

# Mycobiota in Dead and Damaged Branches of Silver Birch in Slovakia

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## Abstract

The species composition of the mycobiota in dead and damaged branches of silver birch (*Betula pendula*) was studied. The study material collected at 44 localities in Slovakia during the period of 2009 to 2015 was examined. In total, 27 fungal taxa (11 Ascomycetes and 16 Deuteromycetes) were identified on the basis of fruiting body morphology. Sixteen of them have never been recorded on birch trees in the country. *Prosthemium betulinum*, *Trimmatostroma betulinum*, *Cytospora betulicola*, *Cryptosporella betulae*, *Coryneum lanciforme*, *Myxocyclus polycystis*, *Pleomassaria siparia*, and *Disculina betulina* were the dominant colonisers of dead branches. The spectrum of fungi colonising dead and dying branches of *B. pendula* was compared in the following different types of stands: public parks and inter-block spaces of greenery, private gardens, forests, and tree alleys alongside roads. The average number of fungal taxa on birch trees growing in different habitats was not significantly different.

**Keywords:** *Betula pendula*, mycobiota, frequency of occurrence, types of stands, Slovakia, urban trees.

## Introduction

Silver birch (*Betula pendula* Roth) belongs to woody plants growing naturally as part of the forest ecosystem and plays an important role in the regeneration of the landscape (Hynynen et al. 2010). Birch is relatively tolerant of heavy metals in the environment and therefore it can grow in soil contaminated by pollutants and in areas with damaged environment (Kozlov 2005). This ornamental tree is often planted in urban and suburban agglomerations due to undemanding growth requirements and overall tree habitus. A complex set of abiotic (temperature and precipitation extremes) and biotic factors (fungi, insects) including human activity in urban greenery maintenance play an important role in the survival of trees in the urban environment. Unfavourable climatic fluctuations adversely affect the health state of trees in urban areas (Omasa et al. 2005). Correct identification of factors that contribute to reduction of the vitality of trees in the urban environment is a key point in the process of elimination of negative elements in the environment and subsequent revitalisation. Birch wood as a raw material suitable for paper and plywood

production must be without noticeable decomposition and other damage caused by fungi or insects. Fungal pathogens are important agents which initiate and contribute to wood-destroying processes affecting tree stability (Schwarze et al. 2000, Terho et al. 2007, Blinkova and Ivanenko 2016, Ostrovský et al. 2017). Saprophytic and endophytic species usually occur as predominant fungi on dying and dead branches of *Betula* spp. (Kowalski and Gajosek 1998, Barengo et al. 2000, Terho and Hallaksela 2008). Birch trees are susceptible to multiple fungal canker disease including *Botryosphaeria*, *Cytospora*, *Hypoxyton* and *Nectria* cankers (Sinclair et al. 1987, Bernadovičová 2008, Grigaliunaite et al. 2010). Trees weakened by drought may be attacked by fungus *Melanconium betulinum* which causes a progressive dieback of upper branches of birches (Pirone et al. 1960). In Slovakia, Bernadovičová (2008) recorded an increasing number of birch trees affected by *Cytospora* canker. During the assessment of the status of woody plant health in urban greenery of Slovak towns, Juhásová et al. (2003, 2004) and Adamčíková et al. (2011) recorded six fungal taxa associated with damaged branches of birch trees. In Slovak forests, living birch trees are commonly at-

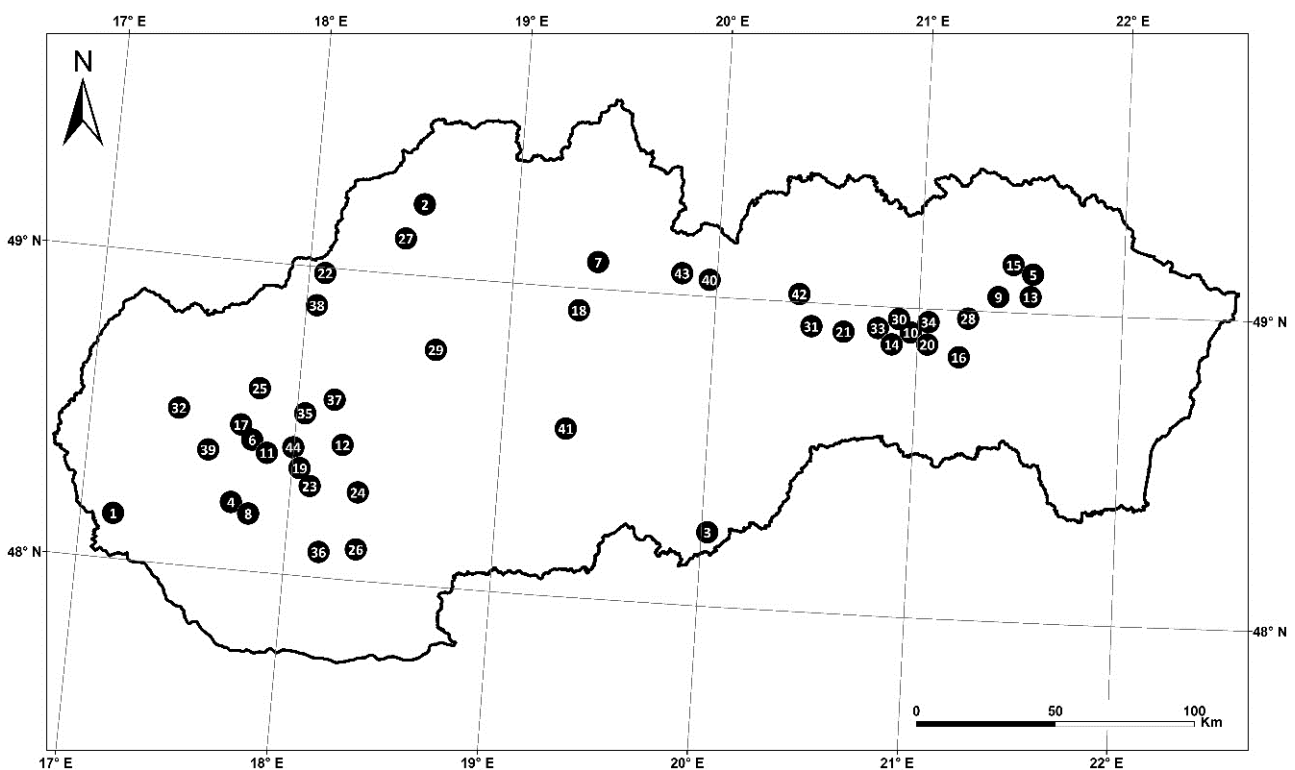
tacked by *Taphrina* fungi causing twig deformation (Bacigálová 1997), and wood decay fungi (Zúbrik et al. 2008). Birches are prone to numerous pests, diseases and environmental problems and are relatively short-lived, especially in hot, dry areas where they may survive for only 20 years. Dry and broken birch trees are frequently found in the urban environment in Slovakia. Such trees present a particularly significant danger during heavy rain and gusting winds. Broken branches and twigs are a gateway for fungi that decay wood structures.

This work is a follow-up to our earlier papers (Pastirčáková and Pastirčák 2010a, 2010b), in which primary results of the spectrum of fungi colonising dead and dying birch branches in Slovakia were published. The aim of the present study was to determine a species composition of fungi colonizing dead and damaged branches of *B. pendula* growing in different types of stands in Slovakia.

**Material and Methods**

*Study sites and sampling*

From 2009 to 2015, during the growing seasons, when dead and damaged branches are visible in the foliage crown, study material was collected from *Betula pendula* trees growing in different types of stands (forest, public parks and inter-block spaces of greenery, private gardens, and tree alleys along the road) at 44 localities in Slovakia (Figure 1). The branches with necrotic lesions, decay of epidermis, or fruiting bodies of fungi clearly visible with the naked eye were selected. Five 30-cm-long and approx. 1.5-2 cm thick sections of 4- or 5-year-old dead and damaged branches in the lower part of the crown were cut from each tree. The samples were placed separately in paper bags and brought to the laboratory for analysis. The number of samples from each site (Table 1) differed depending on the number of symptomatic birch trees. 70 samples were collected in public



**Figure 1.** Map showing the location of sampling sites in Slovakia.

Sampling sites in Slovakia: 1 – Bratislava-Ružinov, 2 – Bytča, 3 – Dechtáre, 4 – Galanta, 5 – Gíraltovec, 6 – Hlohovec, 7 – Ivachnová, 8 – Kajal, 9 – Kapušany pri Prešove, 10 – Kluknava, 11 – Kl’áčany, 12 – Kostol’any pod Tríbečom, 13 – Kračúnovce, 14 – Kropachy, 15 – Kuková, 16 – Kysak, 17 – Leopoldov, 18 – Liptovská Lužná, 19 – Lužianky, 20 – Margecany, 21 – Markušovce, 22 – Nemšová, 23 – Nitra, 24 – Nová Ves nad Žitavou, 25 – Piešťany, 26 – Podhájska, 27 – Považská Bystrica, 28 – Prešov, 29 – Prievidza, 30 – Richnava, 31 – Smižany, 32 – Smolenice, 33 – Spišské Vlachy, 34 – Štefánská Huta, 35 – Šitáre, 36 – Šurany, 37 – Topol’čany, 38 – Trenčín, 39 – Trnava, 40 – Važec, 41 – Vígl’aš, 42 – Vydreník, 43 – Východná, 44 – Zbehy

**Table 1.** Number of fungal taxa found on *Betula pendula* branches at sampling sites in Slovakia

Locality	Type of stand	Number of samples	Number of fungal taxa	Locality	Type of stand	Number of samples	Number of fungal taxa
Bratislava-Ružinov	P	2	2	Nitra	A, P	4, 11	6, 12
Bytča	P	1	5	Nová Ves nad Žitavou	P	1	3
Dechtáre	P	1	3	Piešťany	G, P	3, 5	6, 8
Galanta	P	1	5	Podhájska	P	1	3
Giraltovce	P	2	7	Považská Bystrica	P	1	3
Hlohovec	P	2	4	Prešov	P	1	4
Ivachnová	F	1	4	Prievidza	P	1	3
Kajal	P	3	5	Richnava	P	1	4
Kapušany pri Prešove	G	2	6	Smižany	P	2	4
Kluknava	A, F	5, 14	8, 9	Smolenice	P	1	1
Kľačany	P	1	4	Spišské Vlachy	P	1	3
Kostolány pod Tribečom	F	2	7	Štefánská Huta	F, P	2, 1	2, 3
Kračúnovce	F, P	1, 1	1, 2	Štítáre	P	2	4
Krompachy	F, P	2, 3	4, 10	Šurany	P	1	5
Kuková	F, P	5, 2	8, 6	Topoľčany	P	6	8
Kysak	P	2	4	Trenčín	P	1	5
Leopoldov	P	6	10	Trnava	P	2	3
Liptovská Lužná	F	1	3	Važec	F	5	7
Lužianky	P	1	3	Víglaš	A	1	5
Margecany	P	1	8	Vydrník	A	1	5
Markušovce	P	1	5	Východná	F	1	3
Nemšová	P	1	4	Zbehy	A	5	11

A – tree alley along the road, F – forest, G – private gardens, P – public parks and inter-block spaces of greenery.

parks and inter-block spaces of greenery, 34 samples in forest stands, 16 in tree alleys along the road, and 5 in private gardens. A total of 125 samples were analysed.

#### Identification of fungi

Identification was made by macro- and microscopic analysis of fruiting bodies formed on/in the bark and in the phloem of the branch. For microscopic analysis, hand-cut sections and slides were prepared from fruiting bodies. Microscopic observations were made in tap water. In sections the morphological characteristics of fruiting bodies (ascmata, conidiomata) and fungal spores were evaluated. Observations were made with a stereo microscope (Olympus SZ61, Tokyo, Japan) and standard light microscope (Olympus BX51, Tokyo, Japan). The fungi detected on material examined were identified on the basis of their morphological characteristics using taxonomic manuals for fungi (Grove 1935, 1937, Wehmeyer 1973, Sutton 1980, Barr 1982, Ellis and Ellis 1997, Tanaka et al. 2005).

#### Data analysis

The frequency of occurrence of the fungal taxa was calculated as:

$$\text{Frequency of occurrence (\%)} = \frac{\text{number of samples in which fungal taxon occurred}}{\text{total number of samples examined}} \times 100$$

Differences in number of fungal taxa per sample among the type of stand were tested with one-way ANOVA. Statistical analyses were performed using the statistics software package STATISTICA (StatSoft, Inc. 2011), version 10.

## Results

#### Fungal taxa sporulating on the branches

A total of 27 fungal taxa (11 Ascomycetes, 16 Deuteromycetes) were identified on dead and damaged branches of *B. pendula*. The data overview and the frequency of the recorded fungi is given in Table 2. Sixteen of them have not been recorded on *Betula* in Slovakia so far; these are marked with an asterisk (\*). One to eight fungal taxa per examined sample were found. An average of 3 fungal taxa per examined sample was found (Table 3). The fungus *Prosthemium betulinum* was the dominant species occurring on dead branches (50 % of examined samples). *Trimmatostroma betulinum*, *Cytospora betulicola*, *Cryptosporella betulae*, *Coryneum lanciforme*, *Myxocyclus polycystis*, *Pleomassaria siparia*, and *Disculina betulina* were also the most frequent species on *B. pendula* branches. These taxa were observed in about 25 % of examined samples. Rich fructification of all these fungi was observed on branch segments. Fungi of the genera *Bactrodesmium*, *Diatrypella*, *Melanconium*, *Pseudovalsa*, and *Tubercularia* were recorded in 8-14% of examined samples. About a third of identified fungi occurred in frequency of less than 3%. Although anamorphic fungi were the dominant group, the following fungi have been found in both reproductive stages of their life cycles: *Prosthemium betulinum* with its sexual stage *Pleomassaria siparia*, *Myxocyclus polycystis* with its sexual stage *Splanchnonema argus*, *Coryneum lanciforme* with its sexual stage *Pseudovalsa lanciformis*, *Disculina betulina* with its sexual stage *Cryptosporella betulae*, and *Melanconium bicolor* with its sexual stage *Melanconis stilbostoma*.

**Table 2.** The spectrum of fungi identified on *Betula pendula* branches in Slovakia

Taxon	Number of localities <sup>a</sup>	Number of samples <sup>b</sup>	Frequency <sup>c</sup> (%)	Type of stand <sup>d</sup>
<b>Ascomycetes</b>				
* <i>Cryptosporella betulae</i> (Tul. & C. Tul.) L.C. Mejía & Castl.	21	32	25.8	A, F, P
* <i>Cucurbitaria obducens</i> (Schumach.) Petr.	9	9	7.3	F, P
* <i>Diatrypella favacea</i> (Fr.) Ces. & De Not.	9	10	8.1	A, F, P
<i>Eutypella quatemata</i> (Pers.) Rappaz	2	2	1.6	F
* <i>Leptosphaeria betulina</i> Hazsl.	1	1	0.8	P
<i>Melanconis stilbostoma</i> (Fr.) Tul. & C. Tul.	2	2	1.6	F, P
* <i>Melanomma pulvis-pyrius</i> (Pers.) Fuckel	1	2	1.6	P
<i>Pleomassaria siparia</i> (Berk. & Broome) Sacc.	20	30	24.2	A, F, G, P
* <i>Pseudovalsa lanciformis</i> (Fr.) Ces. & De Not.	4	13	10.5	A, F
<i>Splanchnonema argus</i> (Berk. & Broome) Kuntze	2	4	3.2	F, P
* <i>Valsa ambiens</i> (Pers.) Fr.	1	1	0.8	P
<b>Deuteromycetes</b>				
* <i>Bactrodesmium betulicola</i> M.B. Ellis	11	13	10.5	A, F, P
* <i>Berkleasium concinnum</i> (Berk.) S. Hughes	1	1	0.8	G
* <i>Camarosporium betulinum</i> Died.	2	2	1.6	P
* <i>Coryneum lanciforme</i> (Fr.) Voglmayr & Jaklitsch	17	31	25.0	A, F, G, P
<i>Cytospora betulicola</i> Fautrey	22	36	29.0	A, F, G, P
* <i>Diplodia betulae</i> Westend.	3	3	2.4	F, P
* <i>Disculina betulina</i> (Sacc.) Höhn.	15	30	24.2	A, F, P
<i>Libertella betulina</i> Desm.	4	6	4.8	A, F, P
<i>Melanconium betulinum</i> J.C. Schmidt & Kunze	2	2	1.6	F, P
* <i>Melanconium bicolor</i> Nees	14	17	13.7	A, F, P
<i>Myxocyclus polycystis</i> (Berk. & Broome) Sacc.	11	31	25.0	A, F, G, P
<i>Prosthemium betulinum</i> Kunze	33	62	50.0	A, F, G, P
* <i>Pseudospiropes longipilus</i> (Corda) Hol.-Jech.	4	4	3.2	F, P
* <i>Sporidesmium folliculatum</i> (Corda) E.W. Mason & S. Hughes	1	1	0.8	P
<i>Trimmatostroma betulinum</i> (Corda) S. Hughes	18	41	33.1	A, F, G, P
<i>Tubercularia vulgaris</i> Tode	11	12	9.7	A, F, P

<sup>a</sup>Number of localities on which the fungi were recorded.

<sup>b</sup>Number of samples in which the fungi were present (out of 125 examined samples).

<sup>c</sup>Frequency of the occurrence of fungi in total collection of 125 examined samples.

<sup>d</sup>A – tree alley along the road, F – forest, G – private garden, P – public park and inter-block spaces of greenery.

\*Fungal taxa not recorded on *Betula* to date in the country.

**Table 3.** Number of fungal taxa detected in dead and damaged branches of *Betula pendula*

Type of stand	Number of samples	Total number of fungal taxa	Minimum number of fungal taxa per sample	Maximum number of fungal taxa per sample	Average number of fungal taxa per sample
Tree alley along the road	16	14	1	6	3.69
Forest	34	21	1	6	2.97
Private garden	5	7	1	6	3.60
Public park and inter-block greenery	70	24	1	8	3.31
<b>All stands</b>	<b>125</b>	<b>27</b>	<b>1</b>	<b>8</b>	<b>3.28</b>

***Mycobiota in branches of birch in different types of stands***

The highest total number of fungal taxa (24) was found in public parks and inter-block spaces of greenery and the lowest total number (7 fungal taxa) in private gardens. Mycobiota in birch branches in forest stands was represented by 21 fungal taxa. Fourteen fungal taxa were recorded in tree alleys along the roads (Table 3). The highest number of different fungal species per sample (8 taxa) was found in urban greenery in Krompachy

and Margecany in Eastern Slovakia. Although the highest number of fungal taxa per sample (8) was found on birch trees growing in parks and inter-block greenery, the differences in the average number of fungal taxa per tree among the types of stands were not significant ( $F_{3, 121} = 0.94, p = 0.42$ ; Table 3). Six fungal species (*Coryneum lanciforme*, *Cytospora betulicola*, *Myxocyclus polycystis*, *Pleomassaria siparia*, *Prosthemium betulinum*, *Trimmatostroma betulinum*) were found in all types of stands. The fungi *Berkleasium concinnum*, *Camarosporium betulinum*, *Leptosphaeria betulina*, *Melanomma pulvis-pyrius*, *Sporidesmium folliculatum* and *Valsa ambiens* occurred very rarely and only in public parks, inter-block spaces of greenery and private gardens. *Prosthemium betulinum* was the most frequently occurring species in all types of stands.

**Discussion**

The present results show that the fungal microflora associated with birch branches is very species-rich. In

our earlier papers (Pastirčáková and Pastirčák 2010a, 2010b), ten fungal taxa on birch branches have been recorded. Seventeen other taxa were recorded in the present paper. Two of them, *Eutypella quaternata* (syn. *Quaternaria quaternata*) and *Melanconis stilbostoma*, have already been recorded in Slovakia (Juhásová 2004, Juhásová et al. 2004). Up to sixteen species recorded in present study are not included in the checklist of fungi of Slovakia (Lizoň and Bacigálová 1998). The present paper documents the occurrence of these fungal species on *Betula* for the first time in the country.

Eight fungal taxa dominated the mycobiota in samples examined. *Prosthemium betulinum* and its sexual stage *Pleomassaria siparia*, the most frequently occurring fungi in all types of birch stands in Slovakia, have been reported as endophyte (Kowalski and Kehr 1992), but there are some implications that *Pleomassaria siparia* could be involved in the death of birch twigs (Paavolainen et al. 2000, 2001). Pathogenicity of *Prosthemium betulinum* is still not proven. *Trimmatostroma betulinum*, the second most frequently recorded species, has been detected as a part of endophytic fungal community in silver birch branches (Kowalski and Gajosek 1998, Barengo et al. 2000) but its pathogenicity is still not known. *Cytospora betulicola*, the third most frequently found fungus, is an important plant pathogen causing dieback and canker disease on silver birch in our country (Bernadovičová 2008). *Myxocyclus polycystis* and *Coryneum lanciforme*, also frequently recorded, are considered as saprobic or possibly an endophytic species that act as natural pruning fungi of *Betula* branches (Kowalski and Kehr 1992, Gadgil and Dick 2011). There are no records of these fungi as pathogens. *Cryptosporella betulae* and its asexual stage *Disculina betulina* have been characterized earlier as saprotrophic (Green 2004) or endophytic species (Kowalski and Kehr 1992) but Hanso and Drenkhan (2010) found that this species is an obvious pathogen causing necrotic areas on stems and twigs of young silver birches. *Tubercularia vulgaris* (sexual stage *Nectria cinnabarina* (Tode) Fr.) and *Melanconium bicolor*, recorded in less than 15 % of the samples, are also known plant pathogens that cause birch canker on birch trees (Green 2004, Grigaliunaite et al. 2010).

Serious fungal pathogen *Anisogramma virgultorum* (Fr.) Theiss. & Syd., associated with severe canker disease and crown dieback in birch plantings at upland sites in Scotland (De Silva et al. 2008), has not yet been recorded in Slovakia. The incidence and severity of the disease was much lower at site-natural stands of birch than at planted sites (De Silva et al. 2008). According to Witzell and Karlsson (2002) and Farr and Rossman (2018), the fungus has been recorded in North America (USA) and Europe (Denmark, Finland, Germany, Italy, Sweden,

Switzerland, the United Kingdom, and the European part of Russia).

A number of wood-decay fungi also attack stressed, physiologically weakened, or damaged birch trees. Grigaliunaite et al. (2010) recorded serious damages of birches affected with *Chondrostereum purpureum* (Pers.) Pouzar, *Phellinus igniarius* (L.) Quél., *Stereum hirsutum* (Willd.) Pers., and *Schizophyllum commune* Fr. attacking the living and dying trees. *Cerrena unicolor* (Bull.) Murrill, *Inonotus obliquus* (Fr.) Pilát, and *Kretzschmaria deusta* (Hoffm.) P.M.D. Martin are also very dangerous fungi causing extensive decay of birch trees (Terho et al. 2007). In our country, common damage of birch trees by *Piptoporus betulinus* (Bull.) P. Karst., *Fomes fomentarius* (L.) Gillet, *Armillaria* sp., and *Daedaleopsis confragosa* (Bolton) J. Schröt. was recorded by Bernadovičová (2008) and Zúbrik et al. (2008).

A species composition of fungal communities in branches and twigs of birch trees recorded by Kowalski and Gajosek (1998) and Barengo et al. (2000) was very similar to that obtained during the present study. The most of the fungi that we found on branch segments are typically associated with birch trees and saprobic or endophytic fungi. Certain species occurred in dead or dying tissue independently of type of stands, although their rates of infection may differ. According to Kowalski and Gajosek (1998), the industrial emissions lower the health condition of trees, make the infection for some fungi easier, which initially live as endophytes, and under favourable conditions act as weakness pathogens.

Birches are considered among the shorter-lived trees (Atkinson 1992). Microscopic fungi actively participate in premature dying and breaking of the branches and reduce the overall compactness and aesthetic quality of the birch tree habitus. Butin and Kowalski (1986, 1992) showed a causal relationship between microfungi and detachment of birch branches because of the ability of the fungi to degrade lignin. Based on our observations, leaving the dead branches in the tree crown, or the broken branches on the ground near the tree trunk leads to the multiplication of the fungal pathogens, and infected branches are the source of primary infection of healthy branches. In public parks and inter-block greenery we have rarely recorded damage of the trunk and branches due to incorrect pruning and mechanical damage by anthropogenic activities (mowing, pruning, parking near trees), which is a gateway for fungal infection. For these reasons, regular inspection of trees is needed due to elimination of pathogenic fungi spread.

## Conclusions

The species composition of mycobiota in dead and damaged branches of *Betula pendula* in Slovakia was

reported. The results showed that, in necrotic birch tissue, numerous fungi may occur. Although the total number of fungal taxa found in each type of stands was different, the differences in the average number of fungal taxa per tree among the types of stands were not significant. Sixteen fungal species are new for the Slovak mycobiota.

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