

# DIVERSITY OF ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH QUERCUS PETRAEA IN THE NATIONAL PARK FRUŠKA GORA IN THE REPUBLIC OF SERBIA

## RAZNOLIKOST EKTOMIKORIZNIH GLJIVA NA HRASTU KITNJAKU (QUERCUS PETRAEA) U NACIONALNOM PARKU FRUŠKA GORA U REPUBLICI SRBIJI

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### SUMMARY

Although sessile oak is one of the most important deciduous forest tree species in Europe, data on the diversity of ectomycorrhizal (ECM) fungi on sessile oaks in the Republic of Serbia are scarce. The aim of this study was to provide the first insight into the diversity of ECM fungi on sessile oak in Serbia. Two sites Info center and Brankovac, located in National Park Fruška gora were chosen. ECM fungi were identified combining morphological and anatomical characterization with molecular analysis of nuclear rDNA internal transcribed spacer (ITS) region. All vital ECM root tips were counted, diversity indices were calculated, and ECM fungi were classified into the exploration types. The granulometric and chemical composition of soil were analyzed as well. At both sites, 26 different ECM fungal taxa were recorded in total, 17 taxa were observed at the site Info center and 12 taxa at Brankovac. ECM communities consisted of a few abundant taxa and a larger number of rare taxa. *Lactarius quietus*, *Cenococcum geophilum*, and *Tomentella sublilacina* were recorded at both sites. High abundance of contact and short-distance exploration types recorded in studied stands suggests that soils are sufficiently rich in total nitrogen and organic matter. Values of diversity indices recorded in studied sessile oak stands from Fruška gora were lower in comparison to those obtained in stands of different oak species across Europe which is likely induced by drought. To get a more thorough insight into the diversity of ECM fungi on sessile oak, research should be continued at more sites and seasonal dynamics should be included.

**KEY WORDS:** ectomycorrhiza, *Quercus petraea*, morpho-anatomical characterization, molecular identification, exploration types

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## INTRODUCTION

### UVOD

Sessile oak (*Quercus petraea* (Matt.) Liebl.), together with pedunculate oak (*Q. robur* L.), is one of the most economically important deciduous forest tree species in Europe (Ducouso and Bordacs 2004, Eaton *et al.* 2016, Kesić *et al.*, 2018). Their wood is of good quality and is valued for many purposes such as construction, as well as production of furniture and barrels. Also, oaks have an important ecological role because their acorns are a valuable food source for many animals. They are heliophilic, light-demanding trees which affect regeneration of many other tree species and enrich forest biodiversity. Although sessile oak prefers fertile and moist soils, it has a very large ecological niche and is quite tolerant of drought and poor soil (Ducouso and Bordacs 2004, Eaton *et al.* 2016). In the Republic of Serbia, the area under the forests of sessile oak occupies 173.200 ha and it makes up 7.9% of the total forest area (Banković *et al.* 2008). This species is widespread in Serbia and appears from Fruška gora and Vršачke planine in the north to Kozjak, Kozarnik and Metohija in the south, and from Mount Tara in the west to Stara Planina in the east. It occurs in lowlands (outside floodplains), in hilly and lower mountainous areas, and spreads from 200 m a. s. l., on the northern border of the area, to 1,200 m a. s. l., on the southern and eastern border of distribution in Serbia (Stojanović *et al.* 2007).

In most temperate and boreal forest ecosystems, the establishment, growth and survival of trees depend on association with ectomycorrhizal (ECM) fungi. ECM fungi successfully take water and nutrients from the soil and translocate them to plants from which they receive photosynthetically produced carbohydrates in return (Smith and Read, 2008). Mycorrhizas are one of the essential components of the forest ecosystem stability. Particularly, they have stabilizing effects on forest trees that are under environmental stress. Common mycelial networks have an especially important role in forest regeneration, succession, and resistance against different stress factors (Selosse *et al.* 2006). Such networks connect plants of the same or different species and can affect the physiological and ecological processes of plants (Selosse *et al.* 2006). Also, mycorrhizal mycelium connects forest trees and ground vegetation with decomposers in forest soil, which affects the sustainability, productivity, and vitality of the forest (Kraigher 1996). Moreover, mycorrhizal fungi can improve plant tolerance to abiotic stress factors such as heat, drought, or presence of heavy metals, as well as increase resistance to pathogens and boost plant immunity (Smith and Read 2008, Smith *et al.* 2010, French 2017). The functional compatibility of the symbionts in ectomycorrhiza is species-specific and depends on both partners while stress tolerance and adaptation of individual ectomycorrhizae on different environmental conditions depend on species of ECM fungus and its morphological, physiological

and ecological characters (Kraigher 1996). Therefore, information on the ECM community structure can provide valuable information about the physiology of forest trees and the functioning of forest ecosystems (Kraigher *et al.* 2011).

Classification of ectomycorrhizae based on the exploration types (ETs) connects morphology of ECM fungi, especially the amount and differentiation of emanating elements, with their ecology (Agerer 2001). Contact ET is represented by ectomycorrhizae with a smooth mantle and without rhizomorphs. Short-distance ET is characterized by a voluminous cover of emanating hyphae but without rhizomorphs. Medium-distance ET can be divided into three subtypes. In fringe subtype the fungi often form fans of emanating hyphae and rhizomorphs, smooth subtype has rather smooth mantles with only a few emanating hyphae, while mat-forming fungi have only a limited range of exploration. Long-distance ET is characterized by smooth ectomycorrhizae with few but highly differentiated rhizomorphs (Agerer 2001).

Since data on the diversity of ECM fungi on oaks in the Republic of Serbia are scarce, especially those studied underground, the aim of this study was to make the first insight into the diversity of ECM fungi on sessile oak in Serbia by choosing two sessile oak stands situated in National Park Fruška gora.

## MATERIALS AND METHODS

### MATERIJALI I METODE

#### Sampling site and procedures

Sampling was conducted in two sessile oak (*Quercus petraea* (Matt.) Liebl.) stands situated on the ridge of the mountain Fruška gora, at sites near Info center (N 45° 09' 14.9", E 19° 50' 40.2 and 483 m a. s. l.) and near viewpoint Brankovac (N 45° 09' 18.7", E 19° 45' 1.9 and 471 m a. s. l.). Other woody species present with a minor share were: *Acer campestre* L., *Sambucus nigra* L., *Cornus sanguinea* L., *Fraxinus ornus* L., *Tilia argentea* Desf. ex DC., and *Rubus idaeus* L. at the site Info center; and *Sambucus nigra* L., *Rubus idaeus* L., *Acer campestre* L., and *Tilia argentea* Desf. ex DC. at the site Brankovac.

Although Fruška gora spreads out in the zone with a moderate continental climate, the climate within the mountain area has subcontinental characteristics because of the change of the climatic characteristics along the height gradient and the forest cover. An exception is the climate of the mountain's ridges, with cold winters and chilly summers. The lowest mean value of air temperature occurs in January and amounts - 0.6° C and the highest mean in July with 21.4° C, while the mean annual temperature is 11.2° C. According to the average monthly sum of precipitation in this area, the months with the most precipitations are May and June, while the driest are September and October. (<https://www.npfruskagora.co.rs/en/hidrology-and-climate/>). At the weather station Iriški venac for period 1965-1990 average pre-

precipitation amount per season is as follows: winter 180 mm, spring 198 mm, summer 252 mm, and autumn 161 mm, the average amount of precipitation in the vegetative period is 443.6 mm and average annual amount of precipitation is 791.4 mm (Babić 2015).

Soil sampling was performed at the end of October 2017, in the absence of snow cover, and ten soil samples were taken per site. The sampling was performed with a standardized soil corer (Kraigher 1999) (4 cm in diameter and 18 cm in length, making a total sample volume of 274 mL), 0.5-1 m from the target tree, as far as possible from non-target trees. Soil samples were stored at 4°C for up to two months. Prior to analyses, each sample was submerged in cold tap water to loosen the soil structure. All roots were carefully washed from the soil. Using a dissecting microscope Olympus SZX 10 (Olympus Corp., Tokyo Japan) with magnifications 10-63× (light source: Olympus Highlight 3100, daylight filter), all fine roots were separated as vital ECM root tips or as old, nonturgid and unidentifiable. Vital ECM root tips were categorized into different morphotypes of ectomycorrhizae based on their morphological and anatomical characteristics. Later ones were assessed using a microscope (Olympus BX 53\*, Olympus Corp., Tokyo Japan) with magnifications 100-1000×. Morphotypes of ectomycorrhizae were described following the methodology given by Agerer (1991) and Kraigher (1996). If it was possible, a fungal partner was identified by comparison with published descriptions in Agerer *et al.* (2006), Agerer (2008), or Agerer and Rambold (2020).

Morphotypes of ectomycorrhizae were also classified into the ETs as proposed by Agerer (2001). All vital ECM root tips were counted. The granulometric and chemical composition of soil were analyzed as well.

### Molecular identification of ectomycorrhizal fungi

Confirmation of fungal partners in ectomycorrhiza using molecular methods was based on PCR amplification of fungal nuclear rDNA internal transcribed spacer (ITS) region. Total genomic DNA was extracted from ECM root tips using a DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). If DNA extraction of representative root tips of some morphotype of ectomycorrhiza was not successful and morpho-an-

atomical identification was insufficient to determine the ECM fungus, this ECM morphotype was labeled as an “unidentified” type. Amplifications were performed with ITS-1F (Gardes and Bruns 1993) and ITS 4 primer pair (White *et al.* 1990). The amplification reaction was performed in Eppendorf Master cycler (Eppendorf AG, Hamburg, Germany). Negative controls with no fungal DNA were run for each experiment to check for any contamination. The PCR mixture for one sample was composed of 2.5 µL of 10× Gold Buffer, 2 µL of deoxynucleotide triphosphates (0.2 mM each), 0.6 µL of each primer (10 µM each), 2 µL of MgCl<sub>2</sub> (2.0 mM), 15 µL of sterile distilled water, 0.3 µL of Taq polymerase (5 U µL<sup>-1</sup>), and 2 µL of a DNA extract. Thermal cycling conditions were as follows: initial denaturation and polymerase activation at 95°C for 5 min; 13 cycles at 94°C for 45 s, 55°C for 55 s and 72°C for 45 s.; 13 cycles at 94°C for 45 s, 55°C for 55 s and 72°C for 120 s; 12 cycles at 94°C for 45 s, 55°C for 55 s and 72°C for 180 s and a final extension at 72°C for 10 min. Amplified DNA fragments were separated and purified from the agarose gel using the QIAquick gel extraction kit and QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) and sent for sequencing in Macrogen Europe B.V. Species, genus, or family of ECM fungi were determined by comparing the sequences to those deposited in GenBank (NCBI 2020) and UNITE (Nilsson *et al.* 2018) database.

### Data analysis

Diversity indexes were calculated per sample and per site (i. e. by pooling the ECM community data) following the formulas given by Atlas and Bartha (1981): (i) Species richness (d) = (S-1)/log<sub>10</sub>N, where S is the number of ECM fungal taxa and N is the number of all mycorrhizal tips; (ii) Shannon-Weaver's diversity index (H) = C/N(NlogN - Σn<sub>i</sub>logn<sub>i</sub>), where C=2.3, N is the number of all mycorrhizal tips and n<sub>i</sub> is the number of mycorrhizal tips of an individual ECM fungal taxon.

## RESULTS REZULTATI

According to the granulometric and chemical composition of soil samples taken in studied sessile oak stands, soil on

**Table 1.** Granulometric and chemical composition of soil from sessile oak (*Quercus petraea* (Matt.) Liebl.) stands located at two sites in National Park Fruška gora

**Tablica 1.** Granulometrijski i kemijski sastav tla u sastojinama hrasta kitnjaka (*Quercus petraea* (Matt.) Liebl.) na dva lokaliteta u Nacionalnom parku Fruška gora

Site/Lokacija	Granulometric composition/Granulometrijski sastav						Chemical composition/Kemijski sastav					
	Coarse sand (%)/ Krupni pijesak (%)	Fine sand (%)/ Sitan pijesak (%)	Silt (%)/ Prah (%)	Clay (%)/ Glina (%)	Total sand (%)/ Ukupan pijesak (%)	Total clay (%)/ Ukupna glina (%)	Texture class/ Teksturna klasa	pH/pH	Calcium carbonate (%)/ Kalcijev karbonat (%)	Humus (%)/ Humus (%)	Nitrogen (%)/ Dušik (%)	Carbon (%)/ Ugljik (%)
Info center/ Info centar	10.9	37.7	38.5	12.9	48.6	51.4	loam/ ilovača	5.23	1.18	2.11	0.11	1.20
Brankovac/ Brankovac	9.6	31.9	45.1	13.4	41.4	58.6	loam/ ilovača	5.46	1.75	2.52	0.13	1.41

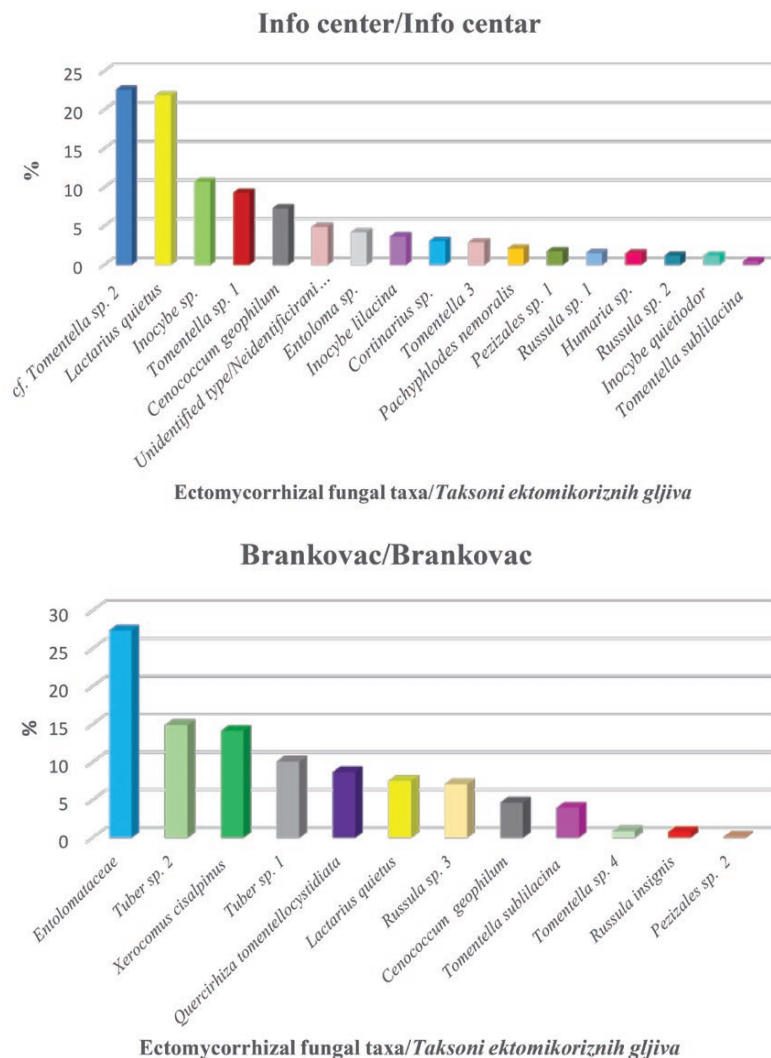
**Table 2.** Total and average values of the number of ectomycorrhizal fungal taxa, vital ectomycorrhizal root tips, and diversity indices in sessile oak (*Quercus petraea* (Matt.) Liebl.) stands located at two sites in National Park Fruška gora

**Tablica 2.** Ukupne i prosječne vrijednosti broja taksona ektomikoriznih gljiva, vitalnih ektomikoriznih korijenskih vrhova i indeksa raznolikosti u sastojinama hrasta kitnjaka (*Quercus petraea* (Matt.) Liebl.) smještenih na dva lokaliteta u Nacionalnom parku Fruška gora

Parameter/Parametar	Info center/Info centar		Brankovac/Brankovac	
	Total value per site/Ukupna vrijednost po lokaciji	Average value per sample/Prosječna vrijednost po uzorku	Total value per site/Ukupna vrijednost po lokaciji	Average value per sample/Prosječna vrijednost po uzorku
Number of ectomycorrhizal fungal taxa/ Broj taksona ektomikoriznih gljiva	17	2.8±0.6	12	3.3±0.4
Number of vital ectomycorrhizal root tips/ Broj vitalnih ektomikoriznih korijenskih vrhova	1719	171.9±53.5	1258	129±27.2
Species richness index/Indeks bogatstva vrsta	4.94	0.86±0.21	3.54	1.10±0.21
Shannon-Weaver index/Shannon-Weaver-ov indeks	2.27	0.69±0.18	2.09	0.87±0.13

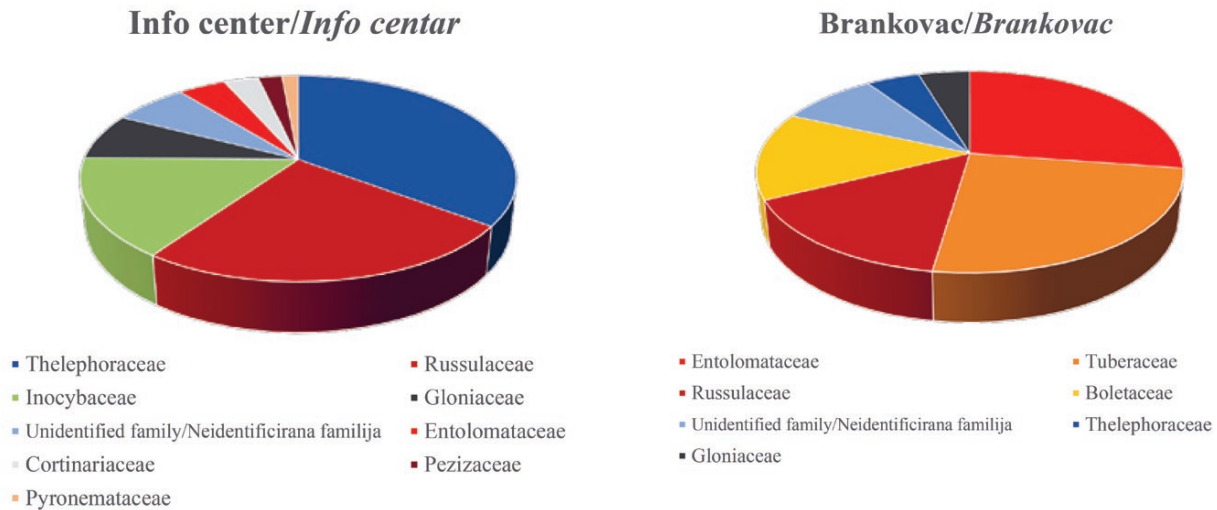
both sites is acidic and belongs to textural class loam with high silt content (Table 1) suggesting relatively strong water absorption by soil particles and poor water availability for

plants. On the other hand, soils in both stands belong to moderate humous soils and are well provided with nitrogen (Table 1).



**Figure 1.** Relative abundance of ectomycorrhizal fungal taxa (based on the number of ectomycorrhizal root tips belonging to the particular ectomycorrhizal fungal taxon in relation to all ectomycorrhizal root tips) in sessile oak stands from sites **A** Info center and **B** Brankovac

**Slika 1.** Relativna brojnost taksona ektomikoriznih gljiva (na temelju broja ektomikoriznih korijenskih vrhova koji pripadaju određenom taksonu ektomikoriznih gljiva u odnosu na sve ektomikorizne korijenske vrhove) u sastojinama hrasta kitnjaka sa lokaliteta **A** Info centar i **B** Brankovac



**Figure 2.** Relative abundance of taxonomic families of ectomycorrhizal fungi based on the number of ectomycorrhizal root tips belonging to a particular family in relation to all ectomycorrhizal root tips in sessile oak stands from sites **A** Info center and **B** Brankovac

**Slika 2.** Relativna brojnost taksonomskih porodica ektomikoriznih gljiva na temelju broja ektomikoriznih korijenskih vrhova koji pripadaju određenoj porodici u odnosu na sve ektomikorizne korijenske vrhove u sastojinama hrasta kitnjaka sa lokaliteta **A** Info centar i **B** Brankovac

The total number of ECM fungal taxa recorded at the site Info center was somewhat higher than the one recorded at the site Brankovac (Tab. 2, Supplement Tab. 1). At both sites, a similar number of ECM fungal taxa was recorded per soil sample. The total number of vital ECM root tips counted in the sessile oak stand at Info center was almost 50% higher than at Brankovac. Although the total number of recorded ECM fungal taxa was higher at the site Info center, the average number of ECM fungal taxa and values of diversity indices calculated per soil sample were higher at the site Brankovac (Tab. 2).

In soil samples taken in sessile oak stands at sites Info center and Brankovac, 26 ECM fungal taxa were recorded in total (Fig. 1). Nine ECM fungi were identified to a species level, 13 to a genus level, one to a family level, two to an order level, and one ECM fungus remained unidentified. Among all observed ECM fungi only *Tomentella sublimilacina*, *Lactarius quietus* and *Cenococcum geophilum* were recorded at both sites. At the site Brankovac, the most abundant ECM fungi were cf. *Tomentella* sp. 2 and *Lactarius quietus* with relative abundance higher than 20%, then *Inocybe* sp. which made association with 10% of all root tips. At the site Brankovac dominating ECM fungus, identified as *Entolomataceae* sp., was associated with almost 30% of all ECM root tips, while *Tuber* sp. 2 and *Xerocomus cisalpinus* had relative abundance higher than 10%. At both sites three most abundant ECM fungi associated with more than 50% of all ECM roots (Fig. 1).

At the site Info center dominant fungal taxonomic families were *Thelephoraceae*, *Russulaceae*, and *Inocybaceae* which together encompassed more than 75% of all vital ECM root tips (Fig. 2A). Moreover, these three families were the most

species-rich families at this site (Fig. 3A). On the other hand, at site Brankovac the most abundant families were *Entolomataceae*, *Tuberaceae*, *Russulaceae*, and *Boletaceae* and their individual relative abundances were above 10% (Fig. 2B). However, family *Russulaceae* was the most species-rich at this site followed by *Tuberaceae* and *Thelephoraceae* (Fig. 3B). At both sites, ECM fungi belonging to the phylum *Basidiomycota* dominated the ECM community. ECM fungi belonging to *Ascomycota* were more abundant at the site Brankovac in comparison with Info center (data not shown).

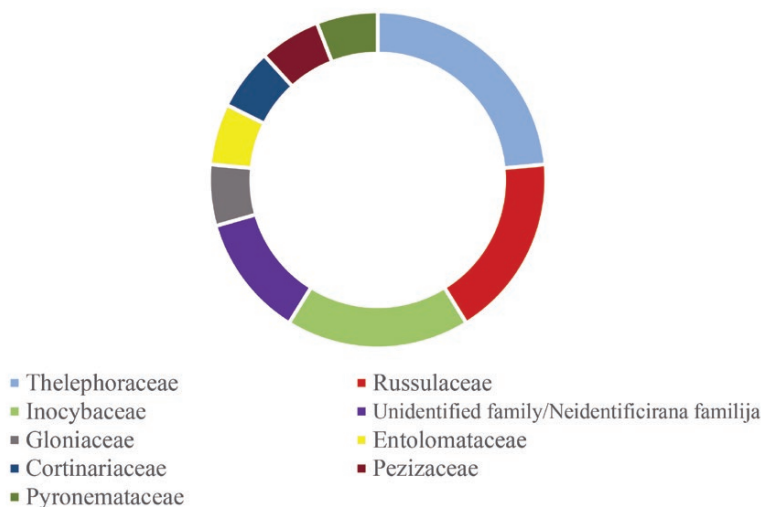
At the site Info center short distance ET dominated, followed by contact ET and medium-distance ET, while long-distance ETs were not found at all. On the other hand, at the site Brankovac the most abundant was contact ET followed by short-distance ET and medium-distance ET, while long-distance ET was the least abundant (Tab. 3).

**Table 3.** Relative abundance ( $\pm$  standard error) (%) of ectomycorrhizal exploration types in sessile oak (*Quercus petraea* (Matt.) Liebl.) stands located at two sites in National Park Fruška gora

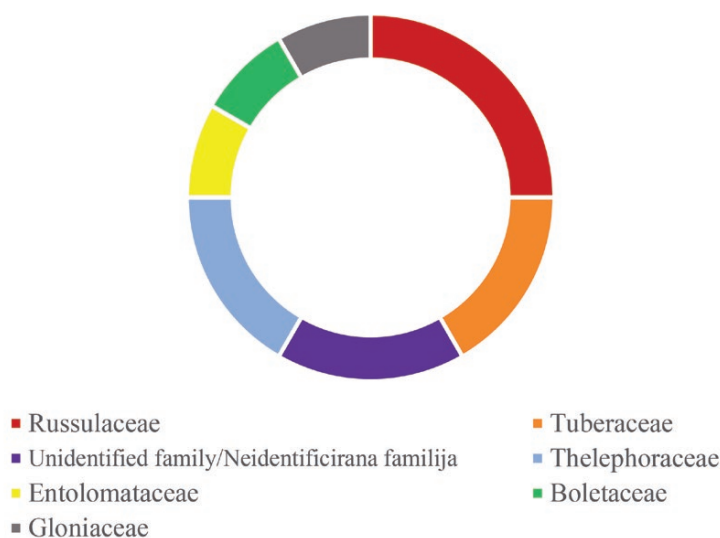
**Tablica 3.** Relativna brojnost ( $\pm$  standardna pogreška) (%) ektomikoriznih eksploracijskih tipova u sastojinama hrasta kitnjaka (*Quercus petraea* (Matt.) Liebl.) smještenih na dva lokaliteta u Nacionalnom parku Fruška gora

Exploration type/ Eksploracijski tip	Info center/ Info centar	Brankovac/ Brankovac
Contact/Kontaktni	33.8 $\pm$ 11.8	43.3 $\pm$ 9
Short-distance/Kratkih udaljenosti	51 $\pm$ 12.0	23.9 $\pm$ 8.4
Medium-distance smooth/ Srednjih udaljenosti glatki	9 $\pm$ 9	0
Medium-distance fringe/ Srednjih udaljenosti sa resama	6.2 $\pm$ 4.6	22.8 $\pm$ 7.7
Long-distance/Dugih udaljenosti	0	10.0 $\pm$ 6.9

### Info center/*Info centar*



### Brankovac/*Brankovac*



**Figure 3.** Species richness of taxonomic families of ectomycorrhizal fungi based on the number of ectomycorrhizal taxa belonging to a particular family in relation to all ectomycorrhizal taxa in sessile oak stands from sites **A** Info center and **B** Brankovac

**Slika 3.** Bogatstvo vrsta taksonomskih porodica ektomikoriznih gljiva na temelju broja ektomikoriznih taksona koji pripadaju određenoj porodici u odnosu na sve ektomikorizne taksoni u sastojinama hrasta kitnjaka sa lokaliteta **A** Info centar i **B** Brankovac

## DISCUSSION RASPRAVA

In two sessile oak stands in National Park Fruška gora studied in autumn, 26 different ECM fungal taxa were recorded in total, of which 14 taxa were observed only at the site Info center, nine taxa only at the site Brankovac and three ECM fungal taxa on both sites. However, an analysis of ectomycorrhizas across nine European countries at long-term and intensively monitored oak plots, showed that the average richness was 55, ranging from 24 fungal taxa in the Netherlands to 83 in Romania (Suz *et al.* 2014). In a temperate oak forest in northeast France, 75 fungal symbionts were

recorded in 15 months (Courty *et al.* 2008). In two stands of *Q. robur* and *Q. petraea* in Austria, 46 and 38 ECM morphotypes were differentiated within 3 years (Kovacs *et al.* 2000). The number of ECM fungi recorded in our study was considerably lower in comparison to these studies. Differences in the obtained number of ECM taxa could be due to the lower number of soil samples taken in our study and the fact that sampling was done only in one season. Abundant species are prone to be present all year long, some species occur only in a particular season or month, while some ECM fungi are present all year long but with fluctuating abundance (Courty *et al.* 2008). On the other hand, species

richness recorded in our research was similar to the one obtained in three declining pedunculate oak stands in western Poland where a total of 18 ECM fungal species was revealed and ECM fungal species richness ranged from 11 to 15 taxa (Bzdyk *et al.* 2019). Also, in the forest of the Tauern mountains in Central Germany, 18 different ECM genera and species were determined on sessile oak (Schirkonyer *et al.* 2013), while in Ireland 21 ECM taxa were found on native oaks *Q. petraea* and *Q. robur* (O'Hanlon and Harrington, 2012).

On average, three ECM fungi were recorded in soil samples from sites Info center and Brankovac. This is considerably lower in comparison to oak stands in Austria where eight morphotypes were found averagely (Kovacz *et al.* 2000) and in the forest of *Q. ilex* in Spain where on average seven species were recorded (de Roman and de Miguel, 2005). Shannon-Weaver index per soil sample at sites Info center and Brankovac with values 0.7 and 0.9, respectively, is considerably lower compared to the ones recorded in oak stands in Austria where vital trees had Shannon-Weaver index 1.3-1.5 (Kovacz *et al.* 2000) and in the *Q. ilex* forest in Spain where this index was 1.2 in the burned stand and 1.3 in the control stand (de Roman and de Miguel 2005). However, if we compare total values of Shannon-Weaver index calculated for sites Info center and Brankovac (2.3 and 2.1, respectively) with the ones obtained by Kovacz *et al.* (2000) in Austria for aggregated soil samples taken under vital trees (in the range 2.6-2.7) we can conclude that these values are comparable.

The low average number of ECM fungal taxa and value of Species richness and Shannon-Weaver index per soil sample could be explained by the lack of precipitation, which is characteristic for the studied sites during September and October, when sampling was conducted. Also, the summer season in 2017 was one of the warmest for the period 2010-2017 (Galić *et al.* 2018). Drought caused dying out of a large number of roots and consequently high number of old, non-turgescence, and nonmycorrhizal roots were observed in soil samples (data not shown), while the number of ECM fungal taxa per soil sample was decreased. Bzdyk *et al.* (2019) suggest that the number of vital and non-vital ECM roots is influenced by short-term factors such as local droughts, but these factors do not influence total ECM diversity which is supposed to be more susceptible to long-term trends.

At both sites, ECM community was made up of a few abundant taxa and a larger number of rare taxa. This is in accordance with numerous studies (Courty *et al.* 2008, O'Hanlon and Harrington 2012, Suz *et al.* 2014, Rosinger *et al.* 2018, Bzdyk *et al.* 2019). It is assumed that a large number of taxa present at low frequencies may constitute biological insurance for adaptation to changing environmental conditions. Namely, a more diverse ECM community that includes rare ECM species is important in case if ecological conditions change, as some dominant ECM fungi might not be adapted

to the new conditions (Penna *et al.* 2010, O'Hanlon and Harrington 2012).

Ectomycorrhizal fungi determined as *Lactarius quietus*, *Cenococcum geophilum*, and *Tomentella sublilacina* were recorded at both studied sites from Fruška gora. Similarly, the most abundant ECM fungi on temperate oaks across nine European countries were *Lactarius quietus*, *Cenococcum geophilum*, and *Russula ochroleuca* (Suz *et al.* 2014). Moreover, in a temperate oak forest *Lactarius quietus*, *Tomentella sublilacina*, *Cenococcum geophilum*, and *Russula* sp.1 were abundant and present all year long (Courty *et al.* 2008). *Lactarius quietus* is an oak specialist which was recorded as one of the most abundant and frequently occurring ECM fungi on temperate oaks across Europe (Voříšková *et al.* 2013, Bzdyk *et al.* 2019). *Cenococcum geophilum* is a species complex that is the most widely distributed making symbiosis with over 200 tree hosts (LoBuglio 1999). *C. geophilum* is also known for its ability to withstand extreme conditions, especially drought, but it is frequently found on oaks across Europe (de Roman and Miguel 2005, Schirkonyer *et al.* 2013).

Families *Russulaceae*, *Cortinariaceae* and *Thelephoraceae* were reported to be dominant in temperate and Mediterranean oak forests (Courty *et al.* 2008, Richard *et al.* 2011). Moreover, research of ectomycorrhizae in temperate oak forests across Europe revealed that the most abundant family was *Russulaceae*, followed by *Gloniaceae*, *Thelephoraceae*, and *Cortinariaceae* (Suz *et al.* 2014). A higher number of ECM fungal taxa belonging to the phylum Basidiomycota in comparison to *Ascomycota* obtained in our study is concordant to many previous studies (Richard *et al.* 2011, Suz *et al.* 2014; Bzdyk *et al.* 2019 etc.)

Classification of ectomycorrhizae based on ETs connects ECM fungal morphology with their ecology (Agerer 2001). Each exploration type has different functional traits and different capabilities to take up and translocate nutrients and to store carbon (Hobbie and Agerer 2010). Furthermore, it was proven that ETs have site indication value, particularly in relation to nutrients availability and soil composition (Rudawska *et al.* 2011).

At both sites on the mountain Fruška gora, contact and short-distance ETs dominated. According to Bzdyk *et al.* (2019), the contact ET is considered well adapted to soil layers rich in organic matter and to relatively nutrient-rich conditions. In contrast, ETs with abundant mycelial biomass, such as the long-distance ET, were found to be well adapted to nutrient-poor conditions, especially poor in nitrogen (N) (Hobbie and Agerer 2010). Usually, mycorrhizae with contact, short- and medium-distance smooth ETs seem to use labile, mainly inorganic N, while medium distance fringe and mat, and long-distance ETs are believed to use organic N and have degradation enzymes with the strongest potential activities (Lilleskova *et al.* 2011, Suz *et al.* 2014).

Mean annual temperature, pH, and tree species significantly influenced the distribution of the different ETs on root tips of beech, spruce and pine stands across Europe (Rosinger *et al.* 2018). Contact or short-distance ETs of ECM fungi had mostly broad environmental ranges. In contrast, long-distance ETs of ECM fungi were particularly restricted to sites with high mean annual temperatures and low precipitation. Shorter distance ETs are favored in colder climates where soils were richer in total N and plant carbon loss is smaller. On the other hand, long-distance ETs of ECM fungi that dominated communities in warmer and less fertile environments are more carbon demanding (Rosinger *et al.* 2018, Defrenne *et al.* 2019). A high abundance of contact and short-distance ETs recorded in analysed stands on Fruška gora suggests that these soils are sufficiently rich in total nitrogen and organic matter which is concordant with the measured content of humus and nitrogen in the soils.

A potential positive role of ECM functional diversity is indicated by finding the highest diversity of ETs in stands with the best tree health status (Bzdyk *et al.* 2019). Furthermore, a high diversity of ETs can make communities more resilient to environmental change (Suz *et al.* 2014). Thus, it is important to conserve the diversity of ECM fungi and also the diversity of their ETs in our forests.

## CONCLUSIONS ZAKLJUČCI

This work presents results of the first study of the diversity of ECM fungi on sessile oak in Serbia. In two sessile oak stands situated in National Park Fruška gora, 26 ECM fungal taxa were described and identified mostly with molecular methods. Fourteen taxa were observed only at the site Info center, nine taxa only at the site Brankovac while *Lactarius quietus*, *Cenococcum geophilum*, and *Tomentella sublimacina* were recorded at both sites. Contact and short-distance exploration types dominated in studied stands suggesting that soils are sufficiently provided with total nitrogen and organic matter. Lower values of diversity indices in sessile oak stands from Fruška gora in comparison to different oak species across Europe can be explained by drought which lasted during the summer at studied sites. In order to get a more thorough insight into the diversity of ECM fungi on sessile oak in Serbia, research should be continued at more sites and seasonal dynamics should be included as well.

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## SAŽETAK

Hrast kitnjak je jedna od ekonomski i ekološki najvažnijih vrsta listopadnog šumskog drveća u Evropi. U većini ekosustava umjerene klimatske zone zasnivanje, rast i preživljavanje stabala ovise o njihovoj zajednici sa ektomikoriznim (ECM) gljivama. Pri tome informacije o strukturi ECM zajednice mogu pružiti dragocjene informacije o funkcioniranju šumskih ekosustava. Međutim, podaci o razno-

likosti ECM gljiva na hrastovima u Republici Srbiji su rijetki. Cilj ovog istraživanja bio je pružiti prvi uvid u raznolikost ECM gljiva na hrastu kitnjaku u Srbiji. Odabrane su dvije lokacije Info centar i Brankovac, smještene u Nacionalnom parku Fruška gora. ECM gljive su identificirane kombiniranjem morfološke i anatomske karakterizacije s molekularnom analizom nuklearne rDNA ITS regije. Prebrojani su svi vitalni ECM korijenski vrhovi, izračunati indeksi raznolikosti i ECM gljive su klasificirane u eksploracijske tipove. Analiziran je i granulometrijski i kemijski sastav tla. Na obje lokacije zabilježeno je ukupno 26 taksona ECM gljiva, 17 taksona zabilježeno je na lokaciji Info centar i 12 taksona na Brankovcu. ECM zajednice sastojale su se od nekoliko brojnih ECM gljiva i većeg broja rijetkih ECM gljiva. Na obje lokacije zabilježeni su *Lactarius quietus*, *Cenococcum geophilum* i *Tomentella sublilacina*. Na lokaciji Info centar dominirale su porodice Thelephoraceae, Russulaceae i Inocybaceae, dok su na lokaciji Brankovac najzastupljenije familije bile Entolomataceae, Tuberaceae i Russulaceae. Velika brojnost kontaktnih i eksploracijskih tipova kratkih udaljenosti zabilježena u proučavanim sastojinama sugerira da su tla dovoljno bogata ukupnim dušikom i organskim tvarima. Vrijednosti indeksa raznolikosti zabilježene u proučavanim sastojinama hrasta kitnjaka s Fruške gore bile su niže u usporedbi s onima dobivenim u sastojinama različitih vrsta hrasta diljem Europe, što je vjerojatno uzrokovano sušom. Da bi se dobio temeljitiji uvid u raznolikost tipova ektomikoriza na hrastu kitnjaku, trebalo bi nastaviti s istraživanjima na više mjesta i uključiti sezonsku dinamiku.

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**KLJUČNE RIJEČI:** ektomikoriza, *Quercus petraea*, morfo-anatomska karakterizacija, molekularna identifikacija, eksploracijski tipovi