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Diversity of the family *Russulaceae* in the Scots pine forests of Záhorská nížina (SW Slovakia)

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The species diversity of the family *Russulaceae* was studied at four representative sites located in forests dominated by *Pinus sylvestris* on sandy soils in the Záhorská nížina lowland in the years 2010–2012. The diversity varies from 7 to 10 species per site. There is a high variation in species composition and frequency rates between the studied sites. At all four sites, members of two *Russulaceae* genera were represented: *Lactarius* with two recorded species in total, while *Russula* with 19 species. The species diversity of *Russulaceae* in the area of Záhorská nížina is estimated based on data from the representative sites, revision of herbarium material, and published data. Nineteen species were identified in the studied herbarium material, and together with the data from the representative sites this amounts to a total of 27 *Lactarius* and *Russula* species associated with *Pinus* and reported from the area. Published reports on the occurrence of three more species of these two genera, reported to be associated with pine trees in the area, have not been confirmed.

Key words: Scots pine, ectomycorrhiza, fungal diversity, *Russulaceae*, *Pinus sylvestris*.

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Diverzita čeľade *Russulaceae* bola študovaná na štyroch reprezentatívnych lokalitách s dominanciou *Pinus sylvestris* na piesočnatých pôdach na území Záhorskej nížiny v rokoch 2010–2012. Diverzita sa pohybovala v rozmedzí 7 až 10 druhov na reprezentatívnu lokalitu. Výrazné rozdiely medzi jednotlivými lokalitami boli pozorované v druhovom zložení a frekvencii výskytu. Na všetkých študovaných plochách boli zaznamenané dva rody čeľade *Russulaceae*: *Lactarius* s celkovo dvoma nájdenými druhmi a *Russula* s celkovo 19 druhmi. Zisťovanie diverzity *Russulaceae* na území Záhorskej nížiny bolo založené na údajoch získaných z reprezentatívnych lokalít, revíziou herbárového materiálu a excerpciou publikovaných dát. Devätnásť druhov zistených revíziou herbárového materiálu predstavuje spolu s našimi údajmi 27 druhov rodov *Lactarius* a *Russula* viazaných na borovicu zaznamenaných v študovanej oblasti. Publikované údaje o výskyte ďalších 3 druhov udávaných z borín v tejto oblasti neboli potvrdené.

INTRODUCTION

The genera *Lactarius* Pers., *Lactifluus* (Pers.) Roussel and *Russula* Pers., encompassing hundreds of species, represent the majority of species diversity within the family *Russulaceae* Lotsy and are the only known epigeic members of the family in Europe. Species of all three genera are ectomycorrhizal fungi associated with various host trees and many are widely distributed throughout Europe from the lowlands to arctic and alpine habitats (Sarnari 1998).

The checklist of non-vascular and vascular plants of Slovakia (Marhold & Hindák 1998) lists 71 species of the genus *Lactarius* and 107 species of the genus *Russula*. However, this publication has not been updated with recent data, e.g. Adamčík et al. (2006) and Ronikier & Adamčík (2009a) reported on the occurrence of another 6 *Russulaceae* species for Slovakia. Our study deals with the diversity of *Russulaceae* in pine forests (dominated by *Pinus sylvestris*) of the Záhorská nížina lowland (SW Slovakia). These forests include habitats on sand dunes with acidic soils (Kollár et al. 1996).

A comprehensive study focusing on the diversity of macrofungi of Záhorská nížina is not available. Most data on the occurrence of *Russulaceae* in the area were published by Aurel Dermek in illustrated field guides to the identification of macrofungi (Dermek & Pilát 1974, Dermek 1976, 1977, Dermek & Lizoň 1980). Other data are included in brief reports on mycodiversity (e.g. Dermek 1978, Záhorovská & Jančovičová 1997). Recently, partial results of a long-term study of fungi in permanent plots in the area were published in theses by students of Comenius University Bratislava (Caboň & Adamčík 2011, Horinková & Jančovičová 2012). The aim of our study was to estimate the species diversity of *Lactarius* and *Russula* in Záhorská nížina based on a revision of herbarium material and our research at representative sites.

MATERIAL AND METHODS

Study area. The Záhorská nížina lowland is situated in western Slovakia. Our research was focused on its specific part called Bor, covered by large pine forests. The study area has a mostly undulated lowland character with an average altitude of about 200 m a.s.l. The whole area is composed of eolean siliceous sands dominated by podzols and arenosols very poor in nutrients (Ružička 1960). The mean annual temperature is 9–9.6 °C, but during the vegetation period it is 14–15 °C, while annual precipitation is in the range of 550–650 mm, with prevailing evapotranspiration (Krippelová & Krippel 1956).

Our study is based on data collected at four selected sites representing the variability of pine forests in Záhorská nížina. The research was carried out during

the years 2010, 2011 and 2012. The sites were selected in an effort to minimalise the presence of any other ectomycorrhizal host plants.

Because of difficulties with collecting hypogeic fungi which did not allow us to estimate the frequency of basidiomata at the sites as defined below (Adamčík et al. 2006), hypogeic *Russulaceae* species are not included in this study. Species identification within the genus *Russula* is based on Romagnesi (1967), Knudsen et al. (2008), and Sarnari (1998, 2005), within the genera *Lactarius* and *Lactifluus* on Heilmann-Clausen et al. (1998). The data obtained from the representative sites were supplemented by revised herbarium material originating from pine forests in the area.

Representative sites. The four representative sites are at least 1 ha large and were delimited in the field based on vegetation type. The brief descriptions of the plots below are based on our field observations (Tab. 1). Altitude, aspect, and coordinates of the seven vegetation plots (each 400 m^2) used for phytosociological description were measured with Garmin Oregon 450 equipment (WGS-84 system) in the centre of the plots.

Plant communities of all four representative sites are representatives of the alliance *Dicrano-Pinion* (Libbert 1932) Matuszkiewicz 1962 (classification in accordance with the Zürich-Montpellier school; Braun-Blanquet 1964, Westhoff & van der Maarel 1978). The age of the forests was estimated according to forest vegetation maps deposited at regional state forest offices. The nomenclature of vascular plants, lichens and bryophytes follows Marhold & Hindák (1998).

List of studied representative sites and relevés.

Krížnica near Malacky – site with almost completely plain relief, 90-year old planted pine trees in a monoculture with the highest tree (E_3) and moss (E_0) cover, but with the lowest cover of lichens.

Rel. 1: 22 Jun 2008, secondary woodland close to military tower, coord. 48°29'27.00" N, 17°05'26.00" E.

Rel. 2: 7 Jun 2009, pine monoculture 100 m NW between public road and military tower, coord. 48°29'48.00" N, 17°05'58.40" E.

Horné Valy near Tomky, in the direction of Červený rybník pond – partially ruderalised site close to urban settlement, with 61-year old planted pine trees, the lowest diversity of vascular plants and lichens, delimited at S and W margin by *Robinia pseudoacacia* stand, at N margin by moist depression with *Frangula alnus* stand, at E margin by forest road.

Rel. 3: 9 Jun 2009, central part of the site, coord. 48°34'17.30" N, 17°07'18.60" E.

Kalaštov near Borský Svätý Mikuláš – ca. 100-year old forest with relatively large central meadow, rather dispersed trees, high E_2 cover with numerous young trees and high cover of grasses in herb layer.

Rel. 4: 4 Jun 2009, coord. 48°38'54.70" N, 17°14'39.60" E.

Rel. 5: 4 Jun 2009, coord. 48°38'54.70" N, 17°14'38.20" E.

Bulkovec near Borský Svätý Mikuláš – semi-natural, sparse forest, 95- to 105-year old pine forest with large sand dune near W margin and uneven relief, highest cover of lichens among the study sites.

Rel. 6: 4 Jun 2009, sparse forest N of Bulkovec cottage, coord. 48°39'01.60" N, 17°15'16.70" E.

Rel. 7: 13 Aug 2009, forest near swamp by Bulkovec cottage, coord. 48°38'50.50" N, 17°14'27.90" E.

Tab. 1. Phytosociological illustration of Scots pine forests at four representative sites.
Abbreviations of representative sites: **KR** – Kríženica, **HV** – Horné Valy, **KA** – Kalaštov, **BU** – Bulkovec.

Relevé nr.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Representative site	**KR**	**KR**	**HV**	**KA**	**KA**	**BU**	**BU**
Altitude (m a.s.l.)	211	205	184	194	192	202	205
Aspect	–	–	S	SW	E	–	N
Slope (°)	–	–	3	3	1	–	2
Tree cover (%)	75	60	55	45	35	40	30
Shrub cover (%)	–	20	1	20	40	5	5
Herb cover (%)	10	7	10	10	10	13	10
Moss and lichen cover (%)	95	95	90	90	90	80	95
trees and epiphytes							
Pinus sylvestris E_3	4	4	3	3	3	3	2
Pinus sylvestris E_2	.	2	.	2	3	2	1
Viscum album subsp. *laxum* E_3	+	1	+	+	+	+	+
Pinus sylvestris juv.	1	1	+	2	2	2	.
Quercus petraea agg. juv.	+	r	1	+	+	+	.
Frangula alnus juv.	r	.	.	.	r	r	+
herbs and small shrubs							
Rumex acetosella	+	.	+	1	+	+	+
Hypericum perforatum	+	.	.	.	+	.	.
Calluna vulgaris	2	+	.	r	.	.	.
Melampyrum pratense	.	+	+	.	.	.	.
Hieracium pilosella	+	.	.	.	1	.	.
Solidago virgaurea	+	.	.	.	r	.	.
Thymus serpyllum	.	.	.	.	+	.	+
graminoid plants							
Festuca ovina agg.	1	2	2	1	1	1	.
Festuca vaginata subsp. *dominii*	.	.	+	1	+	.	+
Calamagrostis epigejos	1	+	.	+	+	.	+
Carex ericetorum	+	.	1	1	+	.	.

Relevé nr.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Representative site	**KR**	**KR**	**HV**	**KA**	**KA**	**BU**	**BU**
Agrostis vinealis et *capillaris*	+	+	.	+	1	+	.
Luzula campestris agg.	r	r	.	+	.	.	.
Koeleria glauca	.	.	.	1	+	.	.
Corynephorus canescens	.	.	.	+	+	.	.
Avenella flexuosa	.	.	.	.	r	.	2
mosses							
Pleurozium schreberi	4	3	3	2	2	3	4
Dicranum polysetum	+	3	3	3	.	3	2
Dicranum scoparium	.	1	.	+	4	+	2
Hypnum cupressiforme	.	.	2	.	1	1	+
Leucobryum glaucum	1	.	1	.	.	+	1
Hylocomium splendens	1	2	.	.	.	.	.
Ceratodon purpureum	.	.	.	1	1	.	.
Scleropodium purum	1	.	.	.	.	.	+
Polytrichum formosum	+	.	+	.	.	.	.
lichens							
Cladonia furcata	.	2	2	1	1	2	3
Cladonia pyxidata	.	1	+	+	+	+	1
Cladonia rangiformis	.	.	2	2	2	2	2
Cladonia rangiferina	.	.	.	2	3	2	2
Cladonia gracilis	.	.	1	2	+	1	2
Cladonia macilenta subsp. *macilenta*	.	+	.	+	+	r	1
Cladonia fimbriata	+	1	.	+	.	.	+
Cladonia phyllophora	.	.	+	+	.	+	+
Cladonia arbuscula subsp. *mitis*	.	.	1	2	1	.	.
Cladonia subulata	+	.	.	+	.	.	+
Cetraria islandica	.	.	.	.	1	.	1
Cladonia chlorophaea	.	.	.	+	.	.	+
Saccomorpha icmalea	.	.	.	+	.	.	+
Cladonia pleurota	.	.	.	.	.	+	+

In one relevé only:
Rel. 1: *Danthonia decumbens* 2, *Melampyrum sylvaticum* agg. 1, *Rosa canina* agg. juv. +, *Mycelis muralis* +, *Viola reichenbachiana* +, *Rubus caesius* juv. +, *Carex pallescens* +, *C. fritschii* +, *Tilia cordata* juv. r, *Genista tinctoria* r, *Euphorbia cyparissias* r, *Carpinus betulus* juv. r, *Fragaria vesca* +, *Hieracium murorum* +, *Cladonia digitata* +; Rel. 2: *Plagiomnium affine* 1, *Pohlia nutans* +, *Cladonia ciliata* +; Rel. 3: *Robinia pseudoacacia* E_3 1, *R. pseudoacacia* E_2 +, *R. pseudoacacia* juv. r, *Frangula alnus* E_2 +, *Linaria genistifolia* +; Rel. 4: *Festuca filiformis* +, *Spergula morisonii* +, *Cladonia cenotea* +, *C. macilenta* subsp. *floerkeana* r; Rel. 6: *Quercus petraea* agg. E_3 1, *Cladonia coccifera* +, *C. glauca* +, *C. rei* +, *C. squamosa* +; Rel. 7: *Quercus robur* E_2 +, *Betula pendula* juv. +, *Anthoxanthum odoratum* +, *Cladonia arbuscula* subsp. *squarrosa* 1, *C. cornuta* +, *C. coniocraea* +.

Each site was visited at least three times during fructification season in 2010, 2011 and 2012. Each *Russulaceae* species was documented with at least one herbarium specimen from each collecting site, deposited in SAV herbarium (Slovak Academy of Sciences). The frequency of basidiomata was estimated using the following scale (Adamčík et al. 2006): 1 = infrequent (1 collection with 5 or less basidiomata); 2 = moderately frequent (1 collection with more than 5 basidiomata or more collections with less than 5 basidiomata); 3 = frequent (more collections and at least 1 with more than 5 basidiomata). Preliminary data gathered at the representative sites in 2010 were published by Caboň & Adamčík (2011).

Revision of supplementary herbarium material. Abbreviations of herbaria mentioned in this study follow Holmgren et al. (1990). Loans of herbarium material for this study were requested according to references in the literature. Most published data about occurrence of *Russulaceae* in the Záhorská nížina lowland refer to specimens deposited in the BRA herbarium. Two publications, Záhorovská & Jančovičová (1997) and Horinková & Jančovičová (2012) refer to specimens deposited in SLO. In addition, we also included material from SAV collected by the authors outside of the representative sites in earlier years. Only specimens collected in pine forests (associated exclusively with *Pinus* according to their herbarium sheet) are included, and exceptionally a few species were included known to have a strict association with coniferous trees (*Pinus sylvestris* is the only autochthonous conifer in the area).

RESULTS

Russulaceae at representative sites

In total, 21 species of *Russulaceae* were collected at the representative sites (excluding collections published by Horinková & Jančovičová 2012). Only two of them belong to the genus *Lactarius* and 19 to the genus *Russula*. All collected species are listed with their frequency rates in Tab. 2. Ten species were recorded at Bulkovec and Kalaštov, nine at Krížnica, and seven at Horné Valy.

Lactarius rufus and *Russula xerampelina* were collected at all four sites, and *R. silvestris* and *R. vesca* at three sites. The only species with a frequency of 3 at more than one site was *L. rufus*. Four more species had the frequency 3 at one site only: *R. amoenolens*, *R. badia*, *R. caerulea* and *R. vesca*. Four species had a frequency of 2 at two sites: *R. amethystina*, *R. sardonia*, *R. silvestris* and *R. xerampelina*. *R. integra* was collected at two sites with frequencies 2 and 1, respectively. Three species, *R. cyanoxantha*, *R. firmula* and *R. paludosa*, were collected only at the Kalaštov site with a frequency of 2, and one species, *R. pectinatoides*, was collected with a frequency of 2 only at Krížnica. Some spe-

cies seem to be rare, being collected at only one site with a frequency of 1: *L. deliciosus*, *R. acrifolia*, *R. atrorubens*, *R. cessans*, *R. postiana*, *R. risigallina* and *R. vinosa*.

Tab. 2. List of pine-associated *Lactarius* and *Russula* species collected at four representative sites in Záhorská nížina in 2010–2012 and/or confirmed by revision of herbarium material originating from the area. Numbers in the columns of the representative sites are frequency rates (see Material and Methods). Plus sign – species known from the area from published reports (see Discussion). Original identifications are in parentheses.

Species	Representative sites				Published reports	Revised herbarium specimens
	Kalaštov	Horné Valy	Bulkovec	Kŕžnica		
Lactarius deliciosus (L.) Gray			1		+	BRA CR15980, BRA CR15981 (as *L. pinicola*), BRA CR15983, BRA CR15985, SLO 829
Lactarius rufus (Scop.) Fr.	3	3	3	3	+	BRA CR15990, BRA CR15991, BRA CR15992, BRA CR15994, BRA CR15995, BRA CR17938, BRA CR18121, SLO 830, SLO 860
Lactarius semisanguifluus R. Heim. & Leclair						SAV F-3572
Russula acrifolia Romagn.	1					
Russula amethystina Quél.	2		2		+	SAV F-3198, SLO 841
Russula amoenolens Romagn.			3			BRA CR18299 (as *R. galochroa*, revised as *R.* cf. *amoenolens*)
Russula atrorubens Quél.		1				
Russula badia Quél.	3		2		+	SAV F-3201, SLO 840
Russula caerulea Fr.		2		3	+	BRA CR16005, BRA CR16006, BRA CR16007, BRA CR16008, BRA CR17855, BRA CR17856, BRA CR17868, BRA CR17869, BRA CR18297, SAV F-2855, SLO 842, SLO 843
Russula cessans A. Pearson			1			SAV F-2851, SAV F-2853, SAV F-3202, SAV F-3210
Russula cyanoxantha (Schaeff.) Fr.	2				+	BRA CR16010, SLO 839
Russula firmula Jul. Schäff.	2				+	
Russula integra (L.) Fr.		1		2	+	SAV F-3195
Russula nigricans Fr.						BRA CR9182, BRA CR18301
Russula paludosa Britzelm.	2					
Russula parazurea Jul. Schäff.					+	SLO 838
Russula pectinatoides Peck				2		
Russula postiana Romell				1		
Russula puellaris Fr.						SAV F-3200
Russula risigallina (Batsch) Sacc.				1		
Russula sanguinaria (Schumach.) Rauschert					+	BRA CR18302, BRA CR18303, BRA CR18304, BRA CR18305, BRA CR18306, SAV F-2854, SAV F-3203, SLO 837

Species	Representative sites				Published reports	Revised herbarium specimens
	Kalaštov	Horné Valy	Bulkovec	Križnica		
Russula sardonia Fr.		2	2		+	BRA CR17758, BRA CR17763, BRA CR17861 (as *R. caerulea*), BRA CR18307, BRA CR18308, SAV F-2852, SAV F-3207, SLO 836
Russula silvestris (Singer) Reumaux	1	2	2			SAV F-3208
Russula sororia (Fr.) Romell						BRA CR17804
Russula vesca Fr.	1		3	1	+	SAV F-3205, SAV F-3211, SLO 832, SLO 833, SLO 834
Russula vinosa Lindblad				1		
Russula xerampelina (Schaeff.) Fr.	1	2	2	1	+	BRA CR17792, BRA CR18310, BRA CR18311, BRA CR18312, BRA CR18313, BRA CR18314, SAV F-2259, SAV F-3656, SAV F-3657, SAV F-3658, SLO 831
Total number of species	10	7	10	9	13	19

Revision of supplementary herbarium material

A number of 83 herbarium specimens originating from pine forests of Záhorská nížina lowland were revised. They represent 19 identified species (Tab. 2). This number includes collections by Horinková (Horinková & Jančovičová 2012) from the site Kalaštov deposited in the SLO herbarium.

After careful re-examination of collections BRA CR15982 and BRA CR15984 (originally identified as *L. deliciosus*) including a comparison with recent material of other pine-associated species of *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn [syn. sect. *Dapetes* (Fr. ex J. Kickx f.) Burl.], absence of scrobicules on the cap and stipe, and a rather fine spore ornamentation correspond better to *L. semisanguifluus* (Nuytinck & Verbeken 2005, 2007). This identification needs to be confirmed by additional field-identified collections, therefore we treat these collections as "*L.* cf. *semisanguifluus*". A specimen originally identified as *R. galochroa* (Fr.) Fr. (BRA CR18299) has a pileipellis structure typical of *Russula* subsect. *Foetentinae* (Melzer & Zvára) Singer, and the spores suggest its affinity to *R. amoenolens*, but recent literature allows distinguishing it from *R. pectinatoides* only by means of field characters (mild taste and paler brown cap for the latter). Specimen BRA CR18309 is the only representative of *R. torulosa* Bres. This species also requires field observations to distinguish it from related species (e.g. *R. sardonia* collected during our studies). Specimens BRA CR18300 (labelled as *R. emetica*) and BRA CR18302 (labelled as *R. sanguinea*) are misidentified, but we were not able to identify them based on micromorphological characters. We could neither confirm the identity of specimen BRA CR15980 (labelled *L. deliciosus*), because it was found to be infected by *Hypomyces* sp.

DISCUSSION

Occurrence of *Russulaceae* in Záhorská nížina

The results of our study on the diversity of pine-associated *Russulaceae* in the Záhorská nížina lowland are also compared with published data from the area: Dermek & Pilát (1974), Dermek (1976, 1977), Dermek & Lizoň (1980), Hagara (1992), Záhorovská & Jančovičová (1997), Adamčík (2001), and Horinková & Jančovičová (2012). Data from M. Horinková's master thesis (Horinková & Jančovičová 2012) originates from the site Kalaštov. This is the only publication with a complete set of herbarium material documenting all 12 published species. M. Horinková collected two species of *Lactarius* and ten species of *Russula* at Kalaštov during her master degree studies, adding five species leading to a total of 13 species recorded at the site.

Among the other 43 published records on 16 species of pine-associated *Russulaceae* from Záhorská nížina, we managed to find corresponding herbarium specimens for only 12 of them: *L. deliciosus* (BRA CR15985, published by Dermek 1977), *L.* cf. *semisanguifluus* (BRA CR15984, published by Dermek 1976 as *L. deliciosus*), *R. caerulea* (BRA CR16007, published as *R. amara* Kučera by Hagara 1992), *R.* cf. *amoenolens* (BRA CR18299, published by Dermek 1977 as *R. galochroa*) and eight specimens of *R. xerampelina* (BRA CR18310, BRA CR18311, BRA CR18312, BRA CR18313, BRA CR18314, SAV F-3656, SAV F-3657, SAV F-3658, published by Adamčík 2001).

The demand for use of fresh material characters in the recent keys to *Lactarius* and *Russula* is a serious problem for the revision of herbarium specimens, therefore we were not able to confirm some other identifications. On the other hand, both genera have a sophisticated classification system based mainly on micromorphological characters, which allowed us to exclude some wrong identifications (see the last paragraph in Results).

Preliminary results of our research from the year 2010 (Caboň & Adamčík 2011) contain a list of 17 *Russula* and *Lactarius* species with 24 frequency rates from the four studied representative sites in the pine forests of Záhorská nížina. In this paper, we have supplemented this list with four new species, and six new or improved frequency rates for already recorded species. Except for six species (*L. semisanguifluus*, *R. nigricans*, *R. parazurea*, *R. puellaris*, *R. sanguinaria* and *R. sororia*) we collected the species from supplementary herbarium material also at the representative sites. Together with revised herbarium material, we confirmed the presence of 27 *Russulaceae* species. Based on frequency data and published records, *L. rufus*, *R. amethystina*, *R. badia*, *R. caerulea*, *R. sardonia*, *R. silvestris*, *R. vesca* and *R. xerampelina* are the most frequent and typical species in pine forests of Záhorská nížina.

Of the 16 species reported in the published literature from pine forests of Záhorská nížina (not counting our preliminary results published in Caboň & Adamčík 2011), three species were not collected during our study of the representative sites and were not confirmed by revision of herbarium material: *L. helvus* (Záhorovská & Jančovičová 1997), *L. sanguifluus* (Dermek & Lizoň 1980), and *R. velenovskyi* (Záhorovská & Jančovičová 1997). According to published data originating from morphological identification of sporocarps and ectomycorrhizae or based on molecular data from the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/), all three species are known to form ectomycorrhiza with *Pinus*. These three species have to be confirmed from the area by recent collections, because of frequent misidentifications and misinterpretations probably caused by imprecise identifications based solely on pictures from field guides (Ronikier & Adamčík 2009b). For example, *L. sanguifluus* is similar to *L. semisanguifluus* and typical of calcareous areas (Heilmann-Clausen et al. 1998). This suggests possible misidentification because this type of bedrock is missing from the studied area.

The results of our research have been influenced by the length and frequency of our visits, climatic factors, size and position of the representative sites, number of observed herbarium specimens, etc. Because of such a complex set of factors, we are not certain how well our list of species corresponds to the real diversity of pine-associated *Lactarius* and *Russula* species. There are other pine-associated species not reported from Záhorská nížina but well-known to form ectomycorrhiza with *Pinus*, e.g. *L. hepaticus* (http://unite.ut.ee/), *R. adusta* (http://unite.ut.ee/), and *R. ochroleuca* (http://www.deemy.de/). Our data shows a considerable variation of species diversity between the studied sites, e.g. four out of nine species collected at Krížnica were not collected at other sites.

Reports of species collected at representative sites and other examined material from pine forests of Záhorská nížina cannot be considered evidence for their ectomycorrhizal association with pine. At all sites oak seedlings were present, which is also an ectomycorrhizal tree (Tab. 1). For this reason, we have searched for information on ectomycorrhizal partnerships in databases of sequences and ectomycorrhizae (http://www.ncbi.nlm.nih.gov/genbank/; http://www.deemy.de/; http://unite.ut.ee/) as well as in monographic publications on *Lactarius* and *Russula* (e.g. Heilmann-Clausen et al. 1998, Sarnari 1998, 2005, Knudsen et al. 2008) for all 27 species reported from pine forests of the Záhorská nížina lowland. All species except for *R. parazurea* and *R. sororia* have been confirmed to form ectomycorrhiza with *Pinus*. Our results do not prove the ability of the recorded fungal species to form ectomycorrhiza, but rather their ability to grow in pine-dominated forests with soil and microclimatic conditions influenced by the decomposition of pine tree parts and overshadowing by pine trees.

Comparison with pine forests in other regions

Publications on the diversity of *Russulaceae* in Slovakia show a much higher diversity of pine-associated *Russulaceae* in other areas. For example, the contribution on the diversity of *Russulaceae* at three phytosociologically defined sites in oak forests of the Štiavnické vrchy Mts. and Pohronský Inovec Mts. (Adamčík et al. 2013) reports 80 taxa of *Russulaceae*, which is a much higher number compared to pine forests.

The relatively low diversity of *Russulaceae* at the studied sites during our research in the Záhorská nížina lowland corresponds with results of ecological studies on permanent plots with dominance of *Pinus* in other parts of Europe. The three examples briefly described below represent studies with the highest numbers of listed *Russulaceae* from pine forests known to us (Bonet et al. 2004, Oria-de-Rueda et al. 2010, O'Hanlon 2011). None of the long-term research projects at permanent plots in pine-dominated forests listed more than 20 species of both genera. The higher number of species reported in our study is undoubtedly influenced by the methods used: all published studies included all epigeic macrofungi and a high number of plots, which decreases the ability of researchers to identify and score the presence of all species growing there.

Bonet et al. (2004) studied species richness and production of fungal sporocarps in forests with dominance of *Pinus sylvestris* (plantations) in the Spanish Pyrenees at 36 plots at an elevation of 900–1500 m a.s.l. They did not specify the bedrock or soil type of the plots, but it was probably very variable and dominated by various mostly calcareous sediments intercepted by thrust tectonics (Williams 1985). They reported more than 6 species of *Lactarius* and more than 11 species of *Russula* (including one unidentified record of *Lactarius* and 15 unidentified records of *Russula*). The most frequent species were *L. deliciosus* and *R. torulosa*. The slope orientation was mostly northern and the age of the pine plantations was classified into 8 categories (up to 84 years old). Besides these factors, apparently also the presence of other mycorrhizal hosts influenced the data. For example, *R. luteotacta*, a species known to be associated exclusively with deciduous trees (Sarnari 1998) was recorded from five plots and was probably associated with oak (present as shrubs).

Similar results are presented by Oria-de-Rueda et al. (2010) from four stands (12 plots) of pine-dominated forests (four different two-needled species of *Pinus*) on calcareous or siliceous soil in the Mediterranean area of Spain. In total, they reported two species of *Lactarius* and 13 species of *Russula*, most of them associated with *P. pinaster*.

O'Hanlon (2011) carried out a study of eight plots in forests dominated by *Pinus sylvestris* on various acidic soil types (mostly podzol and peat) in Ireland. He listed 6 species of *Lactarius* and eight species of *Russula*, the most frequent

species being *L. hepaticus*, *L. rufus*, *L. tabidus* and *R. ochroleuca*. Three of these species were not confirmed in our area at all. This clearly indicates the importance of climate, soil and other abiotic factors in the diversity and composition of fungal species in pine forests. O'Hanlon collected a relatively high number of species only outside the plots in the same type of habitat, which means that a plot of 100 sq. m is not a reliable sample for estimating fungal diversity. This was also the reason why we did not limit our research to plots of a defined size but rather delimited the boundaries of the study sites according to the extent of a particular plant association.

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