

**ACTA  
BOTANICA  
UNIVERSITATIS  
COMENIANAE**

Volume 47

2012  
COMENIUS UNIVERSITY IN BRATISLAVA

**The journal was edited with the title / Časopis bol vydávaný pod názvom**  
Acta Facultatis Rerum Naturalium Universitatis Comenianae, Botanica

**Editor in Chief / Predseda redakčnej rady**

Karol Mičieta; micieta@fns.uniba.sk

**Executive Editor / Výkonný redaktor**

Soňa Jančovičová, jancovicova@fns.uniba.sk

**Editorial Board / Členovia redakčnej rady**

Dana Bernátová, Danica Černušáková, Zuzana Dúbravcová, Katarína Mišíková

**Editor Ship / Adresa redakcie**

Redakcia Acta Botanica, Révová 39, SK-811 02 Bratislava 1; Tel. ++421 2 54411541; Fax ++421 2 54415603

**Published by / Vydavateľ**

© Comenius University in Bratislava, 2012

© Univerzita Komenského v Bratislave, 2012

**ISBN 978-80-223-3332-0**

**ISSN 0524-2371**

## MACROMYCETES OF THE FIALKOVÁ DOLINA NATURE RESERVE (DEVÍNSKA KOBÝLA MTS., SLOVAKIA)

Ondrej Ďuriška\*, Soňa Jančovičová, Ján Miškovic

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany,  
Révová 39, 811 02 Bratislava, Slovakia

Received 18 April 2012; Received in revised form 21 April 2012; Accepted 11 May 2012

### Abstract

Based on published data and data gathered during our 4-year field research (2007–2009, 2011), 149 taxa of macromycetes (18 of Ascomycota and 131 of Basidiomycota) are recently known from the Fialková dolina Nature Reserve. In this paper, the study area is characterized and the list of all recorded macromycetes presented.

**Key words:** Ascomycota, Basidiomycota, diversity, Bratislava

### Introduction

The Fialková dolina was established as Nature Reserve (NR) in 1993 to protect preserved oak-hornbeam forests and rare plant species, especially orchids (Anonymus 2005). Within the Devínska Kobyla Mountains, it belongs to four other protected areas such as the Devínska Kobyla National Nature Reserve (NNR), Štokeravská vápenka NR, Devínska lesostep Nature Monument (NM) and Devínska hradná skala NM. Mycologically, the Devínska Kobyla Mts. is an attractive area and with 428 published taxa of macromycetes it is ranked the sixth phytogeographical unit of Slovakia with the highest fungal diversity (Ripková, Ďuriška 2009). However, there are still poorly researched or even not researched localities in these Mountains and the Fialková dolina NR is one of them; before our research, only four macromycetes were reported from here: *Auricularia auricula-judae*, *Calocybe gambosa*, *Exidia glandulosa* and *Helvella acetabulum* (Záhorovská 1997). In our efforts to know more on the macrofunga of the Reserve, we carried out a 4-year field research (2007–2009 and 2011), the results of which are presented in this paper.

### Material and Methods

**The locality characteristics.** The Fialková dolina NR is situated in the city of Bratislava, the municipal part of Devín. It is bordered by Devínska cesta road and limestone quarry in the south, by cottage settlement in the west, forests in the east and by agricultural soil in the north (Fig. 1). Its area is 20 5879 m<sup>2</sup> and the elevation range 175–250 m a. s. l. Coordinates read ± in the middle of the Reserve from the GPS (WGS 84) are 48° 09' 50.53" N, 17° 00' 16.22" E. As a valley, the Fialková dolina can be divided into three physiogeographic regions: bottom, lower right streambank and higher left streambank. The bottom of the valley (at the NW edge of the Reserve) is broad, formed from carbonate-silicate sediments. It narrows markedly to tens of meters upstream and consists only of stream bed in the narrowest part where highly fissured granite rocks dominate, being schistous or

---

\* Corresponding Author: Ondrej Ďuriška; tajfun21@yahoo.com

transformed into mylonites. Coming from (in the S part of the Reserve), the valley broadens again, creating alluvial plain of carbonated fluvisols. The streambanks are mainly formed from cambisols and pararendzinas (Bizubová, Minár 2005; Kaliská, Nemcová 1988). Climatologically, it pertains to warm and moderately dry climatic region with mild winters; the average number of summer days per year is  $\geq 50$  and daily maximum air temperature  $\geq 25$  °C (Lapin et al. 2002).

**Sublocalities.** For easier localization of collections, along with considering the phytocoenological conditions, we have divided the Reserve into four sublocalities A, B, C, D. The borders of the sublocalities are presented by stream passing through the Reserve from north to south, by imaginary line from west to east (the cross point is an old house in the middle of the Reserve) and real borders of the Reserve (Fig. 1).

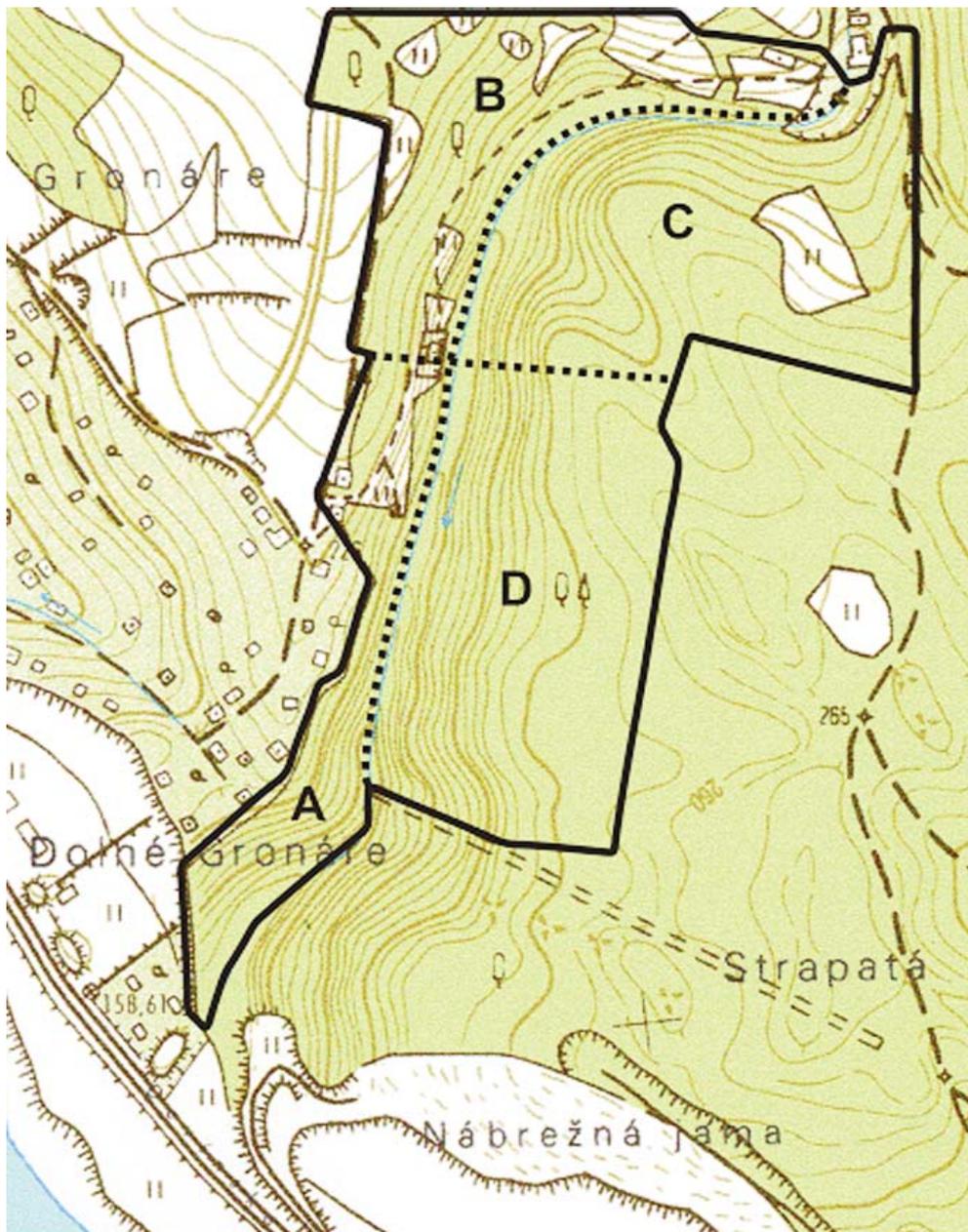


Fig. 1. The Fialková dolina Nature Reserve (Devínska Kobyla Mts., Slovakia)

— borders of the Fialková dolina Nature Reserve

--- borders of the sublocalities A, B, C and D

**Vegetation.** Four phytocoenological relevés were made in the Fialková dolina NR (one relevé per sublocality), following Braun-Blanquet (1964) and Westhoff, Van der Maarel (1978). The nomenclature of vascular plants is based on Marhold (1998); the nomenclature and syntaxonomy of the forest communities on Jarolímek et al. (2008).

**Comparing the diversity of sublocalities.** We used a standard mathematical equation to calculate the similarity of compared sublocalities. In the text below, to express the similarity (in percentage) we used symbol S.

**Period of research.** The years 2007–2009, 2011.

**Collections.** The study is based on 359 own collections. The specimens (241) are kept in the Herbarium of the Department of Botany, Comenius University in Bratislava, Slovakia (SLO); not deposited collections (19) are signified by abbreviation Not. (see *Annotated list of recorded macromycetes* bellow).

**Literature for determination of fungi and nomenclature.** The work *Funga Nordica* (Knudsen, Vesterholt eds. 2008) was mostly used for determination of agaricoid, boletoid and cyphelloid basidiomycetes; the *Nordic Macromycetes* vol. 3 (Hansen, Knudsen 1997) of heterobasidioid, aphylophoroid and gasteromycetoid basidiomycetes; and the *Nordic Macromycetes* vol. 1 (Hansen, Knudsen eds. 2000) of ascomycetes. The nomenclature is unified according to CABI Bioscience Databases: Index Fungorum (Cooper, Kirk 2012); with exception of *Xerocomus* names – they are according Knudsen, Taylor (2008).

## Results

During our 4-year field research (2007–2009, 2011) in the Fialková dolina NR, we have identified 149 taxa (species, infraspecific taxa) of macromycetes (18 of Ascomycota and 131 of Basidiomycota). This total number of taxa is based on 359 collections, out of which 33 remained unidentified (because of difficulties in taxonomy or lack of recent taxonomic works; they are mostly of genera *Clitocybe*, *Cortinarius*, *Entoloma*, *Inocybe*, *Mycena* and *Russula*, and of genera of aphylophoroid fungi). Before our research, (only) four macromycetes (*Auricularia auricula-judae*, *Calocybe gambosa*, *Exidia glandulosa* and *Helvella acetabulum*) were published from the Reserve (Záhorovská 1997) and we have re-collected two: *Auricularia auricula-judae* and *Exidia glandulosa*. All other our records are, therefore, new for the Fialková dolina NR and some of them (59) even for the Devínska Kobyla Mts. (Tab. 1 and *Annotated list of recorded macromycetes*).

**Tab. 1. The summary numbers**

Published taxa (species, infraspecific taxa) before our research		
from the Fialková dolina Nature Reserve	4	Záhorovská (1997)
from the Devínska Kobyla Mts.	428	Ripková, Ďuriška (2009)
Our research (2007-2009, 2011) in the Fialková dolina Nature Reserve		
all collections	359	
identified taxa	149	
not identified collections	33	
new taxa for the Fialková dolina Nature Reserve	147	
new taxa for the Devínska Kobyla Mts.	59	

Particular results of our research were published by Ďuriška (2010; the first author of the paper) focusing on new macromycetes for the Devínska Kobyla Mts. In this paper, we present the complete list of all recorded taxa supplemented with basic ecological data.

Some species of special interest have been recorded in the Fialková dolina NR, too: four red-listed species (Lizoň 2001): *Boletus impolitus*, *Crepidotus crocophyllus*, *Ossicaulis lignatilis*, *Piptoporus*

*quercinus* and one species protected by Regulation of the Ministry of Environment of the Slovak Republic No 93/1999 Coll.: *Boletus impolitus*.

**Vegetation.** A part of our research, it was also to characterize vegetation of the studied area. For easier localization of fungal collections, we have divided the Reserve into four sublocalities A, B, C and D (see Material and Methods and Fig. 1). In addition to collecting fungi, we have listed vascular plants and made phytocoenological relevés in these sublocalities (Relevé 1–4; see below).

Phytogeographically, the Fialková dolina NR belongs to the province of Eupannonian xerotherm flora (Eupannonicum), the Pannonian floral region (Pannonicum), and Devínska Kobyla district (Futák 1984).

The most of area of the Fialková dolina NR is formed by forest communities; out of grasslands, there occur mainly fragments of vegetation *Cirsio-Brachypodium pinnati* Hadač et Klika ex Klika 1951. Although *Carpinus betulus* and *Quercus petraea* agg. are most abundant trees in the Devínska Kobyla Mts., on the slopes above right streambank of the Reserve (sublocalities A and B), there prevail *Acer campestre*, *A. platanoides*, *A. pseudoplatanus*, *Tilia cordata* and *T. platyphyllos* in the treelayer – typical species of the ravine forests of the alliance *Tilio-Acerion* Klika 1955. In the sublocality A, there occur also thermophilic species like *Quercus pubescens* and *Sorbus torminalis*. The shrublayer of both sublocalities is specific for dominant *Staphylea pinnata*; the herblayer is alternatively dominated by two nitrophilous species *Anemone podagraria* and *Mercurialis perennis*; the spring aspect is characteristic by *Corydalis cava*, less by *Ficaria bulbifera*. Due to the presence and abundance of characteristic species of the alliance *Tilio-Acerion* (*Acer platanoides*, *A. pseudoplatanus*, *Mercurialis perennis*, *Tilia platyphyllos* and *Ulmus glabra*) as well the absence of species that dominate the herblayer of oak-hornbeam forests in the Devínska Kobyla Mts. (*Carex pilosa*, *Melica uniflora*), we have classified the forest stands in the sublocalities A and B into the association of the ravine forests *Aceri-Carpinetum* Klika 1941.

In the sublocality C, there occur oak-hornbeam forests of the association *Carici pilosae-Carpinetum* Neuhäusl Novotná et Neuhäuslová 1964. The tree layer is dominated by *Carpinus betulus* and *Quercus petraea* agg. with admixed *Acer platanoides* and *Tilia cordata*. The shrublayer is poorly developed. In the herblayer, there are alternatively dominant *Melica uniflora* and *Carex pilosa*; from the other species, *Galium odoratum*, *Hepatica nobilis* and *Hieracium murorum* prevail. In early spring, the herblayer is composed of *Corydalis pumila* and *Ficaria bulbifera*; the summer aspect is represented by invasive *Impatiens parviflora*.

The sublocality D is dominated by acidophilous oak forests of the association *Luzulo albidiae-Quercetum petraeae* Hilitzer 1932. The treelayer is mainly formed by *Quercus petraea* agg., less by *Pinus sylvestris*, *Sorbus torminalis* and *Tilia cordata*. The shrublayer is poorly developed, with very low total cover (1 %). The herblayer is primarily occupied by *Luzula luzuloides*, *Calamagrostis arundinacea*, *Festuca ovina* agg. and *Hieracium murorum*. The layer of bryophytes is well-developed, too.

**Relevé 1.** Malé Karpaty Mts., Devínska Kobyla Mts., Fialková dolina Nature Reserve, sublocality A, alt. 190 m, N 48° 9' 50.4", E 17° 0' 8.5", SE, 40°, 400 m<sup>2</sup>, E<sub>3</sub> 70 %, E<sub>2</sub> 55 %, E<sub>1</sub> 70 %, E<sub>0</sub> 0 %, 3. IX. 2009, J. Miškovic, O. Ďuriška, S. Jančovičová.  
E<sub>3</sub>: *Tilia cordata* 3, *Carpinus betulus* 2a, *Quercus pubescens* 2a, *Acer platanoides* 1, *Acer campestre* 1  
E<sub>2</sub>: *Staphylea pinnata* 3, *Sorbus torminalis* 1, *Acer campestre* +, *Cornus mas* +, *Crataegus monogyna* +, *Euonymus verrucosus* +, *Fraxinus excelsior* +, *Tilia cordata* +, *Ulmus glabra* +  
E<sub>1</sub>: *Hedera helix* 3, *Mercurialis perennis* 2b, *Acer platanoides* 1, *Hepatica nobilis* 1, *Viola odorata* 1, *Acer campestre* +, *Convallaria majalis* +, *Euonymus europaeus* +, *Galium odoratum* +, *Geum urbanum* +, *Chelidonium majus* +, *Impatiens parviflora* +, *Lamium maculatum* +, *Poa nemoralis* +, *Polygonatum odoratum* +, *Pulmonaria officinalis* +, *Staphylea pinnata* +, *Viburnum lantana* +

**Relevé 2.** Malé Karpaty Mts., Devínska Kobyla Mts., Fialková dolina Nature Reserve, sublocality B, alt. 195 m, N 48° 10' 10.8", E 17° 0' 19.6", SSE, 15°, 400 m<sup>2</sup>, E<sub>3</sub> 90 %, E<sub>2</sub> 15 %, E<sub>1</sub> 70 %, E<sub>0</sub> 0 %, 3. IX. 2009, J. Miškovic, O. Ďuriška, S. Jančovičová.  
E<sub>3</sub>: *Acer platanoides* 3, *Acer pseudoplatanus* 3, *Robinia pseudoacacia* 1, *Tilia platyphyllos* 1, *Corylus avellana* +, *Sambucus nigra* +  
E<sub>2</sub>: *Acer platanoides* 1, *Sambucus nigra* 1, *Acer campestre* +, *Acer pseudoplatanus* +, *Clematis vitalba* +, *Corylus avellana* +, *Euonymus europaeus* +, *Tilia platyphyllos* +

E<sub>1</sub>: *Aegopodium podagraria* 4, *Lamium maculatum* 2b, *Acer platanoides* +, *Acer pseudoplatanus* +, *Euonymus europaeus* +, *Geranium robertianum* +, *Geum urbanum* +, *Hepatica nobilis* +, *Hedera helix* +, *Heracleum sphondylium* +, *Impatiens parviflora* +, *Juglans regia* +, *Mercurialis perennis* +, *Polygonatum odoratum* +, *Pulmonaria officinalis* +, *Robinia pseudoacacia* +, *Salvia glutinosa* +, *Urtica dioica* +, *Fagus sylvatica* r, *Lilium martagon* r

**Relevé 3.** Malé Karpaty Mts., Devínska Kobyla Mts., Fialková dolina Nature Reserve, sublocality C, alt. 232 m, N 48° 10' 8.1", E 17° 0' 34.4", W; 15°, 400 m<sup>2</sup>, E<sub>3</sub> 80 %, E<sub>2</sub> 1 %, E<sub>1</sub> 80 %, E<sub>0</sub> 5 %, 24. IX. 2009, J. Miškovic, O. Ďuriška, S. Jančovičová.

E<sub>3</sub>: *Carpinus betulus* 4, *Quercus petraea* agg. 3, *Acer campestre* 1, *Tilia cordata* 1

E<sub>2</sub>: *Carpinus betulus* +, *Tilia cordata* +

E<sub>1</sub>: *Melica uniflora* 4, *Dactylis polygama* 1, *Galium odoratum* 1, *Hepatica nobilis* 1, *Hieracium murorum* 1, *Impatiens parviflora* 1, *Acer platanoides* +, *Ajuga reptans* +, *Carpinus betulus* +, *Euonymus verrucosus* +, *Fagus sylvatica* +, *Geranium robertianum* +, *Hedera helix* +, *Lamium maculatum* +, *Lathyrus vernus* +, *Ligustrum vulgare* +, *Mycelis muralis* +, *Poa nemoralis* +, *Polygonatum multiflorum* +, *Pulmonaria officinalis* +, *Quercus petraea* agg. +, *Sambucus nigra* +, *Tilia cordata* +, *Viola reichenbachiana* +, *Viscaria vulgaris* +

E<sub>0</sub>: *Atrichum undulatum* +, *Brachythecium velutinum* +, *Dicranella schreberiana* +, *Hypnum cupressiforme* +, *Plagiomnium cuspidatum* +

**Relevé 4.** Malé Karpaty Mts., Devínska Kobyla Mts., Fialková dolina Nature Reserve, sublocality D, alt. 221 m, N 48° 10' 8", E 17° 0' 22.7", SE, 40°, 400 m<sup>2</sup>, E<sub>3</sub> 60 %, E<sub>2</sub> 1 %, E<sub>1</sub> 40 %, E<sub>0</sub> 20 %, 24. IX. 2009, J. Miškovic, O. Ďuriška, S. Jančovičová.

E<sub>3</sub>: *Quercus petraea* agg. 4, *Pinus sylvestris* 1, *Sorbus torminalis* 1, *Tilia cordata* 1

E<sub>2</sub>: *Sorbus torminalis* +, *Tilia cordata* +

E<sub>1</sub>: *Festuca ovina* agg. 3, *Luzula luzuloides* 2b, *Calamagrostis arundinacea* 2a, *Quercus petraea* agg. 2a, *Avenella flexuosa* 1, *Hieracium murorum* 1, *Anthericum ramosum* +, *Convallaria majalis* +, *Fagus sylvatica* +, *Genista tinctoria* +, *Silene nutans* +, *Steris viscaria* +

E<sub>0</sub>: *Polytrichum formosum* 2b, *Hypnum cupressiforme* 1, *Brachythecium velutinum* +, *Dicranum scoparium* +, *Plagiomnium cuspidatum* +

Within our study, we have also compared the occurrence of macromycetes in the sublocalities. In the sublocality A, we have recorded 89 taxa, in B 50 taxa, in C 71 taxa and in D 32 taxa. Only 3 taxa (*Auricularia auricula-judae*, *Exidia glandulosa*, *Phellinus badius*) were common for all sublocalities; 8 taxa were common for triplet of sublocalities A, B, C (*Auricularia auricula-judae*, *Bisporella citrina*, *Bjerkandera adusta*, *Exidia glandulosa*, *Flammulina velutipes* complex, *Phellinus badius*, *Pluteus cervinus*, *Trametes versicolor*); 5 for A, B, D (*Auricularia auricula-judae*, *Exidia glandulosa*, *Phallus impudicus*, *Phellinus badius*, *Xylaria longipes*); 5 for A, C, D (*Auricularia auricula-judae*, *Exidia glandulosa*, *Mycena renati*, *Phellinus badius*, *Polyporus squamosus*); and 4 for B, C, D (*Auricularia auricula-judae*, *Coprinellus domesticus*, *Exidia glandulosa*, *Phellinus badius*). Comparing the couples of sublocalities, we have found out that on the sublocalities A and B were 20 common taxa (S = 28.7 %); on the A and C 16 taxa (S = 20 %); A and D 8 taxa (S = 13.2%); B and C 13 taxa (S = 21.5 %); B and D 8 taxa (S = 19.5 %); C and D 10 taxa (S = 19.4 %). We think that the highest similarity of species composition in the sublocalities A and B can be caused by the similar type of vegetation (see Relevés above).

### The list of recorded macromycetes

1) In the following list, each species is supplemented by these data: sublocality, substrate, host or associated plants, collection date. 2) All specimens are kept in the herbarium SLO; not deposited collections are signified by abbreviation Not. 3) New species for the Devínska Kobyla Mts., including those published by Ďuriška (2010), are marked by point (●). 4) The used abbreviations: f – fallen (trunk or branch), s – standing (trunk or branch), decid. – deciduous tree; *Fg* – *Fagus sylvatica*, *Ca* – *Carpinus betulus*, *Qc* – *Quercus* sp., *Ac* – *Acer* sp., OD – Ondrej Ďuriška, SJ – Soňa Jančovičová.

## Ascomycota

- *Ascocoryne cylichnium* (Tul.) Korf – A, f. trunk of cf. *Tilia* sp., 28. X. 2009, OD.
- *Bisporella citrina* (Batsch) Korf et S. E. Carp. – A, f. branch of decid., 10. X. 2008, OD; – A, f. trunk of decid., 10. X. 2011, SJ; – A, f. trunk of decid., 21. X. 2011, OD; – B, f. trunk of decid., 28. X. 2011, OD, Not.; – C, f. trunk of cf. *Qc*, 10. XI. 2011, OD.
- *Bulgaria inquinans* (Pers.) Fr. – B, f. trunk of cf. *Qc*, 10. X. 2011, SJ; – D, f. trunk of decid., 6. VIII. 2011, OD.
- *Encoelia furfuracea* (Roth ex Pers.) P. Karst. – B, f. branch of decid., 22. I. 2011, OD.
- *Eutypa spinosa* (Pers.) Tul. et C. Tul. – C, f. trunk of *Fg*, 23. IX. 2011, SJ.
- *Helvella solitaria* P. Karst. – A, soil under *Ac* and *Staphylea pinnata*, 6. V. 2011, OD.
- *Hymenoscyphus fructigenus* (Bull.) Gray – A, pieces of wood of decid., 10. IX. 2007, OD.
- *Hypoxylon fragiforme* (Pers.) J. Kickx f. – B, f. branch of *Fg*, 4. VI. 2007, OD; – B, f. branch of *Fg*, 16. III. 2007, OD; – C, f. trunk of *Fg*, 5. VI. 2011, OD.
- *Hypoxylon howeanum* Peck – A, f. branch of decid., 25. X. 2009, OD; – A, f. branch of decid., 17. IV. 2011, OD; – C, f. branch of decid., 24. X. 2008, OD; – C, f. branch of *Qc*, 24. IX. 2009, OD.
- *Chlorociboria aeruginascens* (Nyl.) Kanouse ex Ramamurthi, Korf et L. R. Batra – C, f. branch of decid., 24. IX. 2009, OD.
- *Peziza succosa* Berk. – A, soil, 15. VI. 2007, OD.
- *Rutstroemia bolaris* (Batsch) Rehm – B, f. branch of decid., 17. III. 2009, OD.
- *Sarcoscypha austriaca* (O. Beck ex Sacc.) Boud. – A, f. branch of decid., 16. III. 2007, OD; – A, f. branch of decid., 22. III. 2007, OD; – A, f. branch of decid., 5. IV. 2009, OD; – A, f. branch of decid., 10. XI. 2011, OD.
- *Sarcoscypha coccinea* (Jacq.) Boud. – A, f. branch of decid., 12. XII. 2008, OD.
- *Sarcoscypha jurana* (Boud.) Baral – A, f. branch of decid., 10. III. 2007, V. Kautman; – A, f. branch of decid., 11. III. 2009, OD; – A, pieces of wood, 19. III. 2009, OD; – A, f. branch of decid., 12. III. 2009, OD (6 specimens); – A, f. branch of decid., 19. III. 2009, OD (2 specimens); – A, f. branch of decid., 22. I. 2011, OD (3 specimens); – A, f. branch of decid., 21. X. 2011, OD; – A, f. branch of decid., 22. XI. 2011, OD.
- *Scutellinia nigrohirtula* (Svrček) Le Gal – A, f. branch of decid., 7. IX. 2008, OD, – D, f. trunk of decid., 7. IX. 2008, OD.
- *Xylaria longipes* Nitschke – A, f. trunk of decid., 7. IX. 2008, OD; – A, f. trunk of decid., 10. X. 2008, SJ; – A, f. branch of decid., 21. X. 2011, OD; – B, f. trunk of cf. *Ca*, 16. III. 2007, V. Kautman; – B, f. trunk of cf. *Fg*, 22. III. 2007, OD; – D, f. branch of decid., 7. IX. 2009, OD.
- *Xylaria polymorpha* (Pers.) Grev. – A, f. trunk of decid., 10. X. 2008, OD; – A, f. trunk of *Ca*, 13. XI. 2009, OD; – A, s. trunk of decid., 25. III. 2011, OD; – A, f. trunk of cf. *Acer*, 17. IV. 2011, OD; – B, stump of decid., 9. III. 2009, SJ.

## Basidiomycota

- *Agaricus augustus* Fr. – C, soil, 28. VI. 2007, OD; – D, soil under *Qc*, 10. X. 2007, OD.
- *Agaricus campester* L. – A, soil, 19. IX. 2007, OD.
- *Amanita franchetii* (Boud.) Fayod – C, soil under *Fg*, 10. X. 2007, OD.
- *Amanita muscaria* (L.) Lam. – C, soil, 24. IX. 2009, OD.
- *Amanita rubescens* Pers. – C, soil under *Fg*, 15. VI. 2011, SJ.
- *Auricularia mesenterica* (Dicks.) Pers. – C, f. trunk of decid., 23. IX. 2011, OD.
- *Armillaria cf. gallica* Marxm. et Romagn. – A, pieces of wood, 5. X. 2007, OD; – A, pieces of wood of *Tilia* sp., 5. X. 2007, OD; – A, pieces of wood of decid., 24. X. 2008, OD; – B, pieces of wood of decid., 25. X. 2009, OD; – A, f. trunk of decid., 21. X. 2011, OD; – B, pieces of wood of decid., 28. X. 2011, OD.
- *Armillaria mellea* (Vahl) P. Kumm. – A, pieces of wood of decid., 10. X. 2007, OD; – B, stump of decid., 5. X. 2007, OD; – B, stump of decid., 25. X. 2009, OD.
- *Artomyces pyxidatus* (Pers.) Jülich – A, f. trunk of decid., 5. X. 2007, OD.
- *Auricularia auricula-judae* (Bull.) Quél. – A, live s. branch of *Staphylea pinnata*, 10. X. 2008, OD, Not.; – A, f. branch of decid., 20. V. 2009, OD; – A, f. trunk of decid., 17. IV. 2011, OD; – B, f. branch of decid., 12. III. 2009, OD, Not.; – B, f. branch of decid., 13. XI. 2009, OD; – C, s. branch of *Sambucus nigra*, 2. XI. 2007, OD; – D, f. trunk of decid., 16. III. 2007, I. Kautmanová.
- *Bjerkandera adusta* (Willd.) P. Karst – A, f. trunk of cf. *Ca*, 12. XII. 2008, OD; – A, f. trunk of *Ac*, 24. X. 2008, OD; – A, stump of *Ac*, 18. IV. 2009, OD; – A, f. trunk of decid., 17. IV. 2011, A. Sosňáková; – B, f. trunk of decid., 7. IX. 2008, OD; – B, f. trunk of decid., 8. X. 2009, OD, Not.; – C, f. trunk of decid., 24. X. 2009, OD, Not.
- *Boletus impolitus* Fr. – A, soil under *Fg*, 13. IX. 2007, OD.
- *Boletus reticulatus* Schaeff. – C, soil under *Fg*, 15. VI. 2011, SJ.
- *Bovista pusilla* (Batsch) Pers. – C, soil, 10. IX. 2007, OD.
- *Byssomerulius corium* (Pers.) Parmasto – B, f. branch of decid., 13. XI. 2008, OD.
- *Cantharellus cibarius* Fr. – C, soil under *Fg*, 15. VI. 2011, SJ; – C, soil under *Fg*, 10. VII. 2011, OD.
- *Clitocybe nebularis* (Batsch) P. Kumm. – C, soil, 21. X. 2011, OD.
- *Clitocybe odora* (Bull.) P. Kumm. – A, soil under *Fg*, 28. X. 2011, OD.
- *Clitocybe phyllophila* (Pers.) P. Kumm. – C, f. leaves of decid., 10. X. 2008, OD.

- *Coprinus alopecia* Lasch – A, soil under *Ac*, 5. VI. 2011, OD; – A, pieces of wood, 10. X. 2011, OD.
- Coprinellus disseminatus* (Pers.) J. E. Lange – A, pieces of wood of decid., 15. VI. 2011, SJ.
- *Coprinellus domesticus* (Bolton) Vilgalys, Hopple et Jacq. Johnson – B, f. branch of decid., 12. IV. 2007, OD; – C, pieces of wood of decid., 13. IX. 2007, OD; – D, soil, 15. VI. 2007, OD; – D, f. trunk of *Qc*, 10. XI. 2011, OD.
- *Coprinellus impatiens* (Fr.) J. E. Lange – A, pieces of wood of decid., 10. X. 2008, SJ; – A, soil, 10. X. 2008, SJ; – A, f. branch of decid., 10. X. 2008, OD; – B, soil, 10. X. 2007, SJ.
- Coprinellus micaceus* (Bull.) Vilgalys, Hopple et Jacq. Johnson – A, f. trunk of *Ac*, 10. X. 2008, SJ.
- *Coprinellus xanthothrix* (Romagn.) Vilgalys, Hopple et Jacq. Johnson – A, pieces of wood of decid., 3. IX. 2009, SJ.
- Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys et Moncalvo – A, soil, 10. X. 2008, SJ.
- *Coprinopsis insignis* (Peck) Redhead, Vilgalys et Moncalvo – A, soil under *Ac*, 10. IX. 2007, OD.
- Coprinopsis picacea* (Bull.) Redhead, Vilgalys et Moncalvo – A, soil under *Tilia* sp., 19. IX. 2007, OD; – A, soil, 10. X. 2008, SJ; – C, soil under *Qc*, 28. X. 2009, OD.
- *Crepidotus caspari* Velen. – B, f. branch of decid., 15. VI. 2007, SJ.
- Crepidotus cesatii* (Rabenh.) Sacc. – A, f. branch of decid., 10. IX. 2007, OD – A, f. branch of decid., 2. X. 2008, OD; – A, f. branch of decid., 6. VIII. 2011, OD; – A, f. branch of decid., 23. IX. 2011, OD; – A, f. branch of decid., 10. X. 2011, SJ (2 specimens); – B, f. branch of *Corylus avellana*, 10. X. 2008, SJ; – B, f. branch of decid., 13. XI. 2009, OD.
- *Crepidotus crocophyllus* (Berk.) Sacc. – B, f. trunk of *Tilia* sp., 15. VI. 2007, SJ.
- *Crepidotus epibryus* (Fr.) Quél. – A, f. stem of herb, 10. X. 2008, SJ; – A, f. branch of decid., 10. X. 2008, SJ (2 specimens).
- Cyathus striatus* (Huds.) Willd. – B, pieces of wood of decid., 3. IX. 2009, SJ; – D, pieces of wood of decid., 21. X. 2011, OD.
- Daedalea quercina* (L.) Pers. – C, f. trunk of decid., 4. VI. 2007, OD; – D, f. trunk of *Qc*, 28. VI. 2007, OD; – D, f. trunk of cf. *Qc*, 28. VI. 2007, OD; – D, f. trunk of decid., 30. IX. 2007, OD; – D, f. trunk of *Qc*, 21. X. 2011, OD.
- Daedaleopsis tricolor* (Bull.) Bondartsev et Singer – C, f. trunk of *Fg*, 15. VI. 2011, SJ.
- Daedaleopsis confragosa* (Bolton) J. Schröt. – A, f. branch of decid., 10. X. 2011, OD.
- *Datronia mollis* (Sommerf.) Donk – B, f. branch of *Fg*, 16. III. 2007, OD.
- *Echinoderma perplexum* (Knudsen) Bon – A, soil, 10. X. 2008, SJ.
- Exidia glandulosa* (Bull.) Fr. – A, f. trunk of *Ca*, 19. III. 2009, OD; – B, f. trunk of *Ac*, 2. XI. 2007, OD; – C, f. branch of decid., 24. V. 2008, OD; – D, f. trunk of decid., 20. V. 2009, OD.
- Fistulina hepatica* (Schaeff.) With. – B, base of stump of *Qc*, 3. IX. 2009, SJ; – C, s. trunk of *Qc*, 24. IX. 2009, OD, Not.
- *Flammulaster muricatus* (Fr.) Watling – A, f. branch of decid., 15. VI. 2012, SJ.
- Flammulina velutipes* komplex – A, f. trunk of decid., 12. XI. 2008, OD; – B, f. trunk of decid., 22. III. 2007, OD; – C, f. branch of decid., 2. XI. 2007, OD.
- Fomes fomentarius* (L.) J. Kickx f. – B, f. trunk of decid., 5. X. 2007, OD, Not.; – B, s. trunk of *Fg*, 10. X. 2011, OD, Not.
- Fomitiporia robusta* (P. Karst.) Fiasson et Niemelä, syn. *Phellinus robustus* (P. Karst.) Bourdot et Galzin – C, f. trunk of *Qc*, 15. VI. 2011, OD; – D, s. trunk of *Qc*, 16. III. 2007, OD.
- *Fuscoporia contigua* (Pers.) G. Cunn., syn. *Phellinus contiguus* (Pers.) Pat. – D, f. trunk of *Qc*, 28. VI. 2007, OD.
- *Ganoderma lipsiense* (Batsch) G. F. Atk. – A, f. trunk of *Ca*, 15. VI. 2007, SJ; – A, f. trunk of decid., 2. X. 2008, OD; – C, f. trunk of decid., 13. V. 2007, K. Kusenda.
- Ganoderma lucidum* (Curtis) P. Karst. – A, pieces of wood of decid., 22. III. 2007, OD, Not.; – A, s. trunk of *Ca*, 10. VII. 2011, OD; – C, base of stump of decid., 24. IX. 2009, OD, Not.; – C, base s. trunk of *Ca*, 25. III. 2011, OD.
- Geastrum fimbriatum* Fr. – A, soil under *Ca*, 23. IX. 2011, OD.
- Geastrum triplex* Jungh. – B, soil, 2. X. 2008, OD.
- *Gymnopilus penetrans* (Fr.) Murrill – D, f. trunk of decid., 10. XI. 2011, OD.
- *Gymnopilus brassicolens* (Romagn.) Antonín et Noordel. – A, f. leaves of decid., 5. X. 2007, OD; – A, f. leaves of decid., 2. X. 2008, OD; – A, f. leaves of decid., 10. X. 2008, SJ; – A, soil, 10. X. 2011, SJ.
- Gymnopilus foetidus* (Sowerby) J. L. Mata et R. H. Petersen – A, f. leaves of decid., 10. X. 2011, SJ.
- Gymnopilus peronatus* (Bolton) Antonín, Halling et Noordel. – A, f. leaves of decid., 10. IX. 2007, OD; – C, f. leaves and soil under *Qc*, 13. IX. 2007, OD.
- *Hohenbuehelia auriscalpium* (Maire) Singer – A, pieces of wood of decid., 5. VI. 2011, OD.
- *Hohenbuehelia petaloides* (Bull.) Schulzer – A, f. trunk of decid., 10. IX. 2007, OD; – A, pieces of wood of decid., 10. VII. 2011, OD; – B, pieces of wood of cf. *Ac*, 15. VI. 2007, SJ; – B, f. trunk of decid., 15. VI. 2007, OD; – B, f. trunk of decid., 24. VI. 2008, OD.
- Hymenochaete rubiginosa* (Dicks.) Lév. – C, f. trunk of decid., 13. V. 2007, OD; – D, f. trunk of *Qc*, 12. IV. 2007, OD.
- *Hyphodontia sambuci* (Pers.) J. Erikss. – A, f. branch of *Sambucus nigra*, 15. VI. 2011, SJ.
- Hypholoma fasciculare* (Hunds.) P. Kumm. – A, stump of decid., 4. VI. 2007, OD; – D, f. trunk of decid., 30. IX. 2007, OD; – D, pieces of wood of decid., 30. IX. 2007, OD; – D, f. trunk of *Ca*, 10. XI. 2011, OD.
- Hypsizygus tessulatus* (Bull.) Singer – A, s. trunk of *Fg*, 21. X. 2011, OD.
- Inocybe erubescens* A. Blytt – A, soil under *Ac*, 5. VI. 2011, OD.
- *Inonotus cuticularis* (Bull.) P. Karst. – C, f. trunk of *Fg*, 24. IX. 2009, OD.
- Kuehneromyces mutabilis* (Schaeff.) Singer et A. H. Sm. – B, f. trunk of decid., 15. VI. 2007, SJ; – B, f. trunk of decid., 10. X. 2011, OD.
- Laccaria amethystina* Cooke – D, soil under *Tilia* sp., 8. VIII. 2011, OD.
- *Lactarius azonites* (Bull.) Fr. – C, soil under *Qc*, 5. X. 2007, OD.

- *Lactarius glaucescens* Crossl. – C, soil under *Qc*, 24. VI. 2008, OD.
- Lactarius piperatus* (L.) Gray – D, soil under *Qc*, 15. VI. 2007, SJ; – D, soil under *Ca*, 15. VI. 2011, SJ.
- Laetiporus sulphureus* (Bull.) Murrill – C, f. trunk of *Qc*, 24. IX. 2009, OD; – C, f. trunk of decid., 10. X. 2011, OD, Not.
- Leptinus tigrinus* (Bull.) Fr. – A, f. trunk of *Fg*, 10. X. 2007, OD.
- *Lepiota boudieri* Bres. – A, soil, 19. IX. 2007, OD.
- Lepiota cristata* (Bolton) P. Kumm. – A, soil, 10. IX. 2007, OD; – A, soil under *Fg*, 13. IX. 2007, OD; – A, soil under *Ac*, 19. IX. 2007, OD; – A, soil under *Ca*, 10. X. 2008, OD.
- Lepista nuda* (Bull.) Cooke – B, soil, 10. X. 2008, SJ.
- Lycoperdon lividum* Pers. – C, soil, 2. X. 2008, OD.
- *Macrolepiota konradii* (Huijsman ex P. D. Orton) M. M. Moser – C, soil under *Fg*, 13. XI. 2009, OD.
- Macrolepiota procera* (Scop.) Singer – B, soil, 10. X. 2011, OD, Not.
- *Marasmius curreyi* Berk. et Broome – A, f. leaves of decid., 2. X. 2008, OD; – A, f. leaves of decid., 10. X. 2008, SJ.
- Marasmius rotula* (Scop.) Fr. – A, f. twigs of decid., 10. X. 2011, SJ.
- Merulius tremellosus* Schrad. – B, f. branch of decid., 10. X. 2008, SJ.
- Mycena acicula* (Schaeff.) P. Kumm. – C, pieces of wood of decid., 6. VIII. 2011, OD.
- Mycena crocata* (Schrad.) P. Kumm. – C, f. leaves and pieces of wood of decid., 28. X. 2011, OD.
- Mycena galericulata* (Scop.) Gray – A, pieces of wood of decid., 5. X. 2007, OD; – A, pieces of wood of decid., 10. X. 2007, S. Đurkáčová; – B, f. trunk of decid., 28. X. 2009, OD; – B, f. trunk of decid., 13. XI. 2009, OD.
- Mycena inclinata* (Fr.) Quél. – D, f. trunk of decid., 21. X. 2011, OD.
- Mycena pura* (Pers.) P. Kumm. – B, soil, 10. X. 2008, SJ; – C, f. leaves of decid., 28. X. 2011, OD.
- Mycena renati* Quél. – A, f. trunk of decid., 3. IX. 2009, SJ; – A, f. trunk of decid., 10. X. 2011, OD; – C, f. trunk of decid., 5. VI. 2009, OD; – D, f. trunk of decid., 3. V. 2011, OD.
- Mycena rosea* Gramberg – B, soil, 10. X. 2008, SJ.
- *Mycena speirea* (Fr.) Gillet – A, f. trunk of decid., 24. V. 2008, OD; – A, f. leaves of decid., 20. V. 2009, OD.
- *Ossicaulis lignatilis* (Pers.) Redhead et Ginns – C, f. trunk of *Fg*, 24. IX. 2009, OD.
- *Oudemansiella mucida* (Schrad.) Höhn. – C, f. trunk of decid., 25. X. 2009, OD; – C, f. trunk of *Fg*, 13. XI. 2009, OD; – C, f. trunk of *Fg*, 21. X. 2011, OD.
- Paxillus involutus* (Batsch) Fr. – B, soil, 10. X. 2008, SJ.
- Phallus impudicus* L. – A, soil, 10. X. 2008, OD, Not.; – A, soil, 24. IX. 2009, OD, Not.; – B, soil, 24. V. 2008, OD; – D, soil, 8. X. 2009, OD, Not.
- Phellinus badius* (Berk. ex Cooke) G. Cunn., syn. *Polyporus badius* (Berk.) Schwein. – A, f. trunk of decid., 7. IX. 2008, OD; – A, f. trunk of decid., 2. X. 2008, OD; – A, f. trunk of decid., 9. V. 2009, OD; – A, f. trunk of decid., 3. V. 2011, OD; – B, f. trunk of decid., 13. V. 2007, K. Kusenda; – B, pieces of wood of decid., 5. X. 2007, OD; – C, pieces of wood of decid., 23. IX. 2011, OD; – D, f. trunk of decid., 3. V. 2011, OD.
- Pholiota squarrosa* (Vahl) P. Kumm. – C, f. trunk of *Fg*, 10. X. 2011, OD.
- *Pholiota tuberculosa* (Schaeff.) P. Kumm. – A, f. trunk of decid., 20. V. 2009, OD; – B, f. trunk of decid., 5. VI. 2009, OD.
- *Piptoporus quercinus* (Schrad.) P. Karst. – D, s. trunk of *Qc*, 28. VI. 2007, OD; – D, f. trunk of decid., 28. VI. 2007, OD.
- Pleurotus dryinus* (Pers.) P. Kumm. – A, s. trunk of *Staphylea pinnata*, 10. X. 2008, SJ.
- Pleurotus pulmonarius* (Fr.) Quél. – A, s. trunk of *Fg*, 24. V. 2008, OD.
- Pleurotus ostreatus* (Jacq.) P. Kumm. – C, f. trunk of *Fg*, 15. VI. 2011, SJ.
- Pluteus cervinus* (Schaeff.) P. Kumm. – A, pieces of wood of decid., 10. X. 2011, SJ; – B, f. trunk of *Fg*, 10. X. 2007, OD; – B, f. trunk of decid., 24. VI. 2008, OD; – B, f. trunk of decid., 9. V. 2009, OD; – B, pieces of wood of decid., 20. V. 2009, OD; – B, f. trunk of decid., 3. IX. 2009, SJ; – C, stump of decid., 24. IX. 2009, OD; – C, f. trunk of decid., 24. IX. 2009, OD; – C, f. trunk of decid., 8. X. 2009, OD; – C, f. trunk of decid., 28. XI. 2011, OD.
- *Pluteus dietrichii* Bres. – D, soil, 7. IX. 2008, OD.
- Pluteus ephebeus* (Fr.) Gillet – A, soil, 10. IX. 2007, OD; – A, soil, 13. IX. 2007, OD.
- *Pluteus phlebophorus* (Ditmar) P. Kumm. – C, f. trunk of decid., 15. VI. 2009, SJ.
- *Pluteus plautus* (Weinm.) Gillet – A, f. trunk of cf. *Ac*, 15. IX. 2007, OD; – C, pieces of wood of decid., 15. VI. 2007, OD; – C, f. branch of decid., 10. X. 2008, OD, Not.; – C, f. trunk of decid., 15. VI. 2011, OD.
- *Pluteus romellii* (Britzelm.) Sacc. – A, pieces of wood of decid., 7. IX. 2008, OD; – B, pieces of wood of decid., 15. VI. 2007, OD; – B, f. branch of decid., 10. X. 2008, SJ.
- Pluteus salicinus* (Pers.) P. Kumm. – B, f. trunk of decid., 10. X. 2008, SJ.
- *Pluteus umbrosus* (Pers.) P. Kumm. – A, pieces of wood of decid., 19. IX. 2007, OD.
- *Polyporus alveolaris* (DC.) Bondartsev et Singer – A, f. branch of decid., 24. VI. 2008, OD.
- Polyporus brumalis* (Pers.) Fr. – B, f. trunk of decid., 12. IV. 2007, OD.
- Polyporus squamosus* (Huds.) Fr. – A, pieces of wood of decid., 15. VI. 2011, SJ; – C, f. trunk of decid., 13. V. 2007, K. Kusenda; – C, f. branch of *Tilia* sp., 13. IX. 2007, OD; – C, f. trunk of decid., 10. X. 2011, OD, Not.; – D, f. trunk of decid., 5. VI. 2009, SJ.
- *Porodaedalea pini* (Brot.) Murrill, syn. *Phellinus pini* (Brot.) Bondartsev et Singer – C, s. trunk of *Pinus sylvestris*, 10. IV. 2008, OD.
- *Postia alni* Niemelä et Vampola – B, f. branch of decid., 10. X. 2011, OD.
- Psathyrella piluliformis* (Bull.) P. D. Orton – D, soil, 30. IX. 2007, OD.
- Russula cyanoxantha* (Schaeff.) Fr. – C, soil, 24. VI. 2008, OD; – C, soil under *Fg*, 10. VII. 2011, OD.

- Russula heterophylla* (Fr.) Fr. – C, soil under *Qc*, 15. VI. 2011, SJ.
- *Russula pectinatoides* Peck – A, soil, 15. VI. 2007, OD.
- Schizophyllum commune* Fr. – A, f. trunk of *Fg*, 22. III. 2007, OD; – A, f. trunk of decid., 17. IV. 2011, A. Sosňaková; – B, f. trunk of *Populus* sp., 12. III. 2009, OD.
- *Simocybe centunculus* (Fr.) P. Karst. – C, f. branch of decid., 28. X. 2011, OD.
  - *Simocybe haustellaris* (Fr.) WatlingSinger – A, f. trunk of decid., 15. VI. 2007, OD.
  - *Simocybe sumptuosa* (P. D. Orton) Singer – A, f. branch of decid., 4. VI. 2007, OD.
- Steccherinum bourdotii* Saliba et A. David – A, f. branch of decid., 10. X. 2011, SJ; – B, s. trunk of *Ca*, 19. III. 2009, OD.
- *Steccherinum ochraceum* (Pers.) Gray – A, f. branch of decid., 10. X. 2011, SJ (2 specimens).
- Stereum hirsutum* (Willd.) Pers. – C, f. branch of decid., 24. IX. 2009, OD, Not; – A, f. trunk of decid., 10. X. 2011, OD.
- *Stereum rugosum* Pers. – D, f. branch of *Qc*, 10. XI. 2011, OD.
- Trametes gibbosa* (Pers.) Fr. – A, f. trunk of *Ca*, 28. VI. 2007, OD; – C, f. trunk of *Fg*, 28. VI. 2007, OD; – C, f. trunk of decid., 10. XI. 2011, OD.
- *Trametes trogii* Berk. – A, f. trunk of *Fg*, 24. IX. 2009, OD.
- Trametes versicolor* (L.) Lloyd – A, f. branch of decid., 13. V. 2007, K. Kusenda; – B, f. trunk of cf. *Fg*, 12. IV. 2007, OD; – B, branch of *Corylus avellana*, 17. IV. 2011, A. Sosňaková; – C, f. trunk of decid., 28. VI. 2007, OD, Not.; – C, f. trunk of *Fg*, 10. IV. 2008, OD; – C, f. trunk of *Cerasus avium*, 31. VIII. 2011, OD; – C, f. branch of decid., 10. X. 2011, OD, Not.; – C, f. branch of decid., 10. XI. 2011, OD.
- *Trichaptum biforme* (Fr.) Ryvarden – C, f. branch of *Fg*, 4. VI. 2007, OD; – C, f. branch of *Qc*, 24. IX. 2009, OD; – C, f. trunk of *Fg*, 21. X. 2011, OD.
- *Tubaria romagnesiana* Arnolds – B, pieces of wood of decid., 19. III. 2009, OD; – C, pieces of wood of cf. *Ac*, 13. V. 2007, A. Marko; – C, f. branch of cf. *Tilia* sp., 13. IX. 2007, OD; – C, f. branch of decid., 13. IX. 2007, OD.
- Volvariella gloiocephala* (DC.) Boekhout et Enderle – A, soil, 28. X. 2009, OD; – B, soil, 8. X. 2009, OD.
- *Xerocomus communis* (Bull.) Bon – A, soil under *Ca*, 15. VI. 2007, SJ; – A, soil, 19. IX. 2007, OD.
- Xerocomus chrysenteron* (Bull.) Quél. – A, soil under *Ca*, 10. X. 2008, SJ.
- *Xerocomus porosporus* (Imler ex G. Moreno et Bon) Contu – A, soil under *Fg*, 9. IX. 2007, OD; – A, soil under *Ac*, 19. IX. 2007, OD.
- Xerula radicata* (Relhan) Dörfelt – C, on the base/roots of stump of cf. *Fg*, 13. V. 2007, K. Kusenda; – C, near the f. trunk/roots of *Fg*, 8. X. 2009, OD.

## Acknowledgements

We thank Jan Holec (National Museum, Praha) who kindly helped with identification of *Flammulaster muricatus*. Stanislav Glejdura (Technical University in Zvolen), Viktor Kučera, Slavomír Adamčík (Institute of Botany SAS) and Miroslav Caboň (Comenius University in Bratislava) are also acknowledged for identification of some other fungi, Katarína Mišíková (Comenius University in Bratislava) for identification of mosses. Comments by the reviewer were very helpful. Data included in this work have been granted thanks to the projects VEGA 2/0028/11 and APVV SK-CZ-0052-11.

## References

- Anonymus**, 2005: Enviroportál, informačný systém o životnom prostredí. <http://enviroportal.sk/informacny-system-o-zivotnom-prostredii.php> [accessed 24 April 2012].
- Bizubová, M., Minár, J.**, 2005: Georeliéf a fyzickogeografické komplexy v JZ časti Malých Karpát (Devínska Kobyla, Devínska brána a Bratislavské predhorie). In: Majzlan, O. (ed.), Fauna Devínskej Kobyly, p. 8-15, APOP, Bratislava.
- Braun-Blanquet, J.**, 1964: Pflanzensoziologie. Grundzüge der Vegetationskunde. Springer-Verlag, Wien, New York.
- Cooper, J., Kirk P.**, 2012: CABI Bioscience Databases. <http://www.indexfungorum.org/> [accessed 24 April 2012].
- Ďuriška, O.**, 2010: New records of macromycetes for Devínska Kobyla Mts. Acta Bot. Univ. Comen., 45: 13-23.
- Futák, J.**, 1984: Fytogeografické členenie Slovenska. In: Bertová, L. (ed.), Flóra Slovenska IV/1, p. 418-419, Veda, Bratislava.
- Hansen, L., Knudsen, H.** (eds.), 1997: Nordic macromycetes, vol. 3, Heterobasidioid, aphylophoroid and gasteromycetoid basidiomycetes. Nordsvamp, Copenhagen.
- Hansen, L., Knudsen, H.** (eds.), 2000: Nordic macromycetes, vol. 1, Ascomycetes. Nordsvamp, Copenhagen.
- Jarolímek, I., Šibík, J., Hegedúšová, K., Janišová, M., Kliment, J., Kučera, P., Májeková, J., Micháľková, D., Sadloňová, J., Šibíková, I., Skodová, I., Uhlířová, J., Ujházy, K., Ujházyová, M., Valachovič, M., Zaliberová, M.**, 2008: A list of vegetation units of Slovakia. In: Jarolímek, I., Šibík, J. (eds.), Diagnostic, constant and dominant species of the higher vegetation units of Slovakia, p. 295-329, Veda, Bratislava.

- Kaliská, G., Nemcová, M.**, 1998: Zdôvodnenie ochrany ŠPR Fialkové údolie. Depon. in Administration of the Protected Landscape Area of Malé Karpaty.
- Knudsen, H., Vesterholt, J.** (eds.), 2008: Funga Nordica, Nordsvamp, Copenhagen.
- Knudsen, H., Taylor, A.**, 2008: *Xerocomus* Quéél. In: Knudsen, H., Vesterholt, J. (eds.), Funga Nordica, p. 174-179, Nordsvamp, Copenhagen.
- Lapin, M., Faško, P., Melo, M., Štastný, P., Tomlain, J.**, 2002: Klimatické oblasti. In: László, L. (ed.), Atlas krajiny Slovenskej republiky, p. 95, MŽP SR, Slovenská agentúra životného prostredia, Banská Bystrica, Bratislava.
- Lizoň, P.**, 2001: Červený zoznam húb Slovenska. In: Baláž, D., Marhold, K., Urban, P. (eds.), Červený zoznam rastlín a živočíchov Slovenska, p. 6-13, Ochr. Prír., Bratislava.
- Marhold, K.** (ed.), 1998: Papraďorasty a semenné rastliny. In: Marhold, K., Hindák, F. (eds.), Zoznam nižších a vyšších rastlín Slovenska, p. 333-687, Veda, Bratislava.
- Ripková, S., Ďuriška, O.**, 2009: The current knowledge of funga of the Devínska Kobyla Mts. Acta Bot. Univ. Comen., 44: 41-58.
- Westhoff, V., Van der Maarel, E.**, 1978: The Braun-Blanquet approach. In: Whittaker, R. H. (ed.), Classification of plant communities, p. 289-399, W. Junk, The Hague.
- Záhorovská, E.**, 1997: Huby. In: Feráková, V., Kocianová, E. (eds.), Flóra, geológia a paleontológia Devínskej Kobyly, p. 58-68, Litera, s.r.o., pre APOP, Bratislava.

## Abstrakt

Na základe publikovaných údajov a výsledkov vlastného 4-ročného terénneho výskumu (2007 – 2009, 2011) je z Prírodnej rezervácie Fialková dolina v súčasnosti známych 149 taxónov makromycétov (18 z Ascomycota a 131 z Basidiomycota). V článku uvádzame charakteristiku študovaného územia a zoznam všetkých zistených makromycétov.

**Ondrej Ďuriška, Soňa Jančovičová, Ján Miškovic: Makromycéty Prírodnej rezervácie Fialková dolina (Devínska Kobyla, Slovensko)**

## CONTRIBUTION TO THE KNOWLEDGE OF MACROMYCETES IN THE ZÁHORSKÁ NÍŽINA LOWLAND

Miroslava Horinková, Soňa Jančovičová\*

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany,  
Révová 39, 811 02 Bratislava, Slovakia

Received 25 June 2012; Received in revised form 10 July 2012; Accepted 16 July 2012

### Abstract

The two-year mycological research (2010–2011) in the locality Kalaštov near the village of Borský Peter (Záhorská nížina Lowland) resulted in 59 identified taxa of macromycetes (1 of Ascomycota, 58 of Basidiomycota). In this paper, all recorded macromycetes are listed and provided with basic ecological data.

**Key words:** monoculture, *Pinus sylvestris*, the locality Kalaštov, diversity

### Introduction

Záhorská nížina Lowland – or simply Záhorie – is also known as an attractive mushroom area. Watching crowds of cars along the pine forests, it is a sign the Boletes started to grow. However, besides Boletes many other fungi grow there making Záhorie very rich in species. Collecting published data on occurrence of macromycetes in Slovakia, Adamčík et al. (2003) found out that the Záhorská nížina Lowland belonged – with those of 568 published taxa – among the best mycologically researched. Naturally, this number is by far not definitive and even each field excursion can bring new taxa, as documented by e.g. Adamčík, Hagara (2003), Adamčík, Ripková (2003), Jeppson (2008) and Škubla (2003). We also wanted to contribute to the knowledge of macrofunga of the Záhorská nížina Lowland. We have, therefore, selected a locality in this area – the locality Kalaštov near the village of Borský Peter – and carried out field research here. The results of our 2-year mycological survey are presented in this paper.

### Material and Methods

**The locality characteristic.** With the same name as a nearby hunting lodge, the locality Kalaštov is situated in the cadastre of the village of Borský Peter (see the tourist map Malé Karpaty – Záruby, no. 128, 1 : 50 000, 1994, Harmanec). It has triangular shape with the area about 10 ha and altitude 195 m a. s. l. It is bordered by the forest roads, and the coordinates read more or less in the middle of locality are 48° 38' 54" N and 17° 14' 39" E. Geology and soils. – Airborne silica sand dominates the area and forms several geomorphological structures, most often dunes (Kollár et al. 1996); one of such dunes occurs more or less in the middle of locality Kalaštov. On silica sand, there have been developed Regosols with shallow acidic humus layer as the result of decomposition of needle litter (Dingová 2009). Climate. – The locality pertains to warm climatic region with the mean (m.) annual temperature 9.3 °C and m. annual precipitation 550–650 mm (m. temperature in January ranges from -2.5 to -2 °C,

---

\* Corresponding Author: Soňa Jančovičová; [sona.jancovicova@fns.uniba.sk](mailto:sona.jancovicova@fns.uniba.sk)

in July from 19.6 to 20.2 °C; the most precipitation is in June to August, the least in January to March). During the vegetation period, all the area has water deficit; the evaporation is higher than the precipitation (Halada 1994). During our own research in years 2010–2011, July 2010 was the warmest month (m. temperature was 22.8 °C), and January 2010 the coldest month (m. temperature was -2.1 °C). The most days with precipitation (20 days) was in May 2010, the least (0) in November 2011 (according to our personal observations and measurements). Vegetation. – The forest vegetation of the locality is represented by a nearly one-hundred-year old pine (*Pinus sylvestris*) monoculture with cover of the tree layer 45 %. The shrublayer is also wholly dominated by woody species *P. sylvestris* with height of 2–6 m and cover 20 %. The herb layer is primarily occupied by juveniles of *P. sylvestris*, *Festuca ovina* agg. and *Festuca vaginata* ssp. *dominii*. A well-developed layer of bryophytes is dominated by acidophilous *Dicranum polysetum*; from the lichens, it is predominantly developed by the species of the genus *Cladonia* (Dingová 2009). Anthropogenic influence. – The locality Kalaštov is used for recreational and economic purposes. Mostly local people go there bicycling (the cycle path is here), walking and picking mushrooms. Wood harvesting is planned to be here within the years 1998–2017 (no precise date is known). After cutting, an afforestation process is required to be done by the forest company Lesy SR, š. p. within two years (Struhár pers. com.). In surroundings of the locality, there are several mines as the result of surface sand mining (mining companies are e.g. PD Dojč, SAND s. r. o., Kerko a. s., Košice, Kerkosand s. r. o., Šajdíkové Humence and Najpi s. r. o.).

**Period of research.** Our research went on in the years 2010 and 2011. Altogether, we carried out 18 field excursions, one of them in 2010 (1 October), the rest in 2011 (1, 9 and 23 May; 6 and 20 June; 6, 18 and 30 July; 14 and 29 August; 12 and 26 September; 10, 17 and 24 October; 2 and 21 November).

**Collections.** The study is based on 126 own collections. Out of them, 87 collections (specimens) were deposited in the herbarium of the Department of Botany, Comenius University in Bratislava, Slovakia (SLO). Although provided by the field notes, some frequently occurring and/or easily recognizable fungi were not always backed up by specimens (they were 39 collections).

**Literature for determination of fungi, nomenclature.** The work by Knudsen, Vesterholt eds. (2008) was mostly used for determination of agaricoid, boletoid and cyphelloid basidiomycetes; the works by Hansen, Knudsen eds. (1997) and Jülich (1984) of heterobasidioid, aphylloroid and gasteromycetoid basidiomycetes; and work by Hansen, Knudsen eds. (2000) of ascomycetes. Nomenclature is unified according to CABI Bioscience Databases: Index Fungorum (Cooper, Kirk 2012), with exception of *Xerocomus* names – they follow the work by Knudsen, Taylor (2008); and *Russula* names – they are according to the webpage “Russulales News” (<http://www.mtsn.tn.it/russulales-news/welcome.asp>).

## Results and Discussion

Our 2-year mycological research (2010–2011) was carried out in a nearly one-hundred-year old pine (*Pinus sylvestris*) monoculture in the locality Kalaštov near the village of Borský Peter (Záhorská nížina Lowland). It resulted in 59 identified taxa of macromycetes, out of them 1 of Ascomycota and 58 of Basidiomycota. Altogether, we have observed 126 collections, from which 87 were backed up by specimens and 39 noted (not deposited after their identification; see also Material and Methods). Nine specimens are still unidentified; they are of aphylloroid fungi and agarics of genera *Clitocybe*, *Cortinarius*, *Inocybe* and *Mycena*.

Concerning trophic preferences, most of recorded macromycetes were more or less restricted to *Pinus sylvestris* trees – 36 species formed mycorrhizae [to categorize mycorrhizal fungi, we followed Gryndler et al. (2004)]; one species (*Porodaedalea pini*) grew as parasite (on living standing pine tree) and 13 as saprotrophs (on wood and bark of trunks, stumps or branches of dead pine trees or on pieces of pine wood). *Crepidotus variabilis* was the only wood-inhabiting species that did not produce

basidioma on pine wood, it grew on fallen twigs of *Rosa* sp. The rest of recorded macromycetes were terrestrial – *Aleuria aurantia* grew on naked sandy soil and 8 others on soil among the mosses (Tab. 1).

In the locality Kalaštov, we have also collected some species of special interest: one red-listed species, *Gomphidius roseus* (cf. Lizoň 2001), and four species that probably have not been published from the Záhorská nížina Lowland before: *Gloeophyllum sepiarium*, *Gymnopilus brassicolens*, *Phellodon tomentosus* and *Tricholoma arvernense* [to know it, we have searched the literature compiled by Bacigálová, Lizoň eds. (1999), Adamčík et al. (2003) and Škubla (2003), as well some other work by Adamčík, Hagara (2003), Adamčík, Ripková (2003), Caboň, Adamčík (2011) and Jeppson (2008)].

We realize, comparing our results with those of similar works, e.g. by Dermek 1978 and Záhorská, Jančovičová (1997), that the diversity of macromycetes is rather poor in the locality Kalaštov. It might be caused by 1) climate influences [(in 2011, when most of our field excursions were done, only 47 days with precipitation were counted. In general, the mushroom season 2011 was bad, even the worst within the past years (Anonymus 2011)]; 2) restricted period of research (in field mycology, two years are not enough to get relevant data. Unfortunately, our 2-year research was partially conditioned by the program of Msc. study, the first author of this paper was involved in); 3) anthropic influence (our finding of the occurrence of edible mushrooms of the genera *Boletus*, *Macrolepiota*, *Xerocomus* and *Suillus* was distorted by local mushroom-pickers who used to pick them intensively). Despite these facts, we believe that our results will contribute to the knowledge of macrofunga of the locality Kalaštov (our research was the first of such character in the locality), as well of the Záhorská nížina Lowland.

**Tab. 1. Macromycetes recorded in the *Pinus sylvestris* monoculture in the locality Kalaštov near the village of Borský Peter (Záhorská nížina Lowland) in years 2010–2011**

**I:** the list of taxa; **II:** growth type: **t** – terrestrial, **w** – wood-inhabiting, **m** – mycorrhizal (for details on substrate see Results and Discussion); **III:** collection date(s) (only the 1 of October is of year 2010, the rest of dates are of 2011); **IV:** collector(s): **MH** – M. Horinková; **SJ** – S. Jančovičová; **V:** number of deposited (in SLO)/noted collections

<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>	<b>V</b>
<i>Aleuria aurantia</i> (Pers.) Fuckel	t	17., 24. X.; 2. XI.	MH	1/2
<i>Amanita citrina</i> (Schaeff.) Pers.	m	10., 17., 24. X.; 2., 21. XI.	MH	1/4
<i>Amanita gemmata</i> (Fr.) Bertill.	m	1. X.	SJ	1/0
<i>Amanita muscaria</i> (L.) Lam.	m	10., 24. X.	MH	1/1
<i>Amanita phalloides</i> (Vaill. ex Fr.) Link	m	14. VIII.	MH	1/0
<i>Amanita porphyria</i> Alb. et Schwein.	m	1., 10., 24. X.	SJ, MH	3/0
<i>Amanita rubescens</i> Pers.	m	14. VIII.	MH	1/0
<i>Ampulloclitocybe clavipes</i> (Pers.) Redhead, Lutzoni, Moncalvo et Vilgalys	t	10., 24. X.	MH	1/1
<i>Armillaria mellea</i> (Vahl) P. Kumm.	w	2. XI.	MH	1/0
<i>Boletus erythropus</i> Pers.	m	1. X.	SJ	1/0
<i>Cantharellus cibarius</i> Fr.	m	6. VII.; 1. X.	SJ, MH	1/1
<i>Clitocybe metachroa</i> (Fr.) P. Kumm.	t	17. X.	MH	1/0
<i>Collybia cirrhata</i> (Schumacher.) Quél.	t	10., 17., 24. X.	MH	1/2
<i>Cortinarius semisanguineus</i> (Fr.) Gillet	m	17. X.; 2. XI.	MH	2/0
<i>Crepidotus variabilis</i> (Pers.) P. Kumm.	w	1. X.	SJ	1/0
<i>Cystoderma amianthinum</i> (Scop.) Fayod	t	10., 17. X.	MH	2/0
<i>Galerina pumila</i> (Pers.) M. Lange	t	2., 21. XI.	MH	2/0
<i>Gloeophyllum sepiarium</i> (Wulfen) P. Karst.	w	21. XI.	MH	1/0
<i>Gomphidius roseus</i> (Fr.) Fr.	m	1., 10., 17. X.	SJ, MH	3/0
<i>Gymnopilus picreus</i> (Pers.) P. Karst.	w	10., 17. X.	MH	2/0
<i>Gymnopilus sapineus</i> (Fr.) Murrill	w	10. X.	MH	1/0

Tab. 1. Continuation

I	II	III	IV	V
<i>Gymnopus androsaceus</i> (L.) J. L. Mata et R.H. Petersen	w	10. X.	MH	1/0
<i>Gymnopus brassicolens</i> (Romagn.) Antonín et Noordel.	w	30. VII.	MH	1/0
<i>Gymnopus dryophilus</i> (Bull.) Murrill	t	30. VII.	MH	1/0
<i>Hygrophoropsis aurantiaca</i> (Wulfen) Maire	w	24. X.; 2., 21. XI.	MH	1/2
<i>Hypholoma fasciculare</i> (Huds.) P. Kumm.	w	6., 18. VII.; 24. X.; 2. XI.	MH	2/2
<i>Laccaria laccata</i> (Scop.) Cooke	m	10., 17. X.	MH	2/0
<i>Lactarius deliciosus</i> (L.) Gray	m	1. X.	SJ	1/0
<i>Lactarius rufus</i> (Scop.) Fr.	m	1., 10., 17., 24. X.; 2. XI.	SJ, MH	2/3
<i>Macrolepiota procera</i> (Scop.) Singer	m	2., 21. XI.	MH	2/0
<i>Mycena galericulata</i> (Scop.) Gray	w	2. XI.	MH	1/0
<i>Neolentinus lepideus</i> (Fr.) Redhead & Ginns	w	20. VI.; 18. VII.; 14. VIII.	MH	1/2
<i>Paxillus involutus</i> (Batsch) Fr.	m	10., 24. X.	MH	1/1
<i>Phellodon tomentosus</i> (L.) Banker	m	1. X.	SJ	1/0
<i>Porodaedalea pini</i> (Brot.) Murrill	w	17. X.	MH	1/0
<i>Rhizopogon luteolus</i> Fr. et Nordholm	m	1. X.	SJ	2/0
<i>Rhodocollybia butyracea</i> f. <i>asema</i> (Fr.) Antonín, Halling et Noordel.	t	30. VII.; 21. XI.	MH	2/0
<i>Rickenella fibula</i> (Bull.) Raitelh.	t	30. VII.; 14. VIII.	MH	1/1
<i>Russula amara</i> Kučera	m	14. VIII.; 17. X.	MH	2/0
<i>Russula amethystina</i> Quél.	m	10. X.	MH	1/0
<i>Russula badia</i> Quél.	m	17. X.	MH	1/0
<i>Russula cyanoxantha</i> (Schaeff.) Fr.	m	6. VII.	MH	1/0
<i>Russula</i> cf. <i>parazurea</i> Jul. Schäff.	m	20. VI.	MH	1/0
<i>Russula sanguinaria</i> (Schumach.) Rauschert	m	2. XI.	MH	1/0
<i>Russula sardonina</i> Fr.	m	1. X.	SJ	1/0
<i>Russula vesca</i> Fr.	m	10. X.	MH	1/0
<i>Russula xerampelina</i> (Schaeff.) Fr.	m	10. X.	MH	1/0
<i>Sarcodon imbricatus</i> (L.) P. Karst.	m	1. X.	SJ	1/0
<i>Scleroderma citrinum</i> Pers.	m	20. VI.; 18. VII.; 29. VIII.; 12., 26. IX.; 17., 24. X.	MH	1/6
<i>Suillus bovinus</i> (Pers.) Roussel	m	1., 10. X.	SJ, MH	2/0
<i>Suillus variegatus</i> (Sw.) Kuntze	m	1. X.	SJ	1/0
<i>Tapinella atrotomentosa</i> (Batsch) Šutara	w	6., 18., 30. VII.; 14. VIII.	MH	1/3
<i>Thelephora terrestris</i> Ehrh.	m	30. VII.; 14., 29. VIII.; 12. IX.; 17. X.	MH	1/4
<i>Trichaptum fuscoviolaceum</i> (Ehrenb.) Ryvarden	w	20. VI.	MH	1/0
<i>Tricholoma arvernense</i> Bon	m	1. X.	SJ	1/0
<i>Tylopilus felleus</i> (Bull.) P. Karst.	m	20. VI.; 6., 30. VII.; 14. VIII.; 10. X.	MH	2/3
<i>Xerocomus badius</i> (Fr.) J.-E. Gilbert	m	20. VI.; 1., 10. X.	SJ, MH	3/0
<i>Xerocomus chrysenteron</i> (Bull.) Quél.	m	10., 17. X.	MH	1/1
<i>Xerocomus subtomentosus</i> (L.) Quél.	m	1. X.	SJ	1/0

## Acknowledgements

Our thanks go to Slavomír Adamčík (Institute of Botany SAS) and Miroslav Caboň (Comenius University in Bratislava) for identification of some *Russula* collections. Comments by the reviewer were very helpful. Data included in this work have been granted thanks to the projects VEGA 2/0028/11 and APVV SK-CZ-0052-11.

## References

- Adamčík, S., Hagara, L., 2003: Makroskopické huby. In: Stanová, V., Viceníková, A. (eds.), Biodiverzita Abrodu – stav, zmeny a obnova, p. 49-86, Daphne – Inštitút aplikovanej ekológie, Bratislava.
- Adamčík, S., Kučera, V., Lizoň, P., Ripka, J., Ripková, S., 2003: State of diversity research on macrofungi in Slovakia. Czech Mycol., 55: 201-213.
- Adamčík, S., Ripková, S., 2003: *Boletopsis grisea*. Catathelasma, 4: 31-34.
- Anonymus, 2011: Nahuby.sk. [http://www.nahuby.sk/diskusie.php?parent\\_message\\_id=1615113](http://www.nahuby.sk/diskusie.php?parent_message_id=1615113) [accessed 8 June 2012].
- Bacigálová, K., Lizoň, P. eds., Adamčík, S., Gáper, J., Glejdura, S., Janitor, A., Šimonovičová, A., 1999: Huby. In: Marhold, K., Hindák, F. (eds.), Zoznam nižších a vyšších rastlín Slovenska, CD-ROM ed., Bratislava.
- Caboň, M., Adamčík, S., 2011: Diverzita *Russulaceae* na vybraných lokalitách Borskej nížiny. In: Anonymus, Zborník recenzovaných príspevkov ŠVK Prif UK 2011, CD-ROM ed., p. 86-91, Univerzita Komenského v Bratislave, Bratislava.
- Cooper, J., Kirk P., 2012: CABI Bioscience Databases. <http://www.indexfungorum.org/> [accessed 28 August 2012].
- Dermek, A., 1978: Príspevok k mykoflóre na okolí Brodského, Čárov, Gbelov, Kopčian a Smolinského (západné Slovensko). Česká Mykol., 32: 215-225.
- Dingová, A., 2009: Synúzie lišajníkov a ich cenologická väzba na viatych pieskoch Borskej nížiny. Pre-PhD. thesis depon. in Institute of Botany SAS Bratislava.
- Gryndler, M., Baláž, M., Hršelová, H., Jansa, J., Vosátka, M., 2004: Mykorhizní symbióza. Academia, Praha.
- Halada, E., 1994: Regionálny územný plán ekologickej stability, okres Senica. Regioplán, Nitra.
- Hansen, L., Knudsen, H. (eds.), 1997: Nordic macromycetes, vol. 3, Heterobasidioid, aphyllorphoid and gasteromycetoid basidiomycetes. Nordsvamp, Copenhagen.
- Hansen, L., Knudsen, H. (eds.), 2000: Nordic macromycetes, vol. 1, Ascomycetes. Nordsvamp, Copenhagen.
- Jeppson, M., 2008: The genus *Tulostoma* in Slovakia. Catathelasma, 10: 5-19.
- Jülich, W., 1984: Die Nichtblätterpilze. Gallertpilze und Bauchpilze. Kleine Kryptogamenflora, Bd. II b/1. VEB Gustav Fisher Verlag, Jena.
- Knudsen, H., Vesterholt, J. (eds.), 2008: Funga Nordica. Nordsvamp, Copenhagen.
- Knudsen, H., Taylor, A., 2008: *Xerocomus* Qué. In: Knudsen, H., Vesterholt, J. (eds.), Funga Nordica, p. 174-179, Nordsvamp, Copenhagen.
- Lizoň, P., 2001: Červený zoznam húb Slovenska. In: Baláž, D., Marhold, K., Urban, P. (eds.), Červený zoznam rastlín a živočíchov Slovenska, p. 6-13. Ochr. Prír., Bratislava.
- Kollár, D., Ovečková, J., Ovečková, M., 1996: Slovensko-rakúske Pomoravie. Dajama, Bratislava.
- Škubla, P., 2003: Mycoflora Slovaca. Vlastným nákladom, Šaľa.
- Záhorovská, E., Jančovičová, S., 1997: Mykoflóra alúvia rieky Rudavy. Spravodajca slovenských mykológov, 14: 15-19.

## Abstrakt

Počas dvojročného (2010 – 2011) mykologického výskumu na lokalite Kalaštov pri obci Borský Peter (Záhorská nížina) sme zistili 59 taxónov makromycétov (1 z Ascomycota, 58 z Basidiomycota). V príspevku uvádzame zoznam všetkých zistených makromycétov a doplníme základné ekologické údaje.

**Miroslava Horinková, Soňa Jančovičová: Príspevok k poznaniu makromycétov Záhorskej nížiny**



## EFFECTS OF EXPOSITION TO VARIOUS STRESS CONDITIONS ON FILAMENTOUS GREEN ALGA *KLEBSORMIDIUM* (STREPTOPHYTA) STRAINS, ISOLATED FROM BOTH POLAR REGIONS AND SLOVAKIA

Jana Segečová<sup>1\*</sup>, Josef Elster<sup>2</sup>, Lubomír Kováčik<sup>1</sup>

<sup>1</sup>Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany, Révová 39, 811 02 Bratislava, Slovakia

<sup>2</sup>Academy of Sciences of the Czech Republic, Institute of Botany, 379 82 Třeboň; University of South Bohemia, Faculty of Science, Branišovská 31, 370 05 České Budějovice, Czech Republic

Received 13 August; Received in revised form 20 August; Accepted 24 August

### Abstract

Strains of filamentous green alga *Klebsormidium* (Klebsormidiales, Streptophyta) isolated from distinct geographical regions (Antarctic – South Shetlands, King George Island, Arctic – Ellesmere Island, Svalbard, Central Europe – Slovakia) were experimentally exposed to various stress factors. This genus is one of the most widespread green algal genera found around the world and an interesting candidate for investigations of various stress scenarios. The level of resistance was evaluated by vitality. Strains were generally most resistant to freezing. Lyophilization was the most harmful regime for studied strains. Only Arctic strains LUC 9, LUC 11, LUC 14, strain MON 1 from Slovakia and strain LUC 8 from Antarctica indicated high level of resistance to this stress. On the other hand, two Antarctic strains LUC 4 and LUC 5 indicated low level of resistance to environmental stresses.

**Key words:** *Klebsormidium*, green algae, stress conditions, vitality, Antarctic, Arctic, Slovakia

### Introduction

Algae often occur in extremely hostile environments and this is a reflection of their remarkable ability to tolerate various kinds of stresses. They have to negotiate with low concentrations of essential nutrients in natural waters and low availability of light and temperature on one hand. On the other, they have to survive and grow in habitats enriched with salts, toxic metals and pesticides, elevated temperature, UV-B radiation and light intensity. Algae are the principal primary producers of waterbodies – from a small rain puddle to the big oceans. The tolerance of these organisms to diverse stresses assumes tremendous relevance from an ecological standpoint (Fogg 2001).

Species of the genus *Klebsormidium* represent one of the most widespread groups of aeroterrestrial green algae in the world (Morison et Sheath 1985, Lokhorst 1996, Novis 2006, Škaloud 2006, Rindi et al. 2008, Nagao et al. 2008), ranging in distribution from polar to tropical habitats. *Klebsormidium* is characteristic for its morphological and biological simplicity meaning simple construction of the thallus, unbranched uniseriate filament with undifferentiated cylindrical cells that possess a single parietal plate- or disc-shaped chloroplast. Taxonomy of this genus is a challenge because of the morphological simplicity and probable hidden high cryptic diversity. Species identification in *Klebsormidium* is still a difficult task. Many ultrastructural and molecular studies confirm the position of *Klebsormidium* in the streptophyten lineage along with higher plants (Rindi et al. 2008).

*Klebsormidium* has high ecological importance in various marginal and extreme environments (Elster 1999, Elster 2002, Sluiman 2008, Karsten et al. 2010). For several years the algal ecology from

---

\* Corresponding author: Jana Segečová; [jana.segecova@gmail.com](mailto:jana.segecova@gmail.com)

various habitats in Arctic (Elster et al. 2008), in Antarctic and Central Europe (Uher et al. 2004, 2005, 2006) was studied.

The aim of this study was to assess stress resistance of 12 *Klebsormidium* strains isolated from various habitats: aero-terrestrial (tombstone, gravestone, limestone, green sand), terrestrial (edaphic and lithophytic), hydro-terrestrial (glacial stream periphyton, cryoconite and subglacial river sediment) and special habitats (whale bone remains) in distinct geographical regions (the Antarctic – South Shetlands, King George Island; the Arctic – the Ellesmere Island, Svalbard and Central Europe – Slovakia). It is expected that this study will improve knowledge of susceptibility to various stress factors in the genus *Klebsormidium*.

## Material and methods

A total of 12 unialgal strains of *Klebsormidium* used in this study were collected from a variety of aero-terrestrial and (semi-) aquatic freshwater habitats from Arctic, Antarctic and Slovakia. The collection datas and habitat characteristics are listed in Tab. 1.

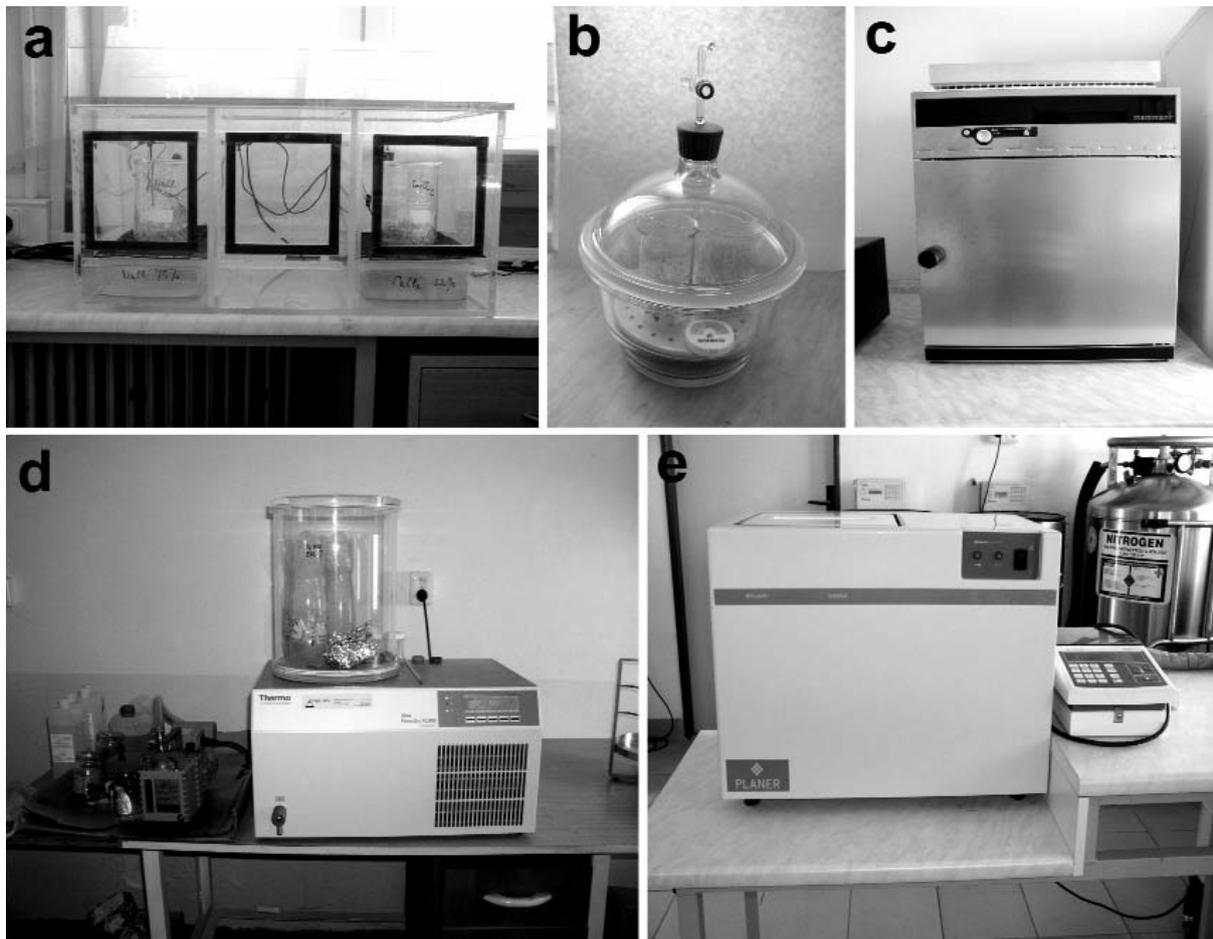
The cultures of these experimental strains were grown (culture of each strain for 1 week and also for 1 month) in Petri dishes on agar plates with medium Z according to Zehnder in Staub (1961) in an air-conditioned cultivation room at temperature ca. 20 °C. Illumination was provided by cool fluorescent lamps (48.6  $\mu\text{mol. m}^{-2}.\text{s}^{-1}$ ) with 12:12 h light-dark cycles. Characteristics of each strain were studied under the JENAMED II (Carl Zeiss Jena) optical microscope. Linear drawings and microphotographs were made by using the Leica DM 2500 optical microscope with Nomarski contrast, Leica DFC 290 HD camera and LAS 3.5.0 software.

**Tab. 1. Localities and habitat characteristics of 12 experimental strains of genus *Klebsormidium***

ACRONYM = STRAIN	LOCALITY, HABITAT
MON 1 = BALÁŽOVÁ 2011/01	Slovakia, Bratislava, St. Martin's Cathedral, green growth on the outer circumferential wall of the presbytery
LUC 1 = UHER 2003/06	Slovakia, Bratislava, cemetery Kozia brana, tombstone of Adolf Orkonyi (1877), neogene calcarenite, shadow and humid
LUC 2 = KOVÁČIK-CHS 1998/13	Slovakia, Bratislava, subterranean jewish cemetery (crypt) Chatam Sofer, gravestone of rabi Hirsch Spira (1727)
LUC 3 = ŠRÁMKOVÁ-GO 5/4	Slovakia, cave Gombasecka, in front of the Herényi Hall – right near steps, closely the lamp, limestone substrata
LUC 4 = JANČUŠOVÁ-ANT 2004/1	Antarctica, King George Island, Admiralty Bay, Crepin Point, green sand near the bird nests
LUC 5 = JANČUŠOVÁ-ANT 2004/2	Antarctica, King George Island, Admiralty Bay, Crepin Point, green sand near the bird nests
LUC 6 = KOVÁČIK- ANT 2003/87a	Antarctica, King George Island, Keller Peninsula, green growth on remainder of hale bone near the seashore
LUC 7 = KOVÁČIK -ANT 2003/87b	Antarctica, King George Island, Keller Peninsula, green growth on remainder of hale bone near the seashore
LUC 8 = KOVÁČIK-ANT 2003/31	Antarctica, King George Island, Keller Peninsula, green growth on the rocks near the seashore
LUC 9 = ELSTER 1991/3	Arctic, Canada, Ellesmere Island, produce dark-green mats in streaming water, these mats were most abundant in glaciál stream close to galcial front, central part of Sverdrup Pass, morfine area, Teardrop Glacier
LUC 11 = KAŠTOVSKÁ 2002/45	Arctic, Svalbard, Ny-Alesund, Austre Broeggerbreen, cryoconite sediment
LUC 14 = KAŠTOVSKÁ 2002/5	Arctic, Svalbard, Ny-Alesund, Vestre Broeggerbreen, cryoconite sediment

A small part of algal growth (ca. 1cm<sup>2</sup>) from each strain culture (one week and one month old culture) was placed on the inner wall of an opened glass tube. Tubes with triplicate algal samples of all

12 strains of *Klebsormidium* sp. were exposed to different stress conditions. Five different devices were employed for carrying out the stress experiments (Fig. 1) for 8 different stresses like, desiccation by saturated NaCl in a desiccator (72 hours, air humidity 75%, temperature 24 °C, state approaching the conditions in nature), desiccation by CaCl<sub>2</sub> in a desiccator (72 hours, air humidity 32%, temperature 24 °C), desiccation by silicagel in a desiccator (72 hours, air humidity 0%, temperature 24 °C, heat shock for one hour in oven (Memmert UNB 400, Schwabach, SRN, temperature 45 °C); heat shock for three hours in drying oven (Memmert UNB 400, Schwabach, SRN, temperature 45 °C); lyophilization (in Lyovac GT2, Leybold-Heraeus, SRN, 48 hours); rapid freezing (in Planer Kryo 10, Planer Inc., Sunbury, UK, 10 minutes, program LEN 4 from 20 °C to -40 °C, speed of freezing 6 °C/min.); slow freezing (in Planer Kryo 10, Planer Inc., Sunbury, UK, 9 hours, program DARIA from +20 °C to -40 °C, speed of freezing +0,1 °C/min.).



**Fig. 1.** Photographs illustrate 5 different devices where 12 *Klebsormidium* strains were exposed to 8 types of environmental stresses; a – desiccator with NaCl and CaCl<sub>2</sub>, b – desiccator with silica gel, c – drying oven, d – Lyovac GT2, e – Planer Kryo 10 (Photo: Segečová)

After exposition to these stress conditions samples were overflowed by Z medium and tubes were closed. Then the vitality of the algal biomass was visually checked, the morphological characteristics of cells in the filaments were microscopically monitored and drawings and microphotographs were finally prepared. Microphotographs of algal filaments stained with DAPI were made by using the Zeiss Axiostar Plus fluorescent microscope with Zeiss Axiocam Cc 3 camera and halogen lamp HBO 50/AC. Filaments for DAPI staining were fixed in 1% of glutaraldehyde. Suspension of filaments was dropped

onto a slide and a drop of DAPI solution was added. After 10 minutes stained preparations were examined in fluorescent microscope.

## Results

After exposure to various stress factors the determination of significance of the differences in vitality (Tab. 2) between different strains of algae *Klebsormidium* (Fig. 2) was made by detailed observations of morphology of strains in an optical microscope.

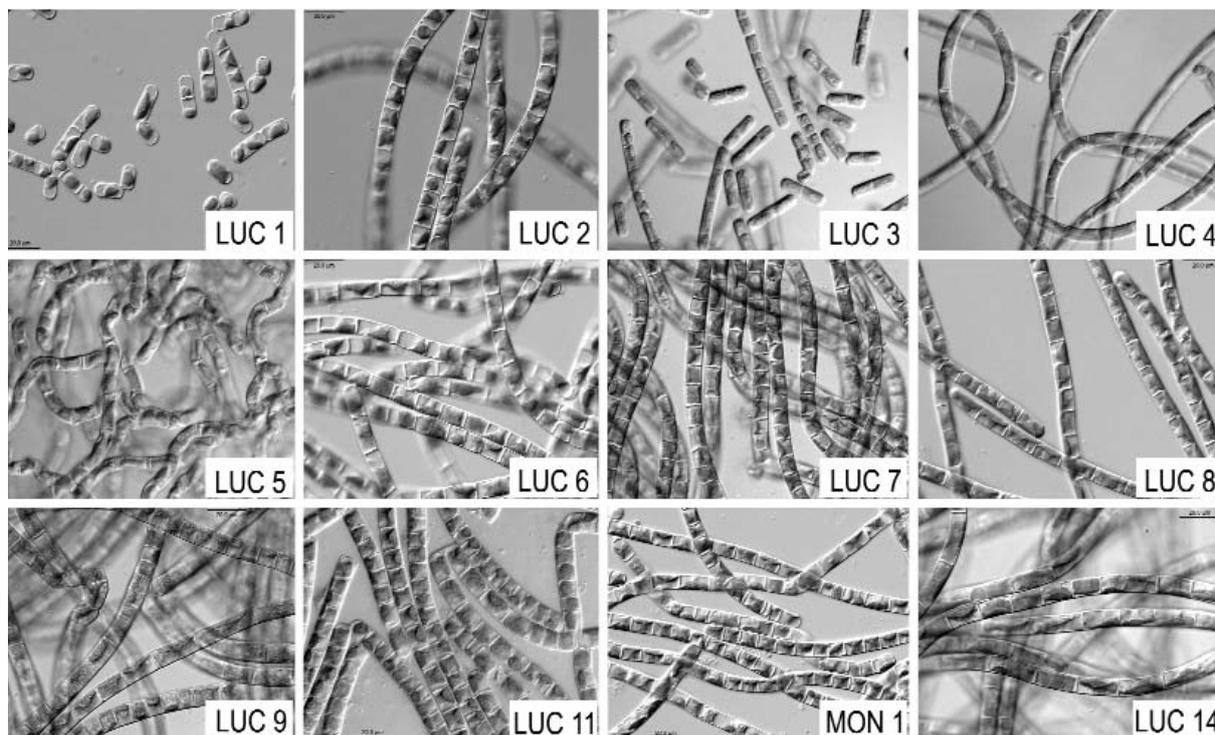


Fig. 2. Morphology of filaments of 12 different *Klebsormidium* strains (Photo: Segečová)

Tab. 2. Differences in vitality after exposure to 8 various environmental stress conditions between 12 different strains of alga *Klebsormidium* sp.

	1. stress	2. stress	3. stress	4. stress	5. stress	6. stress	7. stress	8. stress
MON 1	+	+	+	+	+	-	+	+
MON 1x	+	+	+	+	+	+	+	+
LUC 1	+	+	-	+	-	-	+	+
LUC 1x	+	+	+	+	+	-	+	+
LUC 2	+	+	-	+	-	-	+	+
LUC 2x	+	+	+	+	-	-	+	+
LUC 3	-	-	-	-	-	-	+	+
LUC 3x	+	+	+	+	+	-	+	+
LUC 4	-	-	-	-	-	-	-	-
LUC 4x	+	-	-	+	-	-	+	+
LUC 5	-	-	-	-	-	-	-	-
LUC 5x	+	+	+	+	-	-	+	+
LUC 6	+	+	+	+	+	-	+	+

Tab. 2. Continuation

	1. stress	2. stress	3. stress	4. stress	5. stress	6. stress	7. stress	8. stress
LUC 6x	+	+	+	+	+	-	+	+
LUC 7	+	+	-	+	+	-	+	+
LUC 7x	+	+	-	+	+	-	+	+
LUC 8	+	+	+	+	+	-	+	+
LUC 8x	+	+	+	+	+	+	+	+
LUC 9	+	+	+	+	+	+	+	+
LUC 9x	+	+	+	+	+	+	+	+
LUC 11	+	-	-	+	-	-	+	+
LUC 11x	+	+	+	+	+	+	+	+
LUC 14	+	+	+	+	+	-	+	+
LUC 14x	+	+	+	+	+	+	+	+

+ = recovery of strain after exposure to stress factor

- = no recovery of strain after exposure to stress factor

strain with indication x = one week old strain culture of genus *Klebsormidium* exposed to stress factor

strain without indication x = one month old strain culture of genus *Klebsormidium* exposed to stress factor

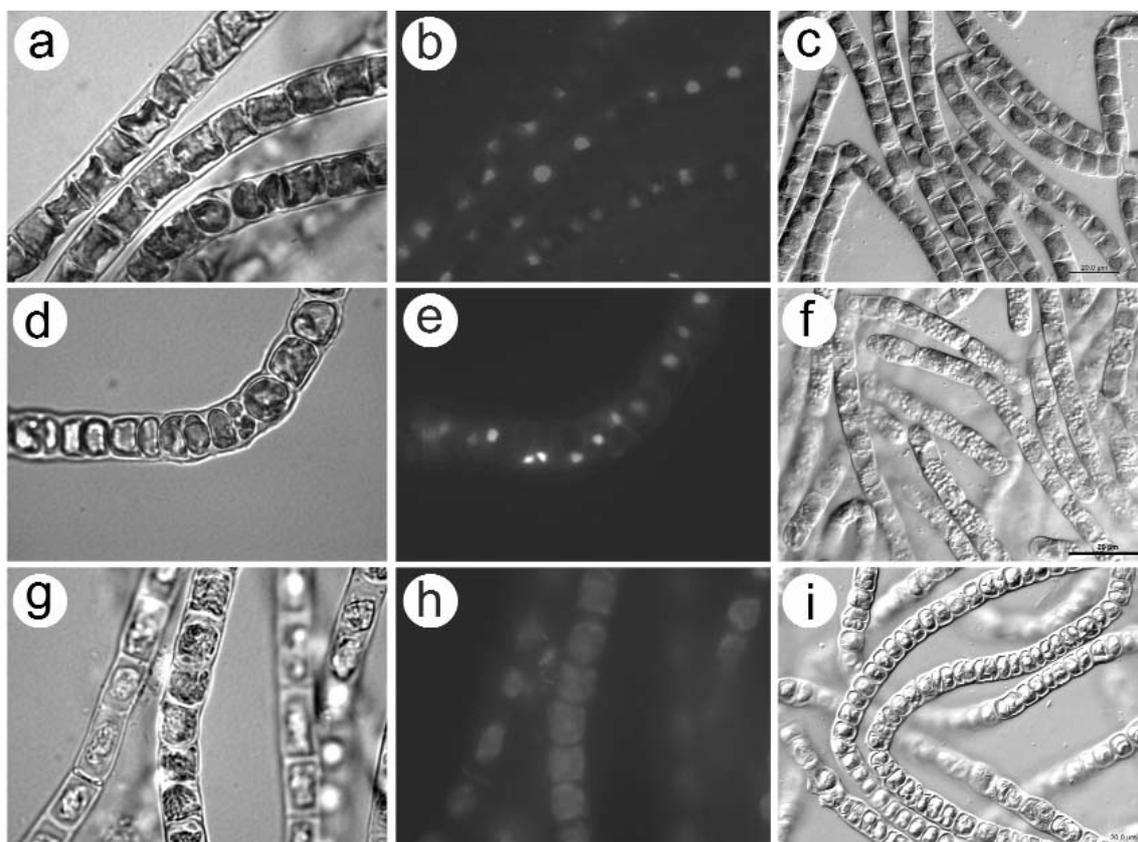


Fig. 3. Characters of the *Klebsormidium* filaments – strain LUC 11 in relation to the culture age; a – detail of one week filament, b – DAPI staining of one week filament, c – clump of one week filaments, d – detail of one month filament, cells filled with lipid globules, e – DAPI staining of one month filament, f – clump of one month filaments, g – detail of dead three month filament, h – DAPI staining of dead three month filament, i – clump of three month filaments

The most resistant strains to 8 environmental stress conditions were strain MON 1 from Slovakia (one week culture), LUC 8 from Antarctica (one week culture), LUC 9 from Arctic (one week and one month culture), LUC 11 from Arctic (one week culture) and LUC 14 from Arctic (one week culture).

At least resistant strains were LUC 4 from Antarctica (one month culture) and LUC 5 from Antarctica (one month culture). Slow and rapid freezing represent stress factors to which strains of *Klebsormidium* were generally most resistant. Exposure of strains to lyophilization proved to be the most drastic of all the stress factors and the majority of 12 *Klebsormidium* strain cultures did not survive except for the strain MON 1 from Slovakia (one week culture), LUC 8 from Antarctica (one week culture), LUC 9 from Arctic (one week culture), LUC 9 from Arctic (one month culture), LUC 11 from Arctic (one week culture) and LUC 14 from Arctic (one week culture). Figure 3 illustrates the habit of LUC 11 strain isolated from Arctic, its filament morphology and its DAPI staining in relation to the culture age.

## Discussion

Many algae are adapted to live at temperatures which are extremely high or low by human standards. Stress may occur when the temperature is suddenly changed. The multifarious biochemical processes making up the metabolic network in an organism are affected differently by temperature change so that the pattern is distorted to a greater or lesser extent – individual enzymes may be inactivated or increased in activity, metabolites degraded or altered in concentration, and vital structures such as cell membranes become altered in conformation. So cold stress and desiccation stress strongly influence metabolic processes in the cells (Elster et Benson 2004, Fogg 2001). There is considerable scope for adjustment and the organism may be able to adapt to the new temperature within a short time. If it cannot, then it functions less efficiently, is stressed, and may die if the distortion is extreme. Stress occurs with a drastic change in temperature, availability of sunlight, the tide retreating or advancing, frost or snow (Peschek et Zoder 2001).

The level of survival capacity varies among individual strains or species and depends on the phylogeny and origin of the organism (Elster et Benson 2004). One of the most important stress factors affecting the composition of polar wetland communities is the freezing and desiccation period, connected with water availability (Elster et Benson 2004). Studies based on field or laboratory experiments have shown that some algae are able to tolerate prolonged periods of desiccation (Jacob et al. 1992) and also freezing (Hawes 1990). One early study investigated desiccation responses in *Klebsormidium rivulare* (Morison et Sheath 1985). These authors reported that long-term desiccated cells had a drastically changed appearance, disintegrated organelles, and the cell lumen filled with lipid globules and starch granules in four-week field-desiccated samples. Gray et al. (2007) demonstrated the ability of desert green algae to survive four weeks of desiccation. A recent study has described the eco-physiological effects of desiccation in *Klebsormidium crenulatum* (Karsten et al. 2010). Organisms have also adapted to extremes through a variety of overwintering strategies that range from physiological changes to alterations in geographical distributions (Cockell 2000, Thomas et al. 2008). At temperatures less than or equal to  $-40\text{ }^{\circ}\text{C}$  homogenous ice nucleation occurs and all the water content of the cell freezes (Elster et Benson 2004).

Freezing and desiccation injury resistance have been reported for various *Klebsormidium* isolates from Antarctic, Arctic and Slovakia (Elster et al. 2008). In their experiments a cooling speed of  $4\text{ }^{\circ}\text{C}/\text{min}$ . was chosen. This is on the boundary between vitrification and crystallization. Crystallisation in cell is the arrangement of liquid water molecules into orderly ice structures. The formation of ice destroys membranes, particularly if the crystals are formed intracellularly. Crystallisation is a slow process, which is very common in nature. Vitrification is a process of solidification of the cellular contents into a non-crystalline (amorphous) state. It is a result of rapid freezing (greater than  $3\text{ }^{\circ}\text{C}/\text{min}$ .) which does not occur in nature (Luyet 1966, Fuller 2004).

The strain from a shallow wetland (hydro-terrestrial) habitat at Ellesmere Island (Canadian Arctic, LUC 9) was highly freeze-desiccation stress resistant. On the contrary Arctic strain LUC 11 from soil habitats in Svalbard stood out by its very low freeze-desiccation resistance (Elster et al. 2008).

In our experiments most of the strains survived the freezing at  $-40\text{ }^{\circ}\text{C}$  (at a speed of  $4\text{ }^{\circ}\text{C}/\text{min}$ ) which was not fatal for most of strains. Our types of desiccation stresses were injurious for many

strains. Strain LUC 9 isolated from Arctic was the most resistant *Klebsormidium* strain. Also in another study (Šabacká et al. 2006), desiccation was more injurious than freezing for green algae that showed high mortality.

*Klebsormidium* strains originating from various different geographical zones and from various habitats have similar response to freezing and desiccation stresses and they are particularly resistant to freezing and desiccation injuries.

## Acknowledgements

The study was supported by VEGA grant No. 1/0868/11, by UK grant No. 46/2012 and by The Grant Ministry of Education of the Czech Republic LM 2010009 and Kontakt ME 934. The authors thank the reviewer for comments on this manuscript.

## References

- Cockell, C. S., Stokes M. D., Korsmeyer K. E., 2000: Overwintering strategies of Antarctic organisms. *Environ. Rev.*, 8: 1-19.
- Elster, J., 1999: Algal versatility in various extreme environments. In: Seckbach, J., (ed.), *Enigmatic microorganisms and life in extreme environments*, p. 215-227, Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Elster, J., 2002: Ecological classification of terrestrial algal communities of polar environment. In: Beyer, L., Boelter, M. (eds.), *Geocology of Terrestrial Oases Ecological Studies*, p. 303-319, Springer-Verlag, Berlin, Heidelberg.
- Elster, J., Benson, E., 2004: Life in the polar terrestrial environment with a focus on algae and cyanobacteria. In: Fuller B., Lane N. & Benson E. (eds.), *Life in the Frozen State*. Taylor and Francis Book, CRC Press LLC, Boca Raton, USA, 111-150 pp.
- Elster, J., Degma, P., Kováčik, L., Valentová, L., Šramková, K., Batista Pereira, A., 2008: Freezing and desiccation injury resistance in the filamentous green alga *Klebsormidium* from the Antarctic, Arctic and Slovakia. *Biologia*, 63: 843-851.
- Fogg, G. E., 2001: Algal adaptation to stress – some general remarks. In: Rai, L. C., Gaur, J. P. (eds.), *Algal adaptation to environmental stresses. Physiological, biochemical and molecular mechanisms*, p. 1-20, Springer-Verlag, Berlin Heidelberg New York.
- Fuller, B. J., 2004: Cryoprotectants: The essential antifreezes to protect life in the frozen state. *CryoLetters* 25: 375-388.
- Gray, D. W., Lewis, L. A., Cardon, Z. G., 2007: Photosynthetic recovery following desiccation of desert green algae (Chlorophyta) and their aquatic relatives. *Plant Cell Environ.*, 30: 1240-1255.
- Hawes, I., 1990: Effect of freezing and thawing on a species of *Zygnema* (Chlorophyta) from the Antarctic. *Phycologia*, 29: 326-331.
- Jacob, A., Wiencke, C., Lehmann, H., Krist, G. O., 1992: Physiology and ultrastructure of desiccation in the green alga *Prasiola crispa* from Antarctica. *Bot. Mar.*, 35: 297-303.
- Karsten, U., Lütz, C., Holzinger, A., 2010: Ecophysiological performance of the aeroterrestrial green alga *Klebsormidium crenulatum* (Klebsormidiophyceae, Streptophyta) isolated from an alpine soil crust with an emphasis on desiccation stress. *J. Phycol.*, 46: 1187-1197.
- Lokhorst, G. M., 1996: Comparative taxonomic studies on the genus *Klebsormidium* (Charophyceae) in Europe. *Crypt. Studies*, 5: 1-132
- Luyet, B. J., 1966: Anatomy of the freezing process in physical systems, pp. 115–138. In: Meryman H. T. (ed.), *Cryobiology*, Academic Press, New York.
- Morison, M. O., Sheath, R. G., 1985: Responses to desiccation stress by *Klebsormidium rivulare* (Ulotrichales, Chlorophyta) from a Rhode Island stream. *Phycologia*, 24: 129-145.
- Nagao, M., Matsui, K., Uemura, M., 2008: *Klebsormidium flaccidum*, a charophyceean green alga exhibits cold acclimation that is closely associated with compatible solute accumulation and ultrastructural changes. *Plant, Cell and Environment*, 31: 872-885.
- Novis, P. M., 2006: Taxonomy of *Klebsormidium* (Klebsormidiales, Charophyceae) in New Zealand streams and the significance of low-pH habitats. *Phycologia*, 45: 293-301.
- Peschek, G. A., Zoder, R., 2001: Temperature stress and basic bioenergetic strategies for stress defence. In: Rai, L. C., Gaur, J. P. (eds.), *Algal adaptation to environmental stresses. Physiological, biochemical and molecular mechanisms*, p. 203-258, Springer-Verlag, Berlin Heidelberg New York.
- Rindi, F., Guiry, M. D., López-Bautista, J. M., 2008: Distribution, morphology, and phylogeny of *Klebsormidium* (Klebsormidiales, Charophyceae) in urban environment in Europe. *J. Phycol.*, 44: 1529-1540.
- Šabacká, M., Elster, J., 2006: Response of cyanobacteria and algae from Antarctic wetland habitats to freezing and desiccation stress. *Polar Biol.*, 30: 31-37.

- Škaloud, P.**, 2006: Variation and taxonomic significance of some morphological features in European strains of *Klebsormidium* (Klebsormidiophyceae, Streptophyta). *Nova Hedwigia*, 83: 533-550.
- Sluiman, H. J., Guihal, C., Mudimu, O.**, 2008: Assessing phylogenetic affinities and species delimitations in Klebsormidiales (Streptophyta): Nuclear-encoded rDNA phylogenies and ITS secondary structure models in *Klebsormidium*, *Hormidiella*, and *Entransia*. *J. Phycol.*, 44: 183-195.
- Staub, R.**, 1961: Ernährungsphysiologisch-autökologische Untersuchungen an der planktischen Blaualge *Oscillatoria rubescens* DC. *Schweiz. Z. Hydrol.*, 23: 82-198.
- Thomas, D. N., Fogg, G. E., Convey, P., Fritsen, C. H., Gili, J. M., Gradinger, R., Laybourn-Parry, J., Reid, K., Walton, D. W. H.**, 2008: *The Biology of Polar Regions*. Oxford University Press Inc., 1-394.
- Uher, B., Aboal, M., Kováčik, L.**, 2004: Cyanobaktérie a riasy na monumentoch a budovách v regióne Murcia (Španielsko) [Cyanobacteria and algae on monuments and buildings in Region of Murcia (Spain)]. *Bull. Slov. Bot. Spoločn.*, Bratislava, Suppl. 10: 77-82.
- Uher, B., Kováčik, L., Degma, P., Vozárová, A.**, 2006: Distribúcia cyanobaktérií a rias na stavebnom kameni Presbytéria Dómu sv. Martina v Bratislave (Distribution of cyanobacteria and algae on building stones of Presbyterium of the St. Martin's Cathedral in Bratislava). *Bull. Slov. Bot. Spoločn.*, Bratislava, 28: 11-20.
- Uher, B., Kováčik, L., Kučera, P., Hindáková, A., Pivko, D.**, 2005: Cyanobaktérie a riasy na kamenných substrátoch objektov kultúrno-historického významu v Bratislave (Cyanobacteria and algae on stone of cultural heritage in Bratislava). *Bull. Slov. Bot. Spoločn.*, Bratislava 27: 11-16.

## Abstrakt

Kmeňové kultúry vláknitej zelenej riasy *Klebsormidium* (Klebsormidiales, Streptophyta) izolovaných z odlišných geografických oblastí (Antarktída – Južné Shetlandy, Ostrov kráľa Juraja, Arktída – Ostrov Ellesmere, Svalbard, Stredná Európa – Slovensko) boli podrobené pôsobeniu rôznych stresových faktorov. Tento rod je jedným z najrozšírenejších rodov zelených rias na svete a zaujímavý kandidát na výskum vplyvu rôznych stresov na riasy. Miera rezistencie voči stresu bola hodnotená pomocou určenia vitality. Všeobecne boli kmene najviac odolné voči zmrazovaniu. Lyofilizácia bola pre študované kmene najdrastickejšia stresová podmienka. Len arktické kmene LUC 9, LUC 11, LUC 14, kmeň MON 1 zo Slovenska a kmeň LUC 8 z Antarktídy boli odolné voči pôsobeniu lyofilizácie. Naopak, dva antarktické kmene LUC 4 a LUC 5 vykazovali najnižšiu mieru rezistencie voči environmentálnym stresom.

**Jana Segečová, Josef Elster, Ľubomír Kováčik: Vplyv rôznych stresových podmienok na kmene vláknitej zelenej riasy *Klebsormidium* (Streptophyta) izolované z oboch polárnych oblastí a zo Slovenska**

## PHENOTYPIC CHARACTERISATION OF SOME GREEN ALGAE ISOLATED TO LABORATORY CULTURE FROM SOME WATER BODIES OF HIGH TATRA MOUNTAINS, SLOVAKIA

Sudipta Kumar Das<sup>1,2</sup>, Lubomír Kováčik<sup>1\*</sup>

<sup>1</sup>Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany, Révová 39, 811 02 Bratislava, Slovakia

<sup>2</sup>Utkal University, P.G. Department of Botany, Bhubaneswar, 751004 Odisha, India

Received 21 August; Received in revised form 28 August; Accepted 31 August

### Abstract

Seven green algal species including six coccal algae and one filamentous form (under family Zygnemataceae) were isolated to laboratory culture from 6 different water bodies like, pool, pond and lakes from the High Tatra Mts. region. Their detailed morphology were studied while in culture and documented for the first time from this region.

**Key words:** Phenotypic characterization, Green algae, Laboratory cultures, High Tatra Mts.

### Introduction

The High Tatras Mts. (Vysoké Tatry) are the highest mountains in Slovakia, situated along the Slovak-Polish border at 20° 10' E and 49° 10' N. It is the central part of the Carpathian mountain ranges, consisting of compactly arranged mountain peaks stretched up to 27 kilometers, which are mostly granite rock beds. The freshwater of this region includes small to large mountain lakes (tarns/pleso) of glacial origin, small polls in the inundated zones of the lakes, creeks and waterfalls. Being at an altitude between 1089 to 2189 m a. s. l. these provide a suitable ambience for sub-alpine to alpine vegetation.

Phyco diversity study of this region was started since the middle of last century though emphasized more on the lakes in comparison to other habitats (lakes cover 80 % of the freshwater habitats of the region). Diversity of phytoplankton along with the ecohydrology of the lakes were studied by Juriš (1964a, Popradské pleso); Ertl et al. (1965, Velké and Malé Hincovo pleso); Juriš, Kováčik (1987, 44 lakes); Lukavský (1994, 87 lakes); Fott et al (1999, Nižné Terianske and Starolesnianske pleso) and Nedbalová et al. (2006, Ľadové pleso). During this period several taxonomic reports of new and distinguished taxa, like *Kephyriopsis tatrica* Juriš (Juriš 1964b), *Binuclearia tectorum* (Kütz.) Berger ex Wichmann (Lukavský 1970), *Scytonematopsis starmachii* Kováčik et Komárek (Kováčik, Komárek 1988), *Cyclotella planctonica* Brunthaler (Houk 1991) and *Discostella tatrica* Procházková et al (Procházková et al. 2012) were also made. More detailed documentation of specific groups of algae like desmids (Ružička 1959), dinoflagellates (Popovský 1967) and diatoms (Štefková 2006) from lakes as well as creeks were published. A check list of all the algal species reported from High Tatra Mts. were published by Hindák, Kováčik (1993).

In this work, detailed morphometric analysis of seven green algae (including six coccal and one filamentous), which were isolated to laboratory cultures from lakes and pools were presented which are not reported earlier from this region.

---

\* Corresponding Author: Lubomír Kováčik; [kovacik@fns.uniba.sk](mailto:kovacik@fns.uniba.sk)

## Materials and methods

Sampling of phytoplankton was carried out in a pond in Tatranská Štrba village, two alpine lakes, i.e. Zelené Javorové pleso and Štrbské pleso along with a pool and a peat bog from Zlomisková and Mlynická valley regions respectively, with the help of plankton net of 10 µm pore size. Epilithic algal mats were also collected from the submerged rock surface between Vyšné and Nižné Temno-smrečianske pleso. After brought to the laboratory in a cool ice chest, the algae were isolated and purified through serial dilution method and the pure cultures were grown in Petri dishes on agar plates as well as in test tubes in liquid Z medium according to Zehnder in Staub (1961) in an air-conditioned culture room with 20 °C temperature. Illumination up to 48.6 µmol. m<sup>-2</sup>.s<sup>-2</sup> was provided by cool fluorescent lamps. The phenotypic characteristics were studied in regular time intervals at different growth phases of the algae. Linear drawings and microphotography were made using the Leica DM 2500 optical microscope with Nomarski contrast, fitted with a Leica DFC 290 HD camera and operated by LAS 3.5.0 software. Species determination was done referring standard monographs.

All the strains were deposited at the Culture Collection of Algae at the Laboratory of Algology (CCALA), Třeboň, Czech Republic.

## Results

A total of seven algal species were isolated from the water bodies of High Tatra Mts. including six species of coccal green algae and one species of filamentous green algae under family Zygnemataceae. Though green algae (Chlorophytes) are the dominant group of algae in earlier published records from this region, the following seven species are a significant contribution to the flora being recorded for the first time.

### *Chlorolunula* sp. (Fig. 1a, 3a)

Cells solitary, rarely form groups in natural materials, but not in culture, spherical to slightly ovoid in shape, 7–9 µm in diameter, chloroplast parietal, cup shaped in mature cells but entire in younger ones, with one pyrenoid, asexual reproduction is by autospores, 2 or 4 autospores in each sporangium, released by fracture in the wall, freshly released autospores appear crescent shaped. • Isolated from the phytoplankton of a pool near Zlomisková valley (CCALA 298).

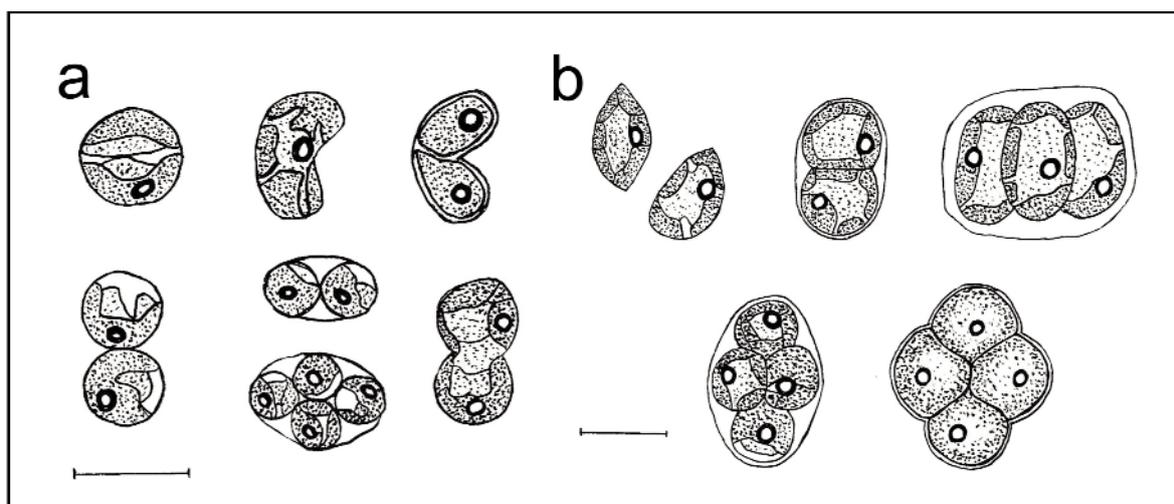


Fig. 1. Linear drawings of (a) *Chlorolunula* sp. and (b) *Enallax* sp. (Scales for all figures are 10 µm)

***Enallax* sp. (Fig. 1b, 3b)**

Cells broadly fusiform to ellipsoidal, coenobia is not seen in cultures, though 4 celled coenobium was found in nature, 12–14  $\mu\text{m}$  long and 7–8  $\mu\text{m}$  broad, ends slightly acute, cellwall is thicker, chloroplast parietal plate like with one large pyrenoid, reproduction is by autospores, 2–4 in each sporangium, released by unequal disruption of the wall. • Isolated from the phytoplankton of a peat bog near Mlynická valley (CCALA 343).

***Desmodesmus* cf. *corallinus* Chodat (Fig. 2a, 3c)**

Coenobia is 2–4 celled, linearly arranged along the length of the cell, cylindrical cells, sometimes slightly oval when mature, with obtuse tip, outer cells are slightly convex inwardly to

give a ‘closely packed’ appearance of the cells in the coenobium, 11–13  $\mu\text{m}$  long and 2.5–3.5  $\mu\text{m}$  broad, cell contents granular, with vacuolated spaces at the poles as a distinctive feature, outer cells have short stout spines at each poles, spineless cells are also found rarely, spines are 1.5–2  $\mu\text{m}$  long. • Isolated from the phytoplankton of a pool near Zlomisková valley (CCALA 442).

***Desmodesmus* cf. *gutwinski* Chodat (Fig. 2b, 3d)**

Coenobia 2–4 celled, linearly arranged, cells are longitudinally oval, rounded to slightly pointed tips, outer cells sometimes convex in the median part, 7–10  $\mu\text{m}$  long and 3–4  $\mu\text{m}$  broad, short caudate spines are present on each pole of the cells, 1–3 lateral spines are also present in the outer cells, spines are 1–1.5  $\mu\text{m}$  long. • Isolated from the phytoplankton of a pond in Tatranská Štrba village (CCALA 448).

***Desmodesmus* cf. *spinosus* (Chodat) Hegewald (Fig. 2c, 3e)**

Coenobia 2–4 celled, arranged parallel length wise, cells broadly elliptical when young and fusiform when mature, ends slightly rotundous to bluntly conical, outer cells sometimes convex, 10–12  $\mu\text{m}$  long and 2.5–5  $\mu\text{m}$  broad, short spines are present at each pole of the cells, spines on the inner cells are shorter than the outer cells, outer cells rarely have one lateral spine, spines are 1–2  $\mu\text{m}$  long. • Isolated from the phytoplankton of Zelené Javorové pleso (CCALA 466).

***Willea* sp. (Fig. 2d, 3f)**

Coenobia 4 celled, flat, cells arranged in one plane with a rhomboid opening at the centre, Cells are ovoid to broadly elliptical, 9–12  $\mu\text{m}$  long and 6–8  $\mu\text{m}$  broad, chloroplast parietal, with one pyrenoid, reproduction by autospores, 2 in each sporangium embedded in the mother cell wall, divisions are always in perpendicular to the mother cell axis. • Isolated from the phytoplankton of Štrbské pleso (CCALA 515).

***Klebsormidium* sp. (Fig. 2e, 3g, 3h)**

Filaments long when young and short when mature, cells cylindrical barrel shaped, 4–10  $\mu\text{m}$  long and 6–8  $\mu\text{m}$  wide, with thick and stratified cell walls in mature cells, chloroplasts one, covers two third part of the cell, sometimes entire, with one pyrenoid, H – shaped segments occasionally found. • Isolated growing on submerged stone surface between Vyšné and Nižné Temnosmrečianske pleso (CCALA 277).

## **Acknowledgement**

This work has been supported by a scholarship awarded to S.K.D. by the National Scholarship Programme of the Slovak Republic (SAIA) for mobility from Utkal University, Bhubaneswar, India to carry out the research at Slovakia. The authors are also thankful to the reviewer for his valuable and necessary suggestions.

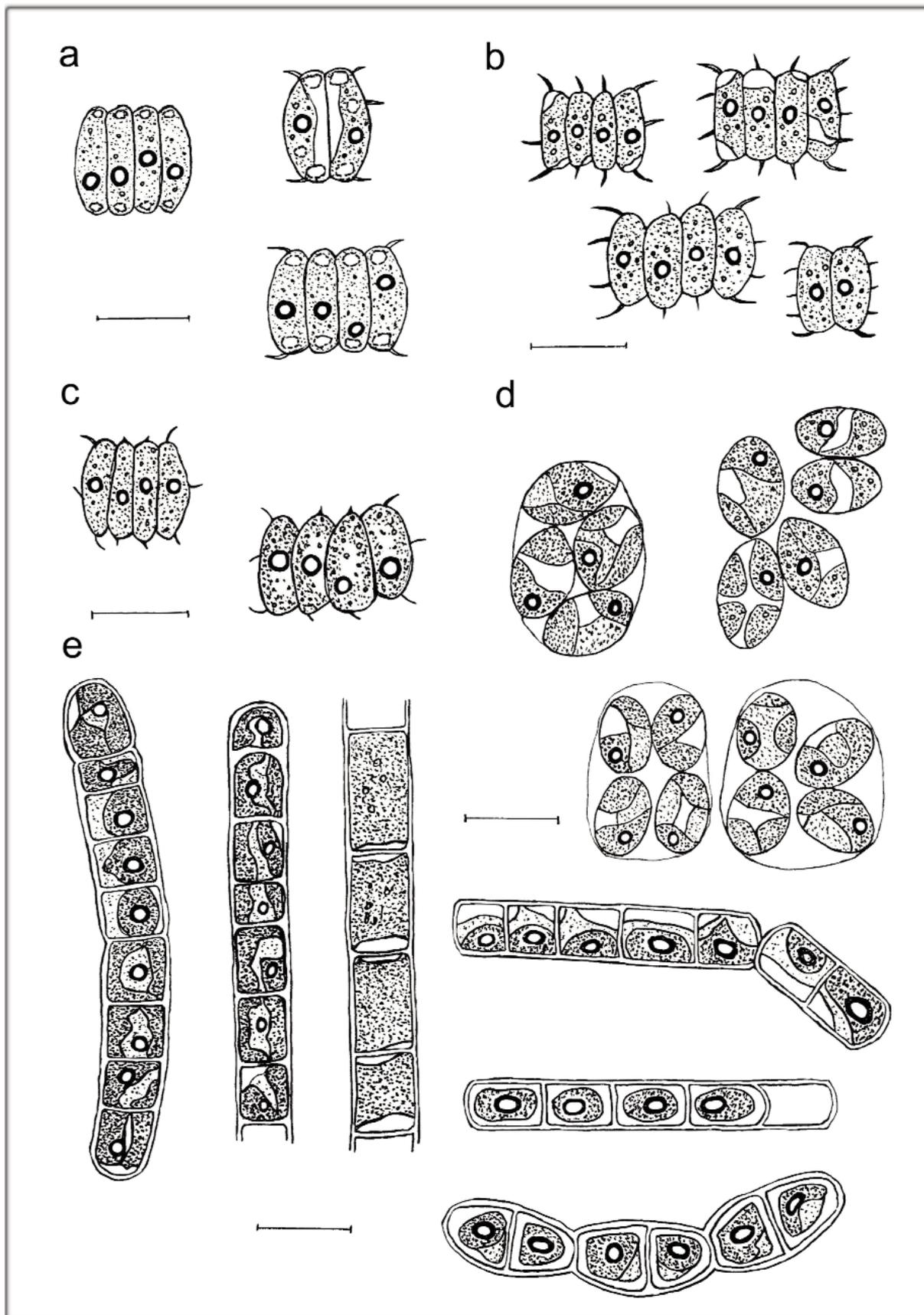


Fig. 2. Linear drawings of (a) *Desmodesmus* cf. *corallinus*, (b) *Desmodesmus* cf. *gutwinskii*, (c) *Desmodesmus* cf. *spinus*, (d) *Willea* sp., (e) *Klebsormidium* sp. (Scales for all figures are 10  $\mu$ m)

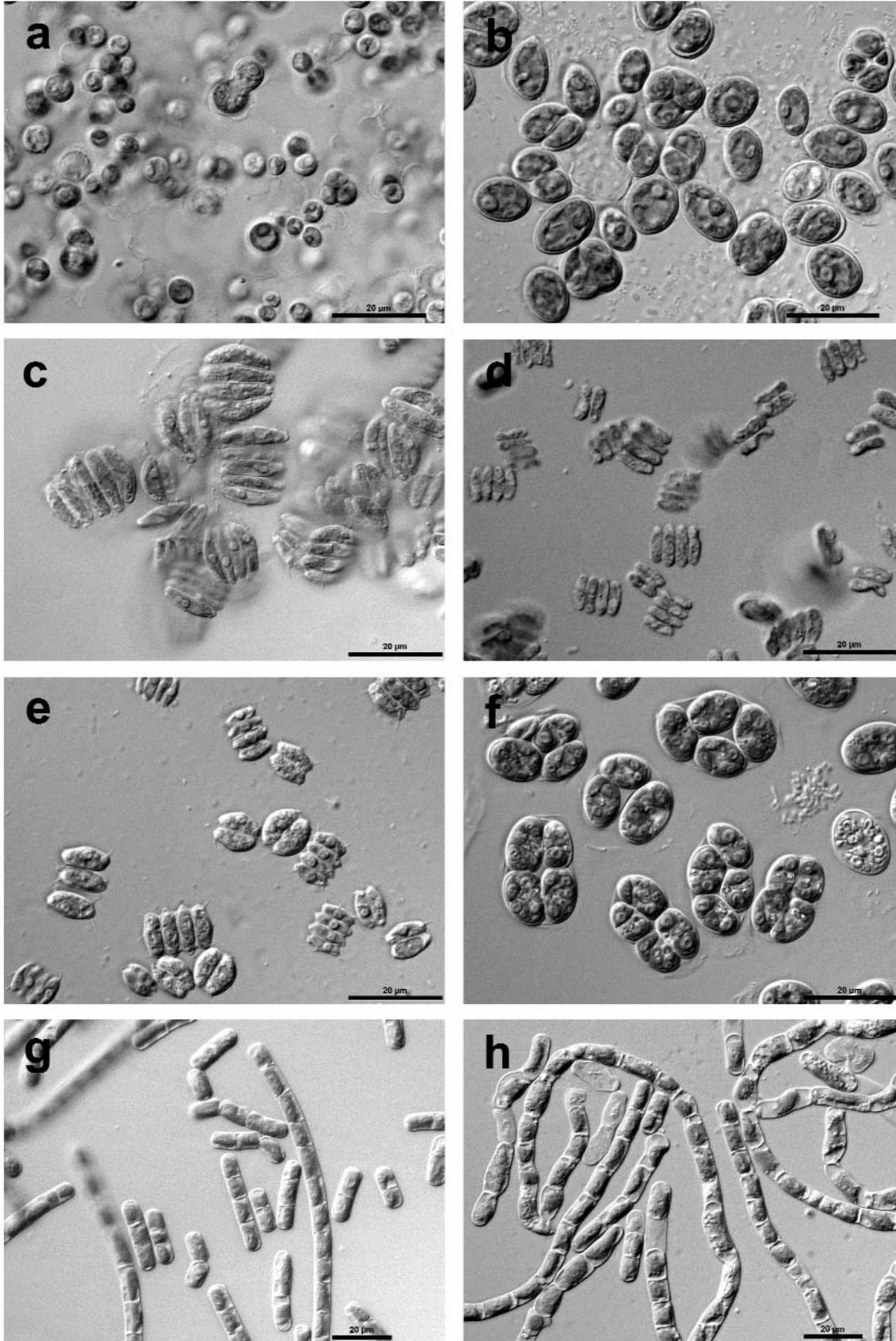


Fig. 3. Microphotographs of (a) *Chlorolunula* sp., (b) *Enallax* sp., (c) *Desmodesmus* cf. *corallinus*, (d) *Desmodesmus* cf. *gutwinskii*, (e) *Desmodesmus* cf. *spinosus*, (f) *Willea* sp., (g, h) young and mature filaments of *Klebsormidium* sp. (Scales for all figures are 20 µm)

## References

- Ertl, M., Juriš, Š., Vranovský, M., 1965: K poznaniu planktónu Veľkého a Malého Hincovho plesa. Sborn. TANAP, 8: 57-69.
- Fott, J., Blažo, M., Stuchlík, E., Strunecký, O., 1999: Phytoplankton in three Tatra Mountain lakes of different acidification status. J. Limno., 58 (2): 107-116.
- Hindák, F., Kováčik, E., 1993: Supis siníc a rias Tatranského Národného Parku. Zborn. TANAP, 33: 235-279.
- Houk, V., 1991: The morphology and taxonomic relationships of *Cyclotella planctonica* Brunthaler (Bacillariophyceae). Algal. Stud., 61: 103-118.
- Juriš, Š., 1964a: Fytoplanktón Popradského plesa. Biologia, 19: 690-704.
- Juriš, Š., 1964b: *Kephyriopsis tatrica* sp. nov.. Phycologia, 4 (1): 52-53.
- Juriš, Š., Kováčik, E., 1987: Príspevok k poznaniu fytoplanktónu jazier vo Vysokých Tatrách. Zbor. Slov. nár. Muz. Prír. Vedy, Bratislava, 33: 23-40.
- Kováčik, E., Komárek, J., 1988: *Scytonematopsis starmachii*, a new cyanophyte species from the High Tatra Mts. (Czechoslovakia). Alogol. Stud., 80: 303-314.
- Lukavský, J., 1970: Morphological variability and reproduction of the alga *Binuclearia tectorum* under natural conditions. Nova Hedw., 19: 189-199.
- Lukavský, J., 1994: Algal flora of lakes in the High Tatra Mountains (Slovakia). Hydrobiologia, 274: 65-74.
- Nedbalová, L., Stuchlík, E., Strunecký, O., 2006: Phytoplankton of a mountain lake (Ladove pleso, the Tatra Mountains, Slovakia): Seasonal development and first indications of a response to decreased acid deposition. Biologia, 61: 91-100.
- Popovský, J., 1967: Einige Dinoflagellaten des Tatra National parks. Sborn. TANAP, 10: 269-276.
- Procházková, L., Houk, V., Nedbalová, L., 2012: *Discostella tatrica* sp. nov. (Bacillariophyceae) – A small centric diatoms from the Tatra Mountain lakes (Slovakia/Poland). Fottea, 12: 1-12.
- Ružička, J., 1959: Krásivky kotliny Siedmich premenov (Belanské Tatry). TANAP, 3: 74-84.
- Staub, R., 1961: Ernährungsphysiologisch-autökologische Untersuchungen an der planktischen Blaualge *Oscillatoria rubescens* DC. Schweiz. Z. Hydrol., 23: 82-198a.
- Štefková, E., 2006: Epilithic diatoms of the mountain lakes of the Tatra Mountains (Slovak). Biologia, 61: 101-108.

## Abstrakt

Sedem druhov zelených rias, z toho šesť kokálnych a jedna vláknitá z čelade Zygnemataceae boli izolované do laboratórnych kultúr a pochádzali zo šiestich rôznych lokalít na území Vysokých Tatier. Preštudovala sa ich detailná morfológia a po prvýkrát bol zdokumentovaný ich výskyt na tomto území.

**Sudipta Kumar Das, Ľubomír Kováčik: Fenotypová charakteristika niekoľkých zelených rias izolovaných do laboratórnych kultúr z rôznych vôd vo Vysokých Tatrách, Slovensko**

## BRYOPHYTES ON THE CEMETERIES IN THE CITY OF ŽILINA (SLOVAKIA)

Katarína Mišíková\*, Jana Cibulková

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany, Révová 39,  
811 02 Bratislava, Slovakia

Received 11 September 2012; Received in revised form 18 September; Accepted 21 September

### Abstract

The contribution gives a brief overview on bryophytes from four selected cemeteries in the downtown and outskirts of the city Žilina. Overall, 46 bryophytes were found - 1 liverwort (Marchantiophyta) and 45 mosses (Bryophyta). In terms of ecological groups, the most abundant are epigeic species growing on slightly shaded lawns and wet bare soil, and epilithic species on partially shaded stone substrates. Size and microclimatic environmental conditions have probably greatest impact on the species diversity of each cemetery.

**Key words:** Urban green areas, cemetery, ecology, Bryophyta, Marchantiophyta

### Introduction

Cemeteries as anthropogenic habitats are characterized by a strong impact of anthropogenic influences, however, distinctive is the reach habitat heterogeneity of urban green areas related with higher bryophyte diversity in comparison with many other man-made habitats (Hohenwalner, Zechmeister 2001; Janovicová et al. 2003; Fudali 2005; Mišíková et al. 2007; Smith et al. 2010).

Bryophytes of the green spaces in sub- and urban areas in Europe are not well studied. The presented paper provides the first information on bryophytes in the area of Žilina, one of the largest cities in the Slovak Republic. The aim of this study is to give a basic overview of the bryophyte diversity on selected cemeteries in the downtown and outskirts of Žilina, together with a basic ecological analysis.

An overview of bryological works in Slovakia and in Europe, dealing with the topic of cemeteries and green spaces in urban areas, is given by Mišíková, Kubinská (2010).

### Material and Methods

Bryophytes were surveyed in a period between April and October 2010, sites were selected in the city center (Old and New cemetery Žilina) well as in the outskirts (Višňové, Bánová) (Fig. 1). Brief description of the cemeteries studied is given in Table 1. Samples found colonising any substrate were collected into paper envelopes and later identified. Substrates included stone surfaces (e.g. graveyards, paths, walls), vegetated land covers (e.g. lawns), living trees and decayed wood. Herbarium specimens are deposited in the SLO herbarium (Comenius University, Faculty of Natural Sciences, Department of Botany). Nomenclature of bryophyte taxa follows Söderström et al. (2002) and Hill et al. (2006) that of vascular plants Marhold et al. (1998). Taxa *Bryum erythrocarpum* agg. and *Schistidium apocarpum* agg. was not possible to identify exactly therefore they are mentioned as aggregate species.

---

\* Corresponding Author: Katarína Mišíková; [katarina.misikova@fns.uniba.sk](mailto:katarina.misikova@fns.uniba.sk)

Tab. 1. Brief comparison of the observed cemeteries in Žilina

Cemeteries	Višňové	Bánová	Old cemetery Žilina	New cemetery Žilina
Downtown/Outskirts	-/+	-/+	+/-	+/-
Date of origin/oldest gravestone*	*1889	after 1806 [1]	1707 [2]	1990 [3]
Area size (ha)	1.18	0.9	3.1	30/4.1**
GPS coordinates	N 49° 9' 55" E 18° 46' 37"	N 49° 11' 41" E 18° 43' 7"	N 49° 13' 23" E 18° 43' 55"	N 49° 13' 27" E 18° 42' 40"
Altitude (m a. s. l.)	442	383	350	404
Location	west slope	west slope	plain	south slope
Dominant plantation	<i>Thuja occidentalis</i> , <i>Picea alba</i> , <i>Larix decidua</i> , <i>Tilia cordata</i>	<i>Thuja occidentalis</i> , <i>Pinus sp.</i> , <i>Picea alba</i> , <i>Juniperus communis</i> , <i>Robinia pseudoacacia</i> , <i>Rosa canina</i>	<i>Betula pendula</i> , <i>Thuja occidentalis</i> , <i>Taxus baccata</i> , <i>Picea alba</i>	<i>Betula pendula</i> , <i>Juniperus communis</i> , <i>Pinus nigra</i> , <i>Thuja occidentalis</i> , <i>Larix decidua</i> , <i>Buxus sempervivens</i>
Stage***	III	III	I	II

\*\*Total area/Observed area

\*\*\*Stage: I – periodically mown /maintained, concrete paths, nearby intense anthropogenic impact (e.g. traffic);

II – periodically mown /maintained, concrete paths, nearby natural or semi-natural habitats (e.g. forests, meadows);

III – occasionally mown, concrete paths, nearby natural or semi-natural habitats (e.g. forests, meadows).

[1] <http://zilina-gallery.sk/picture.php?/4018/category/387> [accessed 9 September 2012]

[2] <http://zilina-gallery.sk/picture.php?/27270/category/108> [accessed 9 September 2012]

[3] <http://zilina-gallery.sk/picture.php?/1817/category/122> [accessed 9 September 2012]



Fig. 1. Observed cemeteries: 1 – Višňové, 2 – Bánová, 3 – Old cemetery Žilina, 4 – New cemetery Žilina

## Results and discussion

Overall, 46 bryophyte species were found, out of them 1 liverwort (Marchantiophyta) and 45 mosses (Bryophyta) (Tab. 2), neither of them is included in the Red List of bryophytes in Slovakia (Kubinská et al. 2001). Species richness varied from 20 to 26 species.

Bryophytes were most abundant on the New cemetery in Žilina (Tab. 3), which is located at the edge of town close to forested area. It has a higher altitude (404 m) and the largest size (30 ha) within the studied cemeteries. On the Old Cemetery in Žilina and the cemetery in Višňové occurred least

bryophyte species represented in equal number (Tab. 3). The regularly maintained Old cemetery is located in the city centre close to the highway with less green areas. It can be one of the reasons for the lower number of bryophytes. The cemetery in Višňové, despite its location close to the mountains, has relatively low species diversity affected probably by its small size. Cemetery in Bánová contrary to the smallest area (0.9 ha) has the highest species number probably due to its location in suburban area, in immediate vicinity of fields and forests. Similarly number of epiphytic bryophytes which respond most sensitive to changes of environment is the highest on this cemetery (Fig. 3). Nevertheless, despite these observations, it seems that for species diversity has a greater impact the microclimatic conditions of cemeteries than their location in the downtown or outskirts of the city. This assumption suggests the study of Mišíková, Kubinská (2010), referring a higher bryophyte number (25 to 43 taxa) found on the historical cemeteries from the centre of cities.

Overall, the number of species found on various cemeteries did not differ substantially. Species composition is clearly different – only 6 (13.1 %) bryophytes were present on all localities, 5 (10.8 %) bryophytes on three of the surveyed cemeteries, 15 (32.6 %) taxa on two and 20 (43.5 %) taxa were found on one cemetery. However, species with a high frequency of occurrence (more than 7 findings on the site) (Tab. 2) were found on all or on most of the surveyed cemeteries.

Most species grows on the partially shaded lawns (2B), on shaded, moist places with herb vegetation (1B) and on partially shaded stone substrates (1B) (Fig. 2). Least species were found on tree trunks (3B) (Fig. 2), bare soil (2D) and on the dead wood (4A) which is often removed from the cemeteries.

Pholiose liverworts (Marchantiophyta, Jungermanniopsida) are less prevalent in the urban areas of Central Europe than mosses due to their sensitivity to changes in humidity, degree of shadowing and high concentration levels of SO<sup>2</sup> (Janovicová et al. 2003; Fudali 2005). The only recorded liverwort *Lophocolea heterophylla* was found on the New Cemetery, where the conditions are favourable (higher substrate and air moisture, shadowing and plantation of trees and shrubs).

The most of the observed taxa are apophytes and pioneer species, occurring frequently in man-made habitats and sites, e. g. *Amblystegium serpens*, *Brachythecium rutabulum*, *Rhynchostegium murale*, *Schistidium apocarpum* agg., *Tortula muralis*, *Barbula unguiculata*, *Fissidens taxifolius*, *Ceratodon purpureus*. This floristic composition is consistent with the conclusions of other studies from Central Europe (Fudali 2001, 2003, 2005; Mišíková, Kubinská 2010; Janovicová et al. 2003). However, results presented in this paper are not definitive, with regard to a short period of field research.

**Tab. 2. List of bryophyte species recorded on the observed cemeteries in Žilina**

Cemetery	Višňové		Bánová		Old cemetery Žilina		New cemetery Žilina	
	ecol. group*	freq. **	ecol. group*	freq. **	ecol. group*	freq. **	ecol. group*	freq. **
<b>Marchantiophyta</b>								
<i>Lophocolea heterophylla</i>							3A	r
<b>Bryophyta</b>								
# <i>Amblystegium serpens</i>	1A 1B 4	++	2B 3A	++	1B 2A	++	1C 3A 3B	++
# <i>Brachythecium rutabulum</i>	1A 2A 3A	++	1B 2A 2B 4A	++	2A 3A	++	1B 3A 4A	++
# <i>Plagiomnium undulatum</i>	2B	r	2C	+	2D	r	2C	++
# <i>Rhynchostegium murale</i>	1A	+++	1C	+	1A 1B	++	1B	+
# <i>Schistidium apocarpum</i> agg.	1A	++	1B	+	1B	+	1B	+
# <i>Tortula muralis</i>	1A 1B	++	1A 1C	++	1A 1B	++	1A 1B	++
<i>Abietinella abietina</i>					2A	r		
<i>Atrichum undulatum</i>	2C	r						
<i>Barbula convoluta</i>					2A	++	2A	+

Tab. 2. Cuntinuation

Cemetery	Višňové		Bánová		Old cemetery Žilina		New cemetery Žilina	
	ecol. group*	freq. **	ecol. group*	freq. **	ecol. group*	freq. **	ecol. group*	freq. **
<i>Brachytheciastrum velutinum</i>	3A	r	3A	r				
<i>Brachythecium salebrosum</i>			1B	r	2A	++		
<i>Bryoerythrophyllum recurvirostrum</i>					1B	r		
<i>Bryum argenteum</i>	2D	+					1B 2A	++
<i>Bryum caespiticium</i>	2A 2C	+					2C	r
<i>Bryum capillare</i>	2C	r			1B	r		
<i>Bryum erythrocarpum</i> agg.			2C	r				
<i>Bryum moravicum</i>			3A	r	3A	r		
<i>Calliergonella cuspidata</i>					2C	r	2B 2C	+
<i>Ceratodon purpureus</i>	1A 2C 4A	+			1B	+	1C 2B 3A	++
<i>Cirriphyllum piliferum</i>			2C	+			2B	r
<i>Cratoneuron filicinum</i>			1C	r				
<i>Ctenidium molluscum</i>			2A 3A	r				
<i>Dicranella varia</i>							2B	r
<i>Didymodon fallax</i>			2B	r	1A	r	1C	r
<i>Didymodon ferrugineus</i>							2B	r
<i>Didymodon rigidulus</i>					1A 1B	++		
<i>Encalypta vulgaris</i>			1A	r				
<i>Fisidens taxifolius</i>			2B 2C	++	2A 2C 2D	++	2B	+
<i>Funaria hygrometrica</i>							2C	r
<i>Herzogiella seligeri</i>			4A	r				
<i>Hypnum cupressiforme</i>	3B	+	3A 3B	++				
<i>Kindbergia praelonga</i>	2C	r	2B	r				
<i>Orthotrichum anomalum</i>	1B	++			1A 1B 1C	++		
<i>Orthotrichum cupulatum</i>	1A	+						
<i>Oxyrrhynchium hians</i>							2B 2C	+
<i>Oxyrrhynchium schleicheri</i>	2C	r						
<i>Plagiomnium rostratum</i>	2D				2D	r	2B 2C	++
<i>Pottia truncata</i>							2A	r
<i>Pseudoscleropodium purum</i>			2C	r			2C	+
<i>Rhynchostegium confertum</i>							1B	r
<i>Rhytidiadelphus squarrosus</i>	2C	r						
<i>Rhytidiadelphus triquetrus</i>	2B	r					2C	r
<i>Sciuro – hypnum populeum</i>			1B	r				
<i>Thuidium philibertii</i>			2B	+			2B	r

**Legend to the Tab. 2**

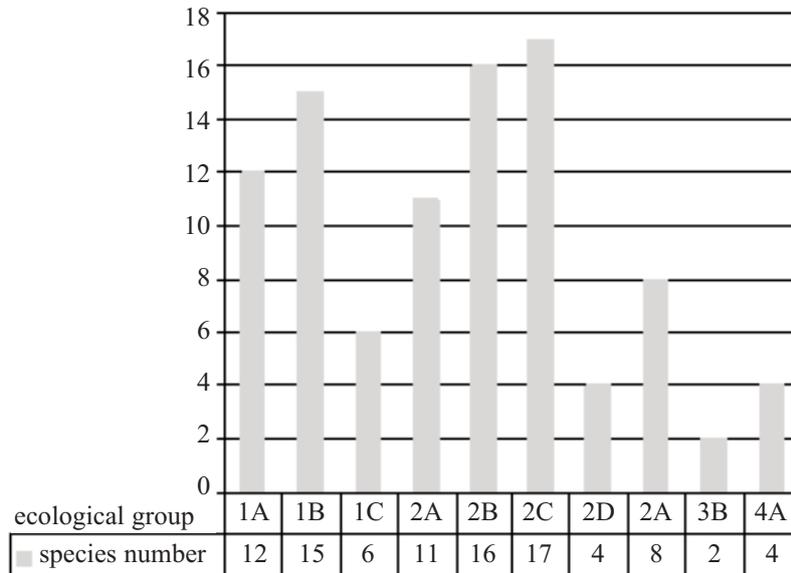
**\*Ecological group:**

1. Epilithic species: A – unshaded stone substrates; B – partially shaded stone substrates; C – shaded wet stone substrates.
2. Epigeic species: A – sunny, dry lawns; B – partially shaded lawns; C – shaded, moist places with herb vegetation; D – open damp soil.
3. Epiphytic species: A – tree bases and trunks up to 40 cm above ground level; B – tree trunks at height 40 cm and 250 cm above ground level.
4. A Epixylic species.

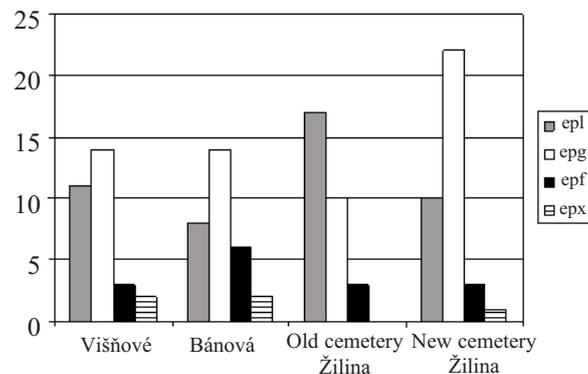
**\*\*Freq.** – occurrence of the species on the observed cemetery: ++ – common species (more than 7 findings at that cemetery), + – occasionally occurring species (2-6 findings on the cemetery), r – rare species (1-2 findings on the cemetery).  
**#** Species found on all four cemeteries.

**Tab. 3. Number of recorded bryophyte species on the observed cemeteries in Žilina**

Cemetery	Višňové	Bánová	Old cemetery Žilina	New cemetery Žilina
Number of recorded bryophytes	20	23	20	26



**Fig. 2. \*Species number in individual ecological groups**  
 \*for abbreviations see Tab. 2



**Fig. 3. Species number in ecological groups on observed cemeteries**  
 epl – epilithic species; epg – epigeic species; epf – epiphytic species;  
 epx – epixylic species

## References

- Fudali, E.**, 2001: The ecological structure of the bryoflora of Wrocław's parks and cemeteries in relation to their localization and origin. *Acta Soc. Bot. Pol.*, 70: 229-235.  
**Fudali, E.**, 2003: Bryophytes of parks and cemeteries of Warszawa town. *Fragm. Floristic. Geobot., ser. Polonica*, 10: 221-240.  
**Fudali, E.**, 2005: Bryophyte species diversity and ecology in the parks and cemeteries of selected Polish cities. Agricultural University of Wrocław.

- Hill, M. O., Bell, N., Bruggeman-Nannenga, M. A., Brugue´ S. M., Cano, M. J., Enroth, J., Flatberg, K. I., Frahm, J.-P., Gallego, M. T., Garilleti, R., Guerra, J., Hedenäs, L., Holyoak, D. T., Hyvönen, J., Ignatov, M. S., Lara, F., Mazimpaka, V., Munõz, J., Söderström, L., 2006: An annotated checklist of the mosses of Europe and Macaronesia. *J. Bryol.*, 28: 198-267.
- Hohenwallner, D., Zechmeister, H. G., 2001: Factors influencing bryophyte species richness and populations in urban environments: A case study. *Nova Hedwigia*, 73: 87-96.
- Janovicová, K., Kubinská, A., Javorčíková, D., 2003: Pečeňovky (Hepatophyta), rožteky (Anthocerotophyta) a machy (Bryophyta) na území Bratislavy, p. 38-98, Botanický ústav SAV, Bratislava.
- Kubinská, A., Janovicová, K., Šoltés, R., 2001: Červený zoznam machorastov Slovenska. In: Baláž, D., Marhold, K., Urban, P. (eds), Červený zoznam rastlín a živočíchov Slovenska, p. 35-47, Ochrana Prírody 20, Suppl.
- Marhold, K., Goliašová, K., Hegedúšová, Z., Hodálová, I., Jurkovičová, V., Kmet'ová, E., Letz, R., Michalková, E., Mráz, P., Peniažteková, M., Šípošová, H., Ťavoda, O. et al., 1998: Papraďorasty a semenné rastliny. In: Marhold, K., Hindák, F. (eds), Zoznam nižších a vyšších rastlín Slovenska, p. 333-687, Veda, Bratislava.
- Mišíková, K., Kubinská, A., 2010: Machorasty historických cintorínov vo vybraných mestách Strednej Európy. *Bull. Slov. Bot. Spoločn.*, Bratislava, 32, 2: 137-145.
- Mišíková, K., Mišík, M., Kubinská, A., 2007: Bryophytes of the forest park Hôrka (Veľký Krtíš town, Slovakia). *Acta Bot. Univ. Comen.*, 43: 9-13.
- Smith, M. R., Thompson, K., Warren, P. H., Gaston, K. J., 2010: Urban domestic gardens (XIII): Composition of the bryophyte and lichen floras, and determinants of species richness. *Biol. Conserv.*, 143: 873-882.
- Söderström, L., Urmi, E., Váňa, J., 2002: Distribution of Hepaticae and Anthocerotae in Europe and Macaronesia. *Lindbergia*, 27: 3-47.

## Abstrakt

Príspevok prináša stručný prehľad machorastov na štyroch vybraných cintorínoch v intraviláne a extraviláne Žiliny. Celkovo bolo zistených 46 druhov machorastov – 1 pečeňovka (Marchantiophyta) a 45 machov (Bryophyta). Z hľadiska ekologických skupín sú najpočetnejšie epigeické druhy rastúce na tienistejších trávnikoch a vlhkej holej pôde, a druhy epilitické na mierne tiených kamenných substrátoch. Na druhovú diverzitu jednotlivých cintorínov má pravdepodobne najväčší vplyv jeho rozloha a mikroklimatické podmienky prostredia.

**Katarína Mišíková, Jana Cibulková: Machorasty cintorínov Žiliny (Slovensko)**

## POLLEN CONCENTRATION IN THE AIR OF BRATISLAVA (SLOVAKIA): A COMPARISON STUDY FROM THE TWO POLLEN MONITORING STATIONS

Jozef Dušička<sup>1\*</sup>, Jana Ščevková<sup>1</sup>, Karol Mičieta<sup>1</sup>, Eva Brutovská<sup>1</sup>, Andrea Sámelová<sup>1</sup>,  
Mária Zámečnicková<sup>2</sup>, Alena Terenová<sup>2</sup>, Jana Lafférová<sup>3</sup>

<sup>1</sup>*Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany, Révová 39,  
811 02 Bratislava, Slovakia*

<sup>2</sup>*Public Health Authority, Trnavská cesta 52, 826 45 Bratislava, Slovakia*

<sup>3</sup>*Regional Authority of Public Health, Department of Environmental Biology, Cesta k nemocnici 1,  
957 56 Banská Bystrica, Slovakia*

*Received 13 September; Received in revised form 10 October; Accepted 15 October*

### Abstract

The focus of our study is the comparison of aeropalynological research of two independent pollen stations localized in different parts of the city area of Bratislava. The sampling sites are placed 5.3 km apart, respectively in the northwestern (Department of Botany of the Comenius University – D.B.) and northeastern (Public Health Office – U.V.Z.) area. Airborne pollen data were collected during the years 2007 – 2010 by using Hirst type volumetric samplers simultaneously in both localities. In our study, we focused on the pollen grains of 10 higher plant taxa (*Alnus* L., *Betula* L., *Carpinus* L., *Taxaceae – Cupressaceae*, *Pinus* L., *Populus* L., *Ambrosia* L., *Artemisia* L., *Poaceae* and *Urticaceae*), which are counted among the strongest pollen allergens in the area of Bratislava, and at the same time are abundantly represented in the spectrum of both pollen stations. From the quantitative point of view, we have observed big differences in the number of pollen grains of the chosen taxa during the individual years, as well as between the individual pollen stations. The biggest difference has been observed with tree and/or shrub species.

**Key words:** aeropalynology, allergology, Department of Botany, Public Health Office, stationary volumetric analysis

### Introduction

Due to the continual increase in the number of allergic diseases in Slovakia (according to the Office of medical information and statistics of the Slovak Republic, the number of patients with allergic rhinitis increased by 100.000 between the years 2005 and 2008), the Pollen information service underwent a lot of changes and development since the first pollen trap has been put in operation until the present state, when there are seven monitoring stations (Bratislava D.B, Bratislava U.V.Z., Trnava, Nitra, Banská Bystrica, Žilina and Košice) in order in the area of the Slovak Republic.

Qualitative and quantitative analysis of the pollen grains in the atmosphere of densely inhabited cities is very important, as the number of people suffering with allergies to pollen grains is increasing yearly (D'Amato et al. 2007). Many European cities have their own pollen calendars with the goal to evaluate the pollen situation in the atmosphere (Gawel et al. 1996; Giner et al. 2002; Abreu et al. 2003; Ballero, Maxia 2003; Gioulekas et al. 2004; García-Mozo et al. 2006; Rizzi-Longo et al. 2007; Docampo et al. 2007; Bilisik et al. 2008; Ščevková et al. 2010). Their application lies mostly in medicine, particularly allergology, where they help to prevent strong allergic reaction of patients by an early application of medicament treatment according to the type of prevailing pollen allergens in the atmosphere in the given phenological season.

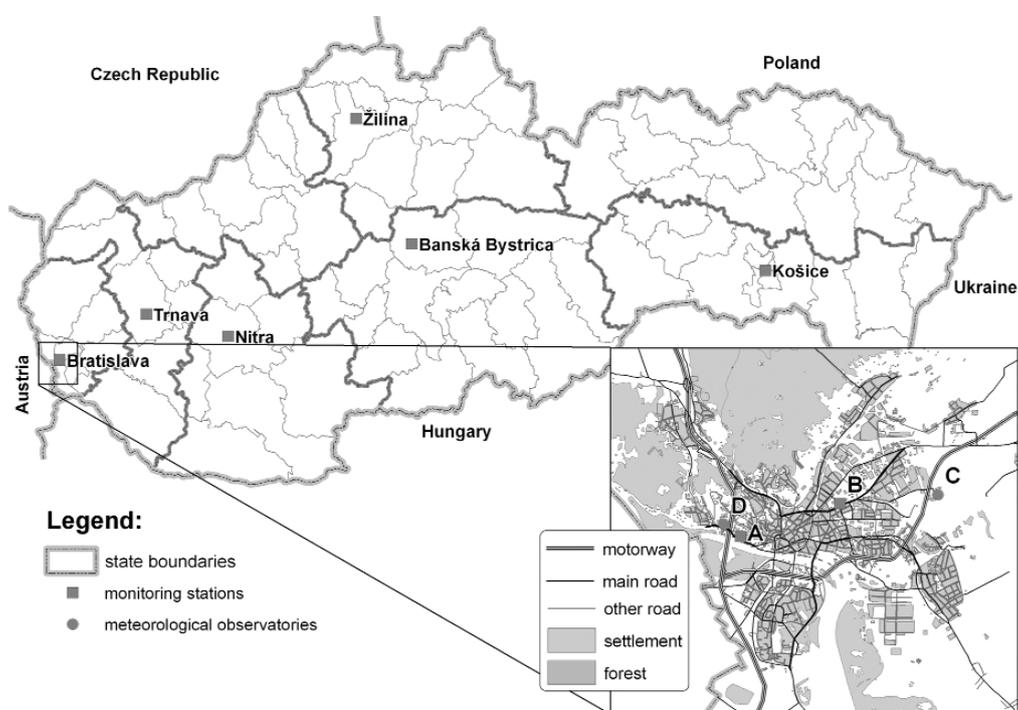
---

\* Corresponding Author: Jozef Dušička; [dusicka@fns.uniba.sk](mailto:dusicka@fns.uniba.sk)

The aim of our study is to compare the results of aeropalynological research of two independent pollen stations in the area of the Slovak Republic's capital, Bratislava, localized in different parts of the city, during the period of four years (2007–2010) and to evaluate the necessity of more monitoring stations within one territorial unit.

## Materials and Methods

The study was carried out in Bratislava, the city in the southwestern part of Slovakia (Fig. 1). Bratislava is situated at the borders of the Danube plain, the Malé Karpaty Mountains and the Záhorská plain. It expands mainly on the alluvial sediments of Danube, that are from a big part covered by urbanized areas, industrial complexes and which are partially agriculturally used. The original vegetation cover has been totally removed, or largely altered due to the anthropic activity. The natural plant communities have been replaced by semi-natural and synanthropic communities.



**Fig. 1. Geographical localisation of pollen monitoring stations (A – D.B., B – U.V.Z.) and meteorological observatories (C – Airport, D – Mlynská dolina) in Bratislava**

The mean yearly air temperature in Bratislava is 10.6 °C, -1.6 °C in the coldest month of the year, January, and 20.5 °C in the warmest month, July. The annual precipitation is in the range between 530 – 650 mm. The annual amount of sunshine reaches the value of 966 hours.

This study compares the data obtained from the pollen sampler placed on the roof of the Department of Botany of the Comenius University (D.B.) in the northwestern part of the city in the height of 10 m above the ground (48°08'N, 17°04'E, 183 m above the sea level) and the pollen sampler situated on the roof of the Public Health Office (U.V.Z.) building in the northeastern part of the city in the height of 15 m above the ground (48°09'N, 17°08'E, 172 m above the sea level) (Fig. 1). The localities are 5.3 km apart.

The daily mean pollen concentrations were monitored from February to October over four vegetation periods (2007–2010) using a Burkard 7-day volumetric pollen trap. This sampler records

pollen concentrations as 2 – h samples, and we converted these values to daily mean pollen concentrations for use in our analysis.

To count the pollen grains in a daily sample we used the 12 – traverse transects method at the D.B. pollen station and the method of four longitudinal transects at the U.V.Z. pollen station. Pollen concentrations were expressed as the number of pollen grains per 1 m<sup>3</sup> of air. The pollen grains were identified according to Erdtman (1969) and Spieksma et al. (1991).

The main pollen season (MPS) of selected pollen types were established according to the method of Nilsson, Persson (1981), defining the main pollen season as the period which begins when the sum of the pollen type concentrations reaches 5 % of the annual total pollen until the time when the sum reaches 95 %.

The data about the average temperature and precipitation (Tab. 1) during the monitored years were collected at two meteorological stations (Mlynská dolina and Airport), localized in the proximity of the pollen traps (Fig. 1). From the four analysed years, the year 2010 was the coldest with the highest precipitation. The warmest year was the year 2007, and the lowest precipitation was recorded in 2008.

**Tab. 1. The mean annual temperature and precipitation measured at two meteorological observatories (Mlynská dolina and Airport) in 2007–2010**

Year	2007		2008		2009		2010	
	Mlynská dolina	Airport						
<b>Temperature (°C)</b>	11.8	12.0	11.6	11.7	11.0	11.3	9.9	10.1
<b>Precipitation (mm)</b>	762.6	597.9	705.6	605.7	781.3	590.8	964.4	794.9

## Results

During the four monitored years (2007–2010), the total number of sampled pollen gains in the atmosphere of Bratislava was 319.556, of which 213.153 pollen grains were measured at the U.V.Z. station and 106.403 at the D.B. station (Tab. 2). With regard to the spectrum of species, no significant differences were observed between the monitoring stations. From the total number of 32 higher plant taxa (21 tree and/or shrub and 11 herbaceous species) only the pollen grains of the *Cyperaceae* family were absent at the U.V.Z. station, and the pollen grains of the genus *Ailanthus* L. were absent at the D.B. station (Tab. 3).

**Tab. 2. Pollen grain sums of the individual taxa measured at two monitoring stations (U.V.Z., D.B.) in 2007–2010**

Pollen taxa	U.V.Z. total	D.B. total
<i>Acer</i>	2.573	1.568
<i>Aesculus</i>	227	677
<i>Ailanthus</i>	201	0
<i>Alnus</i>	5.782	3.595
<i>Ambrosia</i>	12.086	3.769
<i>Apiaceae</i>	243	46
<i>Artemisia</i>	5.507	3.154
<i>Asteraceae</i>	305	193
<i>Betula</i>	30.691	25.888
<i>Carpinus</i>	4.382	3.570
<i>Castanea</i>	416	165
<i>Chenopodiaceae</i>	3.0388	1.620

Tab. 2. Continuation

Pollen taxa	U.V.Z. total	D.B. total
<i>Corylus</i>	2.608	2.107
<i>Cyperaceae</i>	0	606
<i>Fagus</i>	1.564	533
<i>Fraxinus</i>	12.169	1.368
<i>Humulus</i>	974	35
<i>Juglans</i>	1.382	1.909
<i>Larix</i>	23	23
<i>Pinus</i>	15.487	8.688
<i>Plantago</i>	3.930	1.828
<i>Platanus</i>	1.047	97
<i>Poaceae</i>	12.813	6.221
<i>Populus</i>	22.580	3.419
<i>Quercus</i>	13.135	917
<i>Rumex</i>	1.156	664
<i>Salix</i>	3.264	1.067
<i>Sambucus</i>	1.800	1.676
<i>Taxaceae – Cupresaceae</i>	14.517	15.912
<i>Tilia</i>	396	724
<i>Ulmus</i>	1.286	510
<i>Urticaceae</i>	37.221	13.854
<b>Total</b>	<b>213.153</b>	<b>106.403</b>

Tab. 3. Annual total pollen grain count of all pollen taxa (with percentages) measured in the air of Bratislava at two monitoring stations (U.V.Z., D.B) in 2007–2010

Pollen taxa	U.V.Z.					D.B.				
	2007	2008	2009	2010	%	2007	2008	2009	2010	%
<i>Acer</i>	1.494	405	260	414	1.21	878	331	127	232	1.47
<i>Aesculus</i>	37	101	56	33	0.11	299	0	213	165	0.64
<i>Ailanthus</i>	0	26	131	44	0.09	0	0	0	0	0
<i>Alnus</i>	551	716	1.148	3.367	2.71	656	2.648	90	201	3.38
<i>Ambrosia</i>	1.225	3.708	4.793	2.360	5.67	1.213	802	1.051	703	3.54
<i>Apiaceae</i>	35	64	105	39	0.11	27	0	0	19	0.04
<i>Artemisia</i>	838	1.756	1.750	1.163	2.58	1.004	594	954	602	2.96
<i>Asteraceae</i>	59	32	138	76	0.14	74	20	42	57	0.18
<i>Betula</i>	3.783	13.077	2.737	11.094	14.40	8.226	12.123	1.040	4.499	24.33
<i>Carpinus</i>	1.668	1.315	438	961	2.06	1.068	1.711	247	544	3.36
<i>Castanea</i>	114	28	211	63	0.20	106	0	25	34	0.16
<i>Chenopodiaceae</i>	983	875	1.110	420	1.59	486	375	417	342	1.52
<i>Corylus</i>	814	69	955	770	1.22	863	611	223	410	1.98
<i>Cyperaceae</i>	0	0	0	0	0	0	303	303	0	0.57
<i>Fagus</i>	372	730	368	94	0.73	176	122	76	159	0.50
<i>Fraxinus</i>	1.921	7.118	1.833	1.297	5.71	530	381	183	274	1.29
<i>Humulus</i>	21	119	216	618	0.46	3	7	6	19	0.03
<i>Juglans</i>	282	315	604	181	0.65	636	537	436	300	1.79
<i>Larix</i>	4	13	0	6	0.01	23	0	0	0	0.02

Tab. 3. Continuation

	U.V.Z.					D.B.				
	2007	2008	2009	2010	%	2007	2008	2009	2010	%
<i>Pinus</i>	2.589	3.096	5.943	3.859	7.27	2.431	2.023	2.189	2.045	8.17
<b>Pollen taxa</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>%</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>%</b>
<i>Plantago</i>	890	1.054	1.343	643	1.84	425	389	474	540	1.72
<i>Platanus</i>	217	102	315	413	0.49	29	11	11	46	0.09
<i>Poaceae</i>	2.933	3.936	3.614	2.330	6.01	2.527	1.466	1.087	1.141	5.85
<i>Populus</i>	2.558	1.587	7.683	10.752	10.59	1.655	983	225	556	3.21
<i>Quercus</i>	2.601	2.284	7.164	1.086	6.16	185	173	74	485	0.86
<i>Rumex</i>	277	194	265	420	0.54	284	131	158	91	0.62
<i>Salix</i>	1.046	311	1.294	613	1.53	620	274	141	32	1.00
<i>Sambucus</i>	836	574	145	245	0.84	698	465	294	219	1.58
<i>Taxaceae-</i>										
<i>Cupressaceae</i>	2.743	903	5.643	5.228	6.81	8.179	5.005	1.194	1.534	14.95
<i>Tilia</i>	102	131	94	69	0.19	181	223	223	97	0.68
<i>Ulmus</i>	468	162	272	384	0.60	72	311	7	120	0.48
<i>Urticaceae</i>	4.534	10.742	16.350	5.595	17.46	5.320	2.513	2.965	3.056	13.02
<b>Total</b>	<b>35.995</b>	<b>55.543</b>	<b>66.978</b>	<b>54.637</b>	<b>100</b>	<b>38.874</b>	<b>34.532</b>	<b>14.475</b>	<b>18.522</b>	<b>100</b>

From the quantitative point of view, however, we observed great differences between the individual years within the individual pollen stations, as well as differences between the stations. The highest yearly total number of pollen grains (66.978) at the U.V.Z. station was measured in 2009, and the lowest number (35.995) in 2007. The highest yearly total number of pollen grains (38.874) at the D.B. station was measured in 2007, and the lowest number (14.475) in 2008 (Tab. 3).

The highest differences in the pollen quantity at the individual stations were observed with the pollen of tree and/or shrub species. In the case of the taxa *Fraxinus* L., *Populus* L. and *Quercus* L., the number of their pollen grains was several times higher at the U.V.Z. station, compared to the D.B. station (Tab. 2). The opposite situation within the tree and/or shrub species occurred with the taxa *Aesculus* L., *Juglans* L., *Tilia* L. and *Taxaceae – Cupressaceae*, with the pollen quantity measured at D.B. station exceeding the one at the U.V.Z. station (Tab. 2). Among the herbaceous taxa, the highest differences were observed with the genus *Ambrosia* L. and the *Urticaceae* family with significantly higher pollen concentrations measured at the U.V.Z. station compared to the D.B. station (Tab. 2).

In our comparison, we focused on the pollen grains of those plants, which belong to the strongest allergens in the area of Bratislava (Hrubiško 1996) and in the same time were abundantly present in the spectrum of both pollen stations (percentage of total was more than 2 %). It is these 10 taxa: *Alnus* L., *Betula* L., *Carpinus* L., *Taxaceae – Cupressaceae*, *Pinus* L. and *Populus* L. of tree and/or shrub and *Ambrosia* L., *Artemisia* L., *Poaceae* and *Urticaceae* of herbaceous species.

Pollen calendars (Fig. 2) were created for the 10 taxa mentioned above, and the beginning, the end and total duration of their pollen season during the 4 monitored years at both pollen stations were calculated (Tab. 4).

The yearly sums of the pollen grains of genus *Alnus* L. varied from 90 in the year 2009 at the D.B. station to 3.367 in 2010 at the U.V.Z. station. The average yearly sum of the *Alnus* L. pollen captured in the pollen traps of both stations from the atmosphere of Bratislava was 1.172 pollen grains. The duration of the pollen season is approximately the same at both stations, but its beginning was 20 days delayed at the U.V.Z. station compared to the D.B. station, and its duration only half as long as at the D.B. station. The average duration of the pollen season was 26 days, with the longest period of 31 days at both stations in the year 2007.

*Ambrosia* L. pollen grains were more abundant at the U.V.Z. station in each of the monitored years, with the maximum in 2009, exceeding the data from the D.B. station more than five times (Tab. 3). The

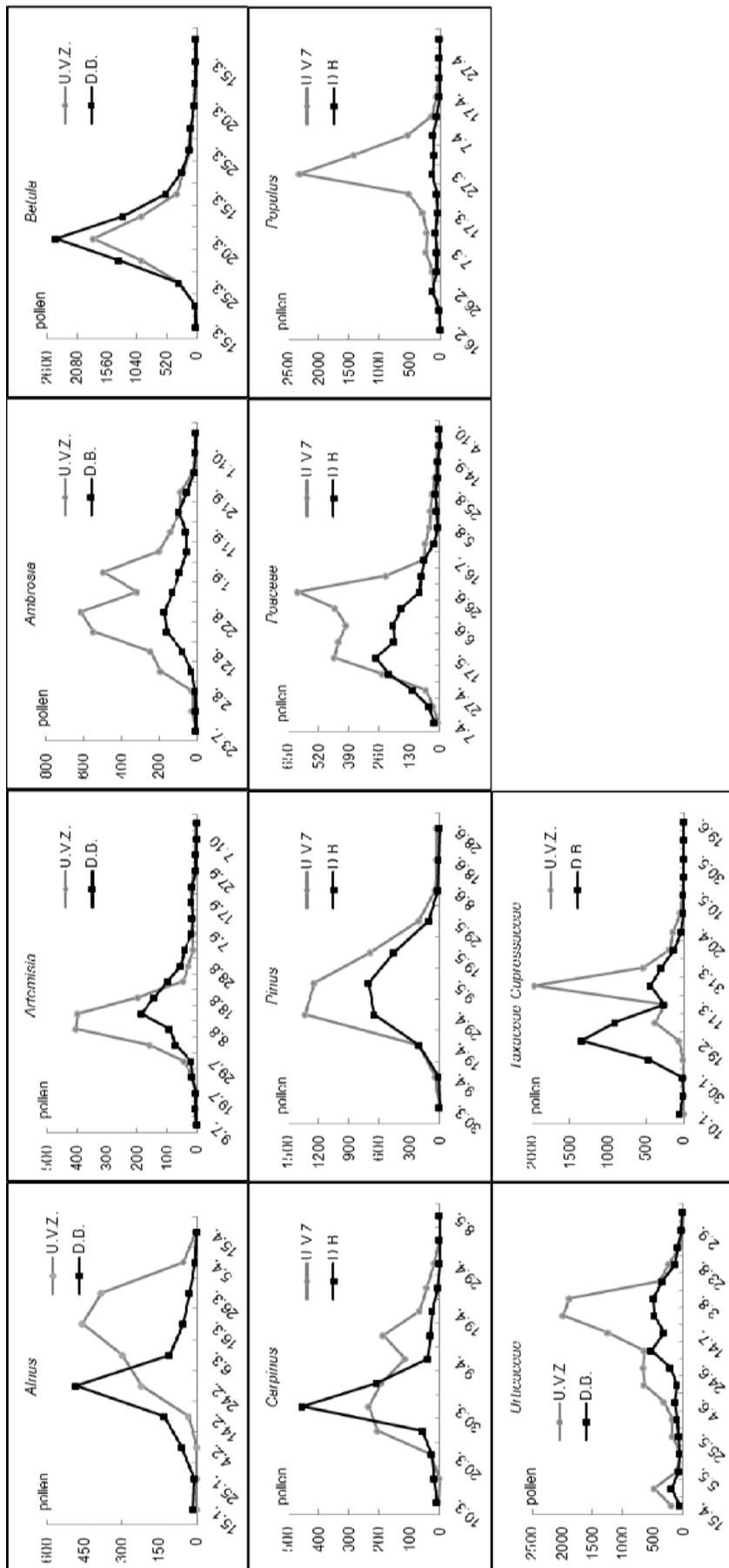


Fig. 2. Pollen calendar for the selected pollen types collected in the Bratislava atmosphere and related 10-day means of the daily mean pollen concentrations

Tab. 4. Characteristics of the main pollen seasons of the selected taxa in the air of Bratislava measured at two monitoring stations U.V.Z. and D.B. in 2007–2010

Characteristics of pollen-producing taxa		Study year											
		2007			2008			2009			2010		
Taxa	Characteristics <sup>a</sup>	U.V.Z.	D.B.	U.V.Z.	D.B.	U.V.Z.	D.B.	U.V.Z.	D.B.	U.V.Z.	D.B.	U.V.Z.	D.B.
<i>Alnus</i>	PS	16 feb-18 mar	10 feb-12 mar	27 feb-10 mar	6 feb-2 mar	7 mar-5 apr	10 mar-3 apr	28 feb-27 mar	8 mar-29 mar				
	SL	31	31	13	26	30	25	28	22				
	Max	60 (22 feb)	71 (12 feb)	172 (28 feb)	457 (23 feb)	145 (29 mar)	9 (16 mar)	397 (18 mar)	21 (19 mar)				
<i>Ambrosia</i>	PS	15 aug-26 sep	15 aug-22 sep	11 aug-10 sep	12 aug-19 sep	15 aug-16 sep	11 aug-21 sep	13 aug-25 sep	11 aug-25 sep				
	SL	43	41	31	39	33	42	44	46				
	Max	146 (23 aug)	156 (21 aug)	395 (3 sep)	57 (21 aug)	582 (21 aug)	57 (28 aug)	581 (23 aug)	37 (23 aug)				
<i>Artemisia</i>	PS	3 aug-17 sep	3 aug-26 aug	1 aug-25 aug	4 aug-9 sep	2 aug-12 sep	2 aug-15 sep	10 aug-24 sep	1 aug-24 sep				
	SL	46	24	25	37	42	45	47	55				
	Max	68 (16 aug)	129 (14 aug)	258 (12 aug)	60 (15 aug)	170 (15 aug)	53 (15 aug)	120 (15 aug)	35 (21 aug)				
<i>Betula</i>	PS	1 apr-23 apr	2 apr-18 apr	29 mar-27 apr	29 mar-16 apr	6 apr-27 apr	7 apr-3 may	30 mar-26 apr	31 mar-25 apr				
	SL	23	17	30	19	22	27	28	26				
	Max	455 (1 apr)	1.369 (3 apr)	1.691 (1 apr)	2.447 (6 apr)	679 (8 apr)	81 (8 apr)	1.119 (30 mar)	392 (10 apr)				
<i>Carpinus</i>	PS	23 mar-17 apr	22 mar-9 apr	28 mar-26 apr	29 mar-9 apr	8 apr-2 may	15 mar-13 apr	30 mar-26 apr	29 mar-24 apr				
	SL	26	19	30	12	25	30	28	27				
	Max	191 (4 apr)	295 (4 apr)	192 (30 mar)	696 (1 apr)	95 (16 apr)	37 (8 apr)	1.74 (17 apr)	43 (20 apr)				
Taxaceae – Cupressaceae	PS	25 feb-15 apr	9 feb-1 apr	27 feb-12 may	22 feb-17 mar	17 mar-17 apr	16 mar-20 apr	20 mar-24 apr	20 mar-11 apr				
	SL	50	52	76	25	32	36	36	23				
	Max	466 (1 mar)	1724 (6 mar)	82 (29 mar)	1.486 (24 feb)	2.477 (28 mar)	86 (23 mar)	1.711 (25 mar)	332 (24 mar)				
<i>Pinus</i>	PS	29 apr-29 may	28 apr-24 may	29 apr-13 jun	20 apr-30 may	25 apr-25 may	21 apr-29 may	6 may-10 jun	5 may-5 jun				
	SL	31	27	46	41	31	39	36	32				
	Max	259 (23 may)	257 (7 may)	369 (7 may)	145 (7 may)	667 (9 may)	109 (5 may)	599 (7 may)	248 (6 may)				
Poaceae	PS	5 may-23 jul	24 apr-21 jul	15 may-6 aug	27 apr-23 jul	10 may-15 aug	28 apr-3 aug	23 may-23 jul	4 may-9 aug				
	SL	80	89	84	88	98	98	62	98				
	Max	134 (23 may)	158 (23 may)	140 (28 may)	75 (26 may)	126 (26 jun)	25 (15 may)	149 (4 jul)	35 (5 jun)				
<i>Populus</i>	PS	24 feb-14 apr	4 mar-13 apr	27 feb-21 mar	25 feb-24 apr	28 mar-11 apr	28 mar-23 apr	24 mar-6 apr	23 mar-10 apr				
	SL	50	41	24	60	15	27	14	19				
	Max	1.703 (17 mar)	140 (14 mar)	555 (10 mar)	152 (1 mar)	2.139 (5 apr)	33 (8 apr)	4.206 (29 mar)	73 (30 mar)				
Urticaceae	PS	20 apr-28 aug	27 apr-1 sep	15 may-3 sep	28 apr-8 sep	28 apr-22 aug	28 apr-8 sep	9 may-1 sep	11 may-12 sep				
	SL	131	128	112	134	117	135	116	126				
	Max	159 (15 jul)	158 (16 aug)	563 (11 aug)	78 (15 aug)	769 (16 aug)	60 (4 aug)	374 (11 aug)	78 (21 jul)				

<sup>a</sup> PS start and ending dates, respectively, SL length of pollen season, Max maximum daily mean pollen concentrations (pollen/m<sup>3</sup>) and its date (in parenthesis)

yearly average was 2.092 pollen grains. For this genus, the beginning and duration of the pollen season at both stations were almost identical. The average length of a pollen season was 40 days, while the longest lasted for 46 days in 2010 at the D.B. station.

The genus *Artemisia* L. had more or less stable yearly level of pollen at both stations, with just slight deviations. Maximum number of pollen grains was 1.756 at the U.V.Z. station in 2008, the average was 1.083 pollen grains a year. The pollen season of this genus was not so homogenous, especially in the first two years of the monitoring, although its beginnings were almost identical. At the U.V.Z. station it was 22 days longer than at the D.B. station, while in 2008, in the contrary, the pollen season was 12 days longer at the D.B. station. Its average duration was 40 days just like the one of *Ambrosia* L., the longest lasting for 55 days in 2010.

The highest yearly sums of pollen grains among the tree and/or shrub species were observed in the genus *Betula* L., in which several authors observed a two-year period of fluctuation, with alternating years of high and low pollen production (Nilsson, Persson 1981; Atkinson, Larsson 1990), a fact that was confirmed by our observations. The most abundant year for pollen was 2008 with 13.077 pollen grains at the U.V.Z. station, while the average considering both stations was 7.072 per year. The beginnings of the pollen season in individual years were set apart by maximally 1 day at the different pollen stations; therefore its definition is similar at both stations, with the average of 24 days and maximum of 30 days in 2008. In 2007 and 2008 there were slight deviations between the stations, reaching 6 and 11 days in behalf of the U.V.Z. station.

For the genus *Carpinus* L., the abundance of pollen grains at both stations was higher in 2007 and 2008 compared to the years 2009 and 2010. The highest sum of pollen grains – 1.711 was observed at the D.B. station in 2008. The beginnings and duration of the pollen season were very similar at both stations, with the exception of the year 2008, when the difference in length was 18 days in behalf of the U.V.Z. station, and the year 2009 when the pollen season began a month earlier at the D.B. station. The longest pollen season lasted for 30 days; it was in 2008 at the U.V.Z. station and in 2009 at the D.B. station, while the average was 25 days.

The *Taxaceae* – *Cupressaceae* families showed an interesting trend in the yearly sums of the pollen grains. While the yearly sums monitored at the D.B. station dominated above the U.V.Z. station in the years 2007 and 2008, in 2009 and 2010 the situation was reversed with higher total numbers at the U.V.Z. station. The maximum was observed at the D.B. station in 2007 – 8.179 pollen grains. The duration of the pollen seasons was similar at both stations in 2007 and 2009. In 2008 the pollen season lasted for 75 days at the U.V.Z. station, and only 25 days at the D.B. station and in 2010 the difference of 13 days was recorded in behalf of the U.V.Z. station. The average length of a pollen season was 41 days.

For the genus *Pinus* L., the annual sums of pollen grains were higher at the U.V.Z. station in every year monitored, with the highest difference (almost three times more) in 2009. The average was 3.022 pollen grains a year and the maximum 5.943 at the U.V.Z. station in 2009. The beginnings and length of the pollen seasons was similar at both stations, with the average of 35 days and maximum of 46 days at the U.V.Z. station in 2008.

The pollen grains of the *Poaceae* family were more abundant at the U.V.Z. station in every monitored year as well, with the most significant difference in 2009, when there were three times more pollen grains at the U.V.Z. station. The yearly maximum was 3.936 in 2008 at the U.V.Z. station. In each of the four years, the pollen season started at least 12 days earlier at the D.B. station, compared to the U.V.Z. station, but there was no significant difference in the length of the pollen season besides 2010, when the pollen season at the D.B. station was 36 days longer. The average length of a pollen season was 86 days, with the maximum of 98 days, and that even three times: at both stations in 2009, and at the D.B. station in 2010.

The biggest differences of yearly sums of pollen grains from all the studied taxa were observed in the genus *Fraxinus* L. The yearly sums at the U.V.Z. station were significantly higher than at the D.B. station, even 18 times higher in 2008. The annual average was 1.692 and maximum 7.118 at the U.V.Z. station in 2007. The differences in the length of the pollen season were 5 days at most in every year, with the average length of 32 days and maximum of 39 days at the U.V.Z. station in 2007.

Significant differences were observed in the genus *Populus* L. as well. In the first two years, the pollen sums were approximately twice higher at the U.V.Z. station than at the D.B. station, while in 2010, it was almost 20 times and in 2009 34 times more in behalf of the U.V.Z. station. The year with a greatest abundance was 2010 with 10.752 pollen grains at the U.V.Z. station, the average was 3.250 pollen grains. There were only minimal differences in the beginnings of the pollen seasons, but their duration was, with the exception of 2007, longer at the D.B. station, with the maximum difference of 36 days in 2008. The average pollen season lasted for 31 days, with maximum of 60 days at the D.B. station in 2008.

The *Urticaceae* family had higher sums of pollen at the U.V.Z. station in every monitored year with the exception of 2007. The maximum was 16.350 pollen grains at U.V.Z. station in 2009, which exceeded the number from the D.B. station five times. The annual average was 6.384. The duration of the pollen season was the longest from all monitored plant species, with the average of 125 days and maximum of 135 days at the D.B. station in 2009. The pollen seasons were longer at the U.V.Z. station in all years with the exception of 2007, the biggest deviation was 22 days in behalf of the D.B. station in 2008.

The highest sums of pollen grains we recorded in April at both monitoring stations in every monitored year. The mean pollen concentration at the U.V.Z. station was 17.106 and at the D.B. station 9.509 pollen grains (Tab. 5).

**Tab. 5. Monthly sums of pollen grains in the air of Bratislava measured at two monitoring stations (U.V.Z., D.B.) in 2007–2010**

Study year	Monitoring station	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
2007	U.V.Z	800	7.734	12.898	4.935	2.525	2.092	3.444	1.055	75
	D.B.	4.480	6.031	13.836	4.582	1.672	2.234	4.296	898	31
2008	U.V.Z	946	9.625	17.033	7.032	3.756	4.555	8.855	3.101	29
	D.B.	7.183	4.246	14.157	3.453	1.191	1.011	2.204	776	30
2009	U.V.Z	0	6.654	24.236	8.077	3.471	5.434	15.649	1.967	64
	D.B.	0	895	3.787	3.122	1.156	1.289	2.941	950	32
2010	U.V.Z	598	20,715	14.256	4.895	2.452	2.669	6.442	1.216	33
	D.B.	0	2.904	6.255	2.894	1.271	1.501	2.285	924	51
Mean	U.V.Z	586	11.182	17.106	6.235	3.051	3.688	8.598	1.835	50
	D.B.	2.916	3.519	9.509	3.513	1.323	1.509	2.932	887	36
Mean for Bratislava		1.751	7.351	13.307	4.874	2.187	2.598	5.765	1.361	43

## Discussion

The sums of pollen grains in the atmosphere of Bratislava during the monitored years were, with the exception of 2007, significantly higher at the U.V.Z. station than at the D.B. station. During the four years, we detected the total amount of 213.153 pollen grains at the U.V.Z. station and 106.763 pollen grains at the D.B. station. The highest number of pollen grains (66.978) was detected at the U.V.Z. station in 2009 and the lowest (14.475) at the D.B. station in the same year. Together, we detected the pollen grains of 32 higher plant taxa, with 21 tree and/or shrub species and 11 herbaceous species. The pollen of tree and/or shrub species was prevalent at the U.V.Z. station with 70 % of the total sum, while herbaceous species represent 30 % of the sum. At the D.B. station, tree and/or shrub species represented 64 % of the total pollen grains; the remaining 36 % were herbaceous species. The cause for higher levels of the tree and/or shrub species pollen is their higher species diversity compared to the herbaceous plants. In the average for both pollen stations during the four monitored years, the genus *Betula* L. represented the most abundant pollen (17.2 %), followed by the taxa *Urticaceae* (15.5 %),

*Taxaceae* – *Cupressaceae* (9.2 %), *Populus* L. (7.9 %), *Pinus* L. (7.3 %) and *Poaceae* (5.8 %). The same order was published by Ščevková et al. (2010).

The highest yearly sums of pollen were observed in the genus *Betula* L., where several authors reported fluctuations with a two year period of alternating years with low and high pollen production (Nilsson, Persson 1981; Atkinson, Larsson 1990; Ščevková et al. 2010). We can confirm this from our observations, although the time of 4 years is too short for postulating definitive arguments.

The plants with the longest pollen season, 125 days per year in average, are the species from the family *Urticaceae*. This family is represented with two species in Bratislava: *Urtica dioica* L. and *Parietaria officinalis* L. The pollen of these species is most abundant among the herbaceous species of Bratislava.

The differences in the quantity of pollen grains between the two pollen stations in Bratislava during the same time period can be caused by several factors. One of them is certainly the composition of the near vegetation and build-up area, as the pollen traps are placed in different biotopes within the city. Another factor is the difference of microclimatic conditions at the individual stations. The data from SHMÚ (Slovak hydro – meteorological institute) suggest that in each of the monitored years, precipitation was higher in the proximity of the D.B. station compared to the U.V.Z. station, with a difference of 156 mm. The temperature was higher at the U.V.Z. station. The higher temperatures and lower precipitation could influence the higher values of pollen grains measured at the U.V.Z. station.

The differences in pollen quantity between the two stations can lie in the use of two different evaluation methods as well. Cariñanos et al. (2000) compared the two methods used in our study, and found out that the method of four longitudinal transects used at the U.V.Z. station yields slightly higher results of daily pollen concentration as the method of 12 – traverse transect used at the D.B. station.

It is also necessary to keep the human factor in mind, as it can significantly influence the final values of the pollen grains measured.

## Conclusion

There are two pollen stations (U.V.Z. and D.B.) currently working in Bratislava, situated in different parts of the city. In the monitored period 2007–2010, we detected great differences in the quantity of pollen grains in the atmosphere between the pollen stations. At the U.V.Z. station we measured 106.750 pollen grains more than at the D.B. station in the total during the four years. The biggest differences in the sum of pollen grains were detected in the tree taxa *Populus* L., *Fraxinus* L. and *Quercus* L. and herbaceous taxa *Ambrosia* L. and *Urticaceae*. The highest yearly sums of pollen grains were observed in the genus *Betula* L. The longest pollen season among the observed taxa was the one of the *Urticaceae* family. The detected differences in the quantity of pollen grains at the monitored stations can be connected with several factors, like the different biotopes in the surroundings of the monitoring stations, different microclimatic conditions, different evaluating methods, and, last but not least, the human factor. It is apparent that the better the web of monitoring stations within the region, the more accurate data about the pollen situation in the atmosphere of the monitored area we get.

## Acknowledgements

We sincerely thank the SHMÚ (Slovak Hydrometeorological Institute) for the meteorological data. This contribution is the result of the project implementation: Centre of excellence for protection and use of landscape and for biodiversity ITMS 26240120014, supported by the Research and Development Operational Programme funded by the ERDF.

## References

- Abreu, I., Ribeiro, H., Cunha, M., 2003: An aeropalynological study of the Porto region (Portugal). *Aerobiologia*, 19: 235-241.
- Atkinson, H., Larsson, K. A., 1990: A 10 year record of the arboreal pollen in Stockholm, Sweden. *Grana*, 29: 229-237.
- Ballero, M., Maxia, A., 2003: Pollen spectrum variations in the atmosphere of Cagliari, Italy. *Aerobiologia*, 19: 251-259.
- Bilisik, A., Yenigun, A., Bicakci, A., Eliacik, K., Canitez, Y., Malyer, H., Sapan, N., 2008: An observation study of airborne pollen fall in Didim (SW Turkey): 2004–2005. *Aerobiologia*, 24: 61-66.
- Cariñanos, P., Emberlin, J., Galán, C., Domingues-Vilches, E., 2000: Comparison of two pollen counting methods of slides from a Hirst type volumetric trap. *Aerobiologia*, 16: 339-346.
- D'Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., Liccardi, G., Popov, T., Cauwenberge, P., 2007: Allergenic pollen and pollen allergy in Europe. *Allergy*, 62: 976-990.
- Docampo, S., Recio, M., Trigo, M. M., Melgar, M., Cabezudo, B., 2007: Risk of pollen allergy in Nerja (southern Spain): a pollen calendar. *Aerobiologia*, 23: 189-199.
- Erdtman, G., 1969: Handbook of palynology. Copenhagen: Munksgaard.
- García-Mozo, H., Pérez-Badía, R., Fernández-González, F., Galán, C., 2006: Airborne pollen sampling in Toledo, Central Spain. *Aerobiologia*, 22: 55-66.
- Gawel, J., Halota, A., Pisiewicz, K., Kurzawa, R., Radliński, J., Doniec, Z., 1996: Allergenic airborne sporomorphs calendar for Rabka (Southern Poland), 1991–1995. *Annals of Agricultural and Environmental Medicine*, 3: 87-98.
- Giner, M. M., García, J. S. C., Camacho, C. N., 2002: Seasonal fluctuations of the airborne pollen spectrum in Murcia (SE Spain). *Aerobiologia*, 18: 141-151.
- Gioulekas, D., Balafoutis, Ch., Damialis, A., Papakosta, D., Gioulekas, G., Patakas, D., 2004: Fifteen years' record of airborne allergenic pollen and meteorological parameters in Thessaloniki, Greece. *International Journal of Biometeorology*, 48: 128-136.
- Hrubiško, M., 1996: Hypersensitivity to pollen of trees, grasses and weeds in Bratislava. Comenius University in Bratislava, Bratislava.
- Nilsson, S., Persson, S., 1981: Tree pollen spectra in the Stockholm region (Sweden), 1973–1980. *Grana*, 20: 179-82.
- Rizzi-Longo, L., Pizzulin-Sauli, M., Stravisi, F., Ganis, P., 2007: Airborne pollen calendar for Trieste (Italy), 1990–2004. *Grana*, 46: 98-109.
- Ščevková, J., Dušička, J., Chrenová, J., Mičieta, K., 2010: Annual pollen spectrum variations in the air of Bratislava (Slovakia): years 2002–2009. *Aerobiologia*, 26: 277-287.
- Spieksma, F. T. M., 1991: Regional European pollen calendars. In: D'Amato, G., Spieksma, F.Th.M., Bonini, S., (eds.), *Allergenic pollen and pollinosis in Europe*. Oxford, Blackwell.

## Abstrakt

Cieľom tejto štúdie je porovnanie aeropalynologického výskumu dvoch nezávislých peľových staníc, ktoré sa nachádzajú v rôznych častiach Bratislavy. Odberové miesta sú od seba vzdialené 5,3 km, Katedra botaniky Univerzity Komenského v severozápadnej a Ústav verejného zdravotníctva v severovýchodnej časti mesta. Údaje o peľi v ovzduší boli získavané počas rokov 2007 – 2010 s použitím Hirstovho typu volumetrického lapača simultánne na oboch lokalitách. V našom porovnávaní sme sa zamerali na peľové zrná 10 taxónov vyšších rastlín (*Alnus*, *Betula*, *Carpinus*, *Taxaceae – Cupressaceae*, *Pinus*, *Populus*, *Ambrosia*, *Artemisia*, *Poaceae* and *Urticaceae*), ktoré patria k najsilnejším peľovým alergénom na území Bratislavy a zároveň boli najhojnejšie zastúpené v spektre oboch staníc. Z kvantitatívneho hľadiska sme zaznamenali veľké diferencie v počte peľových zrn sledovaných taxónov v rámci staníc počas jednotlivých rokov ako aj medzi stanicami navzájom. Najväčšie rozdiely sme pozorovali predovšetkým pri drevinách.

**Jozef Dušička, Jana Ščevková, Karol Mičieta, Eva Brutovská, Andrea Sámelová, Mária Zámečníková, Alena Terenová, Jana Lafférová: Peľové koncentrácie v ovzduší Bratislavy (Slovensko): porovnávací štúdiá z dvoch peľových monitorovacích staníc**



## EFFECT OF SOME SUGARS ON MELIBIASE ACTIVITY IN LEMON BALM

Marcela Koreňová<sup>1</sup>, Ján Stano<sup>2</sup>, Karol Mičieta<sup>3\*</sup>

<sup>1</sup>Comenius University in Bratislava, Faculty of Pharmacy, Garden of Medicinal Plants, Odbojárov 10, 832 32 Bratislava, Slovakia

<sup>2</sup>Comenius University in Bratislava, Faculty of Pharmacy, Department of Cell and Molecular Biology of Drugs, Odbojárov 10, 832 32 Bratislava, Slovakia

<sup>3</sup>Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany, Révová 39, 811 02 Bratislava, Slovakia

Received 11 October 2012; Received in revised form 25 October; Accepted 30 October

### Abstract

Cell suspension cultures of *Melissa officinalis* L. (lemon balm) were permeabilized by Tween 20, Tween 80, hexadecyl(trimethyl)ammonium bromide and hexadecylpyridinium chloride resp. Melibiase showed pH optimum at 5.2 and temperature optimum at 65 °C. Enzyme hydrolysis was linear 3 h reaching 65–75 % conversion. Melibiase was in a moderate way inhibited by galactose, lactose, raffinose, stachyose and gluconolactone. The cells characterized by high enzyme activity and stability in long-term storage showed convenient physico-mechanical properties.

**Key words:** *Melissa officinalis* L., melibiase, permeabilization, immobilization, effectors

### Introduction

Glycosidases are enzymes involved in many important processes such as digestion, biosynthesis of glycoproteins, and catabolism of glycoconjugates. The study of natural inhibitors of glycosidases was started by Chrzaszcz and Janicki in 1933 using several cereals. These inhibitors have the potential to produce a number of beneficial therapeutic effects and are biotechnologically relevant. They stimulate interest for the potential treatment of metabolic disorders, as antitumor agents, antiobesity drugs, antivitals and immune modulators. The chemotherapeutic value of glycosidase inhibitors are recently discussed (El Ashry et al. 2000a; El Ashry et al. 2000b; Peak et al. 1998; Watson et al. 2001; Asano et al. 2000).

Melibiase ( $\alpha$ -D-galactoside galactohydrolase EC 3.2.1.22) an  $\alpha$ -galactosidase plays an important role in the vital activity of many micro- and macroorganisms. They catalyse the cleavage of  $\alpha$ -bonded galactose units from the unreducible terminus of oligo- and polysaccharides and participate in degradation of various saccharides in bacteria, yeast and fungi that act as carbon and energy sources for growth and vital activity of the organism. Modification or blocking of these processes by powerful selective inhibitors provides also a basis for treating several infectious diseases, cancerous tumors and genetic mutations (Bakunina et al. 2009). This enzyme is used in sugar beet industry for the hydrolysis of raffinose and stachyose. Microorganisms are the preferred sources of melibiase (Kaneko et al. 1990). Although melibiase is generally present also in plants, this source has not been used till now.

In this paper, the enzymatic hydrolysis of terminal  $\alpha$ -galactose linkage of glycosides (lactase activity) by free, as well as glutaraldehyde immobilized lemon balm cells and also the effect of some sugars on this enzyme activity was studied.

---

\* Corresponding author: Karol Mičieta; [micieta@fns.uniba.sk](mailto:micieta@fns.uniba.sk)

## Material and Methods

**Plant material.** Long term callus and tissue cultures were derived from stem of lemon balm (*Melissa officinalis* L.) as it was described previously (Koreňová et al. 2007).

**Cell permeabilization.** Cell suspensions were filtered through a nylon cloth, and 10 g of fresh mass was suspended in 50 ml of 0.15 mol.l<sup>-1</sup> NaCl with 5 % Tween 20, 5 % Tween 80, 0.1 % hexadecyltrimethylammonium bromide and/or 0.1 % hexadecylpyridinium chloride resp. Permeabilization was carried out for 3 h with moderate stirring at 20 °C. The cells were filtered off and washed with 2000 ml distilled water and 3000 ml of 0.15 mol.l<sup>-1</sup> NaCl solution.

**Cell immobilization.** The permeabilized cells were immediately suspended in 40 ml of 0.15 mol.l<sup>-1</sup> NaCl solution and then 5 ml of 25 % glutaraldehyde was added slowly under mild stirring for 2.5 h at room temperature. The immobilized cells were then separated and washed with 2000 ml of distilled water and 2500 ml of 0.15 mol.l<sup>-1</sup> NaCl solution.

**Fresh and dry mass.** Fresh and dry mass of native and immobilized cells was determined gravimetrically. For determination of dry mass, samples were dried to a constant mass at 105 °C.

**Temperature influence.** The influence of temperature on enzymatic activity in immobilized cells and cells from suspension cultures were tested from 20° to 100 °C.

**Storage stability.** The stability of lactase during storage was monitored in the following experiments. The immobilized cells were stored at 4 °C in 0.15 mol.l<sup>-1</sup> NaCl with addition of the following compounds: a) chloramphenicol 50 mg/l, b) chlortetracycline hydrochloride (CLCTC) 50 mg/l, c) (1-methyldodecyl)dimethylamine N-oxide (ATDNO) 100 mg/l (Devinsky et al. 1980), d) sodium azide 200 mg/l, e) frozen in 0.15 mol.l<sup>-1</sup> NaCl.

**Sugar effectors.** The influence of some sugars on enzymatic activity in cells and immobilized cells was tested using galactose, gluconelactone, lactose, stachyose and raffinose respectively in concentrations from 0.1 to 200 mmol.l<sup>-1</sup>.

**Glucose utilization.** The immobilized cells and cells suspension were exposed to initial glucose concentration (200 mg/l) in the cultivation medium (Koreňová et al. 2007). The concentration change of glucose in medium was determined by the method of Trinder (1969).

**Enzyme assay.** The enzyme assay was performed by a modified method of Kim et al. (2002) using p-nitrophenyl- $\alpha$ -D-galactopyranoside ( $\alpha$ -PNG) as substrate. The reaction mixture contained 0.1 g of fresh cells and 0.5 mg of  $\alpha$ -PNG in 2 ml Mc Ilvaine buffer, pH 4.8. The control contained boiled cells. Both mixtures were kept in a period ranging from 20 min to 5 h at 30 °C using a rotatory shaker (80 rpm). Adding 2 ml of 1 mol.l<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> solution stopped the reaction. Released p-nitrophenol was determined spectrophotometrically at 420 nm. Enzyme activity was calculated per 1 g of fresh mass. The enzyme activity was expressed in katals (kat = mols/s). Protein content was determined by the method of Doumas et al. (1981) using bovine serum albumin as standard.

**Cell viability.** Cell viability was determined using the method given by Dixon (1991) with 2,3,5-triphenyltetrazolium chloride (TTC) as well as fluorescein diacetate and with an oxygen electrode, respectively.

## Results and Discussion

**Model object.** Lemon balm (*Melissa officinalis* L.) is one of the oldest and most popular medicinal plants growing in Mediterranean area which has sedative, carminative, antispasmodic, antibacterial, antiviral, anti-inflammatory and antioxidative therapeutic properties (Fialová et al. 2008).

Many synthetic chromogenic substrates are broadly used for histochemical and biochemical studies of hydrolytic enzymes. p-Nitrophenyl- $\alpha$ -D-galactopyranoside and 6-bromo-naphthyl-  $\alpha$ -D-galactopyranoside are suitable for the study of intra- and extracellular melibiase.

**Immobilization and permeabilization of cells.** Glutaraldehyde immobilized lemon balm cells showed only little morphological changes in comparison with cells in suspension. A small plasmolysis and some aggregation of the cells were occurring during immobilization. The cells immobilized with glutaraldehyde did neither consume glucose (Fig. 1) and were not viable since they did not show any respiratory activity and were not stained with fluoresceine or 2,3,5 triphenyltetrazolium chloride.

Immobilized cells enclosed in hydrogels can be cultivated in similar way as cultures in suspensions (Stano et al. 1995).

The permeabilization of the studied lemon balm cells by Tween 20, Tween 80, hexadecylpyridinium chloride (HPCH) and hexadecyl (trimethyl) ammonium bromide (HTAB), resp. lead to the leakage or degradation of proteins.

Enzyme activity after permeabilization showed a moderate increase. By glutaraldehyde cross-linking only a moderate increase in the enzyme activity has been found (Table 1).

By cell-wall permeabilization of yeasts, a very significant increase in the activity of phenylalanine amonialyase (PAL) was observed (Srinivasan-Nagajyothi et al. 1994).

**Characterization of melibiase in free and immobilized cells.** Melibiase in immobilized cells had pH optimum at 5.2 similar to that of the free cells. Melibiase pH optimum from the marine bacterium *Pseudoalteromonas* sp. was at pH 6.0–7.5 (Bakunina et al. 1998). Enzyme hydrolysis of p-nitrophenyl- $\alpha$ -D-galactopyranoside ( $\alpha$ -PNG) was linear for 3.0 h reaching 65–70 % of conversion and then practically stopped. The temperature optimum in both of free and immobilized cells was at 65 °C. These results indicate a relatively high degree of temperature stability of studied hydrolase.

A partially purified  $\alpha$ -galactosidase from gherkin and poppy seedlings (Machová 1994, Budík 1992) as well as this hydrolase activity in free and immobilized lemon balm cells were inhibited by 0.1–0.5 mmol.l<sup>-1</sup> p-chloromercuribenzoic acid. This inhibitory effect can be eliminated with cysteine, dithiothreitol or 2-mercaptoethanol in 5–10 mmole.l<sup>-1</sup> concentrations. These results indicate that –SH groups are essential for activities of studied enzymes. Melibiase is not metal-dependent and has a free –SH group that is important for the catalytic activity (Bakunina et al. 1998).

The cells cross-linked with glutaraldehyde showed a strong decrease of aminopeptidase activity (Stano et al. 2006). In case of plant proteases, cross-linking with glutaraldehyde is not a suitable method for their immobilization. Contrary to these results, the immobilization of many other plant cells by glutaraldehyde seems to be a convenient method for preservation of different biocatalysts such as galactosidases, invertase as well as L-tyrosine - and L-DOPA-decarboxylase (Stano et al. 1995; Neubert et al. 2002). As an alternative, immobilization by using alginate, pectate or other hydrogels has attracted attention (Gill, Ballesteros 2000). The immobilization of enzymes or cells by encapsulation in hydrogels indicates that this classic method is more appropriate for several enzymes (Stano et al. 2006; Hulst, Tramper 1989) than cross-linking by means of glutaraldehyde (Stano et al. 1995).

**Storage stability.** As illustrated in Table 2 the melibiase activity in lemon balm cells (in 0.15 mol.l<sup>-1</sup> NaCl solution with all preservatives tested) is still relatively high during 6 months storage. The observed phenomenon – increase in the activity of the storage remains unclear. It may be due to a gradual dissociation of inhibitory compounds originally inactivating the enzyme. The tested preservatives did not influence the enzyme activity.

Partially purified enzyme preparation of galactosidases from gherkin and poppy seedlings was inhibited by galactose (Machová 1994; Budík 1992). A similar inhibitory effect was observed also in immobilized cells and in cell suspension culture. The results shown in Table 3 indicate that the enzyme inhibition by studied effectors (galactose, raffinose, maltose and gluconelactone) depends on their concentration. The immobilization costs are very low, and no special equipment is required. Aeration, agitation and the kind of cultivation medium had no influence on the biotransformation potential of glutaraldehyde-immobilized cells. Immobilization of the cells did not require enzyme isolation, whereas the specific enzyme activity of biocatalyst remained quite high. The immobilization of cells facilitates continuous flow-through arrangement, enables improved separation of products from the biocatalysts, significantly prolongs the biocatalysts half-life, ensures physical protection from shear

forces, prevents cell aggregation, stimulates secondary-metabolite production, and preserves the activity of multifunctional enzyme systems (Hulst, Tramper 1989). Both the reaction kinetic parameters and physico-mechanical properties of the biocatalysts are fully comparable to those biocatalysts prepared by immobilization of soluble enzymes on insoluble carriers (Hasal et al. 1992).

The preparation of extracellular galactosidase as well as immobilized enzymes or cells has some importance for biotechnological application in food and pharmaceutical research as well as in industry (Bakunina et al. 2009; Hasal et al. 1992; Klewicki 2007). Their application in the structure studies of some biologically active compounds (Chai et al. 2003a, b; Shimoda et al. 2009), in understanding some biological processes (symbiosis, pathogen resistance and genome recognition) (Stochmal et al. 2009; Fayos et al. 2006) are another potential fields of their practical use.

Biotransformation using free or immobilized biocatalysts not only provide an alternative and efficient solution to the synthesis of many compounds, but also offer environmentally clean technologies that profit from very mild reaction conditions (Olennikov et al. 2009; Dostálová et al. 1999; Trelles et al. 2004).

The relatively high activity of studied enzymes points to prospects of applying them, in tests as drugs with inhibitory potential in biosynthesis and biotransformation of some biopharmaceuticals (Chai et al. 2003a, b) of lactose and other nutrients (Kaneko et al. 1990; Stano et al. 2006). From the point of view of “green chemistry” this kind of biocatalysts could play an important role in the biotransformation of natural or synthetic substrates into useful compounds (Oppenheimer et al. 2008).

Oppenheimer et al. (2008) described carbohydrate drugs that are in current use or under development, and technologies using carbohydrates that offer improved diagnostic and drug development. Advance in carbohydrate synthesis, analysis, manipulation through the emerging field in glycochemistry and glycobiology are providing new approaches in therapy.

**Influence of sugar effectors.** In the seeds of *Cucurbitaceae* family the reserve polysaccharide starch is absent. These and other families contain as reserve and transport sugars galactosides of sucrose : raffinose, stachyose, verbascose and ajugose. Partially purified enzyme preparation of galactosidases from gherkin and poppy seedlings was inhibited by galactose (Machová 1994; Budík 1992).

A similar inhibitory effect was observed also in cells and immobilized cells and in our experiments. The results shown in Table 3 indicate that the inhibitory effect of studied effectors (galactose, raffinose, stachyose, lactose and gluconelactone) is concentration dependent.

Biotransformation using free or immobilized biocatalysts does not only provide an alternative and efficient solution to the synthesis of many compounds, but also offer environmentally clean technologies that profit from very mild reaction conditions (Trelles et al. 2004; Chai et al. 2003a, b; Shimoda et al. 2009).

**Tab. 1. Protein content and melibiase activity in free cells, permeabilized cells and glutaraldehyde immobilized cells from 10 days lemon balm cell suspension**

Cells	Protein [mg/g fresh weight]	Activity [nkat/g fresh weight]	Specific activity [nkat/mg protein]
<b>Suspension</b>	1.28±0.042	29.6±0.38	23.1
<b>Permeabilized</b> 0.1% HTAB	0.72±0.040	33.8±0.38	46.9
0.1% HPCH	0.72±0.040	33.6±0.36	46.7
5% Tween 20	0.72±0.041	33.5±0.35	46.5
5% Tween 80	0.72±0.042	33.6±0.32	46.7
<b>Immobilized</b> 0.1% HTAB	0.64±0.044	33.4±0.35	52.2
0.1% HPCH	0.64±0.044	33.5±0.36	52.3
5% Tween 20	0.64±0.042	32.9±0.33	51.3
5% Tween 80	0.64±0.041	32.8±0.32	51.3

HTAB – hexadecyl(trimethyl)ammonium bromide

HPCH – hexadecylpyridinium chloride

**Tab. 2. Stability of melibiase in immobilized lemon balm cells on storage**

Conservance	% of original enzyme activity				
	0 month	1 month	2 months	3 months	6 months
None	100				
CLCTC (50 mg/l)	66	68	70	76	86
ATDNO (100 mg/l)	66	67	70	77	87
Chloramphenicol (50mg/l)	64	67	72	77	88
Sodium azide (200 mg/l)	64	67	70	77	90
Frozen in 0.15 mol.l <sup>-1</sup> NaCl	65	67	71	78	92

CLCTC – chlortetracycline hydrochloride

ATDNO – (1-methyldodecyl)dimethylamine N-oxide

**Tab. 3. Effect of some sugars on the melibiase activity in free cells and immobilized cells of lemon balm**

Effector	Concentration mmol.l <sup>-1</sup>	% of original activity	
		Free cells	Immobilized cells
None		100	100
Galactose	40	37	40
	20	44	47
	10	55	58
	5	70	72
	1	82	84
	0.1	96	97
Raffinose	200	60	62
	100	73	75
	40	87	90
	20	93	95
	1	97	98
Stachyose	200	85	87
	100	89	91
	40	93	94
	20	97	98
Lactose	200	81	83
	100	86	88
	50	92	96
Gluconelactone	100	60	63
	40	84	87
	10	94	97

### Acknowledgement

This work was partially supported by the Grant Agency VEGA (Bratislava) grant No. 1/3289/06. We are grateful to Dr. V. Blanáriková (Department of Cell and Molecular Biology of Drugs, Faculty of Pharmacy Comenius University) for providing the cell cultures.

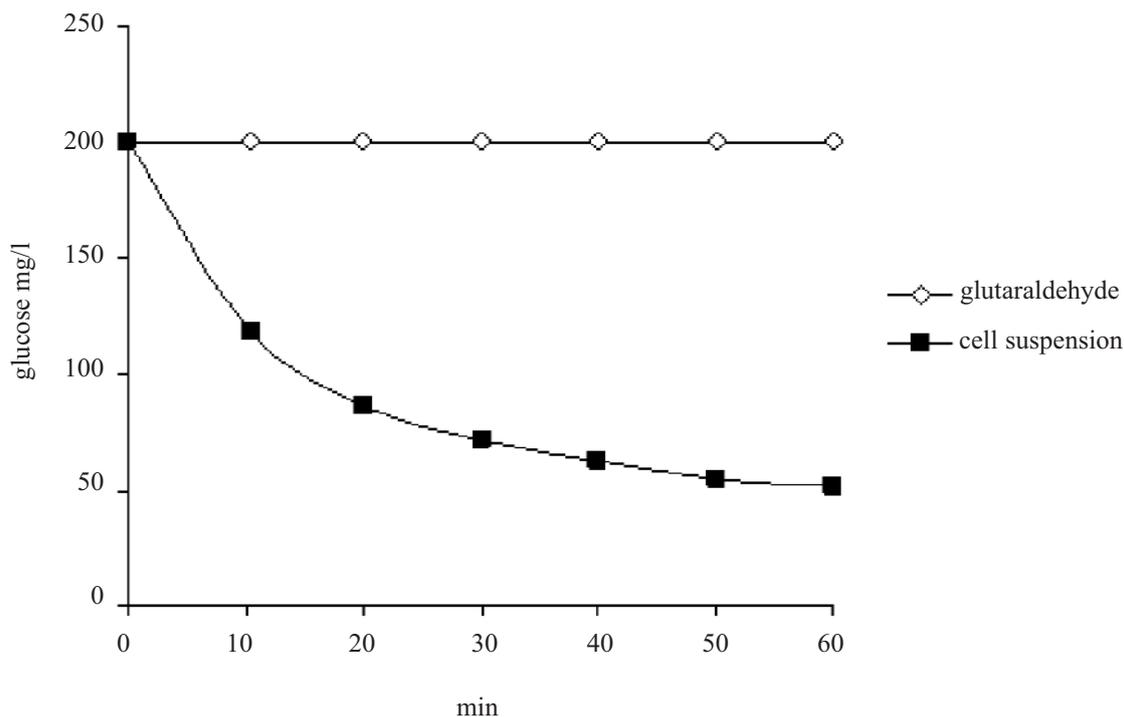


Fig. 1. Time course of glucose consumption in free lemon balm cells and cells immobilized with glutaraldehyde

## References

- Asano, N., Nishida, M., Miyauchi, M., Ikeda, K., Yamamoto, M., Kizu, H., Kameda, Y., Watson, A. A., Nash, R. J., Fleet, G. W., 2000: Polyhydroxylated pyrrolidine and piperidine alkaloids from *Adenophora triphylla* var. japonica (*Campanulaceae*). *Phytochemistry*, 53: 379-382.
- Bakunina, I. Yu., Sova, V. V., Nedashkovskaya, O. I., Kuhlmann, R. A., Likhosherstov, L. M., Martynova, M. D., Mihailov, V. V., Elyakova, L. A., 1998:  $\alpha$ -Galactosidase of the marine bacterium *Pseudoalteromonas* sp. KMM 701. *Biochemistry (Moscow)*, 63: 1209-1215.
- Bakunina, I. Yu., Kol'tsova, E. A., Pokhilo, N. D., Shestak, O. P., Yakubovskaya, A. Ya., Zvyagintseva, T. N., Anufriev, V. F., 2009: Effect of 5-hydroxy- and 5,8-dihydroxy-1,4-naphthoquinones on the hydrolytic activity of  $\alpha$ -galactosidase. *Chem. Nat. Comp.*, 45: 69-73.
- Budík, D., 1992:  $\alpha$ -Galactosidase in poppy cell suspension (*Papaver somniferum* L.) Diploma thesis, Faculty of Pharmacy, Comenius University Bratislava, 96 p.
- Chai, W., Sakamaki, H., Kitanaka, S., Horiuchi, C. A., 2003a: Biotransformation of cycloalkanediones by *Caragana chamlagu*. *Bull. Chem. Soc. Jpn.*, 76: 177-182.
- Chai, W., Sakamaki, H., Kitanaka, S., Saito, M., Horiuchi, C. A., 2003b: Biodegradation of bisphenol A by cultured cells of *Caragana chamlagu*. *Biosci. Biotechnol. Biochem.*, 67: 218-220.
- Chrzaszcz, T., Janicki, J., 1933: "Sisto-amylase", a natural inhibitor of amylase. *Biochem. Z.*, 260: 354-368.
- Devínsky, F., Lacko, I., Mlynarčík, D., Krasnec, L., 1980: (1-methyl-dodecyl)-dimetylamin-N-oxid. CS Patent No. 181477, Praha.
- Dixon, R. A., 1991: Plant cell culture. A practical approach. Oxford, Washington, IRL Press: 1-20.
- Dostálová, J., Krejčová, A., Réblová, Z., Pokorný, J., 1999: Changes of  $\alpha$ -galactosidase content during soaking and cooking of peas. *Bull. Food Res.*, 38: 133-140.
- Doumas, B. T., Bayse, D. D., Carter, R. J., Peters, T., Schaffer, R., 1981: A candidate reference method for determination of total protein in serum. I. Development and validation. *Clin. Chem.*, 27: 1642-1650.
- El Ashry, E. S., Rashed, N., Shobier, A. H. S., 2000a: Glycosidase inhibitors and their chemotherapeutic value. Part 1. *Pharmazie*, 55: 251-256.
- El Ashry, E. S., Rashed, N., Shobier, A. H. S., 2000b: Glycosidase inhibitors and their chemotherapeutic value. Part 2. *Pharmazie*, 55: 331-348.
- Fayos, J., María Bellés, J., López-Gresa, M. P., Primo, J., Conejero V., 2006: Induction of gentisic acid 5-O- $\beta$ -D-xylopyranoside in tomato and cucumber plants infected by different pathogens. *Phytochemistry*, 67: 142-148.

- Fialová, S., Tekeľová, D., Mrlíanová, M., Grančai, D., 2008: The determination of phenolics compounds and antioxidant activity of mints and balms cultivated in Slovakia. *Acta Facult. Pharm. Univ. Comenianae*, 55: 96-102.
- Gill, I., Ballesteros, A., 2000: Bioencapsulation within synthetic polymers. (Part 1): sol-gel encapsulated biologicals. *Trends Biotechnol.*, 18: 282-296.
- Hasal, P., Vojtíšek, V., Čejková, A., Kleczek, P., Kofroňová, O., 1992: An immobilized whole yeast cell biocatalyst for enzymatic sucrose hydrolysis. *Enzyme Microbiol. Technol.*, 14: 221-229.
- Hulst, A. A., Tramper, J., 1989: Immobilized plant cells. A literature survey. *Enzyme Microbiol. Technol.*, 11: 546-558.
- Kaneko, R., Kusakabe, I., Sakai, Y., Murakami, K., 1990: Substrate specificity of  $\alpha$ -galactosidase from *Martierella vinacea*. *Agric. Biol. Chem.*, 54: 237-245.
- Kim, W. D., Kobayashi, O., Kaneko, S., Kakakibara, Y., Park, G. G., Kusakabe, I., Tanaka, H., Kobayashi, H., 2002:  $\alpha$ -Galactosidase from cultured rice (*Oryza sativa* L. var. *Nipponbare*) cells. *Phytochemistry*, 61: 621-630.
- Klewicki, R., 2007: Effect of selected parameters of lactose hydrolysis in the presence of  $\beta$ -galactosidase from various sources on the synthesis of galactosyl-polyol derivatives. *Eng. Life Sci.*, 7: 268-274.
- Koreňová, M., Stano, J., Blanáriková, V., Mičieta, K., 2007: Identification and determination of aminopeptidase activities secreted by lemon balm. *Chem. Nat. Comp.*, 43: 201-203.
- Machová, B., 1994: Identification and study of  $\alpha$ -galactosidase in gherkin seedlings-*Cucumis sativus* L. Diploma thesis, Faculty of Pharmacy, Comenius University, Bratislava, 81 p.
- Neubert, K., Stano, J., Mičieta, K., Kovács, P., Tintemann, H., 2002: Invertase in immobilized cells of *Eschscholtzia californica*. *Biol. Plant.*, 45: 307-310.
- Olennikov, D. N., Roklin, A. V., Tankhaeva, L. M., 2009: Lamiaceae carbohydrates VI. Water-soluble polysaccharides from *Lophanthus chinensis*. *Chem. Nat. Comp.*, 45: 300-303.
- Oppenheimer, S. B., Alvarez, M., Nnoli, J., 2008: Carbohydrate-based experimental therapeutics for cancer, HIV/AIDS and other diseases. *Acta Histochem.*, 110: 6-13.
- Peak, N. S., Kanag, O. J., Lee, H. S., Lee, J. J., Choi, J. J., Kim, T. M., Kim, J. J., 1998: Enzymatic synthesis of 6-0- $\alpha$ -galactopyranosyl-1-deoxynojirimycin using  $\alpha$ -galactosidase from green beans. *Biosci. Biotechnol. Biochem.*, 62: 588-598.
- Shimoda, K., Ishimoto, H., Kamiue, T., Kobayashi, T., Hamada, H., Hamada, H., 2009: Glycosylation of sesamol by cultured plant cells. *Phytochemistry*, 70: 207-210.
- Srinivasan-Nagajothi, A. R., Gowda, L. R., Bhat, S. G., 1994: Phenylalanine ammonia-lyase in permeabilized yeast cells (*Rhodotorula glutidis*). *Biotechnol. Tech.*, 8: 729-734.
- Stano, J., Nemeč, P., Weissová, K., Kovács, P., Kákoniová, D., Lišková, D., 1995: Decarboxylation of L-tyrosine and L-DOPA by immobilized cells of *Papaver somniferum*. *Phytochemistry*, 38: 859-860.
- Stano, J., Mičieta, K., Tintemann, H., Neubert, K., 2006: Bioassay for the determination of the intra- and extracellular activity of aminopeptidases in immobilized tomato cells. *Chem. Biodiv.*, 3: 414-421.
- Stochmal, A., Kowalska, I., Janda, B., Perrone, A., Piacente, S., Oleszek, W., 2009: Gentic acid conjugates of *Medicago truncatula* roots. *Phytochemistry*, 70: 1272-1276.
- Trelles, J. A., Bentancor, L., Schoijet, A., Porro, S., Lewkowicz, E. S., Sinisterra, J., Iribarren, A. M., 2004: Immobilized *Escherichia coli* BL 21 as a catalyst for synthesis of adenine and hypoxanthine nucleosides. *Chem. Biodiv.*, 1: 280-288.
- Trinder, P., 1969: Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Ann. Clin. Biochem.*, 6: 24-32.
- Watson, A. A., Fleet, W. J., Asano, N., Molyneux, R. J., Nash, R., 2001: Polyhydroxylated alkaloids – natural occurrence and their applications. *Phytochemistry*, 56: 265-295.

## Abstrakt

Suspenné kultúry *Melissa officinalis* L. sa permeabilizovali jednotlivo Tweenom 20, Tweenom 80, hexadecyltrimetylamonium bromidom a hexadecylpyridinium chloridom. pH optimum melibiázy je 5,2 a tepelné optimum má pri 65 °C. Enzymová hydrolyza má lineárny priebeh 3 h a dosahuje 65 – 75 % konverzie. Melibiázu parciálne inhibujú: galaktóza, laktóza, rafinóza, stachyóza a glukónolaktón. Imobilizované bunky sa v priebehu dlhodobého uchovávanía vyznačujú vysokou enzymovou aktivitou a vhodnými fyzikálno-mechanickými vlastnosťami.

**Marcela Koreňová, Ján Stano, Karol Mičieta: Účinok niektorých cukrov na aktivitu melibiázy medovky lekárskej**



## Sexagenarian prof. RNDr. Karol Mičieta, PhD. – a botanist, environmentalist, visionary



The official biography of prof. Dr. Karol Mičieta, PhD., is too brief. For each of its sentences a separate chapter could be written.

He was born on November, 19th, 1952, in Žilina. It means that he had a childhood close to Nature. He was interested in collecting butterflies and in aquarium fishes, but botany eventually won. In his diploma work of 1976 he studied flora of the Javorníky mountains near his hometown. In the PhD work of 1980 he focused to the genus *Juncus*.

After completing his studies at Comenius University, he joined the Department of Botany, where Assoc. Prof. Augustín Murín became his scientific supervisor. He remained his most loyal student, which was not easy at all, because Assoc. Prof. Murín was after 1968 proscribed by the political regime. On the other hand, he had far more time for a young botanist and could give him a truly global outlook.

They together established in 1981 a new Department of Karyology of the new Institute of Molecular and Subcellular Biology (later, from 1990 to 1995, the Institute of Cell Biology), Faculty of Science. It was a period of most ambition plans. A completely new methodology for testing of environmental pollution through pollen grains opened a whole world for Karol Mičieta. After the first works published in domestic scientific magazines came in 1996 a groundbreaking article in *Environmental and Experimental Botany* (USA), followed soon by an invitation for an international conference in Lucknow, India. Together with Dr. Gustáv Murín, PhD., they further developed this method and presented their findings during the following years in Malaysia, Japan, the USA and several European capitals with extraordinary positive response from foreign colleagues. Prof. Mičieta has further developed this methodology with the help of a new generation of botanists until today. He is also

a inventor of the original method of retrospective biomonitoring of environment via pollen grains of an old herbarium items even a century back.

In 1995 he returned to the UK Department of Botany, that he actually physically never left, now in the role of head of department in the footsteps of his lifelong teacher professor Murín. As one of the few in that time leadership of science he was able to rapidly adapt to the fundamentally new system of grant funding for research after 1989, thanks to which he has been able to explore further the topics of the use of plants as bioindicators of the environment for the third decade. His reports from systematic and environmental botany contain 416 items, including one monograph, such as articles published in Slovakia and abroad. He is a co-author of several chapters in prestigious foreign monographs published in the USA, the Netherlands, Austria, Poland, Croatia and Ukraine.

In 1998, he qualified for associate professor in the field of botany. In 2007 reached the title of professor of general ecology, ecology of individuals and populations. He is a member of several professional Slovak and foreign scientific societies, expert committees and scientific advisory boards. He participates in many national and international scientific projects. From December 2002 to January 2011 he was the chairman of the Academic Senate of Comenius University. Since February 2011, he has held the post of Rector of Comenius University in Bratislava. Always well-educated, not only in basic scientific issues, but as well as legislative and logistical matters, he can still come up with new approaches to meet current scientific challenges in the changing academic environment in Slovakia. Outstanding scientific personality: botanist, environmentalist, visionary...

### **Šesťdesiatnik prof. RNDr. Karol Mičieta, PhD. – botanik, environmentalista, vizionár**

Oficiálny životopis prof. RNDr. Karola Mičietu, PhD., je príliš stručný. Za každou jeho vetou by sa dala napísať samostatná kapitola.

Narodil sa 19. novembra 1952 v Žiline. To znamená, že mal od mala blízko k prírode. Zaujímal sa o motýle, akvaristiku, nakoniec zvíťazila botanika. Vo svojej diplomovej práci z roku 1976 spracoval botanicky jeho rodisku blízke pohorie Javorníky. V kandidátskej práci z roku 1980 sa venoval problematike rodu *Juncus*.

Po ukončení štúdia na Univerzite Komenského nastúpil na Katedru botaniky, kde bol jeho školiteľom doc. Augustín Murín. Stal sa jeho najvernejším žiakom, čo vôbec nebolo ľahké, lebo doc. Murín bol po roku 1968 režimom proskribovaný. Na druhej strane mal pre vnímavého mladého botanika viac času a odovzdal mu svoj v tej dobe naozaj svetový rozhľad.

Spolu v roku 1981 založili oddelenie Karyológie na Ústave molekulárnej a subcelulárnej biológie (neskôr, 1990 až 1995, Ústav bunkovej biológie) PriF UK. Bolo to obdobie najsmelších plánov. S úplne novou metodikou testovania znečistenia životného prostredia pomocou peľových zŕn sa pred Karolom Mičietom otvoril celý svet. Po prvých prácach publikovaných v domácich periodikách prišiel už v roku 1996 prelomový článok v *Environmental and Experimental Botany* (USA) a zakrátko aj pozvanie na svetovú konferenciu v indickom Lucknow. Túto metódu spolu s Dr. Gustávom Murínom, PhD. ďalej rozpracovávali a odprezentovali aj v Malajzii, Japonsku, USA a viacerých európskych metropolách s mimoriadnym ohlasom u zahraničných kolegov. Túto metodiku rozvíja prof. Mičieta s pomocou novej generácie botanikov dodnes. Je autorom originálnej metodiky retrospektívneho biomonitoringu životného prostredia hodnotením peľových zŕn herbárových položiek späť až o storočie.

V roku 1995 sa vrátil na Katedru botaniky PriF UK, z ktorej fyzicky vlastne nikdy neodišiel, už v úlohe vedúceho katedry po stopách svojho celoživotného učiteľa profesora Murína. Ako jeden z mála sa dokázal po roku 1989 rýchlo vyrovať so vtedy zásadne novým grantovým systémom financovania výskumu, vďaka ktorému rozvíja tému využitia rastlín ako bioindikátorov životného prostredia už tretie desaťročie. Jeho dielo systematického a environmentálneho botanika obsahuje vrátane monografie 416 samostatných prác publikovaných doma i v zahraničí. Je spoluautorom kapitol v prestížnych zahraničných monografiách publikovaných v USA, Holandsku, Rakúsku, Poľsku, Chorvátsku a Ukrajine.

V roku 1998 sa habilitoval na docenta v odbore botanika. V roku 2007 získal titul profesora v odbore všeobecná ekológia, ekológia jedinca a populácií. Je členom viacerých odborných slovenských i zahraničných spoločností, odborných komisií a vedeckých rád. Podieľa sa na mnohých domácich aj zahraničných vedeckých projektoch. Od decembra 2002 do januára 2011 bol predsedom Akademického senátu Univerzity Komenského. Od februára 2011 zastáva post rektora Univerzity Komenského v Bratislave. Vždy rozhladený nielen v odbornej problematike, ale napríklad aj v jej legislatívnom a logistickom zázemí, dokáže prichádzať so stále novými prístupmi k napĺňaniu aktuálnych vedeckých úloh práve v čase zásadnej transformácie akademického prostredia u nás. Výnimočná vedecká osobnosť: botanik, environmentalista, vizionár...

Gustáv Murín

*Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany, Révová 39, 811 02 Bratislava, Slovakia*



**ACTA  
BOTANICA  
UNIVERSITATIS  
COMENIANAE**

Volume 47

Vydala Univerzita Komenského v Bratislave vo Vydavateľstve UK  
Vyšlo v decembri 2012

Technická redaktorka: Alena Zvěřinová

**ISBN 978-80-223-3332-0**  
**ISSN 0524-2371**

