

Current and 20th century distributions of *Vitis sylvestris* in Hungary

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Abstract

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At present, *Vitis sylvestris* C. C. Gmelin is treated as a highly threatened species in Europe, due to the habitat loss and strong human influences. Consequently, the need for distribution maps to aid in the protection of this species is urgent. In this study we analyzed and compared the distribution data from three periods: before 1950, from 1950 to 1990, and from 1990 to the present. We drafted distribution maps of *Vitis sylvestris* and estimated the distribution changes in the last 150 years. Concurrently, we investigated the habitat preferences of *Vitis sylvestris*, and compared the distribution maps of *Vitis sylvestris* C. C. Gmelin and *V. riparia* Michx.

Keywords

distribution map, floodplain forest, *Vitis riparia*, *Vitis sylvestris*

Introduction

Although the current European occurrence of *Vitis sylvestris* C. C. Gmelin (GMELIN, 1806) was documented in 1998 (ARNOLD et al., 1998), we were motivated to map and analyze the distribution of the wild grape, a protected species in Hungary (NÉMETH, 1989; BARTHA, 2000; KIRÁLY et al., 2007), by the conservation priorities of its present habitats. The wild grape appeared during the Atlantic period in the Carpathian basin (JÁRAI-KOMLÓDI, 1966, 1968, 1969; FACSAR and JEREM, 1985), and the studies of the wild grape seed fossils suggest that the wine grape (*Vitis vinifera* L.) has evolved from the wild grape. Already during the Bronze Age, wild grape fruits were collected as food; the wine grape seeds however have only been dated later than the Iron Age (TERPÓ, 1988b, 1988c; GYULAI, 2001, 2009). During the postglacial period, *V. sylvestris* was spreading north from its southern refuges, becoming a common species in European forests, riverbanks, and hillsides (ARNOLD et al., 1998; TERPÓ and BÁLINT, 1987). Large quantities of the wild grape in Hungary were identified on the trees of the Danube floodplain (KERNER, 1863,

1868) between Visegrád [8279.4] and Pilisszentlászló [8379.1]. *V. sylvestris* was likely more common in the 19th and early 20th century than today; Dienes reported about a grape stem as thick as an arm in the Ibrány forest [7896.1] (DIENES, 1939).

Unfortunately, *V. sylvestris* is currently a highly threatened species in Europe – due to habitat loss and strong human influences, consequently, the need for distribution maps to aid in the protection of this species is urgent. In the 20th century, the vast spread of *Vitis riparia* Michx. had a large effect on the distribution and habitat loss of *V. sylvestris* (KEVEY and BARTHA, 2010). The cultivated wine grape (*Vitis vinifera* L.) can spontaneously spread in large areas and hybridize with the native wild grape, leading to a decrease in genetic diversity. The gene stock of the wild-grape stands on Óbuda Island [8480.3] and Pilis [8379.1] differs from the wine-grape cultivars and hybrid species (P. BODOR et al., 2010).

We based our current investigation on previous studies of the wild grape distribution in Hungary (ANDRASOVSKY, 1924–25; KERNER, 1863, 1868; BOROS, 1925, 1930–31, 1936, 1959; TERPÓ, 1962a, 1963, 1966a,

1966b, 1969, 1976a, 1976b, 1977, 1978, 1980, 1986, 1987, 1988a, 1988b, 1988c, 2008; BARTHA, 1991, 1999, 2000; BÖHM, 2007; FACSAR, 2009; FACSAR and UDVARDY, 2006; GÁYER, 1925, 1928; KEVEY, 1983, 1985–86, 1987–88, 1989, 1993a, 1993b, 1996–97, 2001a, 2001b, 2004, 2008; KEVEY and ALEXAY, 1992; KEVEY and HORVÁT, 2000; KEVEY and BARTHA, 2010) and on recent critical assessments and systematization of herbarium data (KEVEY and BARTHA, 2010). We drew distribution maps of *V. sylvestris* and estimated the distribution changes in the last 150 years. Concurrently, we investigated the habitat preferences of *V. sylvestris*, and compared the distribution maps of *V. sylvestris* and *V. riparia*.

Materials and methods

The data collection initially included published studies, Hungarian herbarium data, and field surveys of paper authors encompassing 30 years. We used the Central European Mapping System (NIKLFELD, 1971), operating with geographical longitude and latitude degrees, to construct our distribution maps. *V. sylvestris* distribution patterns were given in grid units of five geographical longitude degree minutes and three geographical latitude degree minutes. Although we did not directly measure the density of the wild grape, we analyzed and compared its distribution patterns before 1950, from 1950 to 1990, and from 1990 to the present, with the aid of the geoinformatic program Digiterra (v. 3.0). We used the geographical macroregion map of Hungary provided by the Landscape Ecological Vegetation Database, the Map of Hungary (MÉTA; MAROSI and SOMOGYI, 1990) and the Hungarian Floramap data (2002–2005) as supports for preparing our distribution map of *V. riparia*.

Results and discussion

We plotted all present, past, and uncertain distribution data for *V. sylvestris* on the geographical macroregion grid map of Hungary (Fig. 1). The majority of these data represent continuous cover on the Great and Small Hungarian Plains; the data in the Transdanubian Medium Mountains and in Southern Transdanubia are scattered, and the data for the Northern Medium Mountains and Western Transdanubia are rare. The data we have got for the period before 1950 concern the floodplain forests of the Danube and the Tisza Rivers, the two largest watercourses in Hungary, and also the Northern (KISS, 1939) and Transdanubian Middle Mountain (KERNER, 1868; BORBÁS, 1879; BOROS, 1936; FEICHTINGER, 1899), Western Transdanubia, Southern Transdanubia, and an area adjacent to the Dráva, the third largest Hungarian river (Fig. 2). The limited amount of data from the mountain areas may be due to the phylloxera disease, which caused the largest losses in this species in Hun-

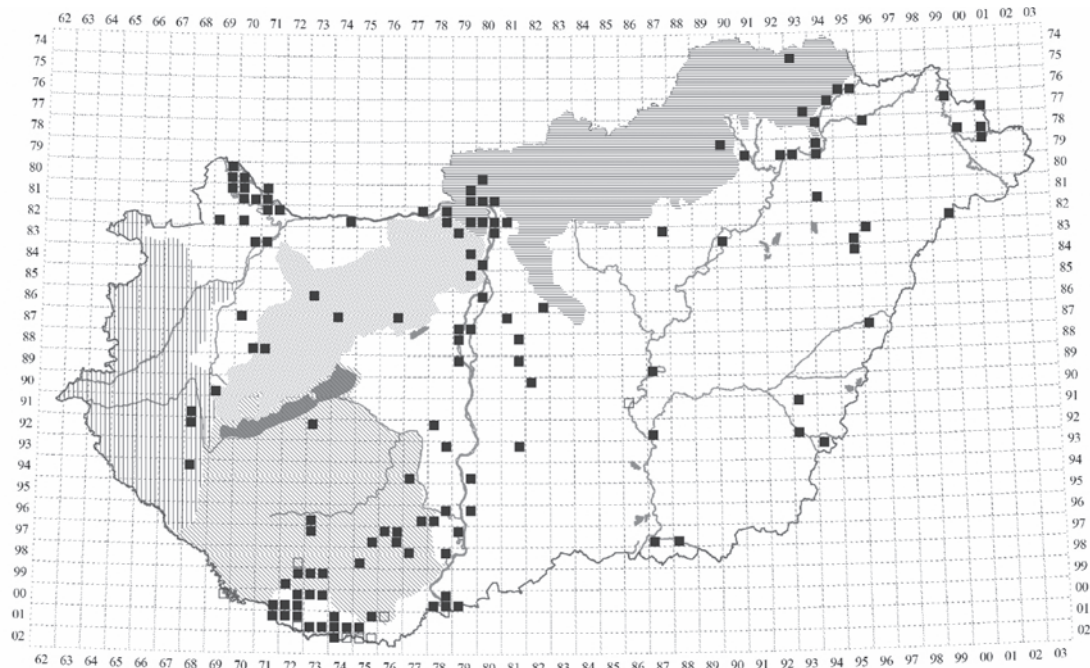
gary in the 1870s. In the floodplain forests, *V. sylvestris* was able to survive in larger quantities because these regions were continuously flooded and phylloxera could not spread rapidly in these areas (TERPÓ, 1962a). For the years 1950–1990, there are available data from the Northern and Transdanubian Middle Mountain, Southern Transdanubia, and many sampling quadrats in the floodplain forests of the Great and Small Plains (Fig. 3). Obviously, there are very few published data along the Tisza river after 1990 (Fig. 4). Despite intensive botanical investigations, the map shows evidence for the decreasing distribution of *V. sylvestris* in Hungary with the exception of two areas: the upper part of the Danube (the Szigetköz) [8170.4; 8170.3; 8271.1; 8271.2] and the areas surrounding the Drava [0171.2; 0172.1; 0173.4; 0174.4; 0274.1; 0071.4; 0072.3; 0073.1; 0073.2]. What is the reason for the decrease of *V. sylvestris* along the Tisza? Most likely the diversion of the river bed (river length reduced by 1/3) caused changes in the surrounding area, which led to the fragmentation and isolation of the floodplain forest habitats. The intensive and rapid spread of *V. riparia*, discussed later, may also have reinforced this effect.

The wild grape is known to occur frequently in three associations: oak-ash-elm forest (*Fraxino pan-nonicae – Ulmetum*), hornbeam-beech forest (*Melitti – Fagetum*), and oak-hornbeam forest (*Quercu robori – Carpinetum*), (TERPÓ, 1988c). We also examined the habitat preferences of *V. sylvestris* (Table 1) over the three chronological periods and separated them into geographical macroregions. The wild grape was present in 135 floodplain forests, but in 69 cases it appeared in different habitats. We concluded that this species prefers the oak-ash-elm forests, as only half as many grapes appeared in mesic broadleaf forests (hornbeam-beech and oak-hornbeam). Seventy-three percent of the habitats occurred on flat land areas in the Great and Small Hungarian Plains; 87% of this amount were floodplain sites (oak-ash-elm), and 13% were not floodplain sites (oak-hornbeam). The Small Plain contains floodplain areas in Szigetköz with wild grape occurring in oak-ash-elm forests as well as oak-hornbeam, white poplar, and white willow forests. Eleven percent of the habitats were found in hilly areas of Western and Southern Transdanubia, with the majority (87%) not in floodplain areas (hornbeam-beech or oak-hornbeam forest). The situation was the same in the Northern and Transdanubian Middle Mountain where 91% of the total 16% observed were not flood area sites (primarily hornbeam-beech and oak-hornbeam).

By the middle of the 20th century, *V. riparia* had been documented in Hungary as a cultivated species along the larger rivers (TERPÓ, 1963). Residents had acknowledged the occurrence of the species on the riverbanks of the Tisza for approximately ten years before 1954, suggesting that *V. riparia* appeared during the Second World War (I'só, 1954). We also created a

grid map of the distribution of *V. riparia* (Fig. 5) highlighting the massive occurrence of the riverbank grape, an effect that may also explain the decrease of the wild grape. Based on comparisons among these distribution-

map, we assume that the native wild grape *V. sylvestris* has been continuously replaced by the adventive *V. riparia*.



■ Distribution of *Vitis sylvestris* in Hungary.

□ Uncertain distribution of *Vitis sylvestris* in Hungary.

Fig. 1. Distribution of wild grape (*Vitis sylvestris*) on the Geographical macroregion map of Hungary.

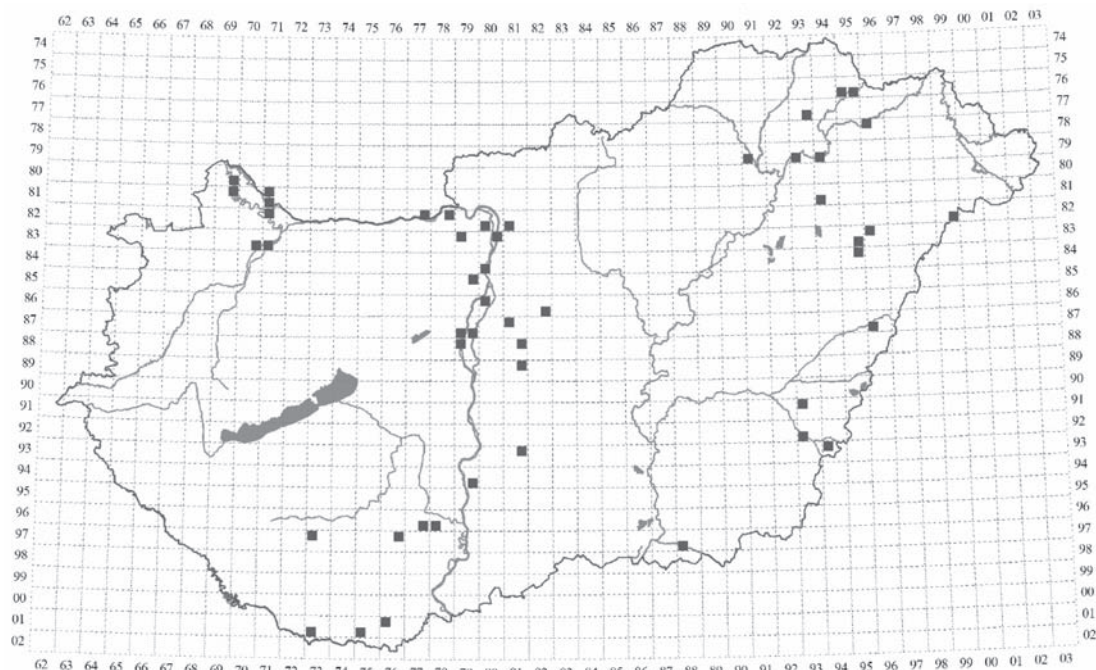


Fig. 2. Distribution map of wild grape (*Vitis sylvestris*) before 1950 in Hungary.

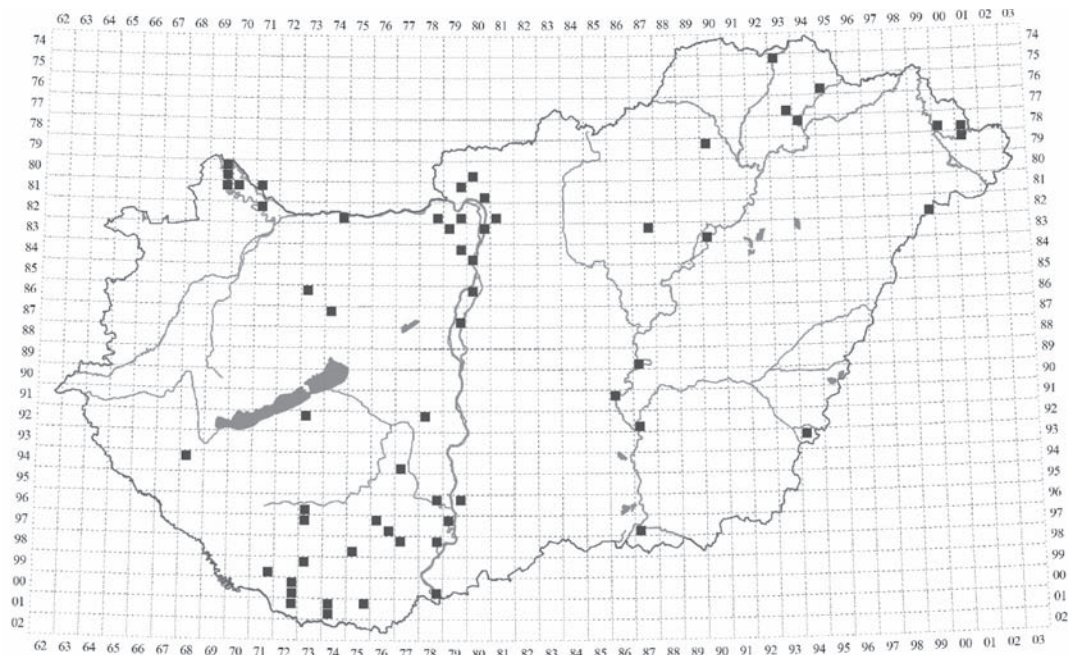


Fig. 3. Distribution map of wild grape (*Vitis sylvestris*) from 1950 to 1990 in Hungary.

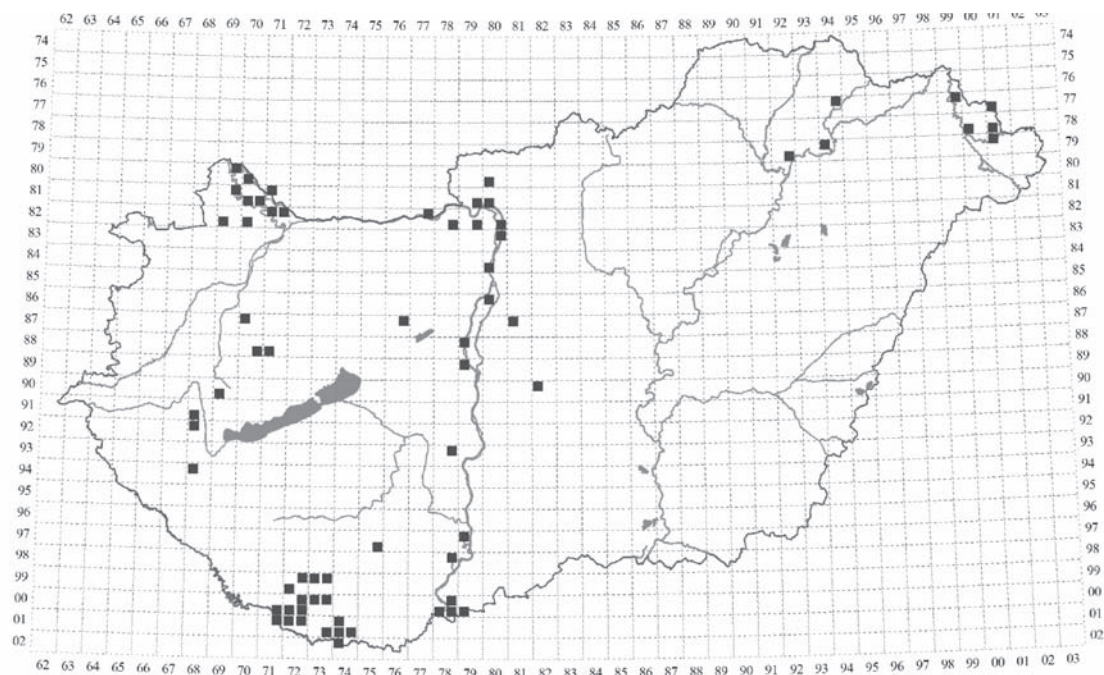


Fig. 4. Distribution map of wild grape (*Vitis sylvestris*) from 1990 to today in Hungary.

Table 1. Habitat preferences of *Vitis sylvestris* in Hungary

Macroregion	Floodplain habitats	Non floodplain habitats	Total
Great Hungarian Plain	95	19	114
Small Hungarian Plain	34	0	34
Northern Middle Mountain	2	11	13
Transdanubian Middle Mountain	1	19	20
Southern Transdanubia	1	18	19
Western Transdanubia	2	2	4
Total	135	69	204

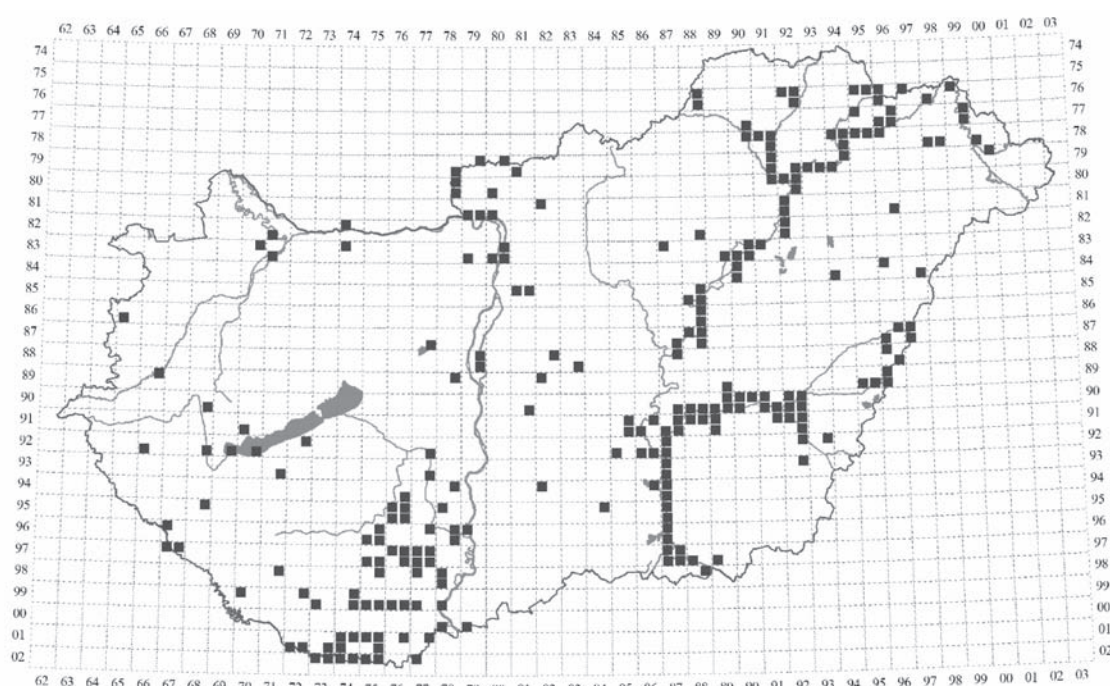


Fig. 5. Distribution map of river grape (*Vitis riparia*) in Hungary.

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Rozšírenie druhu *Vitis sylvestris* v 20. storočí a súčasnosti v Maďarsku

Súhrn

V roku 1998 bol poslednýkrát publikovaný údaj o výskyte viniča lesného (*Vitis sylvestris* C.C. Gmelin) (ARNOLD et al. 1998). Tento fakt nás motivoval k tomu, aby sme podrobne zmapovali výskyt viniča lesného v Maďarsku a mapu neskoršie analyzovali. Vinič lesný patrí do kategórie aktuálne ohrozených druhov Maďarska (NÉMETH, 1989; BARTHA, 2000; KIRÁLY et al., 2007).

Bohužiaľ, v dnešnej dobe počet exemplárov viniča lesného poklesol v dôsledku zníženia počtu biotopov a silného narušenia prírody človekom. Vinič lesný sa v Európe stal ohrozeným druhom, preto sme považovali za dôležité zobraziť jeho výskyt na mape. V 20. storočí sa hojne rozšíril druh *Vitis riparia* (MICHX.), ktorý silne potlačil výskyt viniča lesného. Často zdívie aj vinič hroznorodý a skríži sa s viničom lesným, čo vedie k zhoršeniu jeho genofondu. Údaje o minulom a súčasnom výskyte druhu *Vitis sylvestris* v Maďarsku sme v jednotlivých etapách znázornili graficky. Údaje sme rozdelili do troch etáp (pred rokom 1950, v rokoch 1950–1990 a po roku 1990), čo nám umožnilo jednoduchšie sledovať populáciu viniča lesného. Prieskumy uskutočnené po roku 1990 zreteľne ukazujú, že z okolí rieky Tisa máme len málo údajov, a to preto, lebo v tomto období prebiehala regulácia rieky Tisa. Z porovnania mapových záznamov druhov *Vitis sylvestris* a *Vitis riparia* vyplýva, že populácia pôvodného druhu *Vitis sylvestris* bola postupne vytlačená adventívnym druhom *Vitis riparia*. Okrem toho bola sledovaná aj preferencia prírodných biotopov viniča lesného.

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Natural hybridization in the genus *Abies*: I. Gene exchange in a suture zone of postglacial migration of *Abies alba*

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Abstract

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Patterns of recent migration and gene exchange between divergent lineages of silver fir (*Abies alba* Mill.) were studied in the hybrid zone of genetic lineages associated with different glacial refugia in the Ukrainian Carpathians. Thirty-three silver fir populations from Ukraine, Romania and Slovakia were analyzed using nuclear microsatellites. Based on diploid genotypes, sampled individuals were assigned to either western or eastern lineage using Bayesian inference. A phylogenetic analysis demonstrated the existence of three genetic clusters in the studied area, more or less structured according to genetic lineage and geography. First-generation migrants were concentrated close to the boundary between genetic lineages, whereas F1 hybrids showed a more dispersed distribution. Potential ecological and evolutionary explanations for the observed patterns were shortly discussed.

Keywords

Abies alba Mill., genetic assignment, hybridization, postglacial colonization

Introduction

Evolution has long been considered a simple branching process in which individual genetic lineages split, diverge through accumulation of genetic changes, possibly become extinct, but never join. Darwin himself coined the term “Tree of Life” as a metaphor of this process. In some phylogenetic situations this metaphor is no more valid. In prokaryotes, bacteria can exchange parts of their genomes across species, which is a process called horizontal gene transfer (SNEATH, 2000). In eukaryotes, endosymbiosis also leads to joining previ-

ously separated lineages. Eukaryotic cells contain mitochondria and chloroplasts (plants), having their own genomes of bacterial origins, transmitted from generation to generation (DOOLITTLE, 2000). Hybridization is another example of a reticulate event, quite common in plants; almost one-quarter of plant taxa have probably been involved in hybridization (MALLET, 2005). In these cases evolutionary process is better modeled by a network rather than a tree. Even more serious problem represents the use of tree models in biogeographical studies on the subspecific level. Exchange of genes among regional subpopulations, which sometimes may

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have evolved into separate infraspecific taxa, is absolutely common. Using strictly radiating trees to model biogeographic processes may thus often lead to erroneous conclusions.

The genus *Abies* may represent a good model for the study of reticulate processes. It comprises 59 species organized in two subgenera and 14 sections (FARJON, 2010). However, the issue of subgeneric taxonomy is highly controversial, many taxa recognized as separate species are treated as hybrids or subspecies by different authors (see FARJON and RUSHFORTH, 1989). The section *Abies* is distributed in the northern and eastern Mediterranean area. Five species, namely *A. alba* Mill., *A. nebrodensis* Mattei, *A. cephalonica* Loud., *A. cilicica* Carr. and *A. nordmanniana* Lk. are considered to be separate species, whereas the other taxa (*A. bornmuelleriana* Mattf., *A. equi-trojani* Coode et Cullen, *A. borsii regis* Mattf.) are suspected to be of hybridogenous origin.

Silver fir (*Abies alba* Mill.) is the species with the largest distribution range among Mediterranean firs. The turbulent history of retreats and expansions during the Pleistocene glacials and interglacials produced genetic substructure in the populations of this species. Bordering the European continent from the south by the Mediterranean Sea prevented retaining big population sizes during the glacial periods in most organisms, as the possibilities for a southward retreat were limited. Most species, including silver fir, survived the glacials in refugia, i.e. areas or locations where climates allowing survival and reproduction were preserved, and where trees survived typically in small and fragmented populations. Under the absence of gene flow among refugia, strong differentiation developed, either through genetic drift or by adaptation to regional climates during the ice ages. Migration streams of the postglacial recolonization originating from different refugia may have had strongly differentiated gene pools. Outcomes of crosses between such divergent lineages in the contact zones may be similarly contrasting as those of crosses between closely related species (BURKE and ARNOLD, 2001): both hybrid vigour and outbreeding depression may occur. Information on the patterns of gene exchange among populations of different postglacial origins is thus of utmost importance for practical forestry with respect to gene conservation and seed transfer.

Earlier studies on the Würm and Holocene history of silver fir showed that the sources of recolonization were located in southern Balkans (Greece), northwestern Balkans (Croatia, Bosnia) and the Apennines. Secondary refugia were documented by the presence of macroremains (mainly charcoal) and pollen records also in the Iberian Peninsula, Massif Central, Calabria and elsewhere, but these refugia either remained isolated and developed specific gene pools or were colonized and assimilated by the newcomers (LIEPELT et al., 2009; TERHÜRNE-BERSON et al., 2004). LIEPELT et al. (2002)

showed that there are two maternal lineages of *Abies alba* in Europe (mitochondria are transmitted maternally in most organisms, haplotype distribution reflects thus migration in the diploid stage, i.e. by the adults in animals or by seeds in plants), corresponding to refugial areas (the Apennine and NW Balkan refugia share the mitochondrial haplotype). The studies of GÖMÖRY et al. (2004, 2012) mapped in detail the contact zones: a very narrow and relatively straight contact line was found in the Ukrainian Carpathians, whereas a more complicated boundary was observed in the western Balkans. We reanalyzed the material from the latter study to identify the patterns and extent of the recent gene flow.

Material and methods

We sampled 33 indigenous silver fir populations from the Ukrainian Carpathians and the neighbouring areas of Romania and Slovakia (Table 1). Twigs with 2nd-year needles were collected from approx. 30 trees per population and dried in plastic bags with silica gel until the analysis. Total genomic DNA was extracted from the needles using a modified CTAB protocol following DOYLE and DOYLE (1987). DNA concentration was measured spectrophotometrically. Eleven microsatellite markers according to CREMER et al. (2005) were studied. The PCR reactions were done in 3 multiplex and one singleplex reactions. For multiplex reactions Qiagen Multiplex PCR kit (Qiagen 206143) with Q-solution was used according to manufacturer's instructions in 10 µl mixtures with approximately 50 ng template. Concentration of primers in multiplex A were SF83NED 0.15 µM, SF333NED 0.2 µM, SF1FAM 0.15 µM, SFb4FAM 0.2 µM; in multiplex B SF78PET 0.3 µM, SF331PET 0.3 µM; in multiplex C SF50PET 0.3 µM, SF239FAM 0.2 µM, SFb4FAM 0.1 µM, SF324NED 0.2 µM. Reactions for marker SFg6 contained 1×PCR buffer, 0.8 µg/µl of BSA, 3 mM MgCl₂, 0.2 µM forward and reverse primers, 0.2 µM each dNTP, 0.3 unit *Taq* DNA polymerase (GeneCraft GC 045). The PCR profile for all reactions started with denaturation step at 95 °C for 15 min, followed by 38 cycles of 30 s at 95 °C, 60 s at 55 °C and 90 s at 72 °C. Final extension was at 60 °C for 30 min. PCR products from multiplex A and B and singleplex with SFg6 were mixed in the ratio 1 : 1 : 1 and 1.5 µl of the mixture was mixed with 0.1 µl of size standard and 8.4 µl of formamide, 0.5 µl of an amplification product of multiplex C reaction was mixed with 0.1 µl of size standard and 9.4 µl of formamide. Fragment analysis was performed on ABI PRISM 3130 genetic analyzer. Genotypes were obtained from raw data using GeneMapper 4.0.

We used a distance-based approach to get an initial idea of differentiation patterns. The matrix of pairwise coefficients of differentiation between populations (R_{ST} ; taking into account allele size) was calculated using the

ARLEQUIN v.2.000 software (SCHNEIDER et al., 2000) and subjected to UPGMA clustering using PHYLIP 3.6.a3 (FELSENSTEIN, 2002). The UPGMA tree was visualized using TREEVIEW 1.6.6 (PAGE, 1996).

For the identification of migrants and hybrids, individuals were assigned to populations using multilocus nSSR genotypes. In the earlier study (GÖMÖRY et

al., 2012), individuals were assigned to maternal (i.e., seed-dispersed) lineages associated with the postglacial recolonization using the mitochondrial *nad 5–4* locus. As we were interested in gene flow between maternal lineages, we treated populations with the predominance of the western and/or eastern haplotype as parts of two superpopulations. Actually, distribution range of silver

Table 1. Names, geographical coordinates and sample sizes of the investigated silver fir populations

Name	Longitude	Latitude	Sample size	Mitochondrial lineage
Slovakia				
Kamenica	20°59'	49°12'	33	W
Palota	22°2'	49°16'	35	W
Ukraine				
Kostryna	22°35'	48°56'	38	W
Rozluch	22°58'	49°15'	39	W
Veretskij pereval	23°10'	48°49'	39	W
Pidbuzh	23°14'	49°20'	44	W
Volovets	23°16'	48°45'	37	W
Tukhla	23°28'	48°54'	31	W
Sojmy	23°30'	48°34'	31	W
Truskavec	23°30'	49°17'	40	W
Morzhin	23°53'	49°9'	42	W
Ust' Chorna	23°57'	48°20'	38	W
Rakhinya	24°2'	49°1'	43	W
Kobyletska Polyana	24°5'	48°7'	12	E
Dilove	24°13'	47°56'	38	E
Yasinya	24°19'	48°14'	39	E/W
Rosil'na	24°24'	48°46'	35	W
Lugi	24°32'	48°2'	41	E
Mykulychyn	24°37'	48°25'	36	W
Rybne	24°37'	48°57'	44	E
Beli Oslavi	24°42'	48°30'	41	W
Krivopilskij pereval	24°44'	48°12'	39	E
Knyazhdvir	24°54'	48°33'	36	W
Bagne	25°14'	48°13'	38	E
Putila	25°17'	48°0'	37	E
Migove	25°24'	48°10'	37	E
Budynets	25°39'	48°5'	37	E
Korchivtsi	25°47'	47°57'	35	E
Romania				
Baiut Marmaros	24°0'	47°40'	35	E
Sant	24°49'	47°28'	30	E
Lunca Bradului	25°8'	47°1'	34	E
Campulung Moldovenesc	25°34'	47°33'	34	E
Slatioara	25°40'	47°28'	31	E

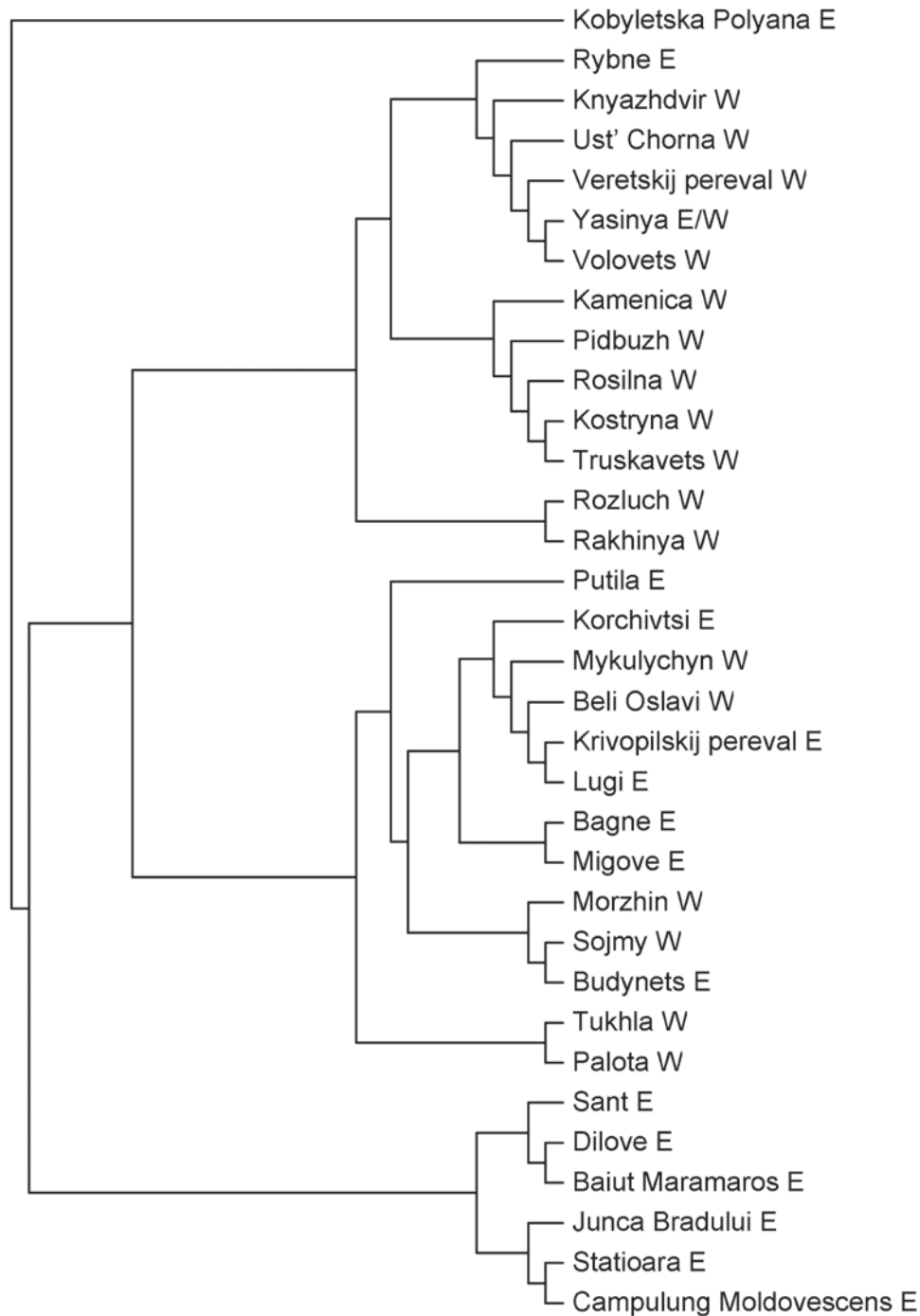


Fig. 1. An UPGMA tree based on pairwise coefficients of differentiation R_{ST} between populations.

fir in the Ukrainian Carpathians is fragmented, with limited gene exchange among fragments, so that such superpopulations do not conform to the ecological or genetic definition of the term 'population'; they were used as purely operational concepts. Further, they are referred to as 'lineages'. As the population Yasinya contains both haplotypes at almost equal proportions, it could not be assigned to either lineage, and was excluded from further analyses. We used Bayesian inference

to assess the likelihood that an individual originated from the one or the other lineage, using the methods of RANNALA and MOUNTAIN (1997) and BAUDOUIN and LEBRUN (2000), as implemented in the program Gene-class 2 (PIRY et al., 2004). The score of the i th individual to belong to the j th lineage ($j = 1, 2$) was calculated as $S_{ij} = L_{ij}/(L_{i1} + L_{i2})$, L_{ij} being the likelihood. Individuals displaying the score higher than 90% to belong to the opposite lineage were considered first-generation

migrants. Trees displaying the score between 40% and 60% were considered F1 hybrids. Geographical patterns of the occurrence of hybrids and migrants were visualized in the ARCVIEW 3.2 environment (ESRI, Redlands, CA).

Gene exchange patterns not follow linear geographical or environmental gradients but reflect curvilinear colonization routes from different glacial refugia along the Carpathian arc and spatial distribution of populations exchanging genes through pollen flow. Therefore, we determined the meeting point of postglacial colonization streams as the point at the main Carpathian ridge lying at the boundary between populations belonging to different lineages (Yablunetskij pereval, 24°27' E, 48°18' N) and expressed the geographical gradient by the distances of the analyzed populations from this point (negative towards the West, positive towards the East). The distribution of the proportions of F1 hybrids and first-generation migrants along the longitudinal gradient (distances from the meeting point) was modeled using the Gaussian curve (procedure NLIN, SAS, 2009):

$$p = p_{\max} \frac{e^{-[(d-c)/\sigma]^2}}{\sigma\sqrt{2\pi}}$$

where p_{\max} is the peak proportion, d is the distance from the meeting point, c is the location where the proportion attains maximum (center of the distribution peak), and σ is the standard deviation (related to the width of the peak). The population Kobyletska Polyana was excluded from this analysis because of a limited sample size.

Results

The distance-based approach (UPGMA tree; Fig. 1) yielded three clusters and one outlier population (Kobyletska Polyana, represented by only 12 trees). Cluster 1 contains solely populations belonging to the western lineage. Cluster 3 consists of Romanian populations (eastern haplotype) and the Ukrainian population Dilove, situated very close to the Romanian border. The central cluster 2 is less consistent, containing almost all Ukrainian populations belonging to the eastern lineage, but also 6 populations where the western mitochondrial haplotype predominates (even the population Palota, located in Slovakia). In spite of the presence of few populations distant from the meeting point, populations close to the boundary between lineages predominate in this cluster; average distance from the meeting point is lower in cluster 2 than in the joint population set of clusters 1 and 3 (one-sided Wilcoxon test, $Z = 1.765^*$; procedure NPAR1WAY, SAS, 2009).

This picture conforms to what showed the Bayesian assignment of individuals into lineages. The two tested methodical approaches (RANNALA and MOUNTAIN,

1997 vs. BAUDOUIN and LEBRUN, 2000) yielded very similar outcomes, which is not surprising, considering that they differ only by the definition of the prior distribution of allele frequencies. The correlation of likelihood scores for the two approaches was 0.92. Nevertheless, geographical distribution of the identified first-generation migrants and F1 hybrids (Fig. 2) indicates that the methodology of BAUDOUIN and LEBRUN (2000) gives slightly more realistic results (less migrants far from the boundary between lineages, smoother distribution of hybrids). Therefore, we refer to these results in the further text.

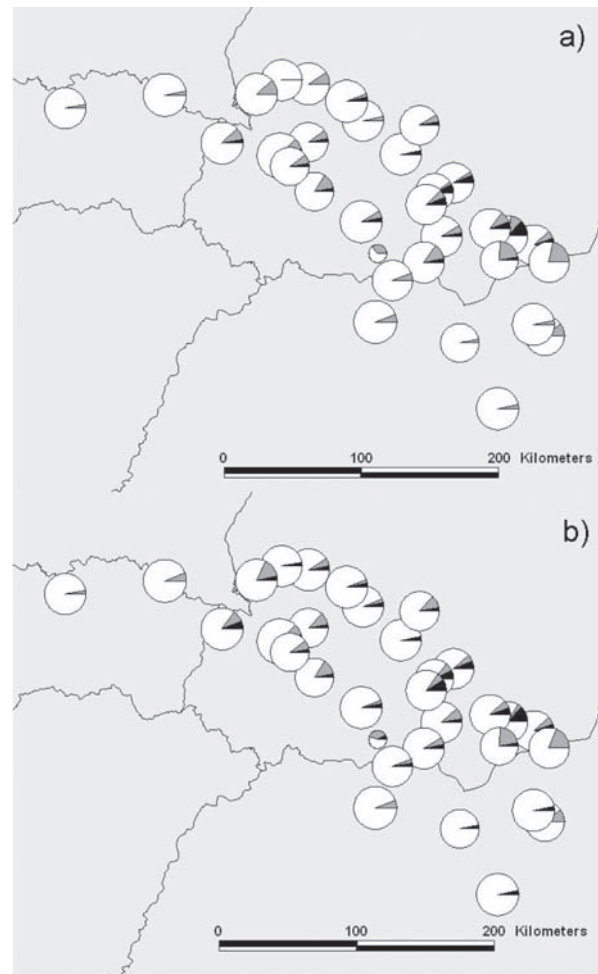


Fig. 2. Geographical distribution of first-generation migrants (black) and F1 hybrids (grey) in silver fir populations: a) Baudouin and Lebrun (2000), b) Rannala and Mountain (1997).

The figures suggest that the proportion of hybrids and migrants is higher in the eastern lineage compared to the western one. The one-sided Wilcoxon test confirmed it for hybrids ($Z = 1.664^*$) but not migrants ($Z = 0.534$ n.s.).

We supposed that the distribution of hybrids and migrants proportion along the gradient of distance

from the meeting point of the streams of postglacial colonization should be unimodal, with the maximum near the boundary of genetic lineages associated with glacial refugia. This assumption proved to be true for the proportion of migrants (Fig. 3a), which fitted significantly with the Gaussian curve with a maximum at -24.6 km (i.e., slightly East of the meeting point). At the same time, the distribution was quite narrow, with $\sigma = 12.6$ km only. First-generation migrants only exceptionally occurred more than 100 km from the meeting point (populations Kostryna, Veretskij pereval, Morzhin, Volovets of the western lineage, none of the eastern lineage; cf. Fig. 2a). On the other hand, no significant fit was found for the proportions of hybrids (Fig. 3b).

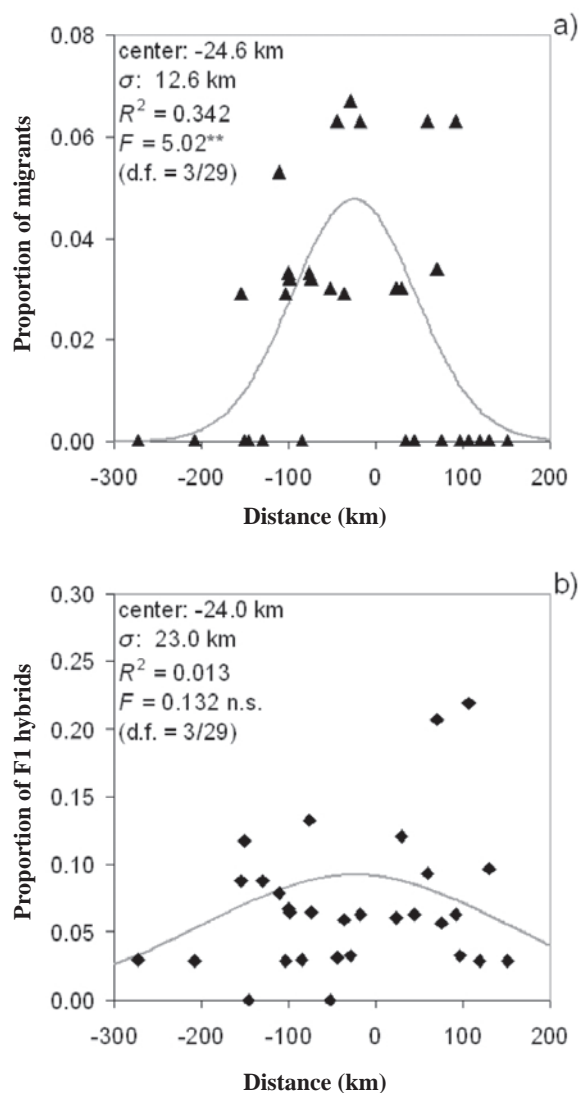


Fig. 3. Distribution of the proportion of first-generation migrants (a) and F1 hybrids (b) along the gradient of distance from the meeting point of maternal lineages fitted to the Gaussian function.

Discussion

Difference in the distribution patterns of hybrids and migrants is associated with different underlying mechanisms of their formation. The occurrence of first-generation migrants is related exclusively to spread by seeds. A 'migrant' in this sense is an individual translocated from one population to another either in the embryonal or in the adult stage, exhibiting thus a genotype corresponding to the gene pool of the source population and thus perceived as alien in the recipient population. Vegetative spread is practically absent in silver fir, so only migration by seeds needs to be considered as a mechanism of migration. The situation of trees identified as 'F1 hybrids' is a bit more complicated. Actually, the designation need not be precise in all cases. Most such trees result very probably from gene flow by pollen, when a local ovule is fertilized by an immigrant pollen grain. However, a different scenario is possible, when a previous-generation migrant is fertilized by local pollen. Naturally, considering a low proportion of immigrants in most population, this case is probably rare. Finally, a close-to-equal representation of domestic and alien genes in the genome of a tree may also result from successive hybridizations and back-crossings. As there is no indication of crossing barriers between the mitochondrial lineages, populations close to the contact zone represent a hybrid swarm, where this possibility cannot be excluded, but the extent of introgression leading to a 50%-to-50% ratio is difficult to estimate.

Genetic assignment of individuals to populations is necessarily associated with errors. Especially in the case of seed migrants occurring in populations distant from the meeting point, the suspect of misidentification is strong. Seed dispersal curves in zoochorous trees have usually been reported to be leptokurtic (or fat-tailed; seeds distributed over very short and very large distances are overrepresented, seeds distributed over medium distances are underrepresented compared to the Gaussian curve), as most seeds are dispersed close to the source, but a part is transported by animals over large distances. This is true also for anemochorous trees to some degree (NATHAN et al., 2001). However, due to more continuous character of the migration front in wind-dispersed plants, long-distance dispersal is much less frequent. AUGSPURGER and KITAJIMA (1992) demonstrated that, once the expanding plant populations meet and a boundary is established, gene flow by seeds over the boundary is negligible because recruitment rates of 'alien' seedlings are low. Long-distance dispersal events are thus not excluded, but are expected to occur exceptionally, mainly when the dispersed seeds do not colonize free patches but land in an established population.

Our study focused on recent gene flow. Naturally, when gene flow effects accumulate over a longer period, genes may be transferred by the stepping-stone mecha-

nism over much larger distances. In a rangewide study, LIEPELT et al. (2002) reported about almost unhindered pollen flow over the whole range. They investigated distribution of a mitochondrial (maternally inherited) and a chloroplast (paternally inherited) gene, which both showed a clinal pattern, but the cline width was over 25° of longitude in the case of the chloroplast gene. This was, however, associated with a methodological failure: their study considered jointly two contact zones of postglacial migration, located actually East and West of the Great Hungarian Plain (the Ukrainian Carpathians and the Dinaric mountains). Treating both zones jointly leads to unrealistically wide clines. Applying the neutral diffusion theory of hybrid zones of BARTON and HEWITT (1985), cline widths observed by LIEPELT et al. (2002) correspond to per-generation dispersal rates of 65 km for seeds and 12,500 km for pollen. Our previous study (GÖMÖRY et al., 2012) focused on the Ukrainian hybrid zone only and found much narrower clines resulting in more realistic dispersal distances (1.5 km for seeds, ~100 km for pollen). These estimates are based on the assumption of the absence of selection pressures, which is fully plausible for mitochondrial and microsatellite markers, commonly considered neutral.

The distribution of the western mitochondrial lineage corresponds quite well to the part of the distribution range of the species where so-called 'silver fir decline' periodically appears (LARSEN, 1986). The syndrome was reported also from Slovakia between 1950s and 1990s and due to its cyclic character is expected to return again. It has been considered the main cause of a steadily decreasing proportion of silver fir in Slovak forests. LARSEN (1986) concluded that the syndrome has a heritable background. If this is true, then the knowledge of the extent of gene flow potentially transferring genes controlling resistance (or decreased susceptibility) to silver-fir-decline causal factors into sensitive western populations is of utmost importance both for gene conservation and legislative regulation of seed transfer.

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Prirodzená hybridizácia v rode *Abies*: I. Výmena génov v kontaktnej zóne postglaciálnej migrácie u *Abies alba*

Súhrn

Predmetom štúdia bol rozsah a obraz recentnej migrácie a výmeny génov medzi evolučne divergentnými genetickými líniami viazanými na pôvod z rozdielnych glaciálnych refúgií u jedle bielej (*Abies alba* Mill.). Jedna z takýchto kontaktných zón bola preukázaná v ukrajinských Východných Karpatoch. Analyzovali sme 33 populácií jedle bielej z Ukrajiny, východného Slovenska a severného Rumunska. Ako genetické markéry boli použité biparentálne dedené jadrové mikrosatelity; tieto úseky DNA sa prenášajú ako semenami, tak aj peľom. Na základe diploidných genotypov sme pre každého jedinca stanovili pravdepodobnosť jeho priradenia k západnej resp. východnej genetickej línii, pričom boli využité postupy založené na Bayesovskej analýze. Fylogenetická analýza preukázala existenciu 3 zhlukov, ktorých zloženie odrážalo ako genetické línie, tak aj zemepisnú pozíciu. Migranti prvej generácie sú vo väčšine prípadov rozmiestnené v blízkosti deliacej čiary medzi genetickými líniami, aj keď ojedinele boli identifikované až do vzdialenosti vyše 100 km. Na druhej strane, F1 hybridy vykazovali oveľa rovnomernejšie rozmiestnenie pozdĺž geografického gradientu migrácie. V krátkosti sú v práci diskutované možné ekologické a evolučné vysvetlenia pozorovaných javov.

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European spruce as an indicator of environmental pollution: an example from the Vysoké Tatry Mts

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Abstract

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Historical variations of the composition of the environmental compartments can be traced by various methods. One of them is dendrochemistry, the quantitative observation of chemical composition of tree rings. Here we report such study on three selected trees from the National Park Vysoké Tatry Mts in Slovakia. Tree rings were analyzed by laser-ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) and we focus on the concentrations of essential (P, K) and toxic (Pb) elements. All three trees belong to European spruce (*Picea abies*) and the historical variations were strongly overprinted by the intrinsic biological processes within the trees. For the essential elements, especially phosphorus, we see a strong enrichment of this element in the youngest tree rings, owing to the preferential channeling of the fluids along these pathways. The pollutants, such as lead, show a signal smeared by the translocation of the elements within the trees. Only in one tree, the declined signal in the past could be related to the decrease of use of the leaded gasoline in Czechoslovakia. If compared to the literature, one could conclude that the national park was located far away from major pollution sources and therefore the results document only the pristine nature within the park, not the variations of the environmental load over the last decades.

Key words

chemical composition, dendrochemistry, lead, phosphorus, tree rings

Introduction

The past variations in the environment are important clues for the sustainable development of the modern society. Yet, these changes are difficult to monitor. Unless samples have been collected in regular temporal intervals over a longer period, the variations must be deciphered from geological or biological records. The former include sediments (MONECKE et al., 2008) or ice cores (BATTLE et al., 1996), the latter tree rings. Chemical composition of the tree rings is the subject of dendrochemistry, with the aim of detecting natural (PEARSON et al., 2006) or man-induced (WATMOUGH and HUTCHINSON, 1999) fluctuations in the environment. Trees can provide a record of their environment over decades or

centuries, reflecting the changes in the concentration of elements but also physical factors (e.g., temperature) of their immediate surrounding. Not all tree species may be suitable for the long-time record as some species exhibit a significant radial translocation of elements across ring boundaries (CUTTER and GUYETTE, 1993).

Materials and methods

In this study, we have examined samples from European spruce (*Picea abies*) trees. The first tree (tree 1) grew near the settlement of Javorina and was cut in 2008. The nearest public road is located about 1 km from the locality where the tree grew. Two trees (tree 2 and 3) grew

in a valley called Tichá dolina and fell during a major storm on November 11, 2004. There are no publicly accessible roads in this valley.

The sections cut from the trees were cut and hand-shaven by a jack plane. From these pieces, stripes of ~1 cm thickness were cut. The thickness of the rings was measured under a binocular, and the stripes were cut to smaller pieces to fit into the laser-ablation chamber of the analytical instrument.

For the analysis, we used a laser-ablation inductively-coupled plasma mass spectrometer (LA-ICP-MS) ThermoFisher Scientific with the LA accessory from Merchantek. The instrument is equipped with a Nd:YAG laser. To remove surface contamination, a pre-ablation run was performed before the data were collected. The material ejected by the laser ablation for the data collection itself was fed into the ICP and analyzed by the mass spectrometer. The elements/masses sought were ^{11}B , ^{24}Mg , ^{27}Al , ^{29}Si , ^{31}P , ^{39}K , ^{44}Ca , ^{55}Mn , ^{56}Fe , ^{65}Cu , ^{66}Zn , ^{88}Sr , ^{111}Cd , ^{137}Ba , and ^{208}Pb . All elements were measured relatively to ^{12}C which was determined quantitatively by a separate set of analyses (see below). To quantify the elemental concentrations, we employed a standard Virginia tobacco leaves (CTA-VTL-2) with certified concentrations for the analyzed elements. The validity of the procedure was tested by comparing the LA-ICP-MS data with a digestion of the wood tissues and subsequent analysis by a conventional ICP-MS in a separate set of experiments (LIPPELT, 2009) in our laboratory.

The total organic carbon (TOC) content was determined by a multiN/C 2100 (Analytik Jena) instrument on a set of similar wood samples and the standard in our laboratory previously.

Natural settings

The primary goal of this study was to evaluate the environmental variations in the trees which grew in the National Park Vysoké Tatry over the last 100 years. The national park was established in 1949 and was strictly protected as a refuge for rare and endemic natural species since then. The national park is one of the smallest Alpine terrains of the world. The main ridge is only 26 km long; the highest peaks reach over 2,600 meters above the sea level and form a natural barrier between the geological and geomorphological units of Inner Western Carpathians (mostly in Slovakia) and Outer Western Carpathians (significantly in Poland). There are no major mountain belts north from the Vysoké Tatry; the flat terrain of Poland extends all the way to the Baltic Sea and allows for a free movement of the air masses. On the other hand, there are numerous mountains south of Vysoké Tatry, and the motion of the air is mostly restricted to the W-E direction of the Podtatranská kotlina, a major valley with high population density and abundant industry. Significant industrial parks existed and exist in the cities of Ružomberok

and Žilina located west from the national park. Because of the prevalent eastward winds and the high air-borne contamination in these areas, the western part of the national park is relatively polluted. The central and eastern parts, however, where the tree samples were collected, belong to the less polluted portions of Slovakia. Despite of the limited input of pollutants from the Slovak industrial sources, both localities are known to be affected by the imission of the metallurgical industry in Katowice, Poland.

Results and discussion

Based on their mutual correlation, the analyzed elements can be divided into two large groups. In all three trees, there was a relatively strong correlation between Ca, Sr, Mg, Mn, Ba, K, Zn, and P. Additionally, Pb and Cd showed a weak correlation with this group of elements. On the other hand, the elements Si, B, Al, Cu, and Fe showed no correlation to any analyzed element. The numerical values of correlation coefficients are listed in Table 1.

The element from the first group showing the strongest affinity to biological matter is phosphorus (Fig. 1). In all the studied trees, phosphorus shows a marked increase toward the last years of the trees' life span. Although this trend could be interpreted as an increase in phosphorus (phosphate) availability during the last years of the trees' life, it is more likely that this trend reflects the translocation of an essential element such as phosphorus into active parts of the trees. CUTTER and GUYETTE (1993) studied an 18-year old spruce and found out that 10–12 rings were active in the process of water transportation. Therefore, translocation of elements can be expected within such a range and a possible environmental change may not be assigned to a specific year. The translocation smears the signal. PROHASKA et al. (1998) studied tree rings from spruce trees which grew under controlled conditions. They observed that not all elements in the tree rings reflected a sudden environmental change (shutdown of the nearby pollution source) for at least 3 years.

Other elements, for example potassium (Fig. 2), show a similar, albeit not as strong increase toward the end of their lives. In general, elements like potassium, magnesium, and other essential elements, show a steady, uniform concentration across the measured profile, as these concentrations are probably maintained at this level by the biological functions of the trees.

We particularly focused on elements recognized as toxic to biota, such as lead. In the trees 1 and 2, lead behaves similarly as K or Mg (Fig. 3), that is, the concentrations are relatively uniform, with fluctuations between the vegetative period and the offseason. The increased concentrations of Pb in the tree 1 and tree 2

Table 1. Correlation coefficients for the elements analyzed in the studied trees

Tree 1	B	Mg	Al	Si	P	K	Ca	Mn	Fe	Cu	Zn	Sr	Cd	Ba	Pb
B	1.00														
Mg	0.51	1.00													
Al	0.28	0.52	1.00												
Si	0.08	0.07	0.14	1.00											
P	0.48	0.92	0.47	0.23	1.00										
K	0.50	0.96	0.49	0.14	0.91	1.00									
Ca	0.51	0.98	0.53	0.07	0.90	0.94	1.00								
Mn	0.50	0.97	0.51	0.12	0.90	0.94	0.97	1.00							
Fe	0.09	0.16	0.14	0.09	0.17	0.17	0.16	0.14	1.00						
Cu	0.25	0.45	0.33	0.27	0.51	0.44	0.44	0.47	0.11	1.00					
Zn	0.46	0.91	0.54	-0.01	0.77	0.86	0.94	0.90	0.16	0.35	1.00				
Sr	0.51	0.98	0.53	0.09	0.92	0.95	0.99	0.97	0.16	0.47	0.92	1.00			
Cd	0.27	0.57	0.29	-0.01	0.47	0.51	0.56	0.58	0.07	0.35	0.53	0.56	1.00		
Ba	0.50	0.98	0.52	0.07	0.91	0.94	0.99	0.97	0.15	0.44	0.91	0.99	0.56	1.00	
Pb	-0.02	-0.05	0.04	0.11	-0.01	-0.03	-0.03	-0.02	0.08	0.02	0.00	-0.02	-0.07	-0.03	1.00

Tree 2	B	Mg	Al	Si	P	K	Ca	Mn	Fe	Cu	Zn	Sr	Cd	Ba	Pb
B	1.00														
Mg	0.08	1.00													
Al	0.08	0.24	1.00												
Si	0.06	-0.47	0.04	1.00											
P	0.04	0.05	0.18	0.12	1.00										
K	0.08	0.67	0.35	-0.34	0.55	1.00									
Ca	0.05	0.81	0.23	-0.31	-0.18	0.45	1.00								
Mn	0.05	0.91	0.21	-0.54	-0.04	0.65	0.87	1.00							
Fe	0.08	0.08	0.07	0.02	0.02	0.04	0.03	0.03	1.00						
Cu	0.01	0.03	0.04	-0.02	0.01	0.06	0.04	0.04	0.00	1.00					
Zn	0.04	0.78	0.27	-0.41	-0.04	0.52	0.82	0.80	0.05	0.04	1.00				
Sr	0.08	0.86	0.28	-0.51	0.12	0.77	0.78	0.88	0.04	0.06	0.79	1.00			
Cd	0.05	0.18	0.11	-0.10	-0.04	0.16	0.17	0.19	0.04	0.03	0.17	0.24	1.00		
Ba	0.06	0.70	0.19	-0.42	-0.09	0.46	0.76	0.75	0.03	0.04	0.71	0.79	0.21	1.00	
Pb	0.04	0.28	0.10	-0.24	-0.08	0.26	0.26	0.35	0.05	0.01	0.33	0.30	0.04	0.27	1.00

Tree 3	B	Mg	Al	Si	P	K	Ca	Mn	Fe	Cu	Zn	Sr	Cd	Ba	Pb
B	1.00														
Mg	0.04	1.00													
Al	0.05	0.33	1.00												
Si	0.04	0.21	0.38	1.00											
P	0.02	0.43	0.33	0.53	1.00										
K	0.07	0.63	0.36	0.44	0.75	1.00									
Ca	0.02	0.47	-0.01	-0.25	-0.07	-0.08	1.00								
Mn	0.02	0.53	0.01	-0.20	-0.05	-0.02	0.87	1.00							
Fe	0.05	0.08	0.07	0.25	0.06	0.06	0.03	0.01	1.00						
Cu	0.04	0.18	0.23	0.20	0.09	0.14	0.07	0.08	0.05	1.00					
Zn	0.05	0.55	0.15	-0.22	-0.15	0.08	0.60	0.65	0.05	0.16	1.00				
Sr	0.03	0.57	0.12	-0.12	0.13	0.12	0.90	0.82	0.04	0.16	0.62	1.00			
Cd	0.05	0.21	0.07	-0.03	-0.12	0.02	0.28	0.27	0.06	0.26	0.38	0.28	1.00		
Ba	0.05	0.42	-0.02	-0.47	-0.31	-0.02	0.64	0.61	0.02	0.12	0.79	0.62	0.31	1.00	
Pb	-0.02	0.07	0.26	0.58	0.26	0.32	-0.40	-0.31	0.06	0.11	-0.37	-0.28	-0.10	-0.60	1.00

cannot be correlated, and therefore probably do not represent a regional or a large-scale influx of lead into the area where the trees grew. On the other hand, tree 3 shows a much smaller Pb concentrations prior to 1920, with occasional spikes of very short duration. The lead concentration starts to increase around 1925 and continues until mid 1950's where it reaches a concentration comparable to that seen in trees 1 and 2. Because the changes in the observed trees are difficult to correlate to each other, it is not easy to assign the observations from all three trees to a single source; for example, the

onset of automobile transportation in Czechoslovakia. In the last decade, however, the lead concentration in the tree rings has been slightly decreasing which could be perhaps explained by the decreased sales of leaded gasoline in Slovakia. An alternative explanation could be the translocation enabling to remove a toxic element such as Pb from the active parts of the trees.

In summary, tree rings have a potential to record the past environmental changes. According to this study, it seems that this potential has been smeared by the translocation of elements within the trees. In a

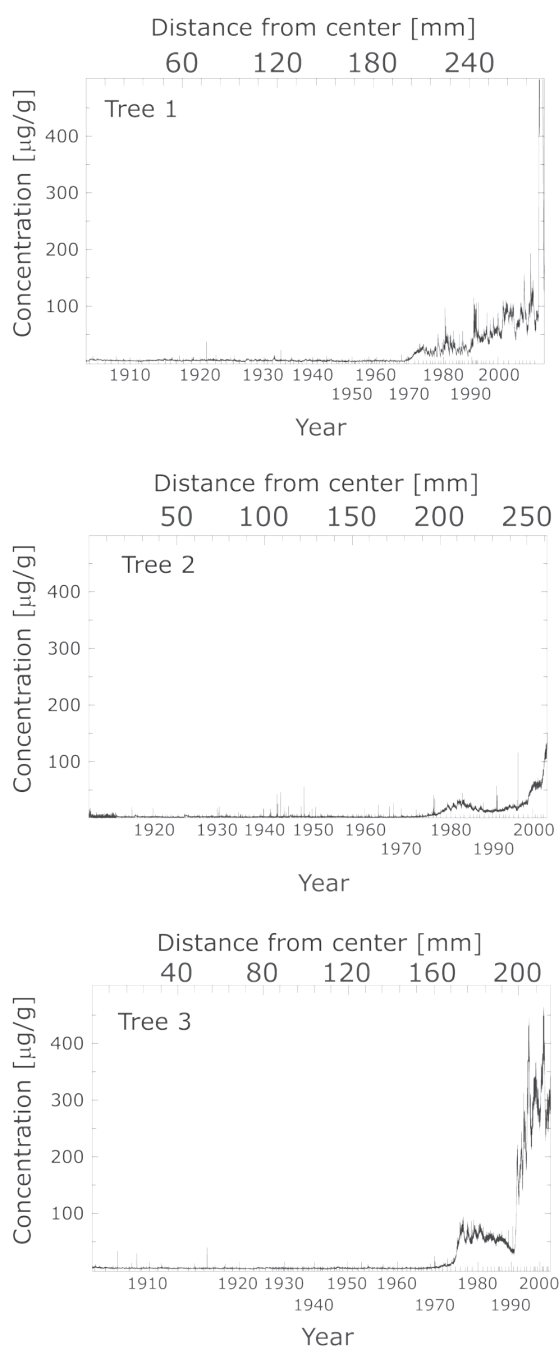


Fig. 1. Concentration profiles of phosphorus in the studied trees.

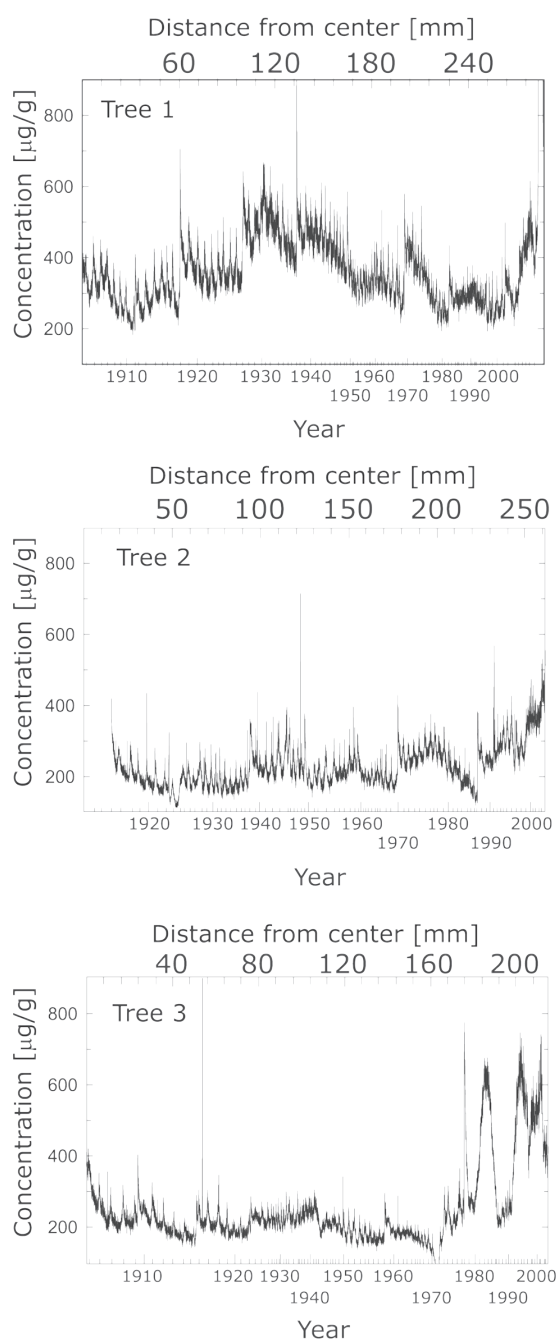


Fig. 2. Concentration profiles of potassium in the studied trees.

single case (tree 3) for one toxic element (Pb), a trend of increase was seen that could be linked to industrial activities. In general, however, the trees studied here do not show any major environmental changes over the past 100 years, either positive or negative. WATMOUGH et al. (1998) have found that the environmental changes associated with Pb can be monitored well in big cities and near major highways, that is, in the immediate vicinity of large sources of this metal. At rural sites, no

fluctuations were seen, similarly to our trees 1 and 2. Therefore, we can conclude that the trees in the Vysoké Tatry Mts have not experienced any local drastic environmental changes. For regional and large-scale changes due to metals, they were located too far away from the pollution sources. Gaseous pollutants, such as SO₂, were not addressed in this study, and need to be monitored by different techniques.

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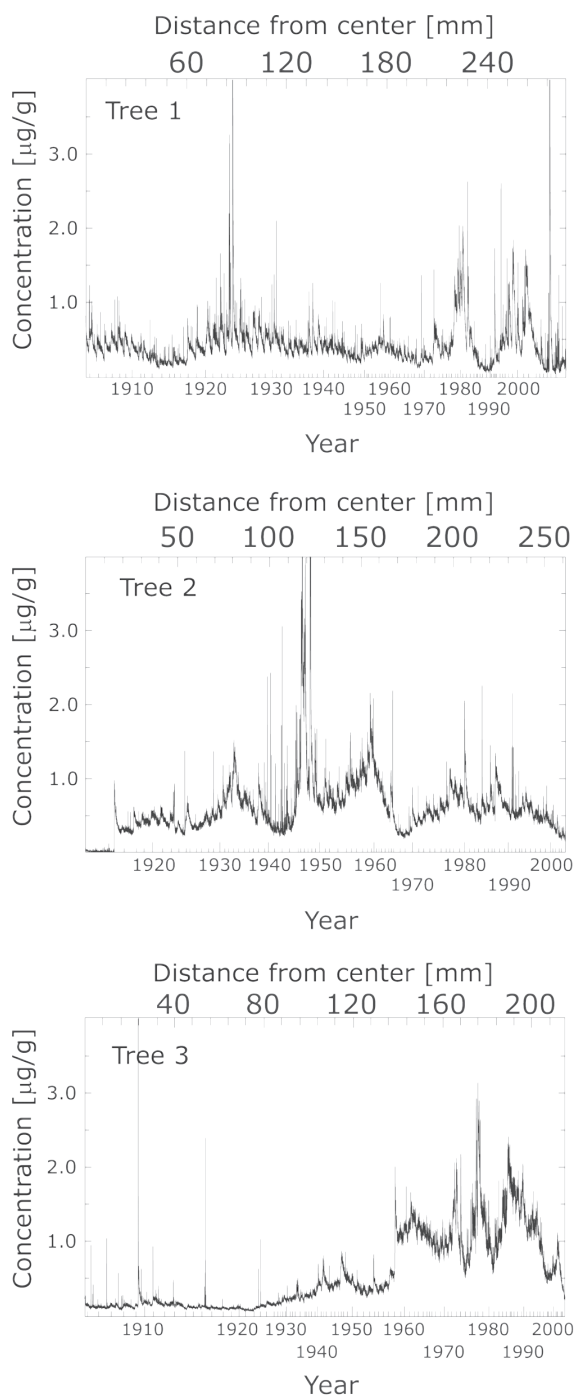


Fig. 3. Concentration profiles of phosphorus in the studied trees.

Smrek ako indikátor znečistenia životného prostredia: príklad z Vysokých Tatier

Súhrn

Vzorky z troch stromov smreka (*Picea abies*) z Vysokých Tatier boli získané ako odrezané disky na analýzu prvkov. Tieto drevené disky boli analyzované pomocou laserovej ablácie a následnej analýzy s hmotnostnou spektrometriou s indukčne viazanou plazmou. Vzorky boli analyzované na viacero prvkov. Sú medzi nimi elementy, ktoré sú nevyhnutnou súčasťou živých organizmov, napríklad P alebo K, ale aj prvky, ktoré znečisťujú životné prostredie a sú používané ako indikátory stupňa znečistenia, napr. Cd alebo Pb.

Jedna vzorka zo smreka vykazuje podstatné zvýšenie olova v dreve počas svojho života. Zvyšovanie sa začína v 20. rokoch 20. storočia a pokračuje až do 60. rokov.

Obsah draslíka sa mení s časom výraznejšie v jednej vzorke smreka. Od začiatku 70. rokov tu pozorujeme drastické zmeny. Obsah draslíka sa prudko zvyšuje okolo roku 1980 a klesá okolo roku 1990. Potom zase stúpa a za posledných 10 rokov sa stabilizoval.

Obsah kadmia, napríklad, sa vo všetkých troch stromoch počas celého ich života prakticky nemení.

Spomedzi prvkov nevyhnutných pre živé organizmy sú výrazné zmeny pozorovateľné najmä v prípade fosforu. Obsah fosforu prudko stúpa v letokruhoch, ktoré predstavujú posledných približne 30 rokov. V tomto prípade však existujú početné štúdie, ktoré ukazujú na to, že stromy majú tendenciu prenášať niektoré potrebné prvky, napríklad fosfor, zo staršieho dreva do mladšieho a pravdepodobne aj ďalej do vyšších nadzemných častí. Zmeny v prípade fosforu teda určite nepredstavujú zmeny v životnom prostredí.

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New, rare and less known slime molds and fungi (Myxomycota, Zygomycota, Ascomycota, Basidiomycota) found in Central Slovakia

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Abstract

MIHÁL, I., BLANÁR, D., GLEJDURA, S. 2012. New, rare and less known slime molds and fungi (Myxomycota, Zygomycota, Ascomycota, Basidiomycota) found in Central Slovakia. *Folia oecol.*, 39: 121–129.

During 2001–2011, we determined several new, rare or little known slime molds and fungi in varied localities in Central Slovakia. As the first reports in Slovakia, we present here the slime molds *Craterium leucocephalum* var. *scyphoides*, *Dictydium cancellatum*, zygomycetous fungus *Entomophthora muscae*, ascomycetous species *Agyrium rufum*, *Tympanis* sp., *Viridispora alata* and basidiomycetous species *Femsjonia peziziformis*. Our unique finding of the fungus *Viridispora alata* is the first finding of this genus and species in the European context (except findings in Madeira). Rare and little known in Slovakia are the ascomycetous species *Hypoxylon ticinense*, *Hypomyces rosellus* and *Eutypella sorbi*. Rare ectomycorrhizal species *Bankera violascens*, *Hydnellum floriforme*, *H. geogenium* and *H. peckii* are Basidiomycetes with a considerable bioindication value, associated with Norway spruce. The species *Cenangium ferruginosum* is an important pathogene on pine trees.

Keywords

Ascomycota, Basidiomycota, Central Slovakia, fungi, Myxomycota, new species, slime molds

Introduction

Mycological surveys, long-term as well as short-term ones, frequently result in registering fungal taxa that are new, very rare or little known from various aspects. Not rarely, these species are either the first findings for the given country or species registered long, for example, 50 or 60 years ago. An evidence for this is given in the work by RÍPKOVÁ and HAGARA (2003), reporting the species *Phlebia ryvardenii* as the first finding for Slovakia, and the species *Hyphodontia latitans*, as a fungus recorded repeatedly after 100 years.

New findings of rare fungi can occur during autecological or mycological studies performed on any

given fungal group. An example is the species *Pseudographis pinicola* recorded as the first finding in Slovakia by JANČOVIČOVÁ et al. (2011) who studied the fungi of the genus *Crepitodus*. In a similar way, MIHÁL and BLANÁR (2011), studying the order Hypocreales, recorded the first findings of the species *Hypomyces cervinigenus*, *Paranectria oropensis* and *Pseudonectria rousseliana*.

More new and rare fungal species are also possible to find either during large-area mycofloristic studies pursued parallelly across several localities or in localities investigated during long time periods. An example is given with the findings of the rare *Auriporia aurulenta*, *Cystolepiota hetieri*, *Gymnopus herinkii*, *Mycoacia nothofagi* and other species collected by KUTHAN et al.

(1999) in the Bukovské vrchy Mts in Eastern Slovakia. Analogically, MIHÁL et al. (2011), pursuing their intensive mycofloristic research in forest stands in the mountain massive Kohút in the Stolické vrchy Mts in Central Slovakia, recorded a number of first findings for the territory of the Slovak Republic, with the species *Ascobolus xylophilus*, *Endogone lactiflua*, *Encoelia fascicularis*, *Scutellinia torrentis*, *Smardae amethystina* and similar.

In this work we describe the occurrence of several new and rare slime mould and fungal species determined during our mycological research in several localities in Central Slovakia. In this way, we intend to enlarge the lists of new, rare and otherwise interesting species of the Slovak mycoflora.

Material and methods

Fruit bodies of the new, rare and otherwise interesting slime molds and fungi were collected in Central Slovakia in the following geomorphological units: Muránska planina mountain plateau; Revúcka vrchovina Mts; Stolické vrchy Mts; Veľká Fatra Mts; and Veporské vrchy Mts; on overall in 11 localities, during 17 field excursions in the years: 2001 (1 excursion), 2008 (1), 2009 (4), 2010 (5) and 2011 (6 excursions). The slime molds and fungi were growing on various substrates, mostly on decaying wood of diverse broadleaved and coniferous species, on fruit bodies of the lichen *Xanthoria parietina*, but also in needle litter and in sandy soils.

The collected material was identified in the laboratory, with the aid of the identification keys designed by ČERVENKA et al. (1972), HAGARA et al. (1999), KEIZER (1998), MOSER (1963), PAPOUŠEK (2004), ROSSMAN et al. (1999), as well as some material provided on the Internet, such as DATABASE (2011a, b) and KRIVOMAZ (2010). The scientific nomenclature and authorised abbreviations have been adopted from the works LIZOŇ and BACIGÁLOVÁ (1998), ROSSMAN et al. (1999), ŠKUBLA (2003), and from the database CABI BIOSCIENCE (2011).

The text contains several acronyms for the names of persons who carried out collection and/or identification: IM (Ivan Mihál), SG (Stanislav Glejdura), DB (Drahoš Blanár), MP (Marián Peiger), names of herbaria: SASZV (mycological herbarium of the Institute of Forest Ecology of the Slovak Academy of Sciences in Zvolen), herb. SG (private herbarium owned by S. Glejdura), herb. DB (private herbarium owned by D. Blanár). The remaining acronyms are: NNR for National Nature Reserve, NR for Nature Reserve, JPRL for forest stand number, and Q 7285a means the number of the basic square and the quadrant letter of the Central-European grid for biota mapping.

Results and discussion

During the research period, we identified in various localities in Central Slovakia a number of rare, for Slovakia little known or new fungal and slime mold species, altogether: 4 mold species, 1 Zygomycota species, 7 ascomycetous and 6 basidiomycetous species. In the list in the following text, we provide short descriptions of these species and the data for the particular findings.

Myxomycota

Craterium leucocephalum var. *scyphoides* (Cooke & Balf. f. ex Massee) G. Lister

The Veporské vrchy Mts; Polomka; NR Fabova hoľa, NE from the mapping spot 1,434.8 (Javorinka Mt), JPRL 408b; Q 7285a; spruce forest; 1,390 m a.s.l.; on decaying parts of a down-lying stem of *Picea abies*; 19. 5. 2009, leg. DB, det. IM, herb. DB, SASZV.

A decorative slime mold from the genus *Craterium* Trentepohl, generating fruit bodies in form of tiny eggs attached with their tops to brownish stalks – columellae, growing in groups as saprophytes on decomposed leaf litter. SCHMID-HECKEL (1988) reports the species *C. leucocephalum* var. *leucocephalum* (Pers.) Ditmar as a common slime mold species growing saprophytically on beech leaf litter in mountainous forests. Our finding is a taxonomic variety of *C. leucocephalum* (Pers.) Ditm. with a more abundant occurrence. The two species differ in irregular opening of the lid shelter tegument and in calcification of the entire fruit bodies' surface.

It is worth to notice that in our conditions, the species of the genus *Craterium* occur rarely, and in mycological surveys, they may often be overlooked. This fact is evident from the very old finding of *C. leucocephalum* from the Štiavnické vrchy Mts in 1900, the author of which is A. Kmeť (ŠKUBLA, 2003). The most recent finding of *C. leucocephalum* from the Slovak slopes of the Biele Karpaty Mts, locality Streženice, 7. 8. 2011, has been reported by O. Roučka (DATABASE, 2011c). Very little investigated taxonomic differences between the two varieties of the *C. leucocephalum* species seem to cause the absence of *C. leucocephalum* var. *scyphoides* records reported from Slovakia so far.

Dictydium cancellatum (E. Jahn) G. Lister

The Veporské vrchy Mts; Pohronská Polhora; NR Fabova hoľa Mt, NWW from the saddle Fabova hoľa, JPRL 134a; Q 7285a; spruce forest; 1,325 m a.s.l.; on decaying wood of a lying stem of *Picea abies*; 24. 4. 2009, 7. 10. 2010, 17. 5. 2011, leg. DB, det. IM, herb. DB, SASZV.

A slime mold species creating fruit bodies exhibiting characteristic shape in form of tiny Japanese lan-

terns protruding from decomposed and decaying wood. *Dictydium cancellatum* produces small red-brownish fruit bodies resembling little heads – peridia, attached to brown-reddish columellae. The columellae often, mainly after the spores' maturity, bend exactly under the peridium. After the spores have been released, there is distinctly visible empty, ribbed peridium that formed the former fruit body. In greater detail, the species *C. cancellatum* has been described by ČERVENKA et al. (1972). In frame of this species, there have been recognized several different taxonomic varieties, such as *C. cancellatum* var. *anomalum* (E. Jahn) G. Lister, *C. cancellatum* var. *fuscum* Lister, *C. cancellatum* var. *purpureum* T. Macbr.

The relevant literature does not report a new finding of this slime mold in Slovakia, which is a surprise – because the morphological traits of the fruit bodies of the genus *Dictydium* are distinct and easy to identify. More frequent occurrence than the documented one of *D. cancellatum* in our natural conditions is almost certain, as the supply of its growing substrate in form of dead wood is very abundant, primarily in floodplain and natural (primeval) forests. The occurrence of this species in the Veporské vrchy Mts can be considered as the first finding for Slovakia.

***Dictydium cernuum* (Pers.) Nees**

The Veporské vrchy Mts; Pohronská Polhora; NR Fabova hoľa Mt, SEE from the mapping spot 1,396.6 (Psica Mt), JPRL 152a; Q 7285a; spruce forest; 1,310 m a.s.l.; on decaying wood of a lying stem of *Picea abies*; 5. 5. 2010, leg. DB, det. IM, herb. DB, SASZV.

The taxon is taxonomically related to the *Dictydium cancellatum* species. The two species also exhibit similar ecological and trophic demands. Similarly as for the preceding slime mold species, there are no records of its occurrence in Slovakia. The only known record of *D. cernuum* is from the locality Sitno in the Štiavnické vrchy Mts, made by A. Kmeť in 1900 (ŠKUBLA, 2003). The occurrence of this species in the Veporské vrchy Mts can be considered as the second finding for Slovakia – following the first one after more than one hundred years.

***Lamproderma columbinum* (Pers.) Rostaf.**

The Veporské vrchy Mts; Pohronská Polhora; PR Fabova hoľa Mt, SEE from the mapping spot 1,434.8 (Javorinka Mt), JPRL 140; Q 7285a; spruce forest; 1,360 m a.s.l.; on decaying wood of a lying stem of *Picea abies*; 24. 4. 2009, leg. DB, det. IM, herb. DB, SASZV.

A slime mold creating little violetish ovum-shaped fruit bodies topping the short black columellae. The morphology of the fruit bodies of this species is somewhat similar to the slime molds *Dictydium*, differing only in the length of columellae. The fruiting bodies

create massive colonies on decaying and decomposed wood. In greater detail, *L. columbinum* has been described in ČERVENKA et al. (1972).

In Slovakia, the species *L. columbinum* has so far been recorded on the Muránska planina mountain plateau, in the locality NNR Hrdzavá (MIHÁL and BLANÁR, 1999) and in the Štiavnické vrchy Mts in the localities Prenčov and Sitno, in which this species was collected by A. Kmeť as long ago as in 1900 (ŠKUBLA, 2003). The occurrence of *L. columbinum* in the Veporské vrchy Mts. can be considered as the third record for the Slovak Republic.

Zygomycota

***Entomophthora muscae* (Cohn) Fresen**

The Revúcka vrchovina Mts; Revúca; N from the mapping spot 708.9 (Hlaviny Mt), locality: Za Peklom; beech forest stand; Q 7386b; 320 m a.s.l.; on a fly imago from the family Asilidae; 13. 9. 2001, leg. DB, det. IM, herb. SASZV.

An ectoparasitic fungus from the family Entomophthoraceae Fres. This species is ectotrophically associated with the Diptera order, where it parasitizes on varied fly species, entering their bodies across their digestive and respiratory systems. The presence of *E. muscae* on a fly's body is manifested through a white plaque on the insect's abdomen, developing later into a connected coat overing the entire body of the attacked fly, with conidiophores formed on the coat surface. More detailed description of the species *E. muscae* can be found in ČERVENKA et al. (1972).

The literature does not mention a prior occurrence of *E. muscae* in Slovakia, despite this obligate parasite on flies is certainly common also in our conditions with abundant occurrence of very diverse fly species. The occurrence of *E. muscae* in the Revúcka vrchovina Mts can be recorded as the first finding in Slovakia.

Ascomycota

***Agyrium rufum* (Pers.) Fr.**

The Revúcka vrchovina Mts; N from Jelšava, locality: Odkalisko; Q 7387c; willow stand on the eastern bank of a sedimentation basin; 305 m a.s.l.; on thallus of *Xanthoria parietina* growing on a branch of *Salix alba*; 12. 11. 2008, leg. DB, det. IM, herb. DB, SASZV.

The species belongs to the order Lecanorales, the members of which often colonize thalluses of a range of lichen species. *A. rufum* forms minute red-brownish fruit bodies growing on bark-free wood of diverse broadleaved woody plants. For a more detailed description of this interesting species, see ČERVENKA et al. (1972).

We found minute fruit bodies of this very interesting fungus growing on wood surface of *Sambucus nigra* branches and on the lichen *Xanthoria parietina*. It seems peculiar that the discussed finding was situated in the extravillane of the magnesite processing plant in Jelšava town in heavy polluted environment next a sedimentation basin for industrial waste water near this plant. The results of prior mycological surveys carried out in the plant's surroundings exhibit a whole range of lichen, moss and fungal species, despite considerable load with airborne pollutants (MIHÁL and BLANÁR, 2007). The literature does not contain a record of *A. rufum* in Slovakia so far, the occurrence recorded in the Revúcka vrchovina Mts can be considered as the first finding.

***Cenangium ferruginosum* Fr.**

The Revúcka vrchovina Mts; Revúca; Revúcke kúpele spa – stream valley of the Dolinský potok, NNE from the mapping spot 664.4; Q 7386b; hornbeam-beech stand with pine; about 415 m a.s.l.; on lying *Pinus sylvestris* branches with a diameter at 0.5–1.5 cm and also on *P. sylvestris* needles (pine branches broken and fallen in the flowering period 2008); 9. 4. 2009, leg. DB, det. SG, herb. SG.

A species belonging to the order Helotiales. *Cenangium ferruginosum* causes necrotic bark disease on branches of varied pine species. The species has been classified as dangerous, representing high risk for health condition of the infected trees (ZÚBRIK et al. 2008). *C. ferruginosum* massive harms the pine stands which are weakened long-term drought. It occurs massively in cyclic patterns, following long lasting rainy or dry periods (BUTIN, 1995; ŠVESTKA et al., 1998). In our conditions, there have not been detected distinct cases of epiphyticia caused by this fungus, so far.

Despite lacking specific data for individual collections (cf. ŠKUBLA, 2003), it can be considered as a relatively rare species, while it is generally declared as a frequent parasite fungus on the pine stands in Slovakia (ČERVENKA et al., 1972; KUNCA and FOFFOVÁ, 2000). At present, its occurrence has only been documented from the Revúcka vrchovina Mts.

***Eutypella sorbi* (Alb. et Schwein.) Sacc.**

The Veporské vrchy Mts; Pohronská Polhora; NR Fabova hoľa, NNW from the mapping spot 1,438.8 (Fabova hoľa Mt), JPRL 131; Q 7285a; spruce forest with rowan interspersed; 1,425 m a.s.l.; on bark of decaying lying branches of *Sorbus aucuparia*; 11. 8. 2009, leg. DB, det. IM, herb. DB, SASZV.

The species is rather rare, and it is characterised through its substrate – bark of dying or dead trees of *Sorbus aucuparia* (RASPE et al., 2000). On this host plant, the saprophyte *E. sorbi* forms clusters of tiny black-coloured fruit bodies breaking the host's bark. In

Slovakia, the occurrence of this fungus is more probable at higher elevations with more abundant occurrence of *Sorbus aucuparia*. A more detailed description of *E. sorbi* can be found in ČERVENKA et al. (1972).

The first record of this substrate-specific species is from the Poľana Mts, locality Ľubietovský Vepor, where the fungus was found by PODLAHOVÁ (1972, in ŠKUBLA, 2003). The most recent finding of *E. sorbi* is from the locality Kohút in the Stolické vrchy Mts (MIHÁL et al., 2011), and the occurrence of this species in the Veporské vrchy Mts is the third finding in Slovakia.

***Hypomyces rosellus* (Alb. et Schwein.) Tul. et C. Tul.**

The Muránska planina mountain plateau; Zlatno; NNR Zlatnica, Postalka (little left-side valley in the central part of NNR); Q 7186d; beech stand with spruce; 825–850 m a.s.l.; on decaying wood of a trunk of *Picea abies*; 23. 6. 2011, leg. DB, det. IM, herb. DB.

An interesting, decoratively coloured fungus, forming pinky to pink-red cottony subiculum, in maturity bearing red perithecia. The fungus is a frequent parasite on a wide range of macromycetes species from the orders Aphyllophorales and Agaricales, but it can also colonise the wood substrate of broadleaved woody plants as a lignicolous saprophyte.

From Slovakia, there have been published only three findings of this species: in the Belianske Tatry Mts (ŠKUBLA, 2003), in the Štiavnické vrchy Mts (MIHÁL et al., 2007) and in the Revúcka vrchovina Mts (MIHÁL and BLANÁR, 2011). The remaining occurrence localities of *H. rosellus* in Slovakia are presented on the Internet, DATABASE (2011d). We may declare that in Slovakia, the species *H. rosellus* has been documented for six localities and it represents, together with our finding, the fourth published occurrence for this species in our country.

***Hypoxylon ticinense* L. E. Petrini**

The Muránska planina mountain plateau; Murán; NNR Šarkanica, Martinova dolina valley; Q 7285d; a stand with *Acer pseudoplatanus*, *Fagus sylvatica* and *Fraxinus excelsior*; 535–550 m a.s.l.; on decomposed wood of a broadleaved hardwood species; 22. 10. 2010, leg. DB, det. IM, herb. DB, SASZV.

An interesting species, in Slovakia included into the Red Check-List of Fungi. The fungus grows as a saprophyte on wood of broadleaved woody plants, with the most findings recorded on woody plants of floodplain forests, such as *Crataegus oxycantha*, *Fraxinus excelsior*, *Negundo aceroides*, *Padus avium*, *Populus alba*, *Salix* sp., *Swida sanguinea*. This relatively rare species has also been recorded in France, Switzerland, Italy, Austria, Bohemia and Croatia. Its occurrence in Slovakia has been reported from 6 localities situated in floodplain forests near Bratislava in the Po-

dunajská lowland (JANČOVIČOVÁ and GLEJDURA, 1999; RIPKOVÁ and HAGARA, 2003). The most recent findings of *H. ticinense* from floodplain forests in surroundings of Bratislava have been put on the web by J. Červenka, 7. 1. 2006, Bratislava – Petržalka, locality Zrkadloový háj and B. Ivičič, 11. 3. 2006, locality a floodplain forest near Bratislava (DATABASE, 2011e).

Our finding from the Muránska planina mountain plateau, represents the seventh published occurrence locality of this species in Slovakia – a new geomorphologic unit at a considerable distance from floodplain forests in the Podunajská lowland – the prior centre of occurrence of the species *H. ticinense*. In our case, *H. ticinense* was growing on decaying wood of a non-identified broadleaved woody plant, in the lower part of the Martinova dolina valley, most probably *Fraxinus excelsior*, *Acer pseudoplatanus* or *Fagus sylvatica*, which are very abundant there. We suppose that the distribution of this species in Slovakia is much larger, primarily in broadleaved forests in Southern Slovakia.

Tympanis sp.

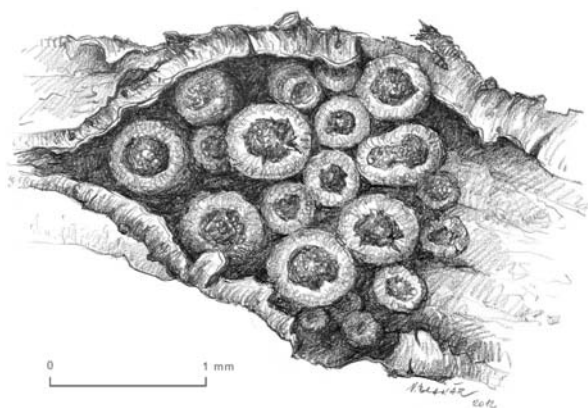


Fig. 1. *Tympanis* sp. – rare and little known fungus growing on *Sorbus aucuparia* (Author of draw V. Blanár).

The Veporské vrchy Mts; Polomka / Pohronská Polhora; NR Fabova hoľa, the mountain saddle of Fabova hoľa, NNW from the mapping spot 1,438.8 (Fabova hoľa Mt), JPRL 131; Q 7285a; spruce forest; 1,357–1,400 m a.s.l.; on lying decaying branches (bark) of *Sorbus aucuparia*; 11. 8. 2009, leg. DB, det. IM, herb. DB, SASZV.

This rare fungus from the family Helotiaceae was found forming tiny fruit bodies protruding from the bark of *Sorbus aucuparia*. YAO and SPOONER (1996) classify the genus *Tympanis* Tode into the order Leotiales, while this order encompasses a large number of species taxonomically related but difficult to differentiate by morphology. KIRK et al. (2008) classify into this order only 28 species, opposite to 86 species reported in other sources (DATABASE, 2011f). The wood substrate provided by *Sorbus aucuparia* is relevant mainly in case

of the species *Tympanis aucupariae* (Pers.) Wallr. and *T. conspersa* (Fr.) Fr., to which evidently belongs also our finding. SCHMID-HECKEL (1988), for example, report *T. conspersa* as a rare saprophytic fungus growing on the bark of *Sorbus aucuparia* in the subalpine zone of the Alps Mts. Similarly, also RASPÉ et al. (2000) report *Sorbus aucuparia* as a host woody plant for *Tympanis conspersa*.

ŠKUBLA (2003) informs that in Slovakia were published records of only rare occurrence and only for two species of this order: *Tympanis piceina* J. W. Groves and *T. pithya* (P. Karst) P. Karst., however, growing on coniferous woody plants. From the Slovak territory has also been recognized the species *Tympanis alnea* (Pers.) Fr., growing on *Alnus glutinosa* wood, collected on 1. 4. 2010 and 2. 2. 2011 by M. Paulfny in the locality Smrečany in the Liptovská basin in Northern Slovakia (DATABASE, 2011g).

Concerning the growing substrate (*Sorbus aucuparia*) on which our findings in the Veporské vrchy Mts were detected, we may suppose that this is the first finding of another species of the genus *Tympanis* in Slovakia, very probably *Tympanis aucupariae* or *T. conspersa*.

Viridispora alata (Samuels) Samuels & Rossman

The Stolické vrchy Mts; Muránska Zdychava; mountain valley of the Števkov potok stream; Q 7285a; 1,300–1,350 m a.s.l.; on sandy soil grown with moss; 20. 8. 2011, leg. SG, det. IM, herb. SG, SASZV.

A very interesting and rare species from the family Nectriaceae, creating minuscule perithecia, classified into the genus *Nectria* (Fr.) Fr. Recently, Samuels and Rossman (in ROSSMAN et al., 1999) have distinguished a new genus of *Viridispora* Samuels & Rossman gen. nov., comprising four species, *V. alata* included. This saprophytic fungus is different from the other three species of this new genus mainly by its greenish coloured spores. ROSSMAN et al. (1999) report that the occurrence of *V. alata* has hitherto been recorded only in tropical forests of Caribic and in the northern parts of South America. This fact, however, does not exclude its cosmopolitan occurrence across Europe, which is documented by our finding from the Stolické vrchy Mts.

The most recent finding of the species *Viridispora alata* outside the American continent has been reported by HIROOKA et al. (2012), who identified it in the laboratory from an isolate obtained from bark of an non-specified woody plant on the island Madeira in the Eastern Atlantic, near Europe and Africa. In context of our finding of *V. alata* in Central Slovakia, the *V. alata* finding on Madeira may point at cosmopolitan character of this species distribution throughout the American and European continent. The occurrence of *V. alata* in this single locality in Slovakia represents the first finding not only in Slovakia but also in the European context.

Basidiomycota

Bankera violascens (Alb. & Schwein.) Pouzar (Fig. 2)

The Veľká Fatra Mts, Vyšná Revúca, Zelená dolina valley, Q 7080d, spruce forest; 850 m a.s.l.; in needles, 11. 8. 2010, leg. et det. SG, herb (SG 4995) – the Stolické vrchy Mts, Muránska Zdychava, locality Števkov diel, Q 7286b; spruce forest; 700 m a.s.l., in needles, 22. 8. 2011, leg. et det. SG, herb (SG 4358).



Fig. 2. *Bankera violascens* – attractive and rare species of mountainous spruce forests (Photo S. Glejdura).

This ectomycorrhizal shaped fungus with a prickly hymenophore is associated with spruce in submountainous and mountainous zones. Since now, there have been published only 5 records of occurrence of this very interesting fungus in Slovakia, namely in the Podtatranská kotlina basin; the High Tatras Mts and the Turzovská vrchovina Mts in the region Kysuce (HROUDA, 2006; ŠKUBLA, 2003).

Femsjonina peziziformis (Léveille) P. Karsten

The Veporské vrchy Mts; Polomka; NR Fabova hoľa, SWW from the mapping spot 1,434.8 (Javorinka Mt), JPRL 408d; Q 7285a; spruce forest; 1,400–1,405 m a.s.l.; on a decaying stump of *Picea abies*; 7. 10. 2010, leg. DB, det. IM, herb. DB, SASZV.

An interesting species from the order Dacrymycetales, forming clusters of tiny yellowish fruit bodies on coniferous wood. The fruit bodies often exhibit bowl shape characteristic for the genus *Peziza* s.l., and this is also pointed out through the genus name of this rare, in surveys evidently bypassed species. More frequent occurrence of this species may be supposed in spruce forests in higher situated zones. So far, there has been no report about the occurrence of *F. peziziformis* in Slovakia. The occurrence of *F. peziziformis* in the Vepor-

ské vrchy Mts observed by us is the first finding for Slovakia.

Hydnellum floriforme (Schaeff.) Banker (Fig. 3)

The Stolické vrchy Mts; Muránska Zdychava; locality Števkov laz; Q 7286b; spruce forest; 700 m a.s.l.; in needles; 20. 8. 2011, leg. SG, det. MP, SG, herb (SG 4015).



Fig. 3. *Hydnellum floriforme* – rare ectomycorrhizal fungus of mountainous spruce forests (Photo S. Glejdura).

A very attractive orange-coloured fungus with a prickly hymenophore. The species is ectomycorrhizal (such as all the species of the genus *Hydnellum*), ecotrophically associated with spruce. By now, there have been published 9 findings in Slovakia. HROUDA (1999) informs that in the Czech Republic, this species occurred at last decades only rarely and only in Southern Bohemia. From the very similar species *Hydnellum aurantiacum*, it can be distinguished based on its farinaceous scent and lighter-in-colour flesh.

Hydnellum geogenium (Fr.) Banker (Fig. 4)



Fig. 4. *Hydnellum geogenium* – ectomycorrhizal symbiont of spruce (Photo S. Glejdura).

The Stolické vrchy Mts; Muránska Zdychava; locality Števkov laz; Q 7286b; spruce forest; 700 m a.s.l.; in needles; 20. 8. 2011, leg. et det. SG, herb (SG 4169).

Sulphur-yellowish shaped fungus with prickly hymenophore, ectomycorrhizally associated with spruce. It has been put on the Red Check-List of Fungi of Slovakia and in the Red Book of Threatened and Rare Plants and Animals of the Slovak and the Czech Republic. In Slovakia, it has been reported from several localities situated in 11 geomorphological units (ŠKUBLA, 2003).

Hydnellum peckii Banker

The Veľká Fatra Mts, Vyšná Revúca, Zelená dolina valley, Q 7080d, spruce forest; 850 m a.s.l.; in needles 11. 8. 2010, leg. et det. SG, herb (SG 4417). – the Stolické vrchy Mts; Muránska Zdychava; locality Števkov laz; Q 7286b; spruce forest; 700 m a.s.l.; in needles; 26. 7. 2011, leg. et det. SG, herb (SG 4263).

Characteristic features of this fungus are: a red liquid produced on the pileus surface in young fruit bodies and the peppery tasting flesh. So far, there have been published 9 findings from 8 geomorphological units of Slovakia (ŠKUBLA, l.c.). The fungus has been put on the Red Check-List of Fungi of Slovakia.

In the locality Stolické vrchy Mts (a grazing ground grown with developing spruce), *H. peckii* occurred together with the above hydneous fungi (family Bankeraceae) *Bankera violascens*, *Hydnellum floriforme*, *H. geogenium* and with the recently published species *Boletopsis leucomelaena* and *Sarcodon imbricatus* (MIHÁL et al., 2011), while in broader surroundings was found sporadically only *S. imbricatus*. Similar accumulated occurrence of relative species growing at a close spacing of a few metres has been also recorded in the Chočské vrchy Mts (locality Svorad), with *Hydnellum caeruleum*, *H. geogenium*, *H. peckii*, *H. suaveopens*, *H. floriforme*, *H. sp.*, *Sarcodon glaucopus* and *Phellodon* sp. (PEIGER, ined.) growing together on a former pasture ground with a developing spruce and bilberry stand. It follows that these species have very similar ecological demands, and that they are potentially associated with pasture grounds with developing or developed spruce cover, with the soil chemistry and nutrition potential different from original older spruce forests.

Tomentella rubiginosa (Bres.) Maire

The Veporské vrchy Mts; Tisovec; locality Roveň, NNW from the mapping spot 1,153.6 (Kľak Mt); Q 7285a; an isolated settlement – a demolished wood construction; 880 m a.s.l.; on decomposed wood of *Picea abies* (processed timber from a wall); 23. 11. 2010, leg. DB, det. IM, herb. DB.

A rare fungus, belonging in the family Telephoraceae, the members of which create resupinate or semi-resupinate fruit bodies in form of soft thin coats on various substrates, mostly as saprophytes on dead wood

and on soil. Analogically, the species *T. rubiginosa* occurs in form of cinnamon-brown fruits on dead wood substrate. SCHMID-HECKEL (1988) describes this fungus as a lignicolous saprophyte, commonly growing on fir and beech wood in mountainous forests.

In Slovakia has been reported only one finding by Adamčík et al. in 1988 (in ŠKUBLA, 2003), however, without data precising the locality and date of findings. Consequently, the occurrence of *T. rubiginosa* in the Muránska planina mountain plateau, can be considered as the second finding and also the first documented finding for Slovakia.

Conclusions

During 2001–2011, we determined a number of new, rare or little known slime mold and fungal species in various localities in Central Slovakia. As the first finding in Slovakia, we report two slime molds *Craterium leucocephalum* var. *scyphoides*, *Dictydium cancellatum*; one zygomycetous fungus *Entomophthora muscae*; three ascomycetous fungi *Agyrium rufum*, *Tympanis* sp., *Viridispora alata*; and one basidiomycetous fungus *Femsjonia peziziformis*. Our unique finding of *Viridispora alata* is, at the same time, the first finding of this species in the European context. The second findings for Slovakia were recorded for the basidiomycetous fungus *Tomentella rubiginosa*, and the slime mold *Dictydium cernuum* – this one with a time gap of more than one hundred years. From rare and little known species, we have recorded also three ascomycetous species *Hypoxylon ticinense*, *Hypomyces rosellus* and *Eutypella sorbi*. Rare ectomycorrhizal fungi *Bankera violascens*, *Hydnellum floriforme*, *H. geogenium* and *H. peckii* are important basidiomycetous indicators for spruce. The species *Cenangium ferruginosum* is an important phytopathogen of pines. Its occurrence reports in Slovakia, however, are only general, and the literature lacks the data concerning their collection.

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Nové, vzácne a málo známe slizovky a huby (Myxomycota, Zygomycota, Ascomycota, Basidiomycota) zistené na strednom Slovensku

Súhrn

Počas krátkodobých alebo dlhodobých mykologických výskumov sa často stáva, že sa determinujú viaceré nové, veľmi vzácne alebo inak málo známe taxóny húb, pričom nezriedka sú to prvonálezy pre krajinu. Počas rokov 2001 až 2011 sme na rôznych lokalitách stredného Slovenska determinovali niekoľko nových, vzácných alebo málo známych slizoviek a húb. Ako prvonálezy pre Slovensko uvádzame slizovky *Craterium leucocephalum* var. *scyphoides*, *Dictydium cancellatum*, zygomycétnu hubu *Entomophthora muscae*, askomycétny druh *Agyrium rufum*, *Tympanis* sp. (*Tympanis aucupariae*?, *T. conspersa*?), *Viridispora alata* a bazídiomycétny druh *Femisia peiziformis*. Náš ojedinelý nález huby *Viridispora alata* zároveň predstavuje aj prvý nález tohto rodu a druhu pre Európu (mimo nálezu *V. alata* na ostrove Madeira). Ako druhý nález pre Slovensko sme zaznamenali výskyt bazídiomycétnej huby *Tomentella rubiginosa*. Podobne, výskyt slizovky *Dictydium cernuum* môžeme tiež považovať za druhý nález pre Slovensko, navyše po viac ako jednom storočí. Medzi vzácnnejšie a málo známe huby u nás patria aj askomycétny druh *Hypoxylon ticinense*, *Hypomyces rosellus* a *Eutypella sorbi*. Vzácne ektomykorízne druhy *Bankera violascens*, *Hydnellum floriforme*, *H. geogenium* a *H. peckii* sú významné bioindikačné bazídiomycéty smreka a sú uvedené v Červenom zozname húb Slovenska a Červenej knihe ohrozených a vzácných druhov rastlín a živočíchov SR a ČR. Druh *Cenangium ferruginosum* je významnou fytopatogénnou hubou borovíc, pričom popri jeho všeobecne uvádzanom výskyte u nás v literatúre absentuje konkretizácia údajov o jeho zberoch.

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EST sequence variation associated with physiological and growth traits in Norway spruce (*Picea abies* [L.] Karst.) provenances

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Abstract

ROMŠÁKOVÁ, I., FOFFOVÁ, E., LONGAUER, R., PACALAJ, M., KMET', J., GÖMÖRY, D. 2012. EST sequence variation with physiological and growth traits in Norway spruce (*Picea abies* [L.] Karst.) provenances. *Folia oecol*, 39: 130–138.

Variation at polymorphic sites (single-nucleotide polymorphisms; SNP) in five EST-derived markers was investigated in Norway spruce (*Picea abies* [L.] Karst.). Three populations originating from different altitudes growing at two climatically contrasting trial plots of the Slovak provenance experiment derived from the IUFRO 1964/68 experiment with Norway spruce were studied. Associations of genotypes at polymorphic sites with physiological and growth traits (chlorophyll *a* fluorescence kinetics, frost resistance, height, diameter and volume growth, vegetative phenology) were tested. Sixty-one SNPs were identified in the analyzed material, representing 1.65% of the total sequence length of 3,702 bp. None of the polymorphic sites showed a significant differentiation between populations. Five sites showed significant association with phenotypic traits after Bonferroni correction, but further 20 SNPs may potentially be of interest for association studies. The outcomes are discussed in the light of local adaptation.

Keywords

frost tolerance, IUFRO 1964/68, *Picea abies*, photosynthesis, provenance research, single nucleotide polymorphisms

Introduction

Natural selection has been considered the main driver of evolution since Charles Darwin published his *Origin of Species* (DARWIN, 1859). Applying the paradigm of selection on the gene pool of a population, changes of the genetic structure of a population in a changing environment are expected due to differential survival of genotypes. This mechanism is supposed to be the biological basis of adaptation, leading to phenotypes providing some kind of advantage under the local environment. In forest trees, local adaptation may be associated with several factors. Climate is generally considered the main driver of adaptation but soil, industrial pollu-

tion, pests and other factors may also provoke selection (GEBUREK, 2000; MÁTYÁS, 1996).

The main tool for the study of adaptation in forest trees is establishing provenance trials. In provenance experiments, a set of populations of a defined origin (called provenances) is planted commonly on a site or, preferably, on a set of sites. This approach eliminates the *in situ* differences in phenotypic traits caused by large-scale environmental effects (macroclimate, parent rock), as plant material is grown under equal conditions, what means that the observed phenotypic differences among populations of different origin are considered to be caused solely by genetic differentiation. At the same time, it allows to identify phenotypic

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responses of different populations to translocation (CLAUSEN et al., 1939; TURESSON, 1925) and to predict the behaviour of tree populations under changing climates (space-for-time substitution; MÁTYÁS, 1996). In addition to numerous regional or national provenance experiments, the International Union of Forestry Research Organizations (IUFRO) coordinated several international experiments, especially with conifers (GIERTYCH and OLEKSYN, 1992; KRUTZSCH, 1992; WEISGERBER and ŠINDELÁŘ, 1992). However, conifers as well as other forest trees are not ideal for studying local adaptation, as they generally exhibit a considerable phenotypic plasticity (MIMURA and AITKEN, 2010) and large-scale gene flow, which is typical for wind-pollinated species, may prevent differentiation (SAVOLAINEN et al., 2007).

Norway spruce is an example of commercially and economically important forest tree species with a large and almost continuous distribution range. Gene-marker studies indicate a considerable genetic differentiation within the range of spruce (HEUERTZ et al., 2006; LAGERCRANTZ and RYMAN, 1990). Significant inter-population differences were also confirmed by provenance trials (GIERTYCH, 1976; KRUTZSCH, 1974). Distinguishing between the effects of natural selection mediated by phenotypic traits and acting on controlling genes and neutral processes such as colonization, gene flow and genetic drift at the rangewide scale is, however, difficult. This is why we studied local adaptation at a small scale, where neutral processes play a negligible role in genetic differentiation.

Traditionally, anonymous or even neutral markers have been used for the study of genetic basis of adaptation. Nowadays, a rapid development in the genomics of forest trees has produced extensive genomic resources leading to the development of new experimental tools for adaptation studies (NEALE and INGVARSSON, 2008; NEALE and KREMER, 2011). In spite of this, there are very few empirical studies aimed at the identification of genetic structure changes of forest tree populations along environmental gradients (ECKERT et al., 2009, 2010; NAMROUD et al., 2008; WACHOWIAK et al., 2009). Our study represents a step in this direction. The objective of our study was identification of single nucleotide polymorphisms in the sequences of EST-derived genetic markers in provenances from climatically contrasting sites and their potential association with fitness-related physiological traits.

Material and methods

The study relies on a spin-off of the IUFRO 1964/68 Inventory Provenance Experiment with Norway spruce (KRUTZSCH, 1974). Eleven Slovak provenances (combined with the material remaining from IUFRO 1964/68 core trials) were planted on a series of 5 plots located in central Slovakia at altitudes of 450 to 1,250 m a.s.l.

A randomized complete block design with 4 blocks and initial number of 49 plants per provenance and block planted at 2-by-2 m spacing was used. During the spring of 2009, tree height and breast-height diameter were measured at all plots. Spring vegetative phenology was assessed for each tree using a 7-point scale (0 – buds closed, 1 – buds swollen and somewhat elongated, 2 – buds elongating, fully ruptured, needles not protruded, scale cup on the apex, 3 – new light-green needles visible and protruding, sprouts soft and short (up to 6 cm long), 4 – protruded needles light green, sprouts extending (approx. 7–12 cm long) but still soft, 5 – needles dark green, sprout elongation terminated, sprouts straight, 6 – fully matured sprouts with dark needles) at two dates; the average phenological score was used of the evaluation.

Three provenances originating from contrasting environments (Beňuš P1; 19 trees, Habovka 49b P12; 20 trees, TANAP P49; 20 trees) planted on the lowermost and uppermost trial sites (Veľký Lom and Mútne-Zákamenné) were chosen for physiological assessments (Table 1), which were performed on branches collected from the upper part of the crown from 10 trees per provenance. We assessed the frost resistance index based on electrolyte leakage after artificial freezing, and the parameters of chlorophyll *a* fluorescence kinetics (rapid phase) characterizing the efficiency of photosystem II. One-year-old needles were used for the measurements (see GÖMÖRY et al., 2010).

Total genomic DNA was isolated from all individuals using a modified CTAB protocol following DOYLE and DOYLE (1987) from 10 mg of silica-dried needles per tree. DNA concentration was verified by spectrophotometry.

We sequenced five polymorphic markers derived from expressed sequence tags (VENDRAMIN pers. comm.), whose putative function was derived from homology with the known *Arabidopsis thaliana* gene: *ATCUL-3a_1*, *ATCUL-3a_2* (cullin-coding genes, coding for small hydrophobic proteins providing scaffold for ubiquitin ligases, controlling many cellular functions), *FY-like_1*, *FY-like_2* (flowering time control protein), and *PgEMB24* (late embryogenesis abundant proteins, protecting other proteins from aggregation from desiccation or osmotic stresses caused by freezing). These markers represent candidate genes for frost tolerance and phenology; we expected therefore that they may respond to selection associated with macroclimate. PCR mixtures (20 µl) contained 1 × PCR buffer, 2 mM MgCl₂, 0.2 µM primers, 0.2 µM dNTP (Fermentas), 0.5 U *Taq* DNA polymerase (BioThermStar™ Hot Start *Taq* DNA Polymerase, GeneCraft), 0.4% BSA and 25 ng template. The thermal profile was as follows: initial denaturation 95 °C 10 min, 35× [denaturation 94 °C 1 min, annealing *T* 1 min (*T* = 60 °C for *ATCUL-3a_1*, *FY-like_2*, *PgEMB24*, *T* = 53 °C for *ATCUL-3a_2*, *FY-like_1*), extension 72 °C 1.5 min], final extension

72 °C 10 min. Amplification success was checked on 1.5% agarose gel. Sequencing was performed by IGA Technology services (Udine, Italy). For all primer pairs, both DNA strands were sequenced. Obtained raw data were evaluated using SeqScape v.2.5 and aligned using Clustal Omega. Sequences were reduced to sites exhibiting single nucleotide polymorphisms (SNPs). For statistical evaluations, each polymorphic site was treated as separate locus.

Genetic differentiation at polymorphic sites was tested by exact probability tests of differences in allelic frequencies using Arlequin 2.000 (SCHNEIDER et al., 2000). Differences among genotypes in phenotypic traits (growth, phenology, photosynthetic parameters, frost resistance) were tested assuming dominance: a two-way analysis of variance was used with fixed effect of test location (Veľký Lom, Mútne-Zákamenné) and random effect of genotype (procedure GLM, SAS 2010). Probabilities associated with the *F*-tests were corrected using sequential Bonferroni correction, and

linkage disequilibria (LD) were tested for the loci showing significant associations with phenotypic traits.

Results

The five analyzed EST-derived markers yielded readable sequences in most trees, naturally, less so in peripheral regions. In the whole analyzed material, 61 SNPs were identified, representing 1.65% of the total sequence length of 3,702 bp. Out of 61 SNPs, 35 were transitions (mainly A↔G, less C↔T) and 26 transversions (mainly A↔T) (Table 2). As we do not dispose of the template sequences (accessions in the GeneBank database), we were unable to localize the identified polymorphisms in genomic regions and assess their putative effects in terms of aminoacid substitutions. This must be emphasized regarding the fact that the polymorphism level might appear quite high, considering that expressed (and thus potentially selected) sequences

Table 1. Characteristics of provenances and trial sites included in the study and overview of the measure phenotypic traits

Object		Longitude N	Latitude E	Altitude [m a.s.l.]	Soil
Provenance					
Beňuš	P1	19°53′	49°50′	700	Dystric Cambisol
Habovka 49b	P12	19°41′	49°15′	1,000	Dystric Cambisol
TANAP	P49	20°15′	49°11′	1,450	Haplic Podsol
Trial site					
Veľký Lom	T1	19°21′	49°20′	450	Eutric Cambisol
Mútne-Zákamenné	T5	19°17′	49°32′	1,250	Dystric Cambisol
Trait	Definition				
Growth					
Height	tree height at 45 years				
Diameter	breast-height diameter at 45 years				
Stem volume	stem volume at 45 years according to volumetric tables (PETRÁŠ and PAJTIK 1991)				
Budburst	mean stage of budburst and shoot development calculated from 2 scoring dates				
Frost tolerance*					
I_{20}	index of frost injury (artificial freezing) at −20 °C				
I_{80}	index of frost injury (artificial freezing) at −80 °C				
Photosynthesis†					
F_0	basic fluorescence; the yield fluorescence in the absence of photosynthetic light, when all reaction centers of photosystem II are open				
F_m	maximum fluorescence				
F_v	variable fluorescence; $F_m - F_0$				
F_v / F_m	the quantum efficiency of open photosystem II centers				
T_m	time required for the increase of fluorescence from F_0 to F_m				
Area	the area above the induction curve between the basic and maximum fluorescence				

* see GÖMÖRY et al. (2010) and FLINT et al. (1967).

† see MAXWELL and JOHNSON (2000).

were studied. Potentially, many identified point mutations may be synonymous or silent (located outside the gene or in an intron), or may result in an aminoacid substitution which does not change protein properties. Anyway, there is a trend in the allelic richness: the number of polymorphisms decreases with altitude (Table 2).

Differences in allelic frequencies may appear big at some polymorphic sites, but this is mostly the case of peripheral sites where frequently unsuccessful am-

plification strongly decreased sample sizes. Exact tests of differentiation did not reveal significant difference at any site.

Several loci were significantly associated with frost tolerance, photosynthetic efficiency and growth (Table 3). As this study was intended only to be a pilot one, all such sites we presented, because they may be prospective for further testing in more detailed studies. However, sequential Bonferroni correction left only

Table 2 Allelic frequencies at polymorphic sites in the studied Norway spruce populations

Marker	Site	Allele*		Population		
				P1	P12	P49
<i>ATCUL-3a_1</i>	91	A	G	0.125	0.125	0.094
	100	A	G	0.094	0	0
	256	A	C	0.029	0	0
	259	A	G	1	0.975	0.971
	268	A	C	0.028	0.025	0
	328	C	T	0	0.026	0.059
	381	C	T	0.972	1	1
	422	G	C	0.028	0	0
	508	A	T	0	0	0.100
	523	A	C	0	0.029	0.067
<i>ATCUL-3a_2</i>	48	A	G	0	0.042	0
	61	A	G	0	0	0.063
	70	A	T	1	0.929	1
	116	A	T	0	0.029	0
	195	G	C	1	0.971	1
	231	A	C	0.969	0.941	0.969
	271	A	G	0	0.029	0
	382	A	G	0.031	0	0
	448	A	T	0.844	0.833	0.846
	649	A	G	0	0	0.038
	737	C	T	1	0.967	1
	834	C	T	0.964	0.967	1
	836	G	C	0	0.033	0
	892	G	T	1	0.967	1
	896	C	T	0.964	1	1
<i>FY-like_1</i>	46	A	C	0.929	1	1
	180	A	T	0.071	0	0
	260	A	T	0.033	0	0
	263	A	T	0.033	0	0
	296	A	G	0.031	0	0
	342	A	T	0.969	1	1
	374	G	T	0.031	0	0
	457	G	T	0.962	1	1
	602	C	T	0.143	0.100	0.500
	616	C	T	0.917	0.722	0.833

Continued Table 2

Marker	Site	Allele*		Population		
				P1	P12	P49
<i>FY-like_2</i>	93	C	T	0	1	0.500
	135	A	G	0.500	0.750	0.500
	157	A	G	1	0.500	1
	164	C	T	0.250	0	0
	165	C	T	0.200	0	0
	166	A	G	0.167	0	0
	167	A	G	0.071	0	0
	177	C	T	0.316	0.094	0.139
	180	A	G	0.474	0.344	0.450
	240	A	G	0.500	0.563	0.525
	249	A	G	0.474	0.313	0.425
	254	C	T	0.500	0.375	0.475
	255	A	G	0.474	0.375	0.425
	288	C	T	0	0	0.050
	330	A	G	0.553	0.625	0.550
	342	A	G	0.316	0.094	0.139
	345	A	G	0	0	0.029
<i>PgEMB24</i>	66	A	T	1	1	0.895
	67	G	C	0	0.025	0
	88	G	T	0	0.025	0
	192	A	T	0.026	0	0
	372	A	C	0.974	1	1
	417	C	T	0.974	0.975	0.975
	490	A	C	0.972	1	1
	495	C	T	0.028	0	0
	531	G	C	0.313	0.389	0.250
n_p				40	33	28

*Frequencies of the first allele of the pair are shown.

five out of 25 sites significant. The affinity to phenotypic traits was very different among loci: whereas the marker *FY-like_1* showed associations exclusively with physiology (namely site 146/ F_v/F_m), the *FY-like_2* locus was associated with growth traits only (site 177/height). Among the remaining markers, *ATCUL-3a_1* showed significant affinity (Bonferroni-corrected) to budburst timing (site 100) and frost resistance (site 508), while *ATCUL-3a_2* was associated with chlorophyll fluorescence (site 737).

No information is available about the localization of the markers used in the study on chromosomes. Anyway, linkage disequilibria were identified only at the within-marker level, namely in *FY-like_2* (data not shown). Naturally, small sample sizes may also have played a role in the outcomes of the LD tests. Nevertheless, the observed general affinity of SNPs in *FY-like_2* to biomass production (stem volume, growth) may re-

sult from the linkage of polymorphic sites and associated selection.

Discussion

In general, local adaptation is often supposed to play an important role in life strategies of plants and empirical studies conform with this suggestion. Recent metaanalyses have demonstrated that fitness of native populations is mostly greater than that of translocated populations. For instance, LEIMU and FISCHER (2008), based on 35 studies, found that local plants performed better than foreign plants in more than 70% of the cases. This finding is based, however, primarily on herbaceous species; in trees, situation may be somewhat different.

The IUFRO-1964/68-derived Slovak provenance experiment has been subject of several studies focusing

Table 3. ANOVA-based significance tests and partial R^2 for single-nucleotide polymorphisms exhibiting significant associations with phenotypic traits

Marker	Site	Trait	Partial R^2	F	P^1	P^2
<i>ATCUL-3a_1</i>	100	I_{80}	0.119	3.78	*	
	100	budburst	0.184	5.18	*	*
	508	height	0.055	4.42	*	
	508	I_{20}	0.134	9.02	**	*
<i>ATCUL-3a_2</i>	195	<i>Area</i>	0.093	4.73	*	
	448	diameter	0.136	5.14	*	
	448	height	0.070	5.43	*	
	448	stem volume	0.164	6.26	*	
	737	F_0	0.297	16.14	***	***
	737	$F_v - F_m$	0.122	5.64	*	
	836	<i>Area</i>	0.099	4.18	*	
	892	<i>Area</i>	0.099	4.18	*	
<i>FY-like_1</i>	146	$F_v - F_m$	0.245	13.03	***	**
	180	$F_v - F_m$	0.137	6.3	*	
	260	<i>Area</i>	0.105	4.34	*	
	263	<i>Area</i>	0.105	4.34	*	
	602	I_{80}	0.118	4.5	*	
<i>FY-like_2</i>	157	budburst	0.400	10.13	*	
	177	diameter	0.106	5.07	*	
	177	height	0.078	7.28	*	*
	177	stem volume	0.142	6.49	*	
	342	diameter	0.137	6.86	*	
	342	stem volume	0.116	5.16	*	
<i>PgEMB24</i>	67	<i>Area</i>	0.075	4.31	*	
	490	I_{20}	0.082	5.84	*	

P – significance of F -tests for loci (sites): ¹significance without Bonferroni correction, ²significance after Bonferroni correction.

on local adaptation. The responses to transfer in fitness-related traits such as growth and survival of the whole set of 11 domestic Norway spruce provenances indicate that the optimum environment is common for all provenances (GÖMÖRY et al., 2011). Physiological traits (those used in this study, i.e. frost tolerance and photosynthesis efficiency) were demonstrated to be related to the trial location rather than provenance (GÖMÖRY et al., 2010), what means that acclimation rather than climate-associated selection is the main driver of the observed inter-population phenotypic contrasts. On the other hand, an analysis of single-nucleotide polymorphisms based on ESTP markers developed for *Picea sitchensis* revealed significant associations with the climate of origin, indicating a potential role of local adaptation (ROMŠÁKOVÁ et al., 2012).

Although we used provenances from the contrasting altitudinal edges of Norway spruce in Slovakia

(700 and 1,450 m a.s.l.), strongly differing in climatic regimes (mean annual temperatures of 2.9 and 5.7 °C, annual precipitations 852 and 1,154 mm, respectively), not a single SNP exhibiting a significant differentiation was identified. Temperature and precipitation are generally considered to be the main climatic drivers of phenotypic variation patterns for forest trees (KÖNIG, 2005), this outcome was thus extremely surprising. The underlying mechanism might be associated with the interference of neutral processes, namely gene flow and genetic drift, affecting genome as a whole including genes with adaptive significance and hindering local adaptation (BLANQUART et al., 2012, LEIMU and FISCHER 2008, SAVOLAINEN et al., 2007). Differentiation of spruce in Slovakia at neutral allozyme loci was reported to be generally small, but anyway considerably higher among artificial forests than among natural stands (GÖMÖRY, 1992). The absence of differentiation at SNPs

supports the hypothesis that neither natural bottlenecks and/or founder events nor establishing the experiment from offsprings of few maternal trees (equivalent to a human-induced founding event) have affected genetic structures in our material. Just opposite, low differentiation levels give a strong hint to extensive gene flow. The available information on pollen flow in spruce species indicate that dispersal distances exceed the distances between the studied provenances (BURCZYK et al., 2004). However, in spite of a small size of the territory the provenances come from, spruce populations could potentially be isolated from each other geographically as well as phenologically. In addition to the photoperiod (playing no role within natural range of spruce in Slovakia because of a negligible latitudinal span), temperature accumulation induces the beginning of flowering (PARTANEN et al., 1998). Altitudinal differences could thus potentially provoke isolation due to different flowering times, which are maintained either epigenetically or by selection. However, as the distribution of spruce in mountainous areas is almost continuous, genes which are not under a direct selection pressure associated with flowering phenology may spread along the whole altitudinal gradient over few generations.

Despite the lack of differentiation, several SNPs exhibited significant associations with phenotypic traits. However, at this stage, it would be too early to speculate about the potential underlying mechanisms. The function of genes included in this study as described in the Material and methods section remains putative, as their locations and suggested functions are only derived from the comparison to homologous sequences in model plants. Comparative transcriptomics of *Populus* and *Arabidopsis* showed that expression patterns of most genes diverged substantially between these two taxa (QUESADA et al., 2008). Even more divergence is expected between phylogenetically more distant *Arabidopsis* (an angiosperm) and *Picea* (a gymnosperm). Therefore, any hypotheses about the physiological basis of the mechanisms by which the identified loci affect phenotypic traits are precocious. It must again be emphasized that the presented study is only a pilot one.

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Variabilita sekvencií EST markérov asociovaných s fyziologickými a rastovými znakmi v provenienciách smreka obyčajného (*Picea abies* [L.] Karst.)

Súhrn

Predmetom štúdie bola premenlivosť polymorfných pozícií (bodové polymorfizmy; SNP) piatich markérov odvodených z EST sekvencií smreka obyčajného (*Picea abies* [L.] Karst.). Sledovali sme tri populácie pochádzajúce z rozdielnych nadmorských výšok vysadené na dvoch klimaticky kontrastných stanovištiach v rámci slovenského provenienčného pokusu odvodeného od experimentu IUFRO 1964/68, pričom sme testovali asociáciu genotypov na polymorfných pozíciách s fyziologickými a rastovými znakmi (kinetika fluorescencie chlorofylu *a*, rezistencia voči mrazu, výškový, hrúbkový a objemový rast, vegetatívna fenológia). V rámci analyzovaného materiálu sme identifikovali 61 SNP, čo predstavuje 1,65 % z celkovej dĺžky analyzovaných sekvencií 3702 bp. Žiadna z polymorfných pozícií nevykazovala významnú diferenciáciu medzi populáciami. Päť bodových polymorfizmov vykazovalo významnú asociáciu s fenotypovými znakmi po Bonferroniho korekcii, ovšem ďalších 20 SNP môže byť potenciálne zaujímavých pre asociačné štúdie. Výstupy sú diskutované v kontexte lokálnej adaptácie.

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Religious motivations for the protection of forest ecosystems

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Abstract

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The shrinking area of forests urges intensification of efforts to restore damaged forest ecosystems and protect the existing ones. Religions may become an important ally in this process. Their ecological potential remains under-exploited. Owing to their ‘ecological advantages’, i.e. their capacity to shape cosmologies (worldviews), their moral authority and broad base of adherents, significant material resources and community-building capacity, religions can help shape ecological lifestyles. All major religious traditions have a lot to offer. Evoking religious motivations of a substantial part of humanity can greatly improve the condition of forests, increase effectiveness of their protection and encourage broad sections of societies to get involved in this task.

Keywords

religion and ecology, religion-based ecological organizations, sacred forest, sacred grove

Introduction

Protection of forest ecosystems is a major challenge that the world faces today. The fragility of these ecosystems, the rapid pace of their depletion, and the time needed to revive them are the reasons why they must be surrounded with special care. It is also necessary to build the broadest possible coalition for the protection of forests and to use all means to this end. The gravity of the problem is reflected by the fact that the UN has declared 2011 the International Year of Forests. The main factors which led the UN to take such a decision include the following: forests cover 31% of the total land area; primary forests account for 36% of forest area; forests are home to 80% of terrestrial biodiversity; forests are home to 300 million people around the world; the livelihoods of 1.6 billion people depend on forests; 30% of forests are used for the production of wood and non-wood products; in 2004, trade in forest produce was estimated at \$ 327 billion dollars (UN, 2011).

Publications on the effectiveness of ecological projects indicate the crucial role of reconciling the so-called Big Conservation with Little Conservation. Lasting ecological effects can only be achieved by using

both the economic resources and technologies held by Big Conservation and the commitment and traditional knowledge that are the assets of Little Conservation (BOUCHER, 2011). A harmonious combination of the two ways to protect nature must lead to a rethinking of the famous slogan of sustainable development, ‘think globally, act locally’. It is in fact impossible to act locally without being able to think globally. The initiators of major ecological projects who think globally, described by Raymond Dasmann as the ‘Biosphere People’, need to understand that the success of their environmental projects depends on the ‘Ecosystem People’. The latter are in fact the guarantors of the effectiveness of environmental projects in their area. They have a vested interest in the condition of the environment in which they live and work, and raise their children. The advantage of the ‘Ecosystem People’ lies in the fact that they know their land and depend on it for their wellbeing. Their identity and their history are part of the landscape. They locate themselves in relation to natural features in the landscape and the history associated with those features (ALCORN, 1995).

A crucial element of the identity of local communities is their religion and its local manifestations as well

as the traditions deriving from it. In building support for environmental projects, appealing to religious motivation to secure a broader and more engaged participation of the local communities greatly increases the chances of success of many environmental initiatives.

This article will examine the opportunities of religion in the field of forest protection stemming from the sacred dimension of the sacred groves. It will also point to religious motives behind the activities of faith-based ecological organizations for the protection of forest ecosystems. Not surprisingly, the current debate on the ecological crisis is held not only by scientists and politicians; alongside private foundations, NGOs, business people and research institutes, religious organizations are its vocal participants (McELROY, 2001; ROLSTON III, 2006).

The ecological potential of religion

Over the last two decades, an ever-increasing commitment to conservation by all the major religious traditions has been noted. This commitment has resulted in a significant increase in the number of pronouncements by religious leaders on the conservation of nature, accompanied by the growing number of religion-based environmental organizations and their active participation in specific environmental actions. This has led to a greater awareness among politicians, scientists and representatives of various international organizations of the role that religions can play in overcoming the ecological crisis.

The cooperation of scientists and religious leaders in work for the environment is today taken for granted (JARSE, 1996). Religions are now widely seen as an important ally in the fight against the ecological crisis. The State of the World Report of 2003 points to five strong assets held by religious leaders and institutions in the environmental context (GARDNER, 2003): 1) capacity to shape cosmologies (worldviews), 2) moral authority, 3) a large base of adherents, 4) significant material resources, 5) community-building capacity.

The greater the role played by religion in the lives of its followers, the greater its ecological advantages. The Gallup research conducted in 143 countries in the years 2006–2008 confirms that religion has a significant impact on the daily lives of a major part of the humanity. Across all populations, the median proportion of residents who said religion is important in their daily lives is 82%. To the question “Is religion an important part of your everyday life?”, 100% respondents in Egypt said “yes”. Similar responses were recorded in countries such as Bangladesh, Sri Lanka, Indonesia, the Congo, and the United Arab Emirates. Almost all African countries scored in the survey in the range of 80–95%. In the countries of South America, with the exception of Argentina, the results were similar to those

from Africa. In the United States, however, the results were at the level of 65%. The study shows that religion matters least in the daily lives of Europeans. Among the eleven countries which scored the lowest in the survey, as many as seven were European countries: Estonia – 14%, Sweden – 17%, Denmark – 18%, Norway – 20%, the Czech Republic – 20%, France – 25%, and Belarus – 27% (CRABTREE and PELHAM, 2009).

The importance of religion for the inhabitants of each country is relatively stable. When comparing the data of similar studies conducted in 44 countries in 2002 by another research institute as part of the Pew Global Attitudes Project, it is evident that for a considerable proportion of the inhabitants of our planet religion continues to play an important role and affects their daily lives (PRC, 2002). The studies show that religious people engage in aid to the needy more often than non-religious people, which is another plus point for religions in encouraging their followers to engage in nature conservation (PELHAM and CRABTREE, 2008).

Sacred groves as ‘religious nature reserves’

One way to protect forest ecosystems is to evoke the institution of sacred trees and sacred groves, which are still present today in some religious traditions. Sacred groves provide the local communities with multiple roles to play, such as religious, secular, political, cultural, medicinal, economic, and psychological. They also have numerous environmental advantages. Sacred groves have well developed forest ecosystems, high degrees of species richness, and a rich biodiversity in general. Sacred groves are an asylum for endemic and endangered plant and animal species; they are store houses of medicinal plants and wild plants that help the gene pool of cultivated varieties. Most of the sacred groves are associated with water bodies such as ponds, streams and rivers. The trees help in retaining the soil fertility by preventing erosion of topsoil, and absorb most of the rainwater from the runoff (JONATHAN, 2008).

Religious motivations for the protection of sacred groves provide a ‘social fence’ that shields them from unreasonable exploitation of the resources within these forest patches. This social fence provides protection for a variety of species and habitats. An appeal to religious motivations and institutions of sacred groves will make the protection of local areas not only more effective but also cheaper, as there will be no need to maintain a large number of security personnel to enforce the law, or complex supervisory institutions. Numerous publications have shown that the recognition of a forest as sacred enhances its safety, especially when it comes to protecting endangered species. Moreover, the sacred groves represent a significant potential for biodiversity (VIRTANEN, 2002). A large proportion of the protected areas which guarantee the conservation of biodiversity is located in regions with a low population density, often

in the mountains or on land unfit for cultivation. Consequently, many species living in lowland areas with a high population density are endangered much more. The bulk of the sacred groves, however, are located on lowlands, which further increases the ecological value of sites protected for religious reasons (BHAGWAT and RUTTE, 2006).

The cult of the sacred grove is known to all Indo-European peoples. There have been numerous studies on the sacred groves in ancient Rome, Greece, Germany, Scandinavia and among the Celts, Balts and Slavs. The most famous sacred grove in the Roman culture is a grove of Ariccia, in which Diana Nemorensis was worshipped. In ancient Greece a significant role was played by the oak grove at Dodona located in the north-western part of the Balkan Peninsula, and near Athens there was the sacred grove of olive trees known as 'the grove of Academe' (FOLKARD, 2009).

According to the Roman historian Tacitus, the Germanic peoples were convinced that forests were the most appropriate places to worship their gods and they treated the sacred groves with extraordinary respect and reverence (TACITUS, 2010). There is a rich bibliography on groves held sacred by the Balts, who considered both the designated areas of forest and the wild animals and birds that lived there as sacred. St. Adalbert, the patron saint of Poland, was presumably martyred in one such grove belonging to the Baltic Prussians, in an act of sacrificial offering by a pagan priest to appease the gods for the sacred grove's violation. Relatively little is known about the worship of sacred groves among the Slavs, but there is no doubt as to the existence of such cults among the Slavic peoples (SŁUPECKI, 1994).

Also the Celts worshipped their gods in sacred groves. These serve as an example of how woodlands considered sacred were protected. The laws concerning the sacred groves of the Celts were similar to those that existed in many other cultures. In the forest areas considered sacred it was not allowed to cut down or damage trees, or even to prune them. Picking berries, nuts or fruit was also forbidden. One could only use or eat what had fallen to the ground. In a sacred grove, fighting or hunting were banned, as was fishing in streams within its bounds, or polluting their waters in any way. It was not allowed to make fires, or even smoke a pipe. A violation of the applicable laws, depending on the motives and the damage caused, was punishable even with death (PULVER, 1994).

With the christianization of Europe, sacred groves disappeared almost completely from the continent. Due to the disappearance of Europe's traditional religions, to appeal for the preservation of forest ecosystems in today's Europe by evoking the tradition of sacred groves would be an anachronism. There are still, however, many forest areas which the local people hold sacred. Most such sites are located in Asia and Africa, especially in India, China, Thailand, Indonesia and Japan, and in Nigeria, Ghana and Kenya.

Prepared in 2005 by WWF and the Alliance of Religion and Conservation, the report *Beyond Belief: Linking faiths and protected areas to support biodiversity conservation* lists a hundred sacred places within protected areas, of which seven are forest areas whose preservation can be explained by the fact that, according to local beliefs, they had been in whole or in part sacred groves or sacred forests. These areas are located in countries such as China, Ghana, Nigeria, Indonesia, Japan and Thailand. The report also lists seven sacred sites outside protected areas, of which three are sacred groves in Ghana, Madagascar and Ethiopia, and the fourth location involves the sacred trees in Võrumaa, Estonia (DUDLEY et al., 2005).

In Japan, for example, sacred groves are closely linked with Shinto Shrines, which are often the only remaining patches of semi-natural vegetation in urban areas. Thus the sacred grove surrounding the Kashima Shrine demonstrates how a site protected for the sake of a religious tradition, eventually gained the status of land protected by state law. In 1956, the Sacred Forest of Kashima covering, together with the surrounding woods, a total area of 1.5 thousand hectares was declared a protected area. The Sacred Forest of Kashima is now home to more than 800 species of trees and features an exceptionally rich bird life. Sacred groves are also present in the Buddhist tradition. Among the Dai people inhabiting the Chinese prefecture of Xishuangbanna there still survives the tradition of sacred groves, which – according to the beliefs of the natives – protect the villages that look after them. It is estimated that only in that province over 100 thousand hectares of forests are protected in this way (DUDLEY et al., 2005).

The largest number of sacred groves are without doubt located in India, as reflected in numerous publications (ENVIS, 2008). Published in 2001, the results of research conducted by the Indian National Science Academy and the Indian Statistical Institute show that more than 50 thousand sacred groves were reported for the survey. Of this huge number only 13,720 were examined. The sheer scope of the study makes it difficult to determine the exact number of sacred groves on the territory of India, but it is estimated that there are some 100–150 thousand (MALHOTRA, 2001). A study published in 1998 covered only 4,415 sacred groves, with a total area of 42 thousand hectares (GOKHALE et al., 1998). However, no study has attempted to offer a more precise number of sacred groves and the area they occupy. On the basis of available data one can only predict that their total surface must be enormous.

For reasons of the numbers and diversity of sacred groves in India, they have been classified into the following categories (ENVIS, 2008): 1) Traditional Sacred Groves – It is the place where the village deity resides, who is represented by an elementary symbol; 2) Temple Groves – Here a grove is created around a temple and conserved; 3) Groves around the burial or cremation grounds.

Currently, however, the sacred groves are facing major challenges. The most important are social and economic change and the changes affecting spiritual life and religious values. Among the socio-economic threats that many parts of the world face today is a decline in the profitability of agricultural production, bringing impoverishment of the rural population; this in turn, combined with population growth and rising immigration, triggers the search for new agricultural land at the expense of sacred groves. Another threat to the sacred groves is the pressure on land from local people for many forms of development, and pressure from the outside, including poaching, legal and illegal logging, mining and pollution. Yet another threat to the sacred groves is the influx of immigrants who are not familiar with local customs, and often are the followers of other religions.

When it comes to change affecting spiritual life and religious values, a major challenge is the expansion of new religions. The tradition of sacred groves probably stems from the beliefs of hunter-gatherer communities and their animistic religions. An example of the disappearance of religions cultivating the sacred groves is Europe, where in the fourth and fifth centuries, with the Christianization, almost all sacred groves were destroyed. A similar process is taking place in India, where the local folk deities have been, and continue to be, replaced with Hindu deities – a process called ‘sanskritization’. In many countries a phenomenon known as ‘westernization’ can be observed which brings a consumerist lifestyle and weakens traditional beliefs that are so important for the continuation of the tradition of sacred groves (BHAGWAT and RUTTE, 2006).

Protection of forests in the activities of religion-based environmental organizations

Religions’ contribution to the protection of forests is not limited to providing support for the protection of forest areas by evoking the tradition of sacred groves. There are many environmental organizations that focus on care for forests. An important feature of the activity of these organizations are their religious motivations. Among the pro-environmentally oriented religious movements and social organizations appealing to religious motivations one can include the Bishnois community, the Chipko movement, the Appiko movement, the Swadhyaya movement, and the Ecological Movement of St. Francis of Assisi. Most of these arose in India, where the tradition of the protection of trees has its own rich history.

The Bishnois Movement

Already in the fifteenth century, under the influence of the teachings of Guru Jambhesvara, a religious group called The Bishnois was established, which strictly pro-

hibited harming trees or animals and encouraged vegetarianism. Out of the 29 principles that Jambhesvara Guru left to his followers, eight rules have been prescribed to preserve biodiversity and encourage good animal husbandry. For them, their tradition, based on the words and life of their guru, is the main reason for their environmentalist activism. This goes much beyond the recognition of bio-divinity based on the Hindu cosmology or Hindu texts. Unlike other Hindu communities, the dharma of Bishnois is not limited just to the Hindu scriptures or rituals but also includes natural resources. In the eighteenth century, 363 Bishnoi people sacrificed their lives to protect desert trees, khejari, which Maharaja Abhay Singh ordered his soldiers to fell. The people who stood up in defence of the trees were led by Amrita Devi, a woman now regarded as the first ecological martyr of India, while the Bishnoi community can be considered as forerunners of today’s ‘tree-huggers’ (JAIN, 2011). In Indian literature, however, there is little mention of the Bishnoi community, which must have inspired the founders of the Chipko movement (KAPLAN et al., 1997).

The Chipko movement

The term ‘Chipko’ is connected with embracing (hugging) trees as a form of protest to protect them from being cut down. The term has its roots in the Garhwali-language word ‘angalwaltha’ meaning ‘embrace’; which was later replaced by the Hindi word ‘chipko’, meaning ‘to stick’ (CHIPKO, 2011). The moral strength of the Chipko movement and its popular support from the public is based on religious convictions. Its members find in the Hindu religion authentic insights and genuine support for the maintenance of the Earth’s ecosystems (JAMES, 2000). Chipko started in the early 1970s as a community initiative in Garhwal Himalaya, where intensive forest logging had taken place, resulting in a catastrophic flood. The logging was done with the permission of state authorities, which failed to foresee the consequences of their decision. The beginnings of Chipko are linked with the events of 1973 when employees of the Symonds Company sent to fell ash trees near the village of Mandal were forced to flee. Mass demonstrations and protests were organized against the authorities, which were accused of causing a flood by endorsing the felling of forests. The event marking the beginning of the Chipko movement was the successful protest of women led by Gaura Devi, head of a women’s organization, who convinced the local women to defend the trees. For four days those women hugged trees in the area of Reni village and thus spared them from logging, despite direct threats from lumbermen and their leaders. The women then announced, “This forest is our mother’s home, will we protect it with all our might.” The protest proved effective, giving rise to a grassroots movement that quickly spread to large areas of India (JAMES, 2000).

One of the founders and early leaders of Chipko, Sunderlal Bahuguna, said: "The solution to present-day problems lies in the re-establishment of a harmonious relationship between man and nature. To keep this relationship permanent we will have to digest the definition of real development: development is synonymous with culture. When we sublimate nature in a way that we achieve peace, happiness, prosperity and, ultimately, fulfilment along with satisfying our basic needs, we march towards culture". (CHIPKO, 2011). Chipko activists have continued to disseminate their message by means of foot marches and environmental camps that continue to the present day. Through their direct presence in the villages and towns they promote the need to protect trees, whose fate is closely linked with the fate of the people living in the area.

Chipko is currently actively involved in protests against the construction of hydroelectric power plants on the Bhagirathi River. The religious ideal of harmony with nature remains a crucial feature of this continuing message. Particularly noteworthy is the attitude of Sunderlal Bahuguna, who took an oath to sacrifice his life to protect the natural environment of the Himalayas. As a result of his hunger strike in 1981, the Government of India introduced a fifteen-year moratorium on all commercial felling in the Uttarakhand region of the Himalaya, and eventually an unconditional ban on the felling of all trees above one thousand meters in elevation; nevertheless commercial penetration has continued to endanger the region (JAMES, 2000). The religious motivation behind the Chipko leaders' efforts is reflected in the statement of their representative at the meeting of the International Alliance against Large Dams, held in Curitiba, Brazil, in 1997: "Indian culture sees divinity in nature. To the rulers, the Ganga is megawatts of power and hectares of irrigated land. To the local people, she is a life-giving goddess" (JAMES, 2000).

The Appiko movement

The success of the Chipko movement, which arose as a form of protest in the Himalayan region, encouraged people from other parts of India to actively protect their forests. Particularly noteworthy is the activity of the Appiko movement in the southern Indian state of Karnataka, especially in the densely forested region of Uttara Kannada, where the local-language term for hugging is 'Appiko'. The Appiko movement adopted Chipko's methods of operation, which focus on activating the local population and holding protests that involve the blocking of logging by hugging trees (KAPLAN et al., 1997).

The protests which led to the rise of the Appiko Movement began in 1983, during efforts to block logging in the Kalas-Kudergod forest. As part of the protest involving the hugging of trees, 120 women and 30 men effectively blocked forest felling in this unique re-

gion, so important ecologically for the whole of southern India. The district is unique in that it traverses five important terrestrial eco-zones. From the west to the east there is the narrow coastal plain, the evergreen and moist deciduous forests of the Western Ghats, dry deciduous forests of the and further east the scrublands, making it one of the important centres of biodiversity in the Western Ghats. Because of the region's importance and the potential consequences of logging for the whole of southern India, Appiko's message had great resonance among the local population; this in turn led to a rapid development of Appiko and its broad social support (SHARMA, 2007).

Appiko operates under the slogan 'Save, grow and sustain'. It does not limit itself to protests in defence of forests, but also conducts a reforestation campaign and education on prudent use of resources. The movement's objectives can be classified into three major areas: 1) The Appiko movement is struggling to save the remaining tropical forests in the Western Ghats; 2) it is making a modest attempt to restore greenery to the denuded areas; 3) it is striving to propagate the idea of rational utilization in order to reduce the pressure on forest resources.

The Swadhyaya movement

The Swadhyaya movement takes a slightly different initiative to protect trees (in Sanskrit Swadhyay means self-study). This new religious movement was founded by the Indian philosopher and spiritual leader Shastri Pandurang Vajinath Athavale. The origins of the movement can be traced to the first public appearances of their founder in 1942, but the beginning of its social-ecological activity dates back to 1979. Today, this movement is active in over 20 countries in Asia, North America, Europe, Africa, the Middle East, Australia and the Caribbean. The essence of this movement is the concept of 'Indwelling God', the presence of divinity in humans and other beings. The ecological dimension of this movement is significant, although Athavale's followers deny that it is an environmental movement. The mission of Swadhyaya is to generate and spread reverence for humans, animals, trees, earth, nature, and the entire universe in general (JAIN, 2011).

Stressing the importance of respect for the trees, the movement's founder Athavale said, "there is a divine power in trees which makes it possible for water and fertilizer to rise from the roots below and reach the top portion against the gravitational force. It is not just the result of 'Keśākarṣaṇa' (capillary action) but it is 'Keśavākarṣaṇa' (Kṛṣṇa's force)." Pankaj Jain, a Swadhyaya scholar, says that in the case of this movement you can talk about 'arboreal dharma', a dharmic environmentalism to worship and nurture the trees for their unique qualities. In his opinion, "this dharmic approach is different from shallow ecology's utilitarian approach,

i.e. to protect ecology for human needs. This is also different from deep ecology's biocentric approach of privileging nature more than human society. The dharmic approach is to connect the humans with the ecology based on the divine relationship between the two, not by separating one from the other" (JAIN, 2011).

It seems that the ecological dimension of Swadhyaya's activity is a by-product of its members' religious respect for the world around them, in which as a whole Swadhyayees perceive the presence of God. As one form of their spiritual practices Athavale recommended to his followers the so-called 'Prayogs' – individual and group 'experiments' – mainly to develop self-esteem and human dignity in individuals. Many of the prayogs, in addition to their spiritual dimension and socio-economic background, have a significant environmental dimension. A prayog initiated by Athavale in 1979 resulted in the setting up of the first tree-temple (Vrkshmandirs). Tree-temples are new 'sacred groves' which have appeared not only in India but also in other countries where there are Swadhyaya communities. Tree-temples are set up on wasteland, and through the persistent efforts of members of the Swadhyaya community working on plots of up to 20 hectares wells are sunk, irrigation systems constructed, soil reconditioned, and trees planted, mostly fruit trees. After they have been set up, tree-temples are cared for by Swadhyaya members who declare themselves as 'pujārī', spiritual custodians of these temples. The fruit from the tree-temples is sold, and the money thus obtained – 'Mahālakṣmī' (divine money) – is distributed among the needy as 'prasada' (divine gift). The popularity of this idea borne out by the fact that today almost every district in Gujarat has such a tree-temple (JAIN, 2011).

St. Francis of Assisi Environmental Movement (REFA)

St. Francis of Assisi Environmental Movement (REFA) is an environmental organization of a distinctively religious character that derives from the Christian religious tradition. The movement was founded in Cracow in 1981 and brings together students and professors, school youth and teachers, naturalists, Franciscans, and inhabitants of villages and towns from different regions of Poland and Europe who as Christians engage in meeting the tough challenges of today's world in the midst of an ecological crisis. The motto of the movement is "the Catholics amongst environmentalists, environmentalists amongst the Catholics". REFA conducts intensive activities in the field of environmental education, but also takes concrete actions to protect nature. Its interesting tree-protection initiative involves setting up the 'gardens of Brother Francis'. These are the surviving fragments of natural or semi-natural ecosystems, e.g. fragments of forests or old parks, or trees clusters, hedges, meadows, ponds, oxbow lakes, bogs

and swamps. The purpose of these gardens is to enhance the protection of natural values of areas that are already covered by one of the forms of legal protection, and to extend such protection to sites that have no official status of protected areas (WOJCIECHOWSKI, 2007).

In the case of REFA, tree protection has a completely different character from that of the other movements discussed above, as it is not based a belief in trees being sacred (as in Hinduism), but stems from the Christian commandment to love one's neighbour, which makes us want to preserve a healthy environment, and from the biblical injunction of stewardship of the earth ordering us to be good custodians of God's creation. An area declared as a garden of Brother Francis is protected from such direct threats as littering, tree felling and destruction of species, and from harmful infrastructure investments. In the cases of particularly valuable sites, efforts are made to put them under legal protection. The specified site becomes a brother Francis garden by means of saying a prayer and blessing it, and placing on it a statue of St. Francis of Assisi, as well as by informing the community about the natural values of the site and awarding it a 'religious' status of a protected place (REFA, 2011).

Conclusions

We more and more often realize that the success of environmental initiatives depends not only on scientific and technological inventions, but also on the state of human minds, which are strongly influenced by very different factors: images, reports, metaphors, feelings, information, statistical data, personal experiences, etc. (POSAS, 2007; LEISEROWITZ, 2006). Religions have a significant and direct impact on many of these factors. Thus, the presence of religion in protecting the forests seems to be relevant and even necessary. Looking at forest ecosystems protection from the religious and moral perspective can significantly broaden the ongoing debate on this issue and contribute to a greater concern for the environment on the part of ordinary citizens. The moral authority of religions and their presence in the life of societies is also an important factor in putting pressure on decision-making bodies when their decisions on the felling of forests raise doubts, or when they lack determination to protect forests.

Different traditions certainly can make different contributions to the protection of forests. It seems that the greatest potential in this field is with the Asian religions which, drawing on the tradition of sacred groves and the activities of numerous faith-based environmental organizations, protect the existing forests and run multiple re- and af-forestation projects. The situation is radically different with religions such as Christianity, Islam and Judaism, which, being monotheistic, do not evoke deities inhabiting the forests and do not

deify trees. The large numbers of Christians and Muslims and the impact of their religion on the daily life of the followers is an important factor in the protection of forest ecosystems. The pronouncements of such religious leaders as the successive popes, the ecumenical patriarch of Constantinople and the World Council of Churches are a voice to be reckoned with for almost all Christians. Pointing to the moral dimension of everyday choices and showing their ecological consequences is a very important tool in shaping eco-lifestyles among Muslims, Christians and Jews. Extremely valuable here is the introduction into everyday usage of such moral concepts as 'ecological sin', which in the case of monotheistic religions is an important factor influencing human choices. An important element in shaping mindsets and lifestyles is the religious education of children and adolescents. The presence of environmental issues in religious education programmes is particularly promising, because it shapes ecological lifestyles of successive generations. Moreover, the rich West, with its dominant Christianity, provides a major economic and social base (e.g. voluntary workers) for actions to protect forests on continents such as Africa, South America and Asia.

Today we better understand that the effectiveness of environmental projects depends on local conditions: social, cultural and religious. Attempts to combine Big and Little Conservation without enlisting religions and tapping on their positive potential seems to be a much more serious omission than sheer negligence. If we seek better protection for forest ecosystems, then appealing to religious motivation and establishing cooperation with religious leaders and faith-based environmental organizations has to become a standard approach.

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Náboženské motivácie ochrany lesných ekosystémov

Súhrn

Neustále sa zmenšujúca plocha lesných porastov núti ľudstvo, aby zvyšovalo úsilie smerujúce k obnove zničených a k ochrane jestvujúcich lesných ekosystémov. Dôležitým spojencom pri týchto snahách sú alebo môžu byť náboženstvá. Ich ekologický potenciál nie je ešte stále dostatočne využitý. Náboženstvá vďaka svojim „ekologickým výhodám“ (schopnosťou formovať kozmologický obraz sveta, morálnou autoritou, veľkým množstvom vyznáčov, značnými ekonomickými prostriedkami, potenciálom vytvárať spoločenstvo) môžu pomôcť pri formovaní ekologického štýlu života. Všetky väčšie náboženské tradície môžu v tomto ohľade veľa ponúknuť. Zameraním pozornosti na náboženské motivácie značnej časti ľudskej populácie môžeme radikálne zlepšiť stav lesov, zintenzívniť účinnosť ich ochrany, ako aj usmerniť široké spoločenstvá, aby sa viac zaangažovali v týchto aktivitách.

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Dens and beds of the brown bear *Ursus arctos* in the Eastern Carpathian region – Poloniny National Park

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Abstract

ŠTOFÍK, J., SANIGA, M. 2012. Dens and beds of the brown bear *Ursus arctos* in the Eastern Carpathian region – Poloniny National Park 2012. *Folia oecol.*, 39: 147–154.

Distribution of dens and beds of the brown bear across the Eastern Carpathians Mts was assessed based on the data about occurrence of bear dens and data assembled by tracking the bears assembled by the year 2012. The spatial analyses were performed the data about 8 (10) ground, 1 tree, 1 camp, 5 breeding and 15 transitory beds. The dens and beds were situated on warmer, SW facing localities (186° , $SD \pm 68^\circ$), at higher altitudes (average of 637 m a.s.l., $SD \pm 118$ m a.s.l.) on relatively steep slopes (14° , $SD \pm 6^\circ$). The hibernation dens (tree and ground) were mostly buried in thickets or in young understory enclaves in older stands. The most part of hibernation dens were in the existing and planned nature reserves of primeval forests (6 in 11). The breeding dens were situated in young conifer stands. The bears emerged from their nests in March, in most cases when distracted.

Keywords

ecology, ethology, distraction, The Eastern Carpathians Mts

Introduction

In Slovakia, the protection of the brown bear has been given a high priority already since 1932, by the Decree 127 203/14-1932, prohibiting the hunting on the bear from 1.9.1932 to 31.12.1934. In that time, the species abundance in Slovakia was estimated only to several tens (FERIANCOVÁ, 1955; ŠKULTÉTY, 1970; JANÍK et al., 1986; HELL and SABADOŠ, 1993). Some works (KOMÁREK, 1955; FERIANCOVÁ, 1955) even suggested absence of bears in the area of Eastern Carpathians during this period. This hypothesis, however, has been rejected after evaluation of diverse data and comparing a rich spectrum of works discussing the area of the Eastern Carpathians (KOMÁREK, 1955; FERIANCOVÁ, 1955; SABADOŠ and ŠIMIÁK, 1981; HELL and SLAMEČKA, 1999; PČOLA, 2002, 2005; FIŇDO et al., 2007; RIGG and ADAMEC, 2007); and the bear's occurrence across this territory is supposed also in periods with low numbers of this species (ŠTOFÍK et al., 2010).

At the beginning of the 21st century, the brown bear population in this territory recorded a significant increase compared to the numbers known from the past and also exhibited a significant extension into the surrounding mountain areas of Vihorlatské vrchy, Beskydské predhoria, Laborecká and Ondavská vrchovina (ŠTOFÍK et al., 2010).

In the territory of the Poloniny National Park (NP), there has been identified an East-Carpathian subpopulation of brown bear (STRAKA et al., 2011b), living here on the boundary of its natural occurrence range (FIŇDO et al., 2007). The number of bears moving across this area has been estimated to 25 individuals (ŠTOFÍK et al., 2010), from which 15 have been confirmed by non-invasive genetic analyses (STRAKA et al., 2011a).

The duration of sleep of bears hibernating in their winter dens varies, and sometimes the animal is forced to interrupt sleeping from various reasons. The females start their denning earlier than the males, the gravid females are the first (DAELE et al., 1990). The males but

also females in northern areas start their denning earlier and finish later; with the gravid females exhibiting the longest denning period; the males emerge from their dens significantly earlier (MANCHI and SWENSON, 2005). The females visit their denning habitats one a month; before the denning, they reduce their moving activities and roam about the denning sites (FRIEBE et al., 2001).

A comparison between two populations in Alaska revealed that the females from the SW parts started their denning by 2 or 3 weeks later than in the N parts. The authors suggest that this delay may be due to different food accessibility (DAELE et al., 1990).

The data concerning dens and beds in the territory discussed in this work have not been processed yet. There exist only pieces of information from the neighbouring Bieszczad about the dens (JAKUBIEC, 2001) and about spring and autumnal beds (FRACKOVIK and GULA, 1996).

The goal of this work is to analyse the bears' denning from the aspects of type, distribution and anthropogenic impact on these activities.

Material and methods

The model territory is situated in the Alpine-Himalayan complex, Carpathian sub-complex, Eastern Carpathian province, Outer Eastern Carpathian province, area

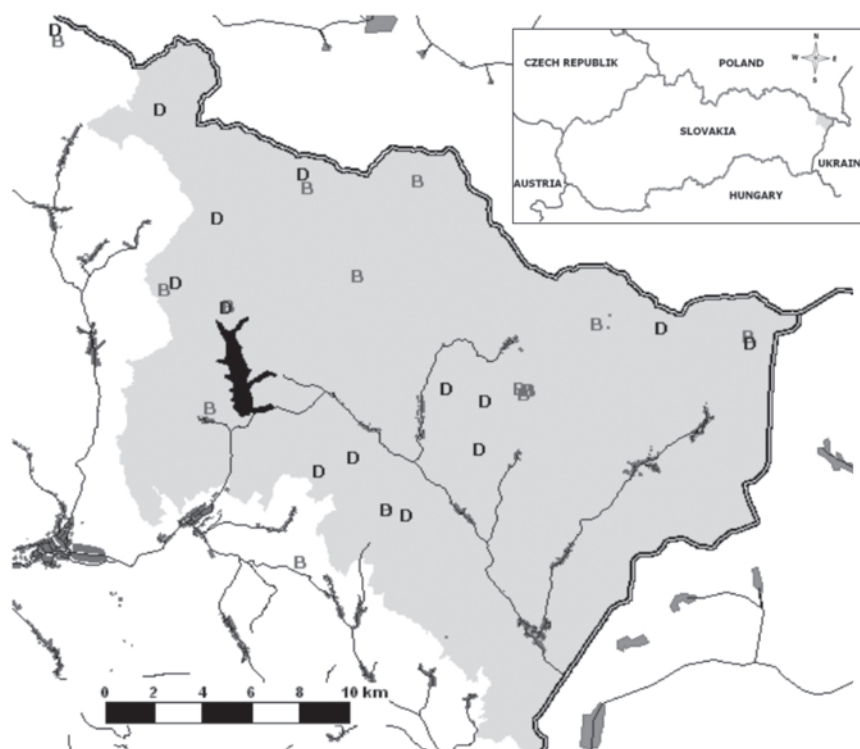
Poloniny (MIKLÓS, 2002). The denning and breeding sites were mostly assessed in the Poloniny National Park (406 km²), Fig. 1.

According to the inventory in 2003, the absolutely dominant landscape element was forest (85.5%), followed by meadows and pastures (8.5%), fields (2.1%), with the other elements occupying only a minimum area (OLAH et al., 2006). The dominant tree species was European beech *Fagus sylvatica*, in higher altitudes with admixture of silver fir *Abies alba*, in lower altitudes forming stands with European hornbeam *Carpinus betulus* and sessile oak *Quercus petraea*.

The territory of the national park is exposed mostly SW, encompassing altitudes from 250 to 1,208 m a.s.l. (average 608 m a.s.l.), with diverse topography (61.5%), inclination 7°–17° (Fig. 2).

The number of days with the average temperature below zero is 78 in southern parts, 96 in northern parts, 118 on mountain ridges. The average depth of snow cover is 30–40 cm in lower parts, about 70 cm in mountains. The average number of days with snow cover is 70 in lower situated and southern parts, 80 in northern parts and over 120 on the ridges (ŠTASTNÝ, 1988).

The data (1974–2012) about disposition of denning sites were obtained from the local residents as well as from bears where also their dens were identified. The data on occurrence, surroundings, altitude and exposition were assembled in the field and recorded with the



B, beds; D, dens.

Fig. 1. Denning and bedding localities in the Poloniny National Park and its surroundings.

aid of a GPS appliance (Garmin etrex Vista H) in the coordinate system WGS 84 (accuracy ± 5 to 20 m).

In the GIS programme GRASS 6.1 provided with the contour line base from SVM 50 (the Base Maps of the Slovak Republic, scale 1:50,000), we have created a digital terrain model for the territory (grid 100 m \times 100 m) and derived strata for morphotype characteristics: exposition (N, NE, E, SE, S, SW, W, NW), sloppiness and stratum of hypsometric degrees (scaled at 100 m). By synthesising the data on inclination (7 classes less than 1°, 1°–3°, 3°–7°, 7°–12°, 12°–17°, 17°–25°, 25°–90°) and exposition (scaled by 30°) we have created morphotype categories classified from 1 to 7, according to the amount of direct incident solar radiation in the vegetation period in kJ: (<535 (1), 536–575 (2), 576–615 (3), 616–665 (4), 666–675 (5), 676–695 (6), 696–< (7)) (MIKLÓS et al., 1997). In the GIS programme GEOMEDA PROFESSIONÁL, we have modelled buffer scaled by 1,000 m from human settlements and main roads connecting the settlements, and assessed the presence of denning and bedding sites in these zones. For comparison, we used analytic tools of GIS for evaluation of selected morphometric parameters and their relative values for the entire area of the Poloniny National Park and its buffer zone (Fig. 2).

Results and discussion

One of the first records of brown bear denning sites for the Slovak Eastern Carpathian region is from 1974 by Poliščuk (JANÍK et al., 1986). A denning female with three cubs in the area of Osadné was reported by Martin Leník. This denning site is probably the same as the one described by PČOLA (2002) in 1997 from surroundings of the village of Nižná Jablonka. These two dens have not been localised in the terrain.

We have got information about 17 dens (15 verified) and 15 transitory beds. Table 1 summarises data on distribution, and supplementary information

for the recognised dens (Fig. 1), occurring mostly in warm, SW facing localities at higher altitudes on steeper slopes (Fig. 3).

The hibernation (underground and one tree) dens (9 in 9) were situated directly in or less than 50 m from forest stands aged over 100 years (Forest stand maps, status 1.1.2010, © National Forest Centre (Slovak Republic)), hidden in thickets and thicket enclaves (7 in 9 dens). Six from 11 hibernation dens were found in the existing primeval forest reserves (or less than 50 m from) and proposed primeval reserves (Table 1). The area of primeval forest reserves in the Poloniny NP represents 5.37% of the total park's area (40,778 ha), with the part proposed to append (BARTUŠOVÁ et al., 2011a, 2011b), their portion should increase to 8.19%.

The data and information of breeding dens occupancy insinuate that they are mostly used by solitary individuals. In winter we recorded tracking paths and transitory beds of males with their cubs, denning, however, was observed only in a tree and in several underground dens (Table 1).

We have not got data on initiation and duration of the denning; on the other hand, we can reliably date the end on 12. 3. 2008 – due to distraction. The bear was probably a female having used the relevant den with her cub in the year 2007. Repeated use of the same den in the next year is not a rule; on the other hand, there are known cases where a male with cubs migrated among several dens in the same year (SCHOEN et al., 1987).

We have recorded the following abandoned dens: 15. 3. 2012, Nature Reserve Havešová – an underground den used by a relatively small bear (abandoned for ca 3 days); 23. 3. 2012, a tree (elm) den near the boundary with Poland, abandoned by a female with three cubs (the tracks were observed on 21. 3. 2012, one day after trimming the road near the den with a bulldozer); 29. 12. 2008 (Monday after a collective hunting the wild boar during the weekend), an abandoned breeding den found on a tracking path; 3. 3. 2009, area of the village of Osadné: a breeding den (by the den we observed

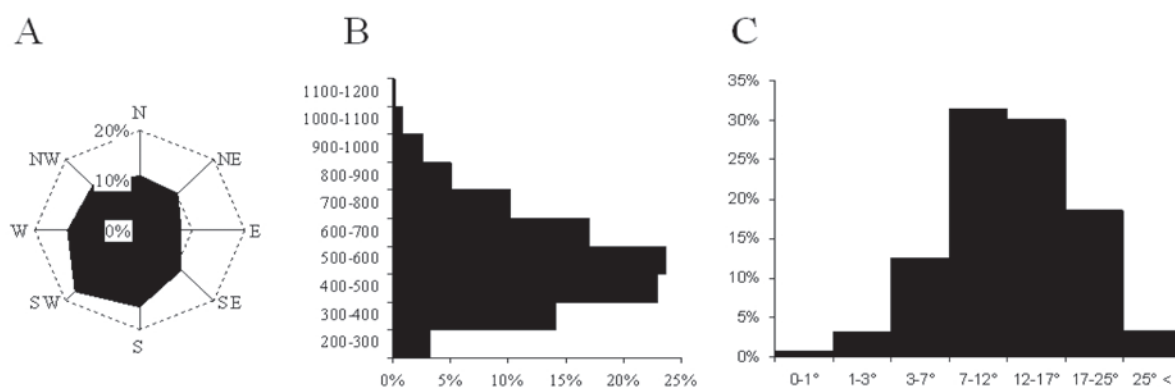
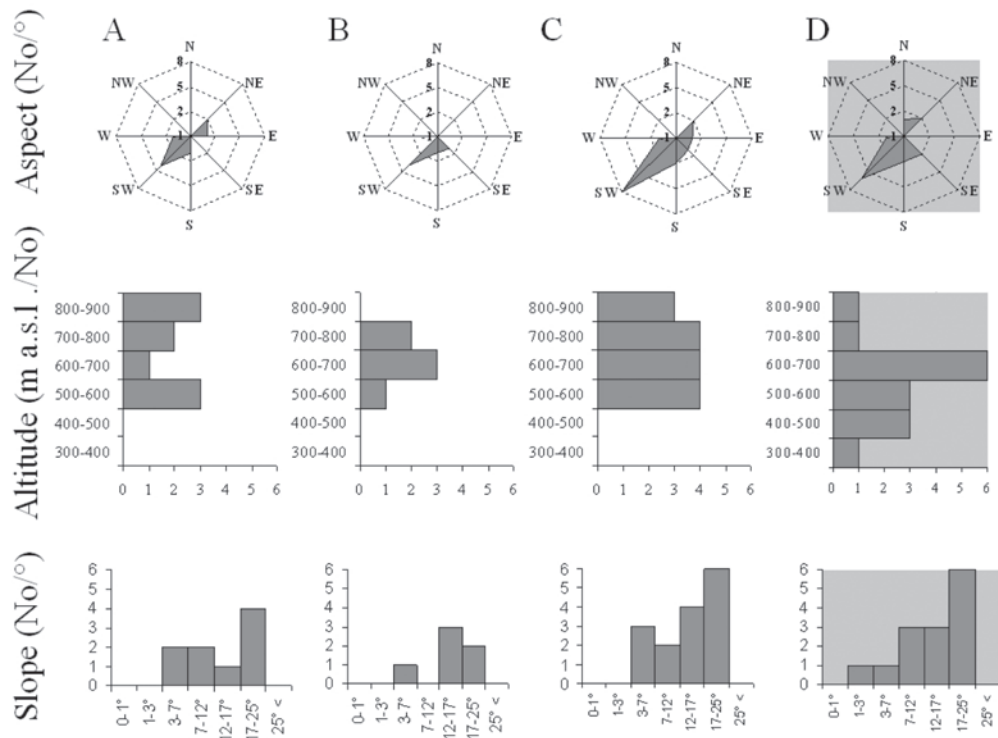


Fig. 2. Selected morphometric parameters of the relief in the Poloniny National Park (relative values).



A, tree den and ground dens; B, breeding dens and camp; C, dens together; D, beds.
Fig. 3. Morphometric parameters of dens and beds.

Table 1. The assessed dens

Dens type (Total)	Site (Reserve)	The cause	End of denning	Source	Year (sex + cubs)
Ground (10)	Nová Sedlica (Stužica)	Distraction		Poliščuk M.	1974 (1)
	Topoľa	Unknown		Micenko M.	1987
	Osadné	Distraction		Lenik M.	1997 (♀ + 3)
	Ostružnica	Unknown		Huťák J.	2000
	Ruské („Černiny“)	Unknown		Sivák J.	2007
	Kolbasov (Havešová)	Distraction	12. 3. 2008	(Blaško J.) Štofík J.	(2007, ♀ + 1) 2008 (1)
	Starina	Unknown		Huťák J.	2009
	Nová Sedlica (Stužica)	Unknown		Sentiván R.	2010
	Kalná Roztoka (Havešová)	Natural	15. 3. 2012	Štofík J.	2012 (1)
	Runina	Unknown		Micenko M.	?
Tree (1)	Nová Sedlica („Borsukov vrch“)	Distraction	20. 3. 2012	Štofík J.	2012 (♀ + 3)
Camp (1)	Ruský Potok	Unknown		Gič M.	1987
Breeding (5)	Ostružnica	Distraction		Blaško J.	2005 (1)
	Príslop	Distraction		Blaško J.	2000 (1)
	Stakčinská Roztoka	Distraction	29. 12. 2008	Štofík J.	2008 (1)
	Osadné	Distraction	3. 3. 2009	Štofík J.	2009 (1)
	Vyšná Jablonka	Natural	4. 3. 2011	Štofík J.	2011 (1)

tracks after snow shoes, indicating, by the season and way of movement, a cast antlers hunter); 4. 3. 2011, area of the village of Vyšná Jablonka, near the boundary with Poland: a breeding den (the bear observed in a bed at a close distance from the den indicated spontaneous emerging from the breeding site); in regions of Ostružnica and Prislop, denning bears two times distracted due to silvicultural activities. All these five breeding dens were situated in young (max. 35 years) stands growing under Norway spruce *Picea abies*.

SCHOEN et al. (1987) report that bears emerge from the winter hibernation from the last days of April to the end of May. This broad interval is probably due to the diverse length of winter period as well as the number of days with snow cover, as the studied territory ranges between 57°–58° N latitude, and the denning period in the southern parts is shorter. This is in accordance with MANCHI and SWENSON (2005), who observed that the beginning of the denning period is linked to the autumnal snow precipitation, so in the northern parts, the males and also females start their denning earlier and emerge from the hibernation later (that means longer hibernation period) than the bears in the southern parts.

27. 2. 1998, near Runina, a bear attacked a man hunting cast deer antlers in the forest (ŠTOFIK and BARTUŠOVÁ, 2009). We suppose that the bear was aroused, as the denning bears may exhibit more aggressive behaviour when the distraction occurs near their denning sites. Such cases are associated with a very high risk for the disturber (SWENSON et al., 1999).

The choice of denning and bedding sites was noticeably affected by these morphometric characteris-

tics: direct solar radiation (9 dens and 5 beds in category 676–695 kJ or 2); SW exposition (8 dens and 6 beds) and steeper slope (6 dens and 6 beds in category 7°–25°).

Similar research in Croatia (22 dens detected by radiolocation) did not approve the influence of aspect on the denning preference – with the most of dens situated in rock caves (HUBER and ROTH, 1997). Similar results were obtained by JUDD et al. (1986). SERVHEN and KLAVER (1983) assessed that the bears avoided NW aspect in their denning behaviour. In Poland, on the other hand, 18 from 28 denning sites exhibited N aspect (JAKUBIEC, 2001).

The mountain ridge of the Carpathians is the boundary between Poland in the north and Slovakia in the south (Fig. 1). We may suppose that the N-facing slopes in Poland and the S-facing slopes in Slovakia may be the underlying cause for the prevalence of N-oriented Polish and S-oriented Slovak dens (Fig. 2). SLOBODJAN (1979) reports that 78% bears in the Carpathians in Ukraine prefer S aspect for denning (cited in JAKUBIEC, 2001), which well corresponds to our results (73%).

The dens were distributed uniformly in categories from 500 to 900 m a.s.l., the beds were also found in lower situated localities, at 375 m a.s.l. (Table 2), more numerous, however in the category 600–700 m a.s.l. (Fig. 3). Denning preferences for higher altitudes and steeper slopes were also observed in Alaska (SCHOEN et al., 1987) and in Sweden (ELFSTRÖM et al., 2008). Our dens, unlike the dens in the neighbouring (JAKUBIEC, 2001), were also situated (5) in lower altitudes (500–600 m a.s.l.).

Table 2. Statistical characteristics of distribution of dens and beds

Characteristics			Beds	Dens	Beds & Dens
Number of subjects			15	15	30
Aspect	[deg.]	Max. / Min.	270° / 45°	270° / 45°	270° / 45°
		Mean (SD)	186° (± 67°)	186° (± 69°)	186° (± 68°)
Altitude	[m a.s.l.]	Max. / Min.	855 / 375	860 / 530	860 / 375
		Mean (SD)	597 (± 119)	678 (± 102)	637 (± 118)
Solar energy	[kJ]	Max. / Min.	700 / 555	700 / 595	700 / 555
		Mean (SD)	671 (± 33)	672 (± 31)	672 (± 32)
Solar energy	[category]	Max. / Min.	6 / 1	5 / 1	6 / 1
		Mean (SD)	2.6 (± 1.1)	2.5 (± 1.1)	2.5 (± 1.1)
Slope	[deg.]	Max. / Min.	23° / 2°	23° / 4°	23° / 2°
		Mean (SD)	13° (± 6°)	15° (± 5°)	14° (± 6°)
Buffer of roads	[m]	Max. / Min.	6,000 / 1,000	6,000 / 1,000	6,000 / 1,000
		Mean (SD)	3,200 (± 1,514)	2,933 (± 1,289)	3,067 (± 1,413)
Buffer of villages	[m]	Max. / Min.	7,000 / 1,000	6,000 / 1,000	7,000 / 1,000
		Mean (SD)	3,467 (± 1,962)	3,133 (± 1,310)	3,300 (± 1,676)

The same also held for beds. In Bieszczady, they were detected at higher altitudes (600–1000 m a.s.l., FRACKOVIÁK and GULA, 1996) than in our study territory where we localised them already from 375 m a.s.l. over the whole winter (XI. – 2, XII. – 5, I. – 3, II. – 2, III. – 3).

The higher numbers of dens and beds in lower altitudes may also result from different periods of assessment and associated forest change patterns (OLAH et al., 2006), and also an increase in the brown bear population in the studied territory (ŠTOFÍK et al., 2011).

Altitude, aspect, sloppiness (Fig. 4) and character of forest stands (primeval) have been recognised as important factors influencing the denning preferences.

The distance from villages and the main roads (Fig. 1) has not been found significant either for denning behaviour or for dens and beds distribution (Table 2). This factor, however, may be affected by increased locomotion and housing activities, as well as building illegal shelters in territories of the villages evacuated due to construction of the water dam Starina. Consequently, distortion of the presented data cannot be excluded.

Another distortion of the information about the denning distractions is supposed intrinsic to the method used – as the data collection relies just on these disturbances. Our list of distractions, in such a way, concerns only possible ways of distractions during denning. By several hibernation dens, we observed also occurrence of transitory beds – probably used also during the growing season (twigs with leaves). The same has been reported by KALAŠ (2004).

In three cases, we could observe the behaviour of distorted bears feeding on thin trees and on bark and rotten wood of dead woody plants. In March, bear droppings contained indigested beech bark and wood residues.

Conclusions

In this work we present data on 10 underground, 1 tree, 1 camp, 5 breeding dens and 15 beds of brown bear in the Slovak Eastern Carpathian area. We have identified the following factors significantly influencing the denning site selection in this region: altitude, exposition, slope and solar energy power. The tree and underground dens were hidden in thickets and thicket enclaves in advance-aged forest stands. We observed a positive impact of establishment and extending of statutory conservation of primeval forest stands for providing habitats promoting denning activities. The breeding dens were situated in young conifer stands. Despite the specific character of breeding dens, we suppose their seasonal use for hibernation.

We did not observe any significant influence of the distance from human settlements and main roads on the disposition of denning sites. This may, however, be due to lively human activities in surroundings of the water dam Starina which required several villages to abandon.

The duration of denning is negatively affected also by distractions by the man: collective hunting, damage by large mechanisms, silvicultural activities, collecting

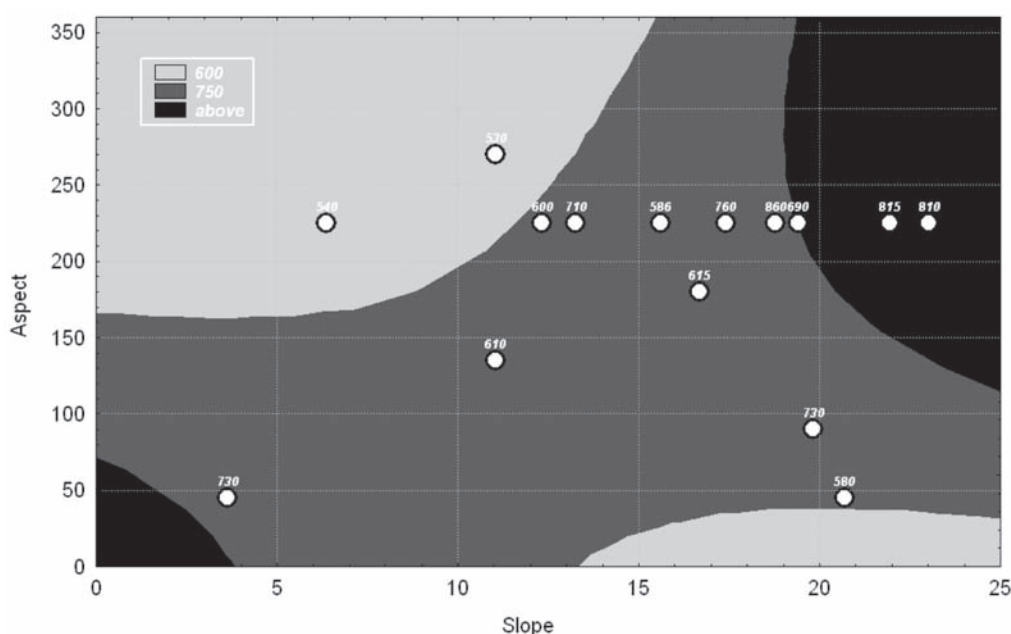


Fig. 4. Influence of selected morphometric parameters on denning preferences (Slope [°] vs. Aspect [°] vs. Altitude [m a.s.l.]).

cast anthers and untimely checking the dens. We observed roaming birds in March, mostly after a distraction.

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Brlohy a ležoviská medveďa hnedého *Ursus arctos* z Východných Karpát – Národný park Poloniny

Súhrn

Umiestnenie brlohov a ležovísk medveďa hnedého v oblasti Východných Karpát sme vyhodnocovali na základe získaných údajov o výskyte medvedích brlohov a údajov zo stopovania medveďov do roku 2012. K priestorovým analýzám boli použité údaje o 8 (10) podzemných, 1 stromovom, 1 stanovom, 5 hniezdnych brlohoch a o 15 prechodných ležoviskách. Brlohy a ležoviská boli situované na teplejších, juhozápadných expozíciách (186° , $SD \pm 68^\circ$), vo vyšších nadmorských výškach 637 m n. m. ($SD \pm 118$ m n. m.) na strmších svahoch (14° , $SD \pm 6^\circ$). Hybernačné brlohy (v strome a pod zemou) sa nachádzali prevažne ukryté v mladinách a mladších enklávach pod staršími porastami. Väčšina z hybernačných brlohov sa nachádzala v rezerváciách a navrhovaných rezerváciách pralesovitého charakteru (6 z 11 brlohov). Hniezdne brlohy sa nachádzali v mladých ihličnatých porastoch. Medvede opúšťali brlohy v marci, no zväčša po vyrušení.

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Distribution of the Southern Oak Bush-cricket *Meconema meridionale* (Orthoptera, Tettigoniidae) in the Czech Republic and Slovakia

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Abstract

VLK, R., BALVÍN, O., KRIŠTÍN, A., MARHOUL, P., HRÚZ, V. 2012. Distribution of the Southern Oak Bush-cricket *Meconema meridionale* (Orthoptera, Tettigoniidae) in the Czech Republic and Slovakia. *Folia oecol.*, 39: 155–165.

Numerous animal species which were originally Mediterranean are seen to be spreading through Central Europe. The bush-cricket *Meconema meridionale* is one of them and it has been known to be spreading mainly in Western Europe. After the first record in late summer of 2008 in the Czech Republic, we checked 44 sites of potential occurrence in the country and 48 in Slovakia. Material was sampled by beating from tree branches and sweeping bushes and small trees. The species was found at 17 localities in the CR and two in Slovakia. The recorded distribution is concentrated in urban environments, connected with traffic or camping. This supports the idea of passive dispersal by cars. The absence of the species in close surroundings of the localities supports the idea of its recent spread over the countries. All the 19 localities are situated in lower altitudes, between 130 and 350 m a.s.l. and the species was found on at least 14 woody species. Morphological parameters of Czech and Slovak specimens are not significantly different from those in original area of distribution.

Keywords

expansion, Czech and Slovak Republics, distribution, invasive behaviour, Orthoptera

Introduction

Many different animal species are reported to extend their distribution ranges towards cooler areas, i.e. northwards in northern hemisphere. It is often assigned to climatic changes or so called global warming (HICKLING et al., 2006). Numerous species which were originally Mediterranean are seen to be spreading through central Europe (e.g. CANNON, 1998; HILL et al., 2011).

Brachypterous, arboreal and arbuticolous non stridulating originally Mediterranean bush-cricket *Meconema meridionale* A. Costa, 1860 is one of them (DETZEL, 1998). The genus *Meconema* Serville 1831 contains two species of small pale green bush-crickets in Europe: macropterous species *Meconema thal-*

sinum (De Geer 1773) is distributed throughout the whole Western Palearctic region, while the brachypterous *M. meridionale* occurred till 1900 or even 1950s only in Italy and some neighboring countries (e.g. BAUR et al., 2006; DETZEL, 1998; HOCHKIRCH, 2001; LUQUET, 1993; ZUNA-KRATKY et al., 2009). Both species are mainly night active. They feed predatorily, mostly on aphids or whiteflies (WEBER and ZIMMERMANN, 1990 in LUQUET, 1993). Their hunting-grounds are trees and bushes, and except of coniferous plants they have been found on many different woody species. In contrast to most bush-cricket groups, their stridulatory apparatus is absent and they communicate by pounding at branches or leaves with their hind legs (DETZEL, 1998; KOČÁREK et al., 2005).

During 1960s, *M. meridionale* has started to expand northwards from its original area of distribution and gradually occupied the northern part of the Alps, the southern part of Germany and northern France (e.g. WEBER and ZIMMERMANN, 1990; JACQUEMIN and RENNER, 1998). During the nineties, it reached central Germany, Belgium, the Netherlands and Hungary (e.g. VAN AS and KLEUKERS, 1994; NIEHUIS and NIEHUIS, 1995; COUVREUR, 1996; SZIRAKI, 1996; NAGY, 2001) and in 2001 it was found in Great Britain (HAWKINS, 2001; SUTTON, 2007). An isolated population was found also in NE Black Sea coast in Bulgaria in 2002 (CHOBANOV, 2003). During the last five years, it occupied northern Germany (Bremen – GRÜNTZ and HOCHKIRCH, 2007; Osnabrück – HUSEMANN et al., 2008; Berlin – SCZEPANSKI, 2008). Furthermore, the species was also introduced to the USA probably with shrubs and trees imported from mainland Europe (in 1957, Long Island, New York), and has since become well established in eastern and western states (CANNING et al., 2007).

The records outside the original area of distribution are mostly from urban habitats, gardens, parks, etc. (LUQUET, 1993). In contrast to most NW European records, only the Hungarian records (NAGY, 2001) represent populations in natural or seminatural habitats situated far from the main traffic routes. The dispersal of this short-winged bush-cricket is suggested to be predominantly passive, mainly by cars, on which it was also observed many times (TRÖGER, 1986; LUQUET, 1993; DETZEL, 1998; SCZEPANSKI and JACOBI, 2005). Dispersal may also be supported by the positive phototaxis of the species (HELVENSEN, 1969): car headlamps switched on make the bush-cricket jump down from a tree or bush. Its spread through Europe is regarded rather rapid for a short-winged species (DETZEL, 1998).

After the first two incidental records of *M. meridionale* in the Czech Republic (BALVÍN et al., 2010) in 2008 and 2009 and analysis of distribution in Austria, a systematic pursuance was carried out in order to detect the actual distribution of the bush-cricket both in the Czech and Slovak Republics. Because of the expected passive dispersal of the cricket (TRÖGER, 1986; LUQUET, 1993; DETZEL, 1998; SCZEPANSKI and JACOBI, 2005) the search was focused on sites where cars and trucks from abroad stop for longer time.

The goals of the present paper are: i) to summarize the first records and present distribution of the species in the Czech and Slovak Republics (further CR and SR); ii) to describe its habitats and discuss the possible ways of its expansion; iii) to compare the morphological data of bush-crickets from newly recorded and original areas of distribution.

Material and methods

In the CR, we checked 44 localities (21 grid mapping squares) during September and October 2009 (Table

1a). Three regions were inspected: Prague and surroundings, southern Bohemia and southern Moravia (Fig. 1a). Among the localities there were 17 camping sites (or caravan parks), 14 resting sites (especially those for trucks – often connected with petrol stations) on major high-ways, eight hotel or other parking places, two streets and one border crossing, garden restaurant and square (green area between branches of the city centre highway).

Abbreviations used in Table 1: CS, (caravan) camping site; HRP, highway resting place; PP, parking place; RS, resting site; UG, urban (street) greenery; UP, urban park; GMSC, grid mapping square code (used for faunistic researches in the CR and SR); AK, Anton Krištín; OB, Ondřej Balvín; PM, Pavel Marhoul; RV, Robert Vlček; VH, Vladimír Hruží.

A similar pursuance was done at 48 localities in the SR (33 grid mapping squares) from August to November 2009–2011 (Table 1b), mainly in the southern half of the country (Fig. 1b). Altogether there were 22 highway resting sites, 15 urban parks and 11 camping sites.

Beside this targeted search for *M. meridionale* a systematic distribution survey of Orthoptera was carried out from April 1994 to November 2011 at 841 sites of 350 squares of the Slovak Fauna Databank (DFS, dimensions of square: N6' and E10', ca. 132 km²) in the SR. Surveyed DFS squares represent 81.4% of all in the territory of the SR (n = 430). Similarly extensive parallel research of orthopterans in natural habitats has been carried out in the CR as well. Up to present, at least 70% of squares of Central European mapping grid system according to EHRENDORFER and HAMANN (1965) have been surveyed in the area of the CR (HOLUŠA in litt.).

The bush-crickets were sampled by beating from woody plant species and sweeping bushes and lower branches of trees at least 30 minutes per site, completed by individual collection. At least in cases of a positive record of the bush-cricket, the altitude, habitat structure and the species of woody species, on which it was found, was recorded. List of all examined specimens together with list of negative inspections is given in Table 1. Most of specimens are kept in author's collections.

Length of body, pronotum, postfemur, posttibia, ovipositor and tegmina were measured (precision 0.1 mm) on collected specimens (13 females and 10 males) and compared with literature data from original area of distribution (HARZ, 1969). We tested the hypothesis that individuals from isolated new established populations have different size than those from original distribution area (CASSEL-LUNDHAGEN et al., 2011).

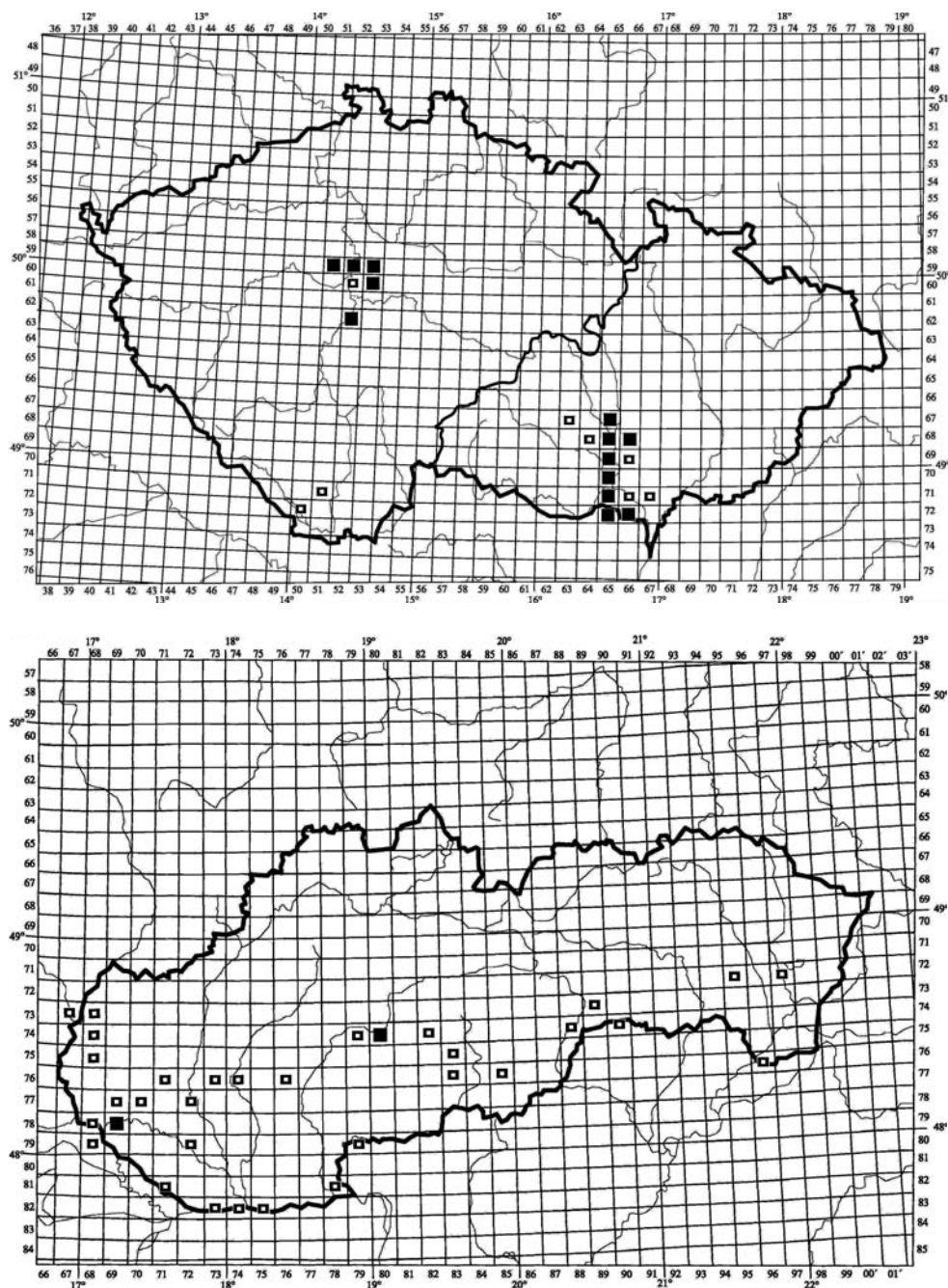


Fig. 1. The distribution of *Meconema meridionale* in the Czech (a) and Slovak Republics (b). Central European mapping grid system according to EHRENDORFER and HAMANN (1965) is used. Black – positive records, white (empty symbols) – negative inspections aimed at *M. meridionale*. For specification of the particular sites see Table 1 (a, b).

Results and discussion

Results

In the CR, *M. meridionale* was observed (one female) for the first time in the camping site in Prague in September 2008. A year later, it was caught (one male) in the camping site at Slapy, about 35 km (SSE) from the first locality. Both these first records of *M. meridi-*

onale in the CR were merely incidental (BALVÍN et al., 2010).

The consequential research in September and October 2009 disclosed other 11 localities with the occurrence of *M. meridionale* in the CR: 5 caravan camping sites, 3 hotel parking places, 2 highway resting places and one border crossing (Table 1, Fig. 1a).

Later, in November 2009, *M. meridionale* was also confirmed by CHLÁDEK (2009) in Brno (city) at other

Table 1a. Surveys of the inspections aimed at *Meconema meridionale* in the Czech Republic, positive records in bold

No.	Date	Locality (CR)	Habitat	GMSC	GPS	m a.s.l.	M/F	Woody species	Collector
1	12.ix.2009	Slapy	CS	6252	49°48'12.8"N, 14°25'16.8"E	280	1/0	<i>Betula</i> sp.	OB
2	18.ix.2009	Praha- Třebonice	CS	5951	50°2'39.0"N, 14°17'2.6"E	350	cca 15	<i>Fraxinus</i> sp., <i>Prunus</i> sp.	PM
3	20.ix.2009	Pasohlávky	CS	7065/7165	48°54'5.7"N, 16°34'31.9"E	170	5/4	<i>Fraxinus</i> sp., <i>Acer</i> sp.	RV
4	22.ix.2009	Brno-Štýřice	Cementery PP	6865	49°10'11.2"N, 16°35'55.8"E	215	neg.	mixed woods and shrubs	RV
5	25.ix.2009	Brno-Staré Brno	Exhibition ground PP	6865	49°11'20.7"N, 16°35'9.5"E	210	neg.	mixed woods and shrubs	RV
6	25.ix.2009	Brno-Staré Brno	Hotel PP	6865	49°11'3.8"N, 16°35'0.5"E	210	2/3	<i>Quercus</i> sp.	RV
7	26.ix.2009	Zlatníky- Hodkovice	CS	6052	49°57'5.1"N, 14°28'29.6"E	370	neg.	mixed woods and shrubs	OB
8	26.ix.2009	Praha-Šeberov	CS	5953/6053	50°0'45.2"N, 14°30'43.1"E	300	0/1	<i>Betula</i> sp.	OB
9	26.ix.2009	Praha-Dolní Počernice	CS	5953	50°5'18.8"N, 14°34'59.2"E	230	neg.	mixed woods and shrubs	OB
10	27.ix.2009	Praha-Bráník	CS	5952	50°1'57.6"N, 14°24'14.5"E	190	neg.	mixed woods and shrubs	OB
11	27.ix.2009	Praha-Slivenec	CS	5952	50°1'10.7"N, 14°21'22.4"E	350	1/0	<i>Malus</i> sp.	OB
12	1.x.2009	Černá v Pošumaví	CS	7250	48°44'15.9"N, 14°6'10.8"E	730	neg.	mixed woods and shrubs	PM
13	1.x.2009	Černá v Pošumaví	CS	7250	48°44'4.6"N, 14°5'53.4"E	730	neg.	mixed woods and shrubs	PM
14	1.x.2009	Černá v Pošumaví	CS	7250	48°43'44.4"N, 14°5'17.7"E	730	neg.	mixed woods and shrubs	PM
15	1.x.2009	Český Krumlov	Town centre PP	7151	48°48'49.6"N, 14°18'47.7"E	490	neg.	mixed woods and shrubs	PM
16	4.x.2009	Dolní Dunajovice	HRP	7165	48°51'43.6"N, 16°36'28.1"E	195	neg.	mixed woods and shrubs	RV
17	4.x.2009	Mikulov	HRP	7265	48°47'51.5"N, 16°37'58.5"E	205	neg.	mixed woods and shrubs	RV
18	4.x.2009	Mikulov	RS at A-CZ border	7265	48°47'24.8"N, 16°38'12.3"E	195	2/2	<i>Tilia</i> sp., <i>Populus</i> sp.	RV
19	4.x.2009	Mikulov	HRP	7165	48°48'29.8"N, 16°37'39.4"E	210	neg.	mixed woods and shrubs	RV
20	4.x.2009	Bavory	HRP	7165	48°49'51.9"N, 16°36'48.6"E	215	neg.	mixed woods and shrubs	RV
21	4.x.2009	Perná	HRP	7165	48°51'33.3"N, 16°36'33.9"E	210	neg.	mixed woods and shrubs	RV
22	5.x.2009	Brno-Bystrc	Boat landing- place PP	6765	49°13'48.8"N, 16°31'3.9"E	240	neg.	mixed woods and shrubs	RV
23	5.x.2009	Rajhrad	HRP	6965	49°5'27.0"N, 16°35'27.5"E	225	neg.	mixed woods and shrubs	RV
24	6.x.2009	Brno-Dolní Heršpice	Shopping park PP	6865	49°9'10.6"N, 16°37'43.6"E	200	neg.	mixed woods and shrubs	RV
25	6.x.2009	Ivaň - Nová Ves	HRP	7065	48°55'29.1"N, 16°32'49.3"E	180	neg.	mixed woods and shrubs	RV
26	7.x.2009	Praha- Motol	Hotel PP	5952	50°4'3.3"N, 14°20'40.8"E	280	2/1	<i>Carpinus</i> sp.	OB
27	7.x.2009	Praha-Motol	CS	5952	50°4'2.6"N, 14°20'46.7"E	280	neg.	mixed woods and shrubs	OB
28	7.x.2009	Praha-Císařská louka	CS	5952	50°3'45.4"N, 14°24'48.0"E	280	neg.	mixed woods and shrubs	OB, PM

Continued Table 1a.

No.	Date	Locality (CR)	Habitat	GMSC	GPS	m a.s.l.	M/F	Woody species	Collector
29	7.x.2009	Praha-Císařská louka	CS	5952	50°3'21.2"N, 14°24'49.0"E	200	0/2	<i>Ligustrum</i> sp.	OB, PM
30	7.x.2009	Přibyslavice	HRP	6763	49°16'14.5"N, 16°16'35.9"E	510	neg.	mixed woods and shrubs	RV
31	7.x.2009	Zálesná Zhoř	HRP	6763	49°16'10.0"N, 16°16'50.0"E	510	neg.	mixed woods and shrubs	RV
32	7.x.2009	Popůvky	HRP	6864	49°11'0.7"N, 16°28'39.9"E	325	neg.	mixed woods and shrubs	RV
33	7.x.2009	Popovice	Motel PP	6865	49°6'20.0"N, 16°36'4.3"E	230	6/2	<i>Acer</i> sp., <i>Prunus</i> sp., <i>Malus</i> sp.	RV
34	7.x.2009	Rajhrad	HRP	6965	49°5'27.0"N, 16°35'27.5"E	225	0/1	<i>Salix</i> sp., <i>Lonicera</i> sp. shrubs	RV
35	8.x.2009	Jiříkovice	HRP	6866	49°11'4.9"N, 16°45'36.6"E	250	1/2	<i>Malus</i> sp., <i>Elaeagnus</i> sp.	RV
36	8.x.2009	Brno-Brno-střed	Hotel PP, bus terminal	6865	49°11'36.5"N, 16°36'52.8"E	200	neg.	mixed woods and shrubs	RV
37	25.x.2009	Nové Mlýny	CS	7166	48°51'23.0"N, 16°43'52.5"E	165	neg.	mixed woods and shrubs	RV
38	25.x.2009	Lednice	CS	7266	48°47'7.3"N, 16°49'35.9"E	170	0/1	<i>Acer</i> sp., <i>Tilia</i> sp.	RV
39	25.x.2009	Ladná	HRP	7167	48°48'37.0"N, 16°53'35.8"E	175	neg.	mixed woods and shrubs	RV
40	25.x.2009	Nosislav	HRP	6966	49°0'37.7"N, 16°41'20.1"E	215	neg.	mixed woods and shrubs	RV
41	viii.2011	Praha-Podolí	UG	5952	50°2'50.3"N 14°25'40.8"E	265	0/2	<i>Aesculus</i> sp.	OB
42	8.ix.2011	Brno-Veverčí	UG	6765	49°12'10.7"N 16°35'34.8"E	250	0/1	<i>Prunus</i> sp.	RV
43	13.ix.2011	Brno-Staré Brno	UG	6865	49°11'14.0"N 16°35'34.3"E	210	0/1	<i>Ailanthus</i> sp., <i>Rosa</i> sp., shrubs	RV
44	x.2011	Praha-Nové město	UP	5952	50°4'18.4"N 14°25'48.4"E	260	0/2	<i>Acer</i> sp. <i>Ligustrum</i> sp., shrubs	OB

Table 1b. Surveys of the inspections aimed at *Meconema meridionale* in the Slovak Republic, positive records in bold

No.	Date	Locality (CR)	Habitat	GMSC	GPS	m a.s.l.	M/F	Woody species	Collector
1	4.ix.2010	Bratislava-Lamač	HRP	7868	48°09'22.3"N, 17°03'15.6"E	168	neg.	<i>Acer</i> sp., shrubs	AK
2	19.ix.2011	Petržalka	RS at AU-SK border	7868	48°07'45.0"N, 17°01'30.9"E	135	neg.	<i>Populus</i> sp.	AK
3	4.ix.2010	Malacky	HRP	7568	48°26'22.1"N, 17°01'5.9"E	159	neg.	<i>Pinus</i> sp.	AK
4	4.ix.2010	Brodské	RS near CZ-SK border	7367	48°41'7.3"N, 16°58'3.9"E	154	neg.	<i>Populus</i> sp.	AK
5	4.ix.2010	Moravský Svätý Ján	RS at A-SK border	7367	48°36'2.3"N, 16°56'25.9"E	153	neg.	<i>Populus</i> sp.	AK
6	4.ix.2010	Závod	HRP	7468	48°32'32.6"N, 17°01'45.9"E	160	neg.	<i>Populus</i> sp.	AK
7	4.ix.2010	Sekule	HRP	7368	48°37'2.4"N, 17°00'49.9"E	156	neg.	<i>Pinus</i> sp.	AK

Continued Table 1b.

No.	Date	Locality (CR)	Habitat	GMSC	GPS	m a.s.l.	M/F	Woody species	Collector
8	19.ix.2010	Triblavina	HRP	7769	48°11'08.7"N, 17°17'9.3"E	139	neg.	shrubs	AK
9	19.ix.2010	Čataj	HRP	7770	48°13'42.4"N, 17°28'22.2"E	140	neg.	shrubs, <i>Populus</i> sp.	AK
10	19.ix.2010	Zeleneč	HRP	7671	48°17'00.0"N, 17°36'45.2"E	146	neg.	shrubs, <i>Populus</i> sp.	AK
11	19.ix.2010	Sereď -Váhovce	HRP	7772	48°13'22.8"N, 17°46'02.9"E	124	neg.	<i>Populus</i> sp.	AK
12	19.ix.2010	Nitra Zobor	HRP	7674	48°20'49.5"N, 18°04'40.4"E	196	neg.	<i>Acer negundo</i> , shrubs	AK
13	19.ix.2010	Olichov	HRP	7676	48°22'15.1"N, 18°28'13.6"E	288	neg.	<i>Quercus</i> sp.	AK
14	19.ix.2010	Žiar nad Hronom	RS	7479	48°34'15.6"N, 18°53'00.0"E	226	neg.	shrubs	AK
15	29.ix.2010	Detva	RS	7482	48°33'20.5"N, 19°24'05.3"E	382	neg.	shrubs, <i>Salix</i> sp.	AK
16	29.ix.2010	Lučenec-Halier	RS	7683	48°25'02.3"N, 19°36'51.9"E	251	neg.	<i>Quercus</i> sp.	AK
17	29.ix.2010	Plešivec	RS	7488	48°33'22.4"N, 20°24'18.6"E	218	neg.	<i>Quercus</i> sp.	AK
18	29.ix.2010	Jablonov nad Turňou	RS	7490	48°36'00.0"N, 20°40'20.9"E	258	neg.	<i>Prunus</i> sp., <i>Vitis</i> sp.	AK
19	29.ix.2010	Dargovský priesmyk	RS	7295	48°44'36.8"N, 21°32'54.8"E	473	neg.	<i>Carpinus</i> , <i>Quercus</i> , <i>Fagus</i> <i>sylvatica</i>	AK
20	15.ix.2011	Slovenské Nové mesto	RS	7696	48°24'45.2"N, 21°40'30.4"E	104	neg.	<i>Quercus</i> sp., shrubs	AK
21	10.x.2011	Šahy	RS at HU-SK border	7979	48°03'52.9"N, 18°59'00.0"E	136	neg.	shrubs, <i>Salix</i> sp.	AK
22	28.ix.2011	Medved'ov	RS at HU-SK border	8171	47°48'00.0"N, 17°39'21.5"E	114	neg.	shrubs, <i>Salix</i> sp.	AK
23	19.ix.2011	Bratislava-Zlaté piesky	UP	7869	48°10'38.2"N, 17°10'52.4"E	130	neg.	<i>Acer negundo</i> , <i>Ulmus</i> sp., shrubs	AK
24	ix.-x. 2009-2011	Nitra	UP	7674	48°18'32.1"N, 18°04'40.4"E	190	neg.	<i>Tilia</i> sp., <i>Populus</i> sp.	AK
25	19.ix.2011	Trnava	UP	7671	48°24'45.6"N, 17°36'01.5"E	146	neg.	<i>Tilia</i> sp., <i>Populus</i> sp.	AK
26	4.ix.2010	Malacky	UP	7568	48°26'45.1"N, 17°00'25.1"E	159	neg.	<i>Tilia</i> sp., <i>Populus</i> sp.	AK
27	22.ix.2011	Bratislava- Horský park	UP	7868	48°10'51.9"N, 17°06'13.2"E	154	neg.	<i>Carpinus</i> <i>betulus</i> , <i>Quercus</i> sp.	AK
28	4.ix.2010	Veľké Leváre	UP, cementery	7468	48°30'32.4"N, 17°00'9.9"E	170	neg.	<i>Tilia</i> sp., <i>Populus</i> sp.	AK
29	22.ix.2011	Rusovce	UP	7968	48°04'12.4"N, 17°09'00.0"E	133	neg.	<i>Platanus</i> sp., <i>Populus</i> sp.	AK
30	19.ix.2010	Sereď	UP	7772	48°17'32.1"N, 17°44'52.9"E	130	neg.	<i>Platanus</i> sp., <i>Populus</i> sp.	AK
31	19.ix.2010	Báb	UP	7673	48°18'52.3"N, 17°53'32.1"E	162	neg.	<i>Carpinus</i> <i>betulus</i> , <i>Quercus</i> sp.	AK

Continued Table 1b.

No.	Date	Locality (CR)	Habitat	GMSC	GPS	m a.s.l.	M/F	Woody species	Collector
32	6.ix.-8. xi.2011	Zvolen	UP	7480	48°34'22.3"N, 19°08'26.5"E	305	0/6	<i>Ulmus</i> sp., <i>Aesculus</i> sp., shrubs	VH, AK
33	10.x.2011	Šahy	UP	7979	48°04'09.3"N, 18°58'13.6"E	138	neg.	<i>Platanus</i> sp., <i>Populus</i> sp.	AK
34	28.ix.2011	Komárno	UP	8274	47°45'07.2"N, 18°08'00.0"E	116	neg.	<i>Platanus</i> sp., <i>Populus</i> sp.	AK
35	28.ix.2011	Štúrovo	UP	8178	47°48'00.0"N, 18°43'32.8"E	111	neg.	<i>Platanus</i> sp., <i>Populus</i> sp.	AK
36	15.ix.2011	Michalovce	UP	7297	48°45'49.2"N, 21°55'09.3"E	115	neg.	<i>Platanus</i> sp., <i>Populus</i> sp.	AK
37	28.ix.2011	Gabčíkovo	UP	8171	47°53'25.1"N, 17°36'22.9"E	116	neg.	<i>Platanus</i> sp., <i>Populus</i> sp.	AK
38	28.viii.2011	Bratislava-Zlaté piesky	CS	7869	48°11'8.9"N, 17°11'11.3"E	130	1/1	<i>Acer negundo</i>	OB
39	28.ix.2011	Jahodná	CS	7972	48°03'45.6"N, 17°43'00.0"E	110	neg.	<i>Populus</i> sp.	AK
40	ix-x.2009- 2011	Zvolen- Neresnica	CS	7480	48°33'42.3"N, 19°08'26.5"E	300	neg.	mixed woods and shrubs	AK
41	15.ix.2011	Ružiná	CS	7583	48°22'52.3"N, 19°34'05.1"E	270	neg.	<i>Populus</i> sp.	AK
42	15.ix.2011	Rimavská Sobota-Cieľ	CS	7685	48°23'32.9"N, 19°58'31.2"E	272	neg.	<i>Quercus</i> sp.	AK
43	15.ix.2011	Gombasek	CS	7488	48°33'15.6"N, 20°28'15.2"E	243	neg.	<i>Quercus</i> sp., shrubs	AK
44	15.ix.2011	Krásna Hôrka	CS	7389	48°09'15.6"N, 20°36'05.2"E	369	neg.	<i>Quercus</i> sp., shrubs	AK
45	28.ix.2011	Patince	CS	8275	47°44'02.9"N, 18°08'16.2"E	110	neg.	<i>Populus</i> sp.	AK
46	28.ix.2011	Štúrovo	CS	8178	47°48'00.0"N, 18°44'39.2"E	111	neg.	<i>Populus</i> sp.	AK
47	19.ix.2011	Senec	CS	7770	48°13'02.5"N, 17°25'52.1"E	137	neg.	<i>Populus</i> sp.	AK
48	28.ix.2011	Zlatná na Ostrove	CS	8273	47°46'00.0"N, 17°59'00.0"E	120	neg.	<i>Populus</i> sp.	AK

three localities (grid mapping squares 6865, 6765). Those findings probably represent already well-established populations in urban environment. Most of the individuals were found already dead under trees or bushes.

During October 2011, other four localities were disclosed incidentally in the CR, two in Prague (garden restaurant and urban park), two in Brno (city streets with urban greenery, Table 1). Those from Prague indicate that *M. meridionale* has already well-established populations in urban environment also in Prague, not only in Brno.

Though an extensive pursuance focused on *M. meridionale* had been carried out in the SR since autumn 2009, the first record of the species was an incidental finding in camping site in Bratislava only 10 km E from

Austrian border in August 2011. Later the bush-cricket was found in urban park 190 km NE from there, in central Slovakia in Zvolen, what is recently the most north-eastern locality in Europe. Beside the localities mentioned, the bush-cricket has not been found elsewhere, despite an intensive faunistic research of orthopteran communities in different habitats in the CR and SR.

We are not able to conclude if *M. meridionale* was introduced into the SR later than the CR; at least the population in Central Slovakia (Zvolen) seemed to be well established. But the comparison of proportions of positive findings to the numbers of inspections focused on the bush-cricket in the CR and SR strongly suggest that *M. meridionale* is still much less abundant and the introductions by car or railway traffic are less frequent in

the SR. It is probably because at least the tourist traffic in the SR is much lower than in the CR (6.5 million foreign tourists in the CR and 1.3 million in the SR in 2010 according to the official agencies Czech Tourism (www.czechtourism.cz) and Slovak Tourist Board (www.sacr.sk)). The camping site Zlaté Piesky in Bratislava (with records of *M. meridionale*) is the largest one in the capital of the SR with frequent foreign customers.

The absence of the bush-cricket in close surroundings of some localities with a positive record of the bush-cricket also supports the idea of its recent spreading over the countries. For example, two camping sites at Praha-Císařská louka were very close each to other (750 m), but the first one was negative and in the second one two females were found. Similarly, the caravan park in Praha-Motol lies just next to a hotel, the bush-cricket was abundant (three individuals caught by few sweeps) on the parking of the hotel while a proper inspection of the caravan park brought no results. Also, only one of two neighboring highway resting places was occupied: highway (to Prague) resting place was positive (one female only) meanwhile highway (to Vienna) resting place lying on the opposite side of the road was negative.

In addition, the individuals were often found in trees or bushes which were closest to the places reserved for trucks, while just several meters away the bush-crickets were absent. Still, large differences in the abundance of the bush-cricket on different sites may suggest that some localities with higher abundance recorded have been occupied for several seasons already. Therefore, *M. meridionale* might have occurred in the CR and SR already few years ago, being overlooked.

All of the 17 detected localities in the CR are situated in lower altitudes, between 200 and 350 m a.s.l. in Central Bohemia and between 170 and 250 m a.s.l. in Southern Moravia. Similarly, two localities in the SR are at 130 m a.s.l. (Bratislava) and 305 m a.s.l. (Zvolen). Average value of the altitudes of all 19 Czech and Slovak localities is about 243.5 m a.s.l. (SD = 59.6).

Discussion

Frequent occurrence in urban parks or other urban greenery and camping sites in the CR and the SR supported hypothesis of urban environments as warm islands for Mediterranean faunal elements (DETZEL, 1998; ZUNA-KRATKY et al., 2009). Only in areas of origin and at few localities in Hungary and in Western Europe it is living in deciduous forests and their edges (DETZEL, 1998; NAGY, 2001; ZUNA-KRATKY et al., 2009).

Inside entire distribution area it is arboricolous and arbusticolous species, occurring on trees and higher shrubs of different species, but feeding on small insects (DETZEL, 1998). In the CR and SR it was found on many diverse woody species (at least 14 species, mainly *Acer*

spp., *Prunus* spp., *Malus* spp. etc., see Table 1). Also in Lower Austria, it has been found on different species of deciduous woody species, not only on Oaks in any case (ZUNA-KRATKY et al., 2009). These facts suggest that there is no preference and the abundance or density of woody plants at localities determines the occurrence of the bush-crickets more than the species of the woody plants.

The records of *M. meridionale* in the CR and SR come mostly from urban environments or they are connected with the car traffic and clearly support the idea of its passive dispersal by cars, especially by caravans and trucks (cf. DETZEL, 1998).

The delay of the first records of *M. meridionale* in the CR and SR and also in Hungary, in contrast with Austria and Germany, was possibly caused by minimum international car transport and border traffic before 1989 (before political changes in former Czechoslovakia). However, the establishment of permanent populations in the area of the CR and SR was very probably even of a later date and was prerequisites by a positive population and range trend in Austria, Germany, Hungary, but also in the Netherlands – a country, from which a considerable number of caravans arrive into the CR every year (personal observations). Hence, we can suppose two ways of introduction into the CR and SR. First, from the western and northwestern sites of origin (Germany, Netherlands) for some local populations, e.g. inhabiting Prague surroundings in the CR and, second, from the southern and southwestern ones, mostly situated in Austria (see in ZUNA-KRATKY et al., 2009) and Hungary (SZIRAKI, 1996; BÁLINT, 2001; NAGY, 2001) for local populations inhabiting Moravia and Slovakia.

In original area of distribution in the Mediterranean (and Submediterranean) it is occurring rarely up to 900 m a.s.l. (Italy, DETZEL, 1998) or 950 m a.s.l. (Switzerland, THORENS and NADIG, 1997). Hence, we confirmed the preference of lowland altitudinal distribution up to 350 m a.s.l. in the new colonized countries. E.g. 86% of all finding sites in Austria are situated under 300 m a.s.l. (ZUNA-KRATKY et al., 2009). However, in Germany, most of finding sites are situated under 300 m a.s.l. too, but some other ones higher, between 400 and 530 m a.s.l. (Baden-Württemberg, DETZEL, 1998) or between 490 and 500 m a.s.l. (Bayern, SCHLUMPRECHT and WAEBER, 2003).

We confirmed that the species belong to so called late bush-cricket species occurring also after the first frosts in November (mainly females even after -5°C). It was already suggested by CHLÁDEK (2009, see above), despite the fact, that most of the individuals collected by himself were already dead. In neighboring Lower Austria, last individual was observed alive even on 5th December 2006 (ZUNA-KRATKY et al., 2009).

Morphological parameters and measurements in this species are less known. Females are supposed to be slightly bigger (body length 11.5–17 mm) than males

Table 2. Morphological parameters in males (M) and females (F) of *Meconema meridionale* in the Czech and Slovak Republics (in mm, mean \pm SD are given)

Sex	Body	Pronotum	Postfemur	Posttibia	Ovipositor	Tegmina
F (13)						
Mean	11.2	3.1	9.3	10.1	7.0	1.9
SD	1.1	0.2	0.6	0.8	0.6	0.2
M (10)						
Mean	11.2	3.0	9.5	10.2	–	1.9
SD	0.7	0.2	0.4	0.4	–	0.3

(11–13 mm) (HARZ, 1969; DETZEL, 1998), but in our study we found no significant differences between sexes (Mann Whitney U test, $p > 0.16$, Table 2). Body size, postfemora and pronotum lengths of isolated Czech and Slovak populations were not different from populations in original area of distribution (HARZ, 1969; DETZEL, 1998; Table 2).

Conclusions

The recorded distribution of *M. meridionale* in the CR and SR is concentrated in urban environments, connected with traffic or camping. This supports the idea of passive dispersal by cars. The absence of the species in close surroundings of the localities supports the idea of its recent spread over the countries. All the 19 localities are situated in lower altitudes, between 130 and 350 m a.s.l. and the species was found on at least 14 woody species without detected preference.

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Rozšíření kobylky juhoeurópskej *Meconema meridionale* (Orthoptera, Tettigoniidae) v České republice a na Slovensku

Súhrn

Viaceré pôvodne mediteránne druhy živočíchov sa šíria do strednej Európy. Kobylka *Meconema meridionale* je jedným z nich a známe je jej šírenie hlavne v západnej Európe. Po prvom zázname v lete 2008 v Českej republike sme tam kontrolovali 44 potenciálnych lokalít a 48 na Slovensku. Materiál sme zbierali smýkaním a sklepaním konárov krov a malých stromov. Druh sme zistili na 17 lokalitách v Českej republike a 2 na Slovensku. Zaznamenaný výskyt je koncentrovaný v mestskom prostredí, spojený s dopravou a kempingom. Tento poznatok podporuje hypotézu o pasívnom šírení automobilmi. Absencia druhu v blízkom okolí lokalít výskytu podporuje myšlienku súčasného šírenia v týchto krajinách. Všetkých 19 lokalít sa nachádza v nízkych nadmorských výškach medzi 130 a 350 m n. m. a druh bol nájdený na min. 14 druhoch drevín. Morfológické parametre nájdených jedincov z Českej republiky a Slovenska neboli odlišné od jedincov z územia pôvodného rozšírenia.

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