2014; RAJA et al. 2017).

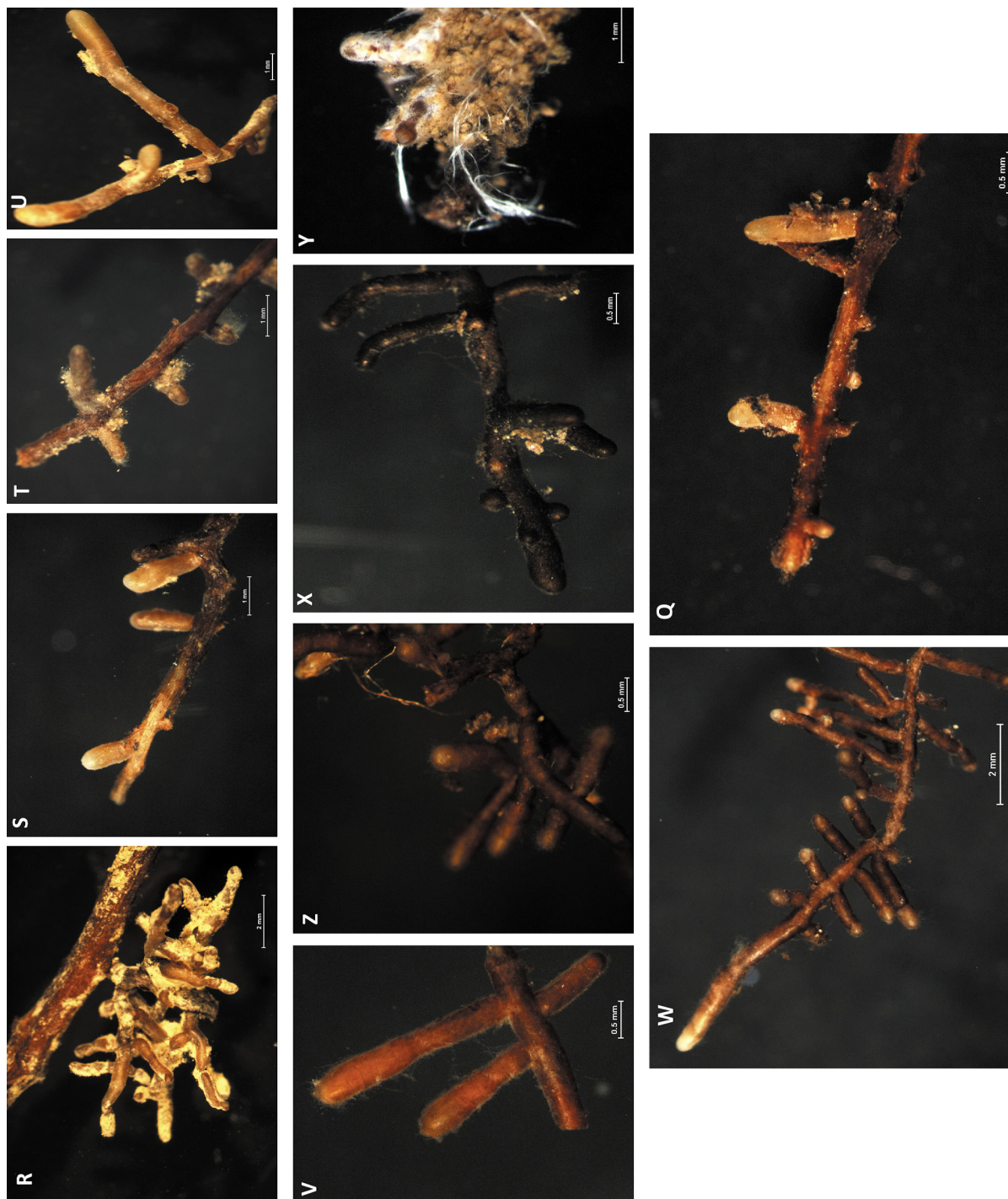
### 3 RESULTS

With the 16 sampling dates pooled together and 243 root samples, this study revealed the 86 ECM symbionts of silver fir in Slovenia, belonging to 32 different genera. Sixty-eight ECM fungal taxa were successfully amplified, sequenced, and identified using genetic tools, meanwhile 18 ECM fungal taxa were identified solely based on morphological-anatomical descriptions. Among 86 identified ECM fungal taxa, 77 (89 %) were identified to species level and 9 (11 %) to genus. In order of abundance, the most abundant ECM fungal taxa were assigned to *Russula*, *Tomentella* and *Lactarius*, with average abundance higher than 10 % per sample. More specifically, the most common ECM taxa with relative abundance higher than 1 % per sample and were in symbiosis with silver fir identified before

were *Amanita rubescens*, *Amphinema byssoides*, *Clavulina corraloides*, *Lactarius salmonicolor*, *Lactarius subdulcis*, *Lactifluus vellereus*, *Neoboletus erythropus*, *Russula chloroides*, *Russula cyanoxantha*, *Russula ochroleuca*, *Russula nigricans*, *Sebacina epigeae*, *Thelephora wakefieldiae*, *Tomentella stuposa*, *Tylospora fibrillosa* and *Xerocomellus pruinatus*. Among 86 identified ECM fungal taxa, 48 ECM taxa were identified in symbiosis with silver fir for the first time. The most abundant (with relative abundance higher than 1 % per sample) were *Elaphomyces granulatus*, *Inocybe assimilata*, *Lactarius tabidus*, *Russula delica*, *Russula heterophylla*, *Russula illota*, *Russula turci*, *Sebacina incrustans*, *Terfezia* sp. and *Tricholoma virgatum* (Figure 1, Table 1).



Figure 1: Most common ECM taxa in symbiosis with silver fir. **A:** *Amanita rubescens* Pers., **B:** *Amphinema byssoides* (Pers.) J. Erikss., **C:** *Clavulina corraloides* (L.) J. Schröt., **D:** *Elaphomyces granulatus* Fr., **E:** *Inocybe assimilata* (Britzelm.) Sacc., **F:** *Lactarius salmonicolor* R. Heim. & Leclair, **G:** *Lactarius subdulcis* (Pers. Ex. Fr.) Gray, **H:** *Lactarius tabidus* (Fr.) Kuntze, **I:** *Neoboletus erythropus* (Pers.), **J:** *Russula badia* Beeli, **K:** *Russula chloroides* (Krombh.) Bres., **L:** *Russula cyanoxantha* (Schaeff.) Fr., **M:** *Russula delica* Fr., **N:** *Russula heterophylla* (Fr.) Fr., **O:** *Russula illota* Romagn., **P:** *Russula ochroleuca* Pers., **R:** *Russula nigricans* Fr., **S:** *Russula turci* Bres., **T:** *Sebacina epigeae* (Berk & Broome) Neuhoff, **U:** *Sebacina incrustans* (Pers.) Tul. & C. Tul., **V:** *Terfezia* sp. (Tul. & C. Tul.) Tul. & C. Tul., **Z:** *Thelephora wakefilediae* Zmitr., **X:** *Tomentella stuposa* (Link) Stalpers, **Y:** *Tricholoma virgatum* (Fr.) P. Kumm., **W:** *Tylospora fibrillosa* (Burt) Donk, **Q:** *Xerocomellus pruinatus* (Fr. & Hök) Šutara.



Slika 1: Najpogostejši ektomikorizni taksoni, ki so se pojavljali v simbiozi z navadno jelko. **A:** *Amanita rubescens* Pers., **B:** *Amphinema byssoides* (Pers.) J. Erikss., **C:** *Clavulina corraloides* (L.) J. Schröt., **D:** *Elaphomyces granulatus* Fr., **E:** *Inocybe assimilata* (Britzelm.) Sacc., **F:** *Lactarius salmonicolor* R. Heim. & Leclair, **G:** *Lactarius subdulcis* (Pers. Ex. Fr.) Gray, **H:** *Lactarius tabidus* (Fr.) Kuntze, **I:** *Neoboletus erythropus* (Pers.), **J:** *Russula badia* Beeli, **K:** *Russula chloroides* (Krombh.) Bres., **L:** *Russula cyanoxantha* (Schaeff.) Fr., **M:** *Russula delicata* Fr., **N:** *Russula heterophylla* (Fr.) Fr., **O:** *Russula illota* Romagn., **P:** *Russula ochroleuca* Pers., **R:** *Russula nigricans* Fr., **S:** *Russula turci* Bres., **T:** *Sebacina epigeae* (Berk & Broome) Neuhoff, **U:** *Sebacina incrustans* (Pers.) Tul. & C. Tul., **V:** *Terfezia* sp. (Tul. & C. Tul.) Tul. & C. Tul., **Z:** *Thelephora wakefieldiae* Zmitr., **X:** *Tomentella stuposa* (Link) Stalpers, **Y:** *Tricholoma virgatum* (Fr.) P. Kumm., **W:** *Tylospora fibrillosa* (Burt) Donk, **Q:** *Xerocomellus pruinatus* (Fr. & Hök) Šutara.

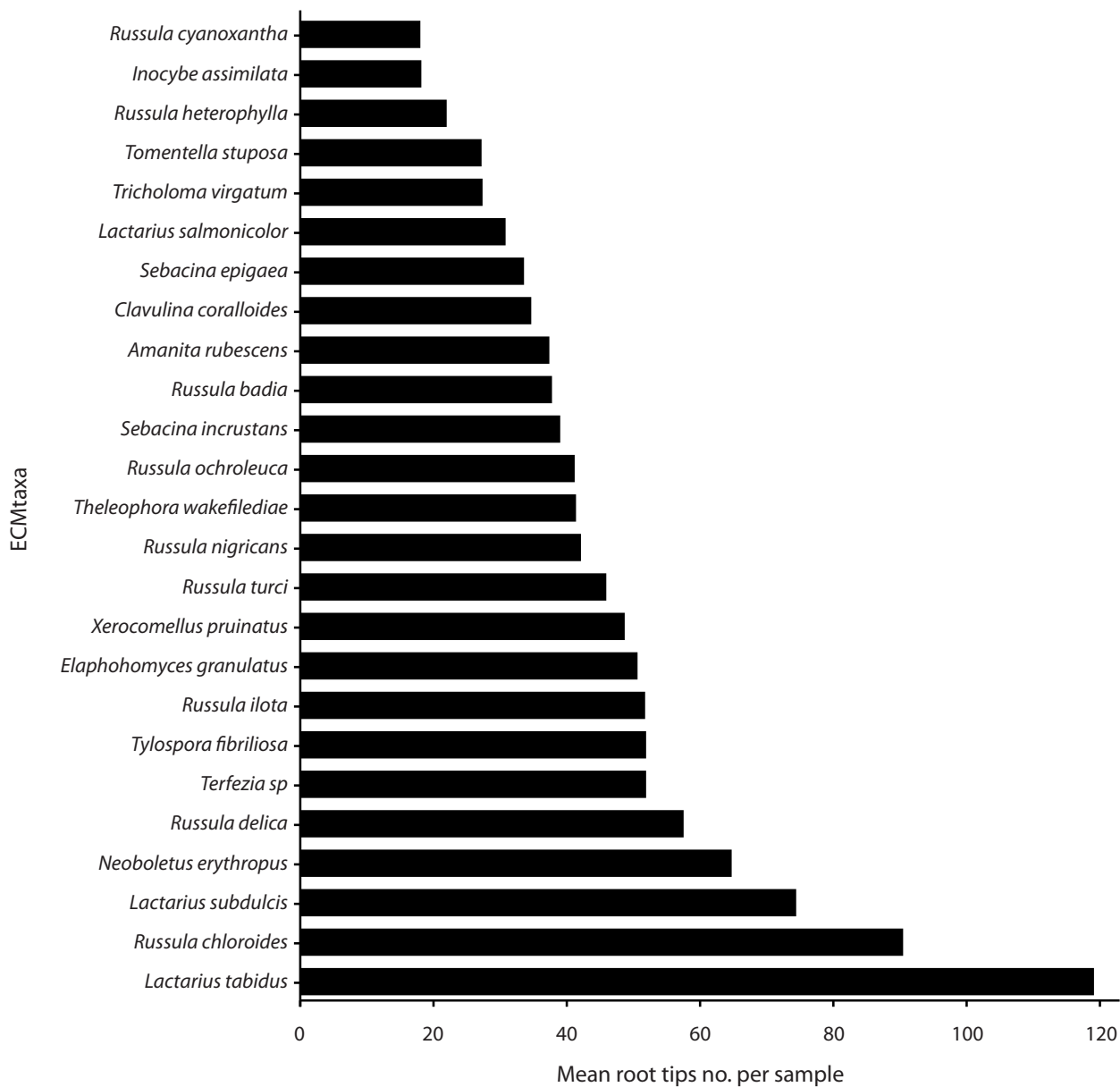


Figure 2: Mean root tips number of individual most common taxa in symbiosis with silver fir.

Slika 2: Povprečno število koreninskih vršičkov posameznega pogostejšega taksona, ki je tvoril simbiozo z navadno jelko.

**Table 1: Most common ECM taxa in symbiosis with silver fir, with morphotype description, accession number and closest GenBank match.****Preglednica 1: Najpogostejši ektomikorizni taksoni v simbiozi z navadno jelko z morfološkimi opisi, pristopno številko in najbližjim GenBank ujemanjem.**

ECM taxon	Morphotype description	Accession no. (representative sequence)	Closest Genbank match	Identities	Similarity	E-value
<i>Amanita rubescens</i> (Pers.)	brown-reddish grainy mantle with hyphae	MN265475	<i>Amanita rubescens</i> MF954678.1	613/615	99.67 %	0.0
<i>Amphinema byssoides</i> (Pers.) J. Erikss.	white to yellow woolly mantle with rhizomorphs	MN265483	<i>Amphinema byssoides</i> MH248042.1	481/481	100 %	0.0
<i>Clavulina corraloides</i> (L.) J. Schröt.	light brown grainy mantle with hyphae	MN265498	Uncultured <i>Clavulina cristata</i> KT020818.1	653/654	99.85 %	0.0
<i>Elaphomyces granulatus</i> Fr.	brown smooth shiny mantle	MN265528	<i>Elaphomyces granulatus</i> KX238835.1	644/644	100 %	0.0
<i>Inocybe assimilata</i> (Britzelm.) Sacc.	beige grainy mantle with substrate	MN265542	<i>Inocybe assimilata</i> MN047063.1	488/488	100 %	0.0
<i>Lactarius salmonicolor</i> R. Heim & Leclair	orange smooth mantle with substrate	MN265578	<i>Lactarius salmonicolor</i> MK028450.1	646/647	99.85 %	0.0
<i>Lactarius subdulcis</i> (Pers. Ex. Fr.) Gray	brown-orange smooth to grainy mantle with hyphae	MN265567	<i>Lactarius subdulcis</i> MN959786.1	638/638	100 %	0.0
<i>Lactarius tabidus</i> (Fr.) Kuntze	brown mantle, with bended unramified ends	MN265596	Uncultured <i>Lactifluus</i> clone MK820103 <i>Lactarius tabidus</i> KR364106.1	680/680 670/674	100 % 99.41 %	0.0 0.0
<i>Lactifluus vellereus</i> (Fr.) Fr.	brown grainy mantle	MN265592	<i>Lactarius vellereus</i> MH125241.1	654/659	99.24 %	0.0
<i>Neoboletus erythropus</i> (Pers.)	dark brown grainy mantle with bended, light brown to orange unramified ends	MN265815	<i>Neoboletus erythropus</i> MK492598.1	458/459	99.78 %	0.0
<i>Russula badia</i> Beeli	brown shiny grainy mantle	MN265655	<i>Russula badia</i> MG679813.1	638/639	99.84 %	0.0
<i>Russula chloroides</i> (Krombh.) Bres.	ocher with substrate	MN265643	<i>Russula chloroides</i> KX034108.1	660/663	99.55 %	0.0
<i>Russula cyanoxantha</i> (Schaeff.) Fr.	white covered with substrate	MN265612	Uncultured <i>Russula</i> clone JF519199.1	621/622	99.84 %	0.0
<i>Russula delicata</i> Fr.	dark brown mantle covered with substrate	MN265678	<i>Russula</i> sp. KM576518.1 <i>Russula delicata</i> AF418605.1	601/604 600/604	99.5 % 99.34 %	0.0 0.0
<i>Russula heterophylla</i> (Fr.) Fr.	ocher grainy mantle with bended unramified ends	MN265625	<i>Russula heterophylla</i> DQ422006.1	556/557	99.82 %	0.0
<i>Russula illota</i> Romagn.	brown orangish grainy mantle with substrate	MN265669	<i>Russula illota</i> MG687367.1	653/655	99.69 %	0.0
<i>Russula ochroleuca</i> Pers.	dark ocher warty mantle with yellow dots	MN265640	<i>Russula ochroleuca</i> MN959793.1	532/534	99.63 %	0.0
<i>Russula nigricans</i> Fr.	brown grainy mantle covered with substrate	MN265606	<i>Russula nigricans</i> voucher MK028892.1	479/480	99.79 %	0.0
<i>Russula turci</i> Bres.	light brown to white mantle	MN265615	<i>Russula turci</i> KF002747.1	654/665	98.35 %	0.0

<i>Sebacina epigeae</i> (Berk & Broome) Neuhoff	light orange to white wooly mantle	MN265696	<i>Sebacina epigeae</i> JQ665513.1	557/565	98.58 %	0.0
<i>Sebacina incrustans</i> (pers.) Tul. & C. Tul.	light brown to white smooth mantle	MN265710	Uncultured <i>Sebacina</i> HE687124.1	578/580	99.66 %	0.0
			<i>Sebacina incrustans</i> MN947392.1	562/581	97 %	0.0
<i>Terfezia</i> sp. (Tul. & C. Tul.) Tul. & C. Tul.	brown mantle with emanating hyphae	MN265716	<i>Pezizaceae</i> sp. KM576471.1	593/593	100 %	0.0
<i>Thelephora wakefilediae</i> Zmitr.	dark brown wooly mantle	MN265765	<i>Tomentella sublilacina</i> KY693713.1	539/539	100 %	0.0
<i>Tomentella stuposa</i> (Link) Stalpers	dark brown to black with emanating hyphae	MN265719	<i>Tomentella stuposa</i> MK602778.1	628/630	99.68 %	0.0
<i>Tricholoma virgatum</i> (Fr.) P. Kumm.	brown wooly mantle, shiny with emanating hyphae and rhizomorphs	MN265777	Uncultured ectomycorrhizal fungus AB828043.1	649/651	99.69 %	0.0
			<i>Tricholoma virgatum</i> DQ389735.1	645/657	98.17 %	0.0
<i>Tylospora fibrillosa</i> (Burt) Donk	light brown with emanating hyphae and cystidia	MN265788	Uncultured <i>Tylospora</i> clone MK820144.1	565/565	100 %	0.0
			<i>Tylospora fibrillosa</i> KP783485.1	563/565	99.65 %	0.0
<i>Xerocomellus pruinatus</i> (Fr. & Hök) Šutara	ocher grainy mantle, shiny with bended unramified ends	MN265793	<i>Xerocomus pruinatus</i> MN959798.1	715/717	99.72 %	0.0

#### 4 DISCUSSION

Using molecular markers, with the 16 sampling dates pooled together from three study sites in Slovenia, we were able to identify 86 different ECM fungal taxa belonging to 32 different ECM fungal genera. Among most abundant ECM fungal taxa in symbiosis with silver fir in Slovenia were *Tomentella stuposa*, *Lactarius salmonicolor*, *Amanita rubescens*, *Russula chloroides*, *Thelephora wakefilediae*, *Xerocomellus pruinatus* etc., for which ectomycorrhizal symbiosis formation with silver fir was already reported by several authors (LAGANÀ et al. 2000, 2002; CREMER 2009; WAŻNY 2014; RUDAWSKA et al. 2016; WAŻNY & KOWALSKI 2017). Identified ECM fungal taxa are also among the more common members of ECM fungal communities of temperate and boreal forest in Europe and many of them exhibit broad host ranges (KENNEDY et al. 2003; NARA 2006). These taxa have been previously reported from ectomycorrhizal root tips studies from different locations in Slovenia (KRAIGHER 1996; PUČKO et al. 2005; GREBENC & KRAIGHER 2007). Observed result confirm high potential for diversity of ECM fungi in silver fir stands, as was already reported in our previous study (UNUK & GREBENC 2017). To compare, 48 morphotypes were recorded in five silver fir forests in

Italy (COMANDINI et al. 2004), 25 morphotypes were recorded in two forest stands in Central Italy (PACIONI et al. 2001) as well as in five different stands on the Taunus Mountains in Germany (SCHIRKONYER et al. 2013). In Polish Pomerania RUDAWSKA et al. (2016) native to the mountainous regions of Europe but has been also widely introduced in the lowlands outside its native range. Like most forest tree species, *A. alba* forms obligate mutualisms with ectomycorrhizal (ECM, recorded 35 ECM taxa in four forest stands, meanwhile 63 and 53 ECM taxa, were recorded on silver fir seedlings from three or six Poland forest stands, respectively (WAŻNY 2014; WAŻNY & KOWALSKI 2017)  $p = 0.0001$ .

However, as many as 48 fungal ECM taxa, were in our study in symbiosis with silver fir identified for the first time, for example *Elaphomyces granulatus*, *Inocybe assimilata*, *Lactarius tabidus*, *Russula delica*, *Russula heterophylla*, *Russula illota*, *Russula turci*, *Sebacina incrustans*, *Terfezia* sp. and *Tricholoma virgatum*, etc. Their occurrence in symbiosis with numerous host trees from family Pinaceae have been previously reported (BUÉE et al. 2011; DING et al. 2011; GAO et al. 2013; KERNAGHAN and PATRIQUIN 2015; ARGÜELLES-MOYAO et al. 2017), however till date there were no



reports about their ectomycorrhizal symbiosis formation with silver fir.

In this study we have identified numerous ECM fungal species associated with silver fir fine roots, where many of them were identified in symbiosis with silver fir for the first time. With this study the range of

fungal species that can form ectomycorrhizal symbiosis with silver fir expanded. A lot of species were identified for the first time, which indicates poor current knowledge of silver fir ectomycorrhizal symbionts and indicates on high potential for diversity of ECM fungi in silver fir stands.

## POVZETEK

Ektomikorizni simbionti navadne jelke so v Sloveniji bili le redko analizirani in identificirani. Namen študije je bil tako analizirati in identificirati ektomikorizne simbionte navadne jelke na treh z navadno jelko prevladujočih območjih v Sloveniji, in sicer na Jelovškem borštu, Ljubelju in Lehnu na Pohorju. Na vsaki izmed lokacij smo postavili eno ploskev, velikosti 20 m x 20 m. Znotraj posamezne ploskve smo izbrali po 5 zdravih in odraslih dreves navadne jelke, katere smo v razmaku enega meseca v času od marca do oktobra v letih 2016 in 2017 vzorčili po protokolu opisanem v KRAIGHER (1996). Drobne korenine smo analizirali in med seboj ločili na podlagi morfološko-anatomskih lastnosti opisanih v AGERER (1987-), do končnega števila 250 analiziranih koreninskih vršičkov na vzorec. Tri do pet vršičkov posameznega morfotipa smo vključili v nadaljnje analize določanja z uporabo ITS DNK barkod. Na skupno treh območjih in 16 združenih mesecih vzorčenja, smo tako določili kar 86 različnih ektomikoriznih simbiontov navadne jelke v Slove-

niji, pripadajočih 32 različnim rodov. Od tega smo kar 68 ektomikoriznih taksonov navadne jelke uspešno pomnožili, sekvencirali in identificirali z uporabo ITS DNK barkodiranja, medtem ko smo 18 ektomikoriznih taksonov identificirali samo na osnovi morfološko-anatomskih značilnosti. Najpogostejši ektomikorizni taksoni navadne jelke prihajajo iz rodov *Russula*, *Tomentella* in *Lactarius*, pri čemer smo kar 48 taksonov v simbiozi z navadno jelko v naši študiji identificirali prvič, na primer *Elaphomyces granulatus*, *Inocybe assimilata*, *Lactarius tabidus*, *Russula delica*, *Russula heterophylla*, *Russula illota*, *Russula turci*, *Sebacina incrustans*, *Terfezia* sp. and *Tricholoma virgatum*, itd.

V študiji smo identificirali veliko število različnih ektomikoriznih simbiontov navadne jelke, pri čemer smo jih kar nekaj v ektomikorizni simbiozi z navadno jelko identificirali prvič, kar kaže slabo poznavanje ektomikoriznih simbiontov navadne jelke in kaže na velik potencial pestrosti ektomikoriznih gliv v sestojih navadne jelke.

## 5 REFERENCES - LITERATURA

- AGERER, R., KRAIGHER, H. & JAVORNIK, B., 1996: Identification of ectomycorrhizae of *Hydnum rufescens* on Norway spruce and the variability of the ITS region of *H. rufescens* and *H. repandum* (Basidiomycetes). *Nova Hedwigia*, 63: 183-194
- AGERER, R. : 1987: *Colour Atlas of Ectomycorrhizae*, 1st-12th edn. Einhorn-Verlag, Schwäbisch Gmünd, Germany
- AGERER, R. & RAMBOLD, G., 2004–2018: DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. In: München, Ger.
- ARGÜELLES-MOYAO, A., GARIBAY-ORIJEL, R., MÁRQUEZ-VALDELAMAR, L.M. & ARELLANO-TORRES, E., 2017: *Clavulina-Membranomyces* is the most important lineage within the highly diverse ectomycorrhizal fungal community of *Abies religiosa*. *Mycorrhiza* 27:53–65. <https://doi.org/10.1007/s00572-016-0724-1>
- BERNDT, R., KOTTKE, I. & OBERWINKLER, F., 1990: Ascomycete mycorrhizas from pot-grown silver-fir seedlings (*Abies alba* Mill.). *New Phytol* 115:471–482. <https://doi.org/10.1111/j.1469-8137.1990.tb00473.x>
- BONČINA, A., DIACI, J. & CENČIČ, L., 2002: Comparison of the two main types of selection forests in Slovenia: Distribution, site conditions, stand structure, regeneration and management. *Forestry*. <https://doi.org/10.1093/forestry/75.4.365>
- BUÉE, M., MAURICE, J.P., ZELLER, B., et al., 2011: Influence of tree species on richness and diversity of epigeous fungal communities in a French temperate forest stand. *Fungal Ecol.* <https://doi.org/10.1016/j.funeco.2010.07.003>

- COMANDINI, O., HAUG, I., RINALDI, A.C. & KUYPER, T.W., 2004: Uniting *Tricholoma sulphureum* and *T. bufonium*. *Mycol Res* 108:1162–1171. <https://doi.org/10.1017/S095375620400084X>
- COMANDINI, O., PACIONI, G. & RINALDI, A.C., 1998: Fungi in ectomycorrhizal associations of silver fir (*Abies alba* Miller) in Central Italy. *Mycorrhiza* 7:323–328. <https://doi.org/10.1007/s005720050200>
- CREMER, E., 2009: Population genetics of silver fir (*Abies alba* Mill.) in the Northern Black Forest – preconditions for the recolonization of windthrow areas and associated ectomycorrhizal communities. *Phillips-Universität Marbg* 1:103. <https://doi.org/10.1017/CBO9781107415324.004>
- DE GROOT, M., ELER, K. & FLAJSMAN, K., et al., 2016: Differential short-term response of functional groups to a change in forest management in a temperate forest. *For Ecol Manage* 376:256–264. <https://doi.org/10.1016/j.foreco.2016.06.025>
- DE ROMÁN, M. & DE MIGUEL, A.M., 2005: Post-fire, seasonal and annual dynamics of the ectomycorrhizal community in a *Quercus ilex* L. forest over a 3-year period. *Mycorrhiza* 15:471–482. <https://doi.org/10.1007/s00572-005-0353-6>
- DING, Q., LIANG, Y., LEGENDRE, P., et al., 2011: Diversity and composition of ectomycorrhizal community on seedling roots: The role of host preference and soil origin. *Mycorrhiza*. <https://doi.org/10.1007/s00572-011-0374-2>
- EBERHARDT, U., OBERWINKLER, F., VERBEKEN, A., et al., 2000: *Lactarius* ectomycorrhizae on *Abies alba*: Morphological description, molecular characterization, and taxonomic remarks. *Mycologia* 92:860–873. <https://doi.org/10.2307/3761582>
- GAO, C., SHI, N.N., LIU, Y.X., et al., 2013: Host plant genus-level diversity is the best predictor of ectomycorrhizal fungal diversity in a Chinese subtropical forest. *Mol Ecol*. <https://doi.org/10.1111/mec.12297>
- GARDES, M. & BRUNS, T.D., 1993: ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- GREBENC, T. & KRAIGHER, H., 2007: Changes in the community of ectomycorrhizal fungi and increased fine root number under adult beech trees chronically fumigated with double ambient ozone concentration. *Plant Biol* 9:279–287. <https://doi.org/10.1055/s-2006-924489>
- KEARSE, M., MOIR, R., WILSON, A., et al., 2012: Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- KENNEDY, P.G., IZZO, A.D. & BRUNS, T.D., 2003: There is high potential for the formation of common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest. *J Ecol* 91:. <https://doi.org/10.1046/j.1365-2745.2003.00829.x>
- KERNAGHAN, G. & PATRIQUIN, G., 2015: Diversity and host preference of fungi co-inhabiting *Cenococcum* mycorrhizae. *Fungal Ecol*. <https://doi.org/10.1016/j.funeco.2015.05.001>
- KLOPČIČ, M. & BONČINA, A., 2011: Stand dynamics of silver fir (*Abies alba* Mill.)-European beech (*Fagus sylvatica* L.) forests during the past century: A decline of silver fir? *Forestry* 84:259–271. <https://doi.org/10.1093/forestry/cpr011>
- KRAIGHER, H., JAVORNIK, B. & AGERER, R., 1995: Ectomycorrhizae of *Lactarius lignyotus* on Norway spruce, characterized by anatomical and molecular tools. *Mycorrhiza*, 3: 175–180.
- KRAIGHER, H., 1996: Tipi ektomikorize - taksonomija, pomen in aplikacije - Types of ectomycorrhizae - their taxonomy, role and application. *Acta Silvae et Ligni* 49:33–66
- KRAIGHER, H., AL SAYEGH PETKOVŠEK, S., GREBENC, T. & SIMONČIČ, P., 2007: Types of ectomycorrhiza as pollution stress indicators: Case studies in Slovenia. *Environ Monit Assess* 128:31–45. <https://doi.org/10.1007/s10661-006-9413-4>
- LAGANÀ, A., ANGIOLINI, C., LOPPI, S., et al., 2002: Periodicity, fluctuations and successions of macrofungi in fir forests (*Abies alba* Miller) in Tuscany, Italy. *For Ecol Manage* 169:187–202. [https://doi.org/10.1016/S0378-1127\(01\)00672-7](https://doi.org/10.1016/S0378-1127(01)00672-7)
- LAGANÀ, A., SALERNI, E., BARLUZZI, C., et al., 2000: Mycocoenology in *Abies alba* Miller woods of Central-Southern tuscany (Italy). *Acta Soc. Bot. Pol.* 69:293–298
- MRAK, T., HUKIĆ, E., ŠTRAUS, I., et al., 2020: Ectomycorrhizal community composition of organic and mineral soil horizons in silver fir (*Abies alba* Mill.) stands. *Mycorrhiza* 30:. <https://doi.org/10.1007/s00572-020-00970-y>
- NARA, K., 2006: Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytol* 169:. <https://doi.org/10.1111/j.1469-8137.2005.01545.x>

- NILSSON, R.H., LARSSON, K.H., TAYLOR, A.F.S., et al., 2019: The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res* 47:D259–D264. <https://doi.org/10.1093/nar/gky1022>
- PACIONI, G., COMANDINI, O. & RINALDI, A.C., 2001: An assessment of below-ground ectomycorrhizal diversity of *Abies alba* Miller in central Italy. *Plant Biosyst - An Int J Deal with all Asp Plant Biol* 135:337–350. <https://doi.org/10.1080/11263500112331350960>
- PORRAS-ALFARO, A., LIU, K.L., KUSKE, C.R. & XIE, G., 2014: From genus to phylum: Large-subunit and internal transcribed spacer rRNA operon regions show similar classification accuracies influenced by database composition. *Appl Environ Microbiol* 80:829–840. <https://doi.org/10.1128/AEM.02894-13>
- PUČKO, M., GREBENC, T., BOŽIČ, G., et al., 2005: Identification of types of ectomycorrhizae on seedlings in a beech provenance trial. *Zb gozdarstva Lesar* 75:87–104
- RAJA, H.A., MILLER, A.N., PEARCE, C.J. & OBERLIES, N.H., 2017: Fungal identification using molecular tools: a primer for the natural products research community. *J Nat Prod* 80:756–770. <https://doi.org/10.1021/acs.jnatprod.6b01085>
- RUDAWSKA, M., PIETRAS, M., SMUTEK, I., et al., 2016: Ectomycorrhizal fungal assemblages of *Abies alba* Mill. outside its native range in Poland. *Mycorrhiza* 26:57–65. <https://doi.org/10.1007/s00572-015-0646-3>
- SCHIRKONYER, U., BAUER, C. & ROTHE, G.M., 2013: Ectomycorrhizal diversity at five different tree species in forests of the Taunus Mountains in Central Germany. *Open J Ecol* 03:66–81. <https://doi.org/10.4236/oje.2013.31009>
- SULZBACHER, M.A., GREBENC, T., CABRAL, T.S., et al., 2016: *Restingomyces*, a new sequestrate genus from the Brazilian Atlantic rainforest that is phylogenetically related to early-diverging taxa in Trappeaceae (Phallales). *Mycologia* 108:954–966. <https://doi.org/10.3852/15-265>
- UNUK, T. & GREBENC, T., 2017: Silver fir (*Abies alba* Mill.) ectomycorrhiza across its areal – a review approach / Ektomikorizni simbionti bele jelke (*Abies alba* Mill.) na naravnem območju razširjenosti – pregled. *Folia Biol Geol* 58:.. <https://doi.org/10.3986/fbg0025>
- UNUK, T., MARTINOVIĆ, T., FINŽGAR, D., et al., 2019: Root-associated fungal communities from two phenologically contrasting silver fir (*Abies alba* Mill.) groups of trees. *Front Plant Sci* 10:1–11. <https://doi.org/10.3389/fpls.2019.00214>
- WAŻNY, R., 2014: Ectomycorrhizal communities associated with silver fir seedlings (*Abies alba* Mill.) differ largely in mature silver fir stands and in Scots pine forecrops. *Ann For Sci* 71:801–810. <https://doi.org/10.1007/s13595-014-0378-0>
- WAŻNY, R. & KOWALSKI, S., 2017: Ectomycorrhizal fungal communities of silver-fir seedlings regenerating in fir stands and larch forecrops. *Trees - Struct Funct* 31:929–939. <https://doi.org/10.1007/s00468-016-1518-y>
- WHITE, T.J., BRUNS, T., LEE, S., TAYLOR, J., 1990: Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In: *PCR Protocols*. Elsevier, pp 315–322
- WOLF, H., 2003: EUFORGEN Technical Guidelines for genetic conservation and use for silver fir (*Abies alba*). *Int Plant Genet Resour Institute, Rome, Italy*. <https://doi.org/10.1016/j.jaci.2010.08.025>
- ŽELEZNIK, P., HRENKO, M., THEN, C., et al., 2007: CASIROZ: Root parameters and types of ectomycorrhiza of young beech plants exposed to different ozone and light regimes. *Plant Biol* 9:298–308. <https://doi.org/10.1055/s-2006-955916>
- ŽELEZNIK, P., VILHAR, U., STARR, M., et al., 2016: Fine root dynamics in Slovenian beech forests in relation to soil temperature and water availability. *Trees - Struct Funct* 30:375–384. <https://doi.org/10.1007/s00468-015-1218-z>

