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E-mail: knizuel@savzv.sk

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Morphological variability among populations of *Harpalus rufipes* (Coleoptera, Carabidae): What is more important – the mean values or statistical peculiarities of distribution in the population?

Viktor V. Brygadyrenko, Daria Y. Reshetniak

Department of Zoology and Ecology, Oles' Honchar Dnipropetrovsk National University, pr. Gagarina, 72, 49010 Dnipropetrovsk, Ukraine; e-mail: brigad@ua.fm

Abstract

Brygadyrenko, V. V., Reshetniak, D. Y. 2014. Morphological variability among populations of *Harpalus rufipes* (Coleoptera, Carabidae): What is more important – the mean values or statistical pecularities of distribution in the population? *Folia oecol*., 41: 109–133.

The paper analyzes the variability of 19 characteristics (14 linear measurements, 4 angular characteristics and density of elytra downiness), as well as 8 morphometric indices for 391 imagoes of *Harpalus rufipes* (De Geer, 1774) collected in 9 forest, field and steppe ecosystems under various degrees of anthropogenic pressure in four administrative districts of Dnipropetrovsk region, Ukraine. The presence of significant ($P < 0.001$) negative asymmetry in females and absence thereof ($P > 0.05$) in males is typical for body length, head length, elytra length, distance between eyes, head width, prothorax width between the front angle and the back angle, elytra width between humeral angles, and maximum elytra width. For all these characteristics, the excess in males is not significant ($P > 0.05$), while in females in most cases it is significantly positive ($P < 0.05$), which is evidence that there is a large number of females with a greater length of the body and greater width of the head, prothorax and elytra. The absence of significant asymmetry ($P > 0.05$) in males and females proves the absence of directional selection in the populations of *H. rufipes* on the density of elytra downiness and value of the prothorax back angle. A significant negative asymmetry was recorded both in males and females for the maximum width of prothorax ($P < 0.001$) and body height ($P < 0.05$), i.e. unidirectional increase in these characteristics takes place in specimens of both sexes. As distinct from the linear measurements, for all 8 considered proportions of the body in specimens of both sexes the excess is significantly positive ($P < 0.001$), suggesting higher constancy of bodily proportions in *H. rufipes* than of absolute size. For most of the linear characteristics, a significant ($P < 0.001$) sexual dimorphism is recorded. No marked differences between the 9 populations studied within the groups of specimens of the same sex are recorded. In the areas where the annual burning of crop residues and litter is observed, differences between males and females in length are two times higher than the differences between males and females for the ecosystems with no such burning. In the driest areas, maximum elytra width – prothorax width ratio is observed in females. The vertex angle of elytra significantly differs in the populations of the various administrative districts. The average density of elytra downiness in males is 13.3% lower than in females. The results of PCA (principal component analysis) have shown that most of the linear characteristics were connected with the sex of the beetle, while variations in the angular characteristics and degree of elytra downiness bore no relationship to the sex of the *H. rufipes* specimens. The results of our research suggest that the mean values of morphometric characteristics in environmental studies may have less diagnostic value than the type of their distribution in the population.

Key words

Carabidae, Coleoptera, *Harpalus rufipes,* morphometrics, population variability, sexual dimorphism

Introduction

Earlier studies of populations of beetles, primarily ground beetles, were focused on identification of the differences between related species (Pizzo et al., 2006, Talarico et al., 2011), presence of sexual dimorphism (Benítez et al., 2010, 2013a, 2013b), fluctuating asymmetry (Benítez, 2013; Bravi and Benítez, 2013; Daloso, 2014) or changes in the average size of the body under the influence of certain environmental factors or geographic location (Alibert et al., 2001; Bonacci et al., 2006; Okuzaki et al., 2010; Giglio et al., 2011; Sukhodolskaya, 2013). The range of fluctuations and patterns of distribution of morphometric characteristics have been examined in a few studies only (Blake et al., 1994; Sota et al., 2000; Okada et al., 2006; Slin'ko et al., 2008; Benítez et al., 2011; Sukhodolskaya and Eremeeva, 2013). Usage of the methods of multivariate statistics (for example, the geometrical morphometric approach), on the one hand, makes the assessment of changes within populations more clear. However, on the other hand, it does not allow the analysis of characteristics taken separately and comparison of one's own results with the data of other authors. In connection with this, taking one of the most dominant species of ground beetle as our example, we would like to show in this study the importance of not only the analysis of mean values of any characteristic and their joint variability, but to emphasize the importance of analyzing the patterns of distribution of characteristics in a population.

Normal distribution of a particular characteristic may indicate the absence of directional selection on the given parameter (Schluter, 2000). Presence of significant asymmetry is one of the indicators of directional selection on a particular parameter in a specific population (Rueffler et al., 2006). Significant values of excess indicate the intensification of stabilizing selection on the defined attribute. A particularly strong change in the statistical parameters of variation should take place in (1) so called twin species (phylogenetically close species occupying a similar geographic range), as well as in (2) species populating various habitat types (forest, meadow, steppe ecosystems), (3) taxa for which sharp changes in numbers are observed as a result of adaptation to the impact of anthropogenic factors (in various types of agrocenoses, urbanized ecosystems, areas close to industrial enterprises etc.) (Svanbäck et al., 2009).

In this context a convenient object of population studies is *Harpalus rufipes* (De Geer, 1774) (Coleoptera, Carabidae). This is an abundant species corresponding to all three characteristics given above:

- In most habitats it is found together with 3–7 species of the genus *Harpalus*, more often dominating among them in its numbers and having the maximum size among the entire group of ground beetles with mixed (vegetable and animal) diet (Niemelä, 1993; Zhang et al., 1997; Lang et al., 1999; Snyder and Wise, 1999; Thomas et al., 2001; Purvis and Fadl, 2002; Irmler, 2003; Monzo et al., 2011).
- In the steppe zone it is a habitat generalist which populates ecosystems of all moisture gradations (from swampy river banks to the driest positions of ridges which divide ravines) and degrees of soil salination (from salt flats and carbonate soils to humic chernozem soils and areas with insignificant acidification of individual soil horizons), various types of phytocenoses (from dry steppe areas with poorly developed herbaceous vegetation to indigenous floodplain and sandy terrace forests with closed canopy of leaves and shrubs), and inhabiting litter horizons at all degrees of development (from its total absence on arable land to a heavy layer of pine needles or leaf litter in broadleaf forests) (Parmenter and Macmahon, 1988; Frampton et al., 1995; Hawthorne et al., 1998; Magura et al., 2001; Brygadyrenko, 2003; Reshetniak and Brygadyrenko, 2013).
- Particularly high populations are reached in natural communities disturbed by man (on fields of nonperennial and perennial agricultural crops, near major traffic arteries, in industrial zones and human settlements) (Davies, 1953; Dunn, 1981; Kutasi et al., 2004).

H. rufipes is capable of undertaking significant flightless and flight migrations, while forming considerable clusters of individuals in the areas with optimal hydro-thermal conditions, and concentrations of food (vegetable and animal) items. Seeds of plants represent a favorite component of the diet of this ground beetle species, which actually causes considerable damage to agricultural crops (Hartke et al., 1998; Gaines and Gratton, 2010; Meiss et al., 2010; Saska et al., 2010; Bohan et al., 2011; Baraibar et al., 2012). Invertebrates are supplements to the diet of *H. rufipes*, and the list of species it consumes in the steppe zone of Ukraine amounts to several dozen (Reshetniak and Brygadyrenko, 2013). Populations of *H. rufipes* are convenient for assessment of morphological variability in various types of ecosystems and under the influence of different anthropogenic factors, but this subject has remained unexplored so far.

Before beginning our research we raised the following hypotheses: (1) the distribution of morphometric parameters in the studied ecosystems would be normal, (2) as anthropogenic transformation of an ecosystem increases significant changes in mean values should occur, with growing asymmetry and excess of morphometric characteristics and indices, and (3) in the conditions of anthropogenically transformed ecosystems differences between *H. rufipes* males and females will become greater.

Material and methods

We studied 9 populations of *H. rufipes*, located in Novomoskovsk (ecosystems 1–6), Pavlograd (7), Dnipropetrovsk (8) and Petrikovka (9) districts of Dnipropetrovsk region, Ukraine (Table 1). The ecosystems from which the ground beetle imagoes were collected have differing degrees of humidity (xerophilous – 5 and 6, mesoxerophilous – 1 and 8, xeromesophilous – 3 and 4, mesophilous – 2 and 7, mesohygrophilous – 9), types of plant community (agrocenoses – 1, 2 and 8, natural forest – 3 and 9, planted forest – 4 and 7 and steppe ecosystems – 5 and 6), types and degrees of anthropogenic transformation (none – 3 and 9, low – 5 and 7, medium – 4 and 6, high – 1, 2 and 8).

Specimens of *H. rufipes* were collected by soil traps; beetles were killed by freezing at –15 ºC during 24 hours in a cooling chamber and laid onto cotton mats, having been previously stretched out (to maintain proportions, orientation of the head and prothorax was tracked). Photographs of the dried insects were taken through binocular stereoscopic microscope MBS-10 with the use of digital camera with the resolution of 5 megapixels in two (top and side) projections. Each beetle was assigned a serial number including the ecosystem number and sex of the specimen (female, male). Measurements were made by digital photographs in the software package TpsDig 2.17 (2013, Rohlf F.J., Ecology & Evolution, SONY at Stony Brook). 14 linear, 8 angular characteristics and pore density on the elytra were measured.

The following linear characteristics were measured: length of head (Lc), prothorax (Lp), elytra (Le), clypeus (Lcl), distance between eyes (Sa1), length of

Table 1. Brief characteristics of the ecosystems (Dnipropetrovsk region, Ukraine) where *H. rufipes* was collected

Ecosystem	District	Coordinates	Type of moisture	Type of ecosystem	Degree of anthropogenic impact
1	Novomoskovsk	48.790374 N, 35.455946 E	Mesoxerophilous	Lucerne field	High: cutting of vegetation, application of fertilizers
2	Novomoskovsk	48.774368 N, 35.419725 E	Mesophilous	Clover field	High: cutting of vegetation, application of fertilizers
3	Novomoskovsk	48.789978 N, 35.449251 E	Xeromesophilous	Ravine soil cover without grass, elm-ash forest	None
4	Novomoskovsk	48.760904 N, 35.462040 E	Xeromesophilous	Acacia forest belt with catchweed and cow parsley on the upland soil	Medium: burning of leaf litter, contamination with domestic waste
5	Novomoskovsk	48.760452 N, 35.452169 E	Xerophilous	Area of zonal steppe vegetation	Low
6	Novomoskovsk	48.779289 N, 35.468220 E	Xerophilous	Area of zonal steppe vegetation	Medium: excessive grazing of cattle, burning of crop residues
7	Pavlograd	48.602401 N, 35.623144 E	Mesophilous	Soil cover without grass, elm-ash-oak plantation with traces of excessive salination of soil	Low: cattle grazing, soil salination
8	Dnipropetrovsk	48.383352 N, 35.068592 E	Mesoxerophilous	Corn field	High: soil replowing, cultivation of row crops, application of fertilizers
9	Petrikovka	48.495128 N, 34.797109 E	Mesohygrophilous	Bottomland maple-ash forest with nettle	None

eyes (La), width of head with eyes (Sa2), prothorax width between the front angles (Sp1) and back angles (Sp3), maximum width of prothorax (Sp2), width of elytra near humeral angles (Se1), maximum width of elytra (Se2), body height at the level of metathorax (Hb). Total body length (Lb) was determined by combining the length of the head, prothorax and elytra (from the forward edge of upper lip to the top of elytra). Linear characteristics were evaluated by photographs with an accuracy of 1 pixel equal to 0.0024 mm for linear measurements up to 2 mm and 0.0048 mm – for linear measurements over 2 mm.

In order to eliminate the influence of the position of each beetle when the photographs were taken, angular values were measured for the right and left parts of the body, for the further calculations their arithmetical mean value was used. The left (A1) and right angle of prothorax (A2), left (B1) and right back angle of prothorax (B2), left (C1) and right humeral angle of elytra (C2), left (D1) and right vertex angle of elytra (D2) were measured. Measurement of angles was made using photographs with an accuracy of 0.1º.

Elytra pore density (P) was assessed from photographs, by counting the quantity of hairs on an area of 0.15 mm^2 between the backward edge of the scutellar groove and the first groove of the elytra. For each beetle hairs were counted on the right and left elytra; for further processing the arithmetical mean values of the above were taken.

Indices (body proportions) were calculated taking into account methods we have used earlier (Sharova, 1981; Faly and Brygadyrenko, 2007; Brygadyrenko and Fedorchenko, 2008; Korolev and Brygadyrenko, 2014). 8 indices were calculated, namely: ratio of body length to its height (Lb/Hb), ratio of arithmetical mean value of the width of head, prothorax and elytra to body length ((Sc + Sp +Se)/3Lb), ratio of prothorax length to its maximum width (Lp/Sp2), ratio of elytra length to prothorax length (Le/Lp), ratio of maximum elytra width to maximum prothorax width (Se2/Sp2), ratio of maximum prothorax width to its width at the backward edge (Sp2/Sp3), ratio of maximum elytra width to the distance between their front angles (Se2/Se1), and elytra length to width ratio (Le/Se).

The results were processed by standard methods of variation statistics (with the calculation of: x – mean value, SD – standard deviation, Min–Max – minimum and maximum values, D – variation range of characteristics, As – asymmetry, Ex – excess) using Statistica software (version 8, StatSoft, USA). Significance of variations between samples was assessed using one-way ANOVA, for multiple comparisons of samples we used the Tukey test (StatGraphics Plus v5.1 package). Data in the text, in tables and on diagrams (Fig. 7 and 8) are represented as the mean value ± standard deviation.

Results

General variability of distribution of characteristics in males and females

Presence of significant ($P < 0.001$) negative asymmetry in females and its absence ($P > 0.05$) in males is typical for body length (Lb, $As_{male} = –0.13$, $As_{female} = –0.88$, Fig. 1), head length (Lc, $As_{male} = –0.16$, $As_{female} = –0.79$, Fig. 1), elytra length (Le, $As_{male} = –0.27$, $As_{female} = –1.30$, Fig. 1), distance between eyes (Sa1, $As_{male} = 0.23$, $As_{female} = –0.62$), head width (Sa2, $As_{male} = –0.25$, $As_{female} = –1.02$, Fig. 2), prothorax width between the front angles (Sp1, $As_{male} = 0.15$, $As_{female} = –0.67$, Fig. 2) and back angles (Sp3, $As_{male} = –0.06$, $As_{female} = –0.80$, Fig. 2), width of elytra between the front angles (Se1, $As_{male} = –0.17$, $As_{female} = –0.89$, Fig. 3), maximum width of elytra (Se2, $As_{male} = 0.05$, $As_{female} = – 0.74$, Fig. 3). For all these characteristics the excess in males is not significant ($P > 0.05$), while in females in most cases (except Sp1 and Sa1) it is significantly positive ($P < 0.05$ and $P < 0.001$). It indicates the presence of selection in females: specimens with greater body length, width of head, prothorax and elytra are more widespread in the population.

Significant ($P < 0.001$) asymmetry in males and insignificant asymmetry ($P > 0.05$) in females was recorded for prothorax length (Lp, $As_{male} = –0.57$, $As_{female} = –0.23$, Fig. 1). It indicates the higher rate of increase in prothorax length in males compared with females.

The symmetry of distribution of characteristics (absence of significant asymmetry) for males and females is evidence of absence of directional selection in the studied populations of *H. rufipes*. Absence of asymmetry ($P > 0.05$) was revealed for pore density of the elytra (P, $As_{male} = 0.16$, $As_{female} = 0.01$, Fig. 3) and value of the prothorax back angle (B, $As_{male} = –0.05$, $As_{female} = 0.14$, Fig. 4). These attributes can be considered among of the most stable for *H. rufipes*.

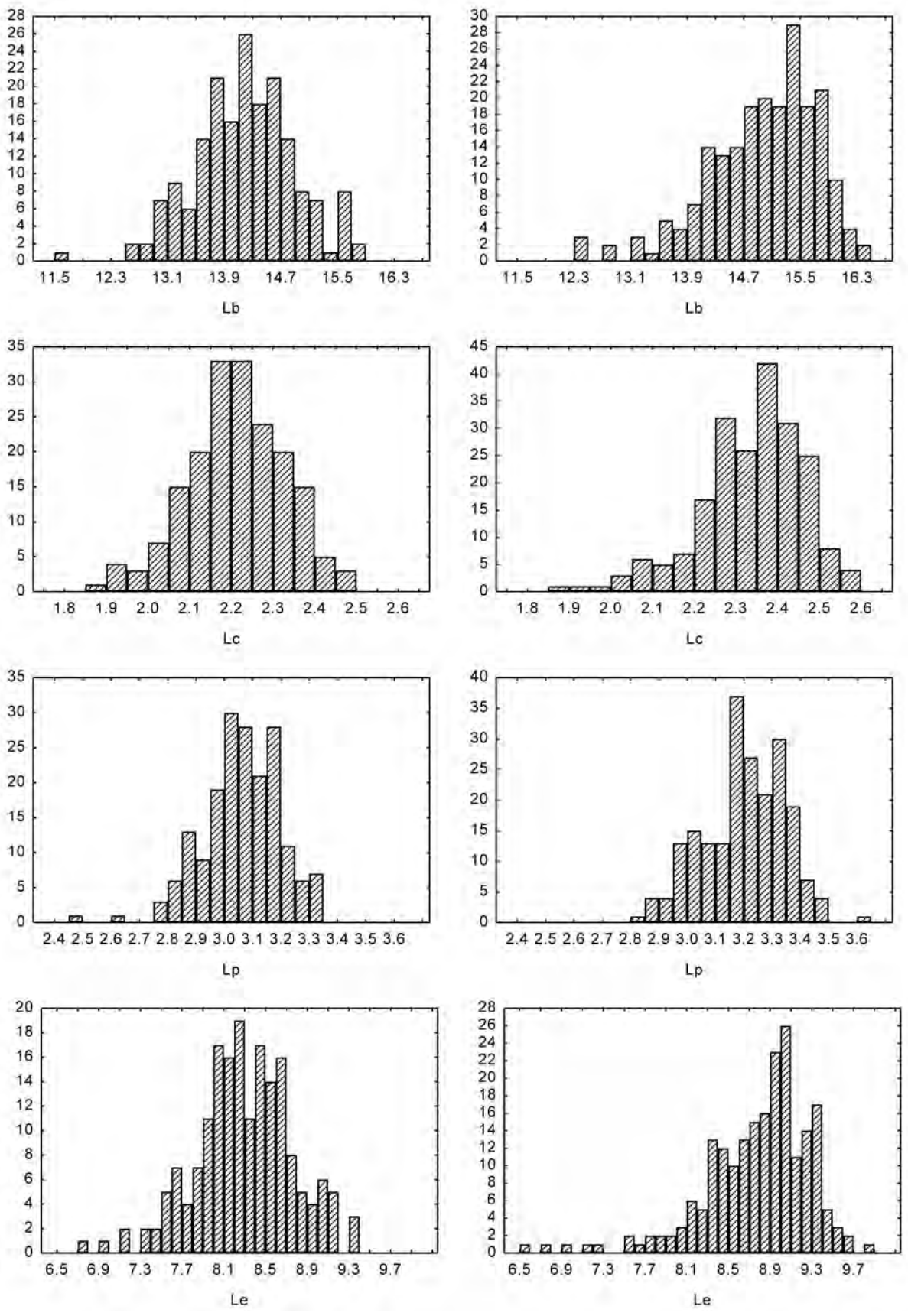

Fig. 1. Variability of length of the body (Lb), head (Lc), prothorax (Lp) and elytra (Le) in *H. rufipes*: on the left – males (n = 183), on the right – females (n = 209); on X-axis – value of characteristics in millimeters, on Y-axis – number of specimens.

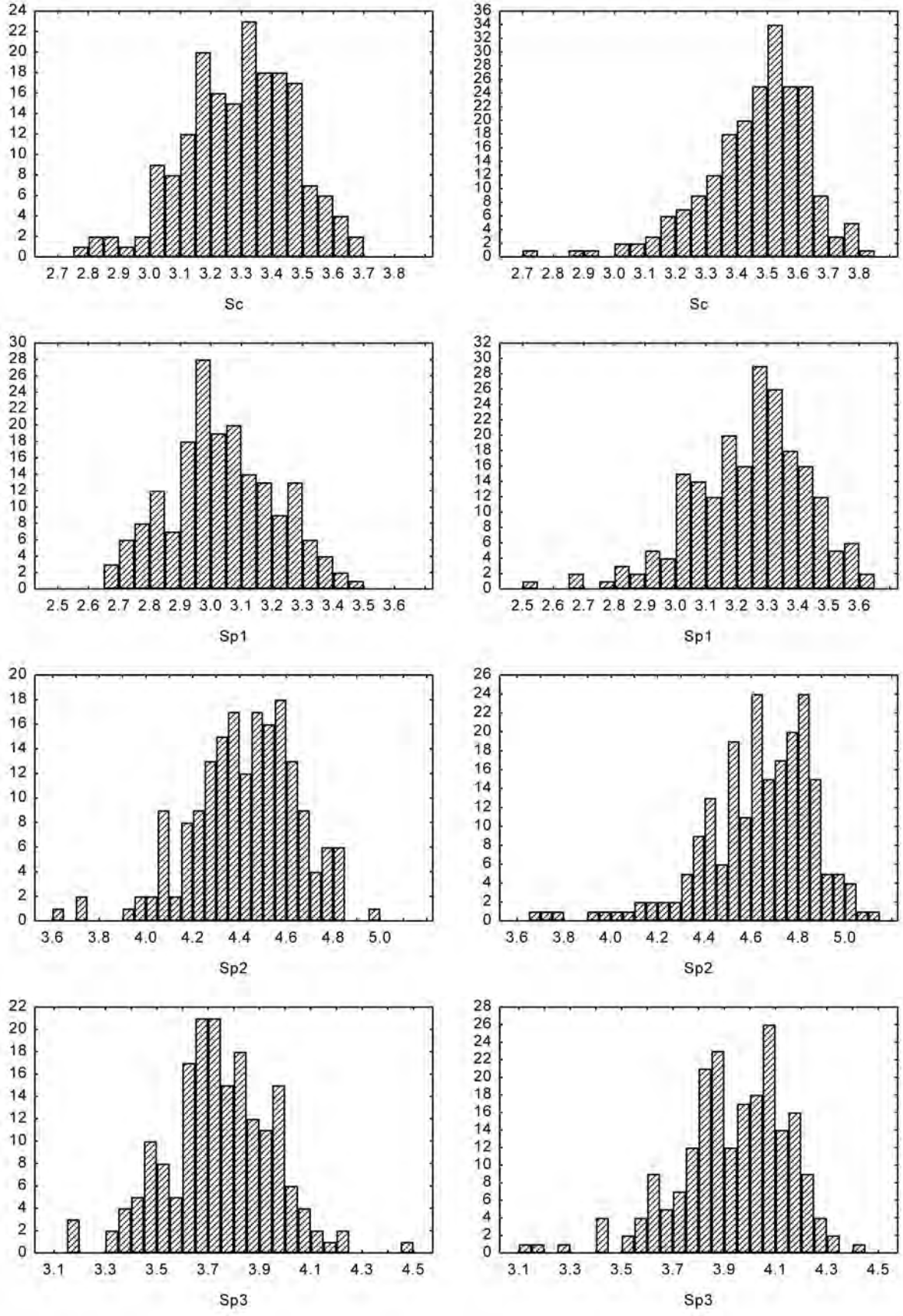

Fig. 2. Variability of head width (Sc), maximum prothorax width (Sp2), prothorax width at the forward edge (Sp1) and backward edge (Sp3) in *H. rufipes*: on the left – males (n = 183), on the right – females (n = 209); on X-axis – values of characteristics in millimeters, on Y-axis – number of specimens.

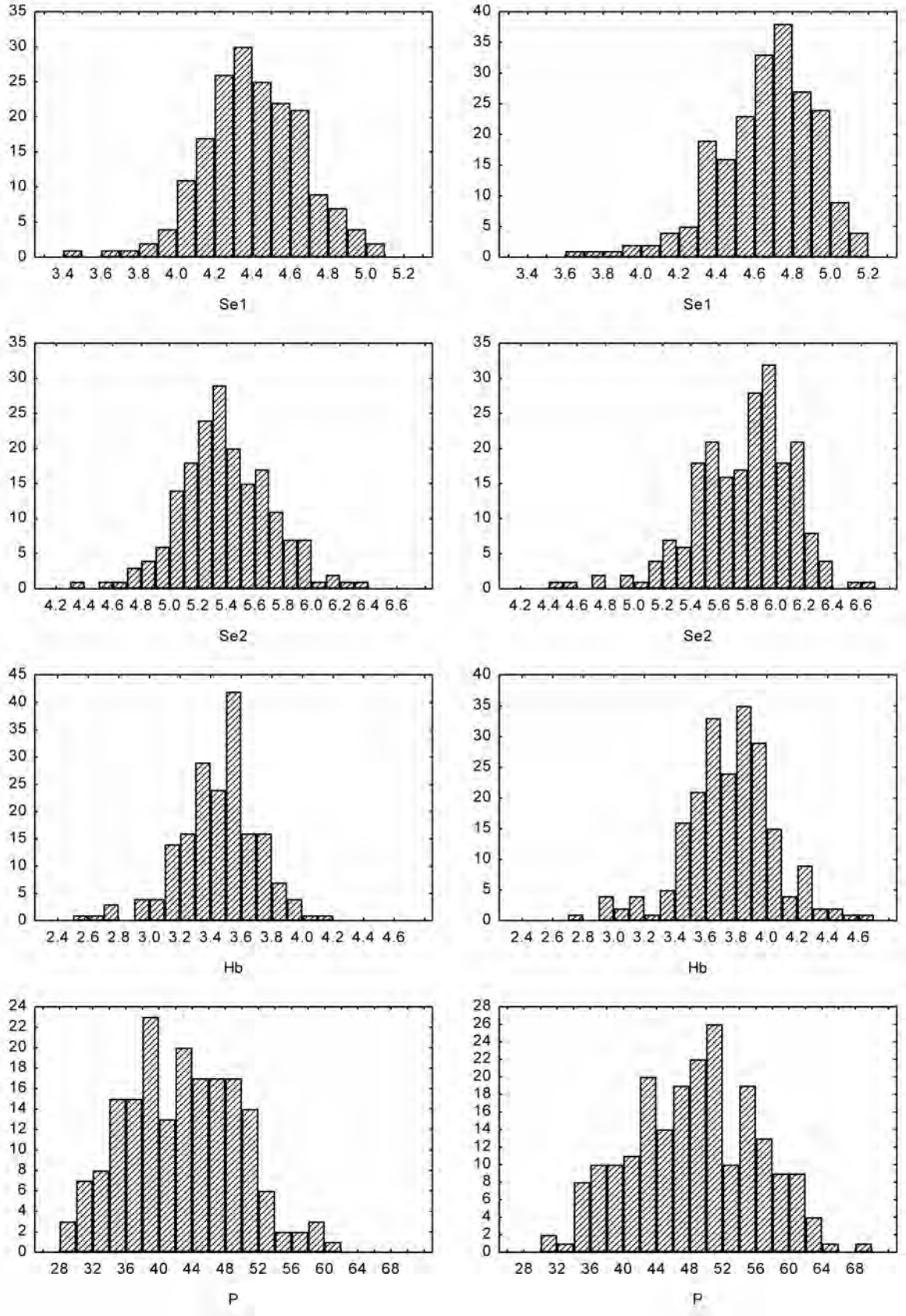

Fig. 3. Variability of distance between humeral angles (Se1, mm), maximum elytra width (Se2, mm), body height (Hb, mm) and quantity of pores on elytron area (P, pc./0.15 mm²) in *H. rufipes*: on the left – males (n = 183), on the right – females (n = 209); on X-axis – for Se1, Se2 and Hb values of characteristics in millimeters, for P – quantity of pores on elytra (pc./0.15 mm²), on Y-axis – number of specimens.

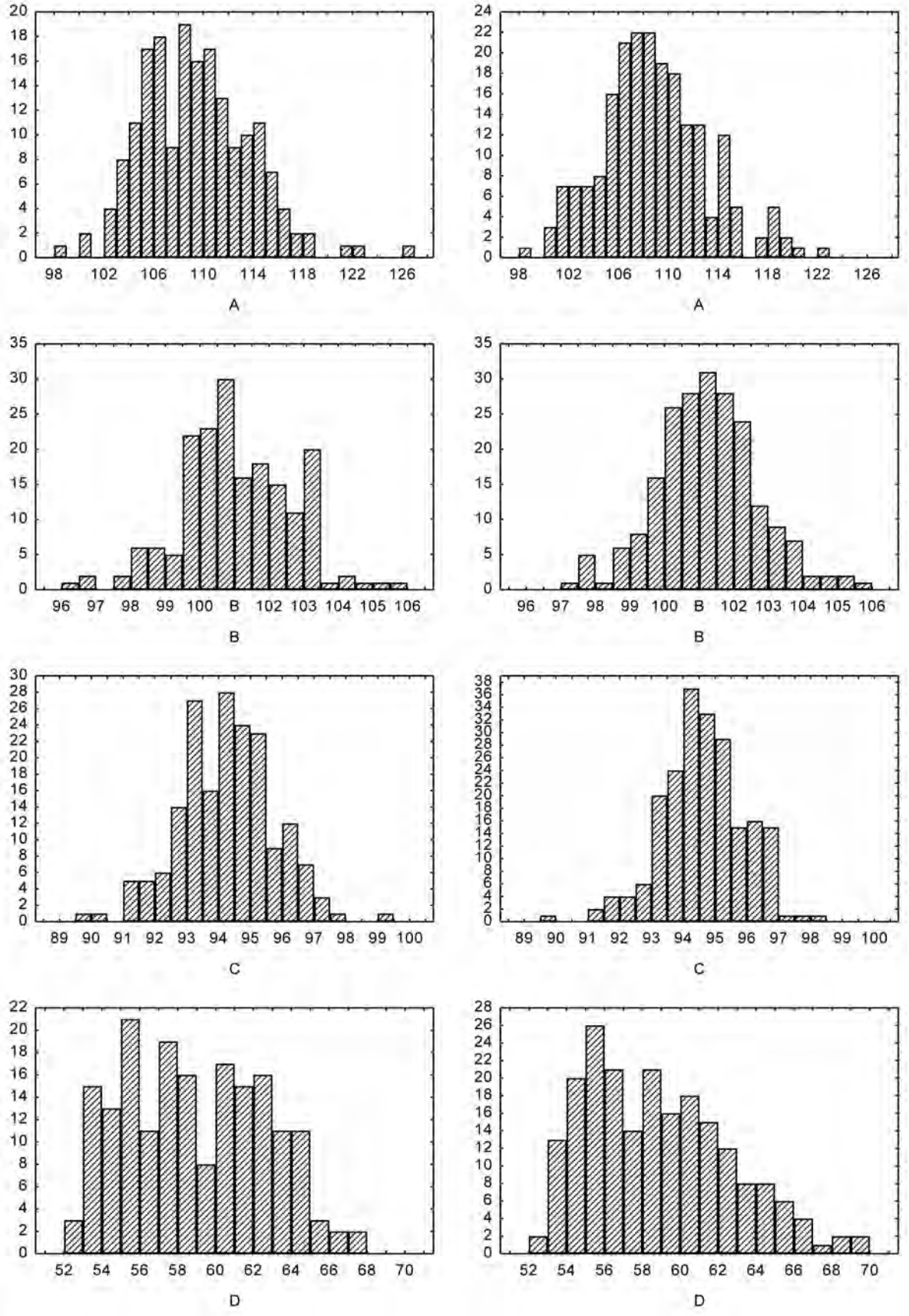

Fig. 4. Variability of front (A) and back (B) angles of prothorax, humeral angles (C) and vertex angles of elytra (D) of H. rufipes: on the left – males (n = 183), on the right – females (n = 209); on X-axis – value of angle in degrees, on Y-axis – number of specimens.

Significant negative asymmetry was also recorded in males and females for the maximum prothorax width (Sp2, $As_{male} = -0.56$, $P < 0.001$; $As_{female} = -1.11$, $P < 0.001$, Fig. 2) and body height (Hb, $As_{male} = -0.49$, $P < 0.01$; $As_{female} = -0.28$, $P < 0.05$, Fig. 3), i.e. unidirectional variation (increase) of the given characteristics occurs in males and females.

General variability of distribution of body proportions in males and females

In contrast to linear measurements, the index (ratio of two linear measurements) is a non-dimensional number which describes the change in body proportions. Both absolute value of the index and variability of its values in the studied populations are important. For all 8 body proportions considered (Fig. 5 and 6) the excess values are significantly positive (more often $P < 10^{-10}$–10^{-14}, and always $P < 0.001$) in both males and in females. It suggests considerably higher constancy of bodily proportions in *H. rufipes* than of absolute size.

Absence of asymmetry in males and females was revealed for the ratio of maximum elytra width to their width between humeral angles (Se2/Se1, $As_{male} = -0.06$, $P > 0.05$; $As_{female} = -0.13$, $P > 0.05$, Fig. 6), i.e. side faces of elytra do not become more parallel or more rounded.

Positive asymmetry ($P < 0.001$) is recorded for the ratio of body length to its height (Lb/Hb, $As_{male} = 1.61$, $As_{female} = 0.85$, Fig. 5) both in males and in females, i.e. specimens with more convex body prevail in the populations.

With regard to the ratio of body width to its length ((Sc+Sp+Se)/3Lb), no asymmetry in males is found ($As_{male} = 0.01$, $P > 0.05$), while it is significantly positive in females ($As_{female} = 1.92$, $P < 0.001$, Fig. 5), which indicates the relative decrease in female body width.

For the ratio of prothorax length to its width asymmetry is significantly positive both in males and in females (Lp/Sp2, $As_{male} = 2.87$, $P < 0.001$; $As_{female} = 2.80$, $P < 0.001$, Fig. 5), i.e. a significant shortening of the prothorax is observed.

Concerning the ratio of elytra length to prothorax length, a significant negative asymmetry (Le/Lp, $As_{male} = -1.47$, $P < 0.001$; $As_{female} = -2.30$, $P < 0.001$, Fig. 5) is recorded, which proves the presence of selection towards the increase in the relative elytra length.

Maximum width of elytra and prothorax in males and in females varies in different ways. For males, positive asymmetry of their ratio is revealed, while it is negative in females (Se2/Sp2, $As_{male} = 0.96$, $P < 0.001$; $As_{female} = -0.38$, $P < 0.05$, Fig. 6), i.e. with regard to maximum prothorax width the males' elytra become gradually narrower, while in females, on the contrary, wider.

As to the ratio of maximum width of prothorax to the width between its back angles, no asymmetry is present, whereas it is negative in females (Sp2/Sp3, $As_{male} = 0.14$, $P > 0.05$; $As_{female} = -0.59$, $P < 0.001$, Fig. 6), i.e. prothorax in females gradually assumes a more distinct heart shape, and in males its shape remains unchanged.

The ratio of the length of elytra to their width features a negative asymmetry both in males and in females (Le/Se, $As_{male} = -0.75$, $P < 0.001$; $As_{female} = -0.31$, $P < 0.001$, Fig. 6), i.e. elytra become longer in relation to their width.

Values of measured characteristics on the different sampling plots

Significant sexual dimorphism is recorded for *H. rufipes* body length in all the populations studied (Table 2). Average body length of females is 5.6% greater than in males; in population 6 (steppe area) – by 1.27 mm, in population 4 (acacia forest belt) – by 1.18 mm. Annual burning of crop residues (in spring and during the period of summer drought) takes place on both plots (Moroz et al., 2011). Minimal differences in average body length are recorded for populations 9 (maple-ash forest) and 5 (steppe area) – 0.50 and 0.56 mm, respectively. These sampling plots are characterized by minimal anthropogenic impact; the litter horizon is maintained here intact throughout the season.

Distribution of males for all 9 studied populations by body length does not differ from the norm (Table 2). Distribution of females by body length in four (sampling plot 2 – clover field, 6 – steppe area, 8 – corn field and 9 – maple-ash forest) of nine populations deviates from the normal distribution. In all four cases a significant negative asymmetry is manifested, i.e. the population has a larger number of specimens with considerable excess over the average body size.

Differences between body length of males on the examined sampling plots (Fig. 7) are significant as well (Table 2). The size of the males is minimal in sampling plots 3 (elm-ash forest), 6 (steppe area) and 4 (acacia forest belt), and maximal in sampling plots 5 (steppe area), 1 (lucerne field) and 8 (corn field). Minimal body length of females (Fig. 7, Table 2) is observed in sampling plots 3 (elm-ash forest) and 9 (maple-ash forest); differences in body length between females of the other sampling plots are not significant.

As a whole, for the combined 9 samples of males and females (Fig. 1) differences in distribution by body length are typical: it is normal in males, while in females a significant positive excess and negative asymmetry of this characteristic are expressed.

A significant ($P = 1.9 \cdot 10^{-21}$, $F = 101.90$, $F_{0.05(1, 390)} = 3.87$) sexual dimorphism (by 5.6%) between males (2.213 ± 0.119 mm) and females (2.337 ± 0.125 mm) is recorded for the head length (Lc). No significant variations between the populations within the group of specimens of the same sex are recorded.

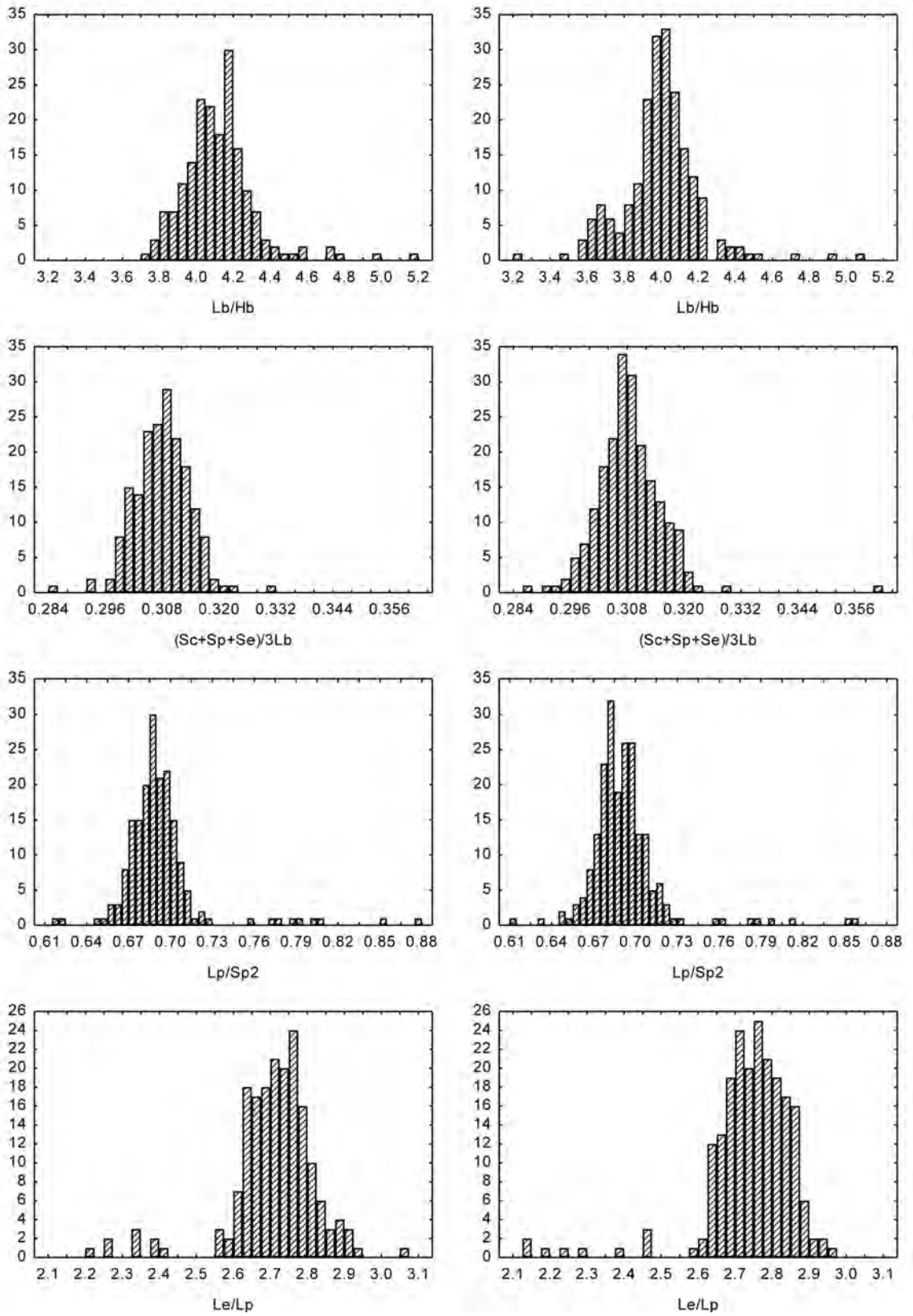

Fig. 5. Variability of morphometric indices of *H. rufipes*: Lb/Hb – ratio of body length to body height, (Sc + Sp + Se)/3Lb – ratio of arithmetical mean value of width of the head, prothorax and elytra to body length, Lp/Sp2 – ratio of prothorax length to maximum prothorax width, Le/Lp – ratio of elytra length to prothorax length; on the left – males (n = 183), on the right – females (n = 209); on X-axis – index value, on Y-axis – number of specimens.

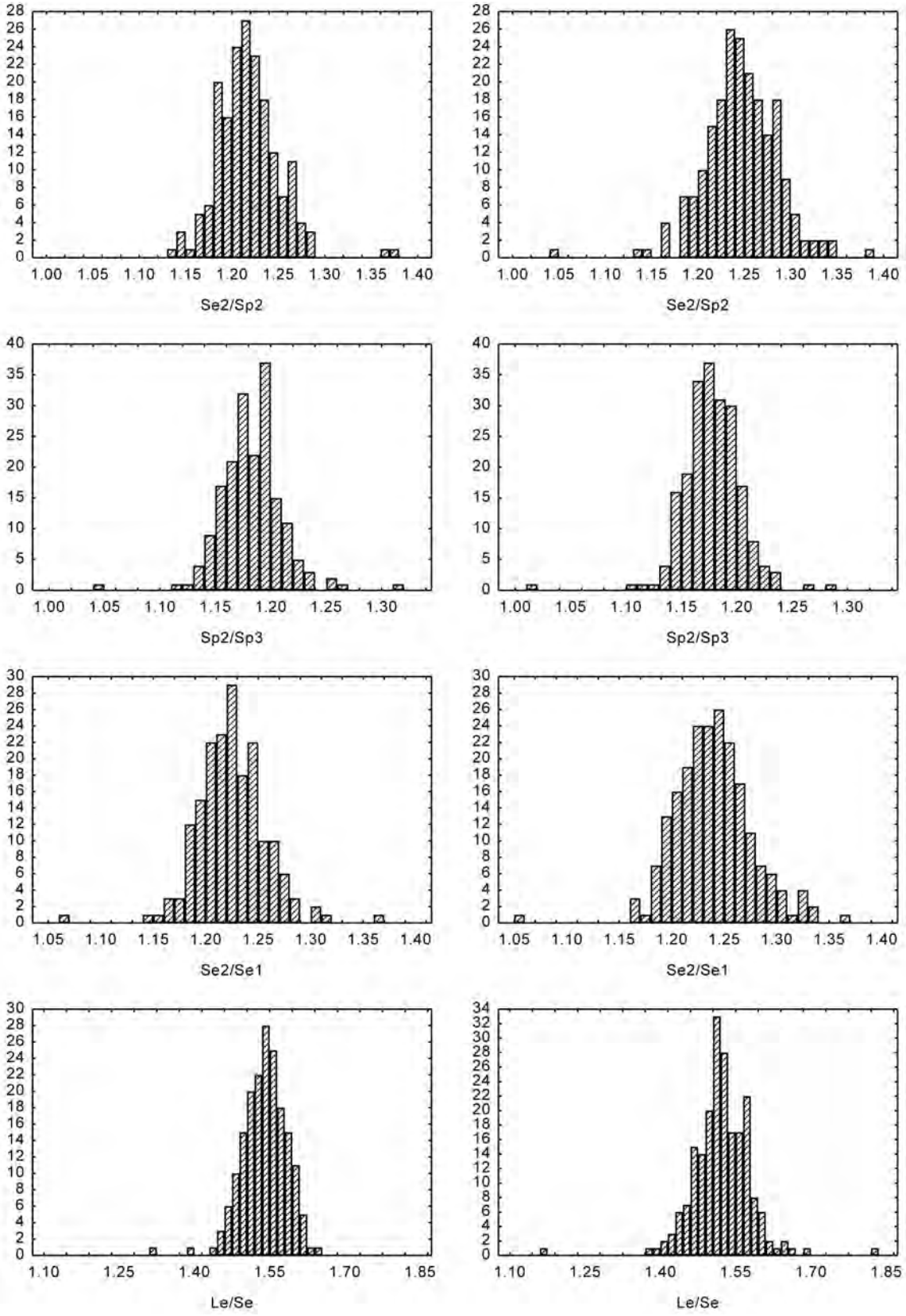

Fig. 6. Variability of morphometric indices of *H. rufipes*: Se2/Sp2 – ratio of maximum elytra width to maximum prothorax width, Sp2/Sp3 – ratio of maximum prothorax width to its width at the backward edge, Se2/Se1 – ratio of maximum elytra width to the distance between their front angles, Le/Se – ratio of elytra length to their width; on the left – males (n = 183), on the right – females (n = 209); on X-axis – index value, on Y-axis – number of specimens.

Table 2. Variability of body length (Lb) in the studied populations of *H. rufipes* (*n* = 392)

Ecosystem	Sex	n	x ± SD [mm]	Min–Max [mm]	D [mm]	Ex ± SD	As ± SD	F	$F_{0.05}$ (df1, df2)	P
1	m	18	14.54 ± 0.63	13.48–15.59	2.12	–0.84 ± 1.02	0.30 ± 0.53	8.42	4.09 (1, 39)	0.006
	f	23	15.11 ± 0.61	13.52–16.01	2.49	0.86 ± 0.93	–0.87 ± 0.48			
2	m	29	14.13 ± 0.67	12.88–15.62	2.73	–0.28 ± 0.84	0.21 ± 0.43	18.97	4.04 (1, 48)	$7.1*10^{-5}$
	f	21	15.10 ± 0.91	12.38–16.12	3.74	2.64 ± 0.96*	–1.46 ± 0.50**			
3	m	17	13.85 ± 0.88	11.64–15.19	3.55	1.21 ± 1.04	–0.76 ± 0.55	6.02	4.16 (1, 31)	0.020
	f	16	14.54 ± 0.73	13.13–15.90	2.77	–0.49 ± 1.07	0.05 ± 0.56			
4	m	29	13.97 ± 0.54	12.53–14.88	2.35	0.67 ± 0.84	–0.73 ± 0.43	38.42	4.03 (1, 51)	$1.0*10^{-7}$
	f	24	15.15 ± 0.84	12.89–16.38	3.49	1.70 ± 0.91	–1.12 ± 0.47			
5	m	19	14.60 ± 0.58	13.60–15.81	2.21	0.11 ± 1.00	0.07 ± 0.52	8.66	4.07 (1, 43)	0.005
	f	26	15.17 ± 0.67	13.53–16.31	2.78	0.07 ± 0.88	–0.54 ± 0.45			
6	m	7	13.94 ± 0.42	13.36–14.54	1.19	–1.11 ± 1.41	0.25 ± 0.77	39.42	4.54 (1, 15)	$1.5*10^{-5}$
	f	10	15.21 ± 0.40	14.24–15.65	1.40	3.72 ± 1.26*	–1.67 ± 0.68*			
7	m	16	14.09 ± 0.75	12.66–15.76	3.10	0.70 ± 1.07	0.35 ± 0.56	10.85	4.13 (1, 34)	0.002
	f	20	14.99 ± 0.85	13.20–16.26	3.05	–0.15 ± 0.98	–0.66 ± 0.51			
8	m	16	14.49 ± 0.70	13.48–15.66	2.18	–0.76 ± 1.07	0.52 ± 0.56	12.88	4.11 (1, 37)	0.001
	f	23	15.25 ± 0.62	13.70–16.21	2.52	0.80 ± 0.93	–1.00 ± 0.48*			
9	m	32	14.15 ± 0.72	12.86–15.56	2.70	–0.85 ± 0.81	–0.22 ± 0.41	7.98	3.97 (1, 76)	0.006
	f	46	14.65 ± 0.80	12.43–15.88	3.45	1.22 ± 0.69	–0.91 ± 0.35*			
Total	m	183	14.19 ± 0.70	11.64–15.08	4.17	0.37 ± 0.36	–0.14 ± 0.18	110.78	3.86 (1, 390)	$5.7*10^{-23}$
	f	209	14.98 ± 0.77	12.37–16.38	4.00	0.87 ± 0.33**	0.88 ± 0.17***			
Differences between males								3.07	1.99 (8, 174)	0.003
Differences between females								2.80	1.98 (8, 200)	0.006

Numbers of ecosystems and their brief characteristic see in Table 1; sex m – male, f – female; Min–Max – minimum and maximum value of the characteristic; D – variation range equal to Max–Min; As – asymmetry; Ex – excess; *, ** and *** – significance of asymmetry and excess $P < 0.05$, 0.01 and 0.001, respectively.

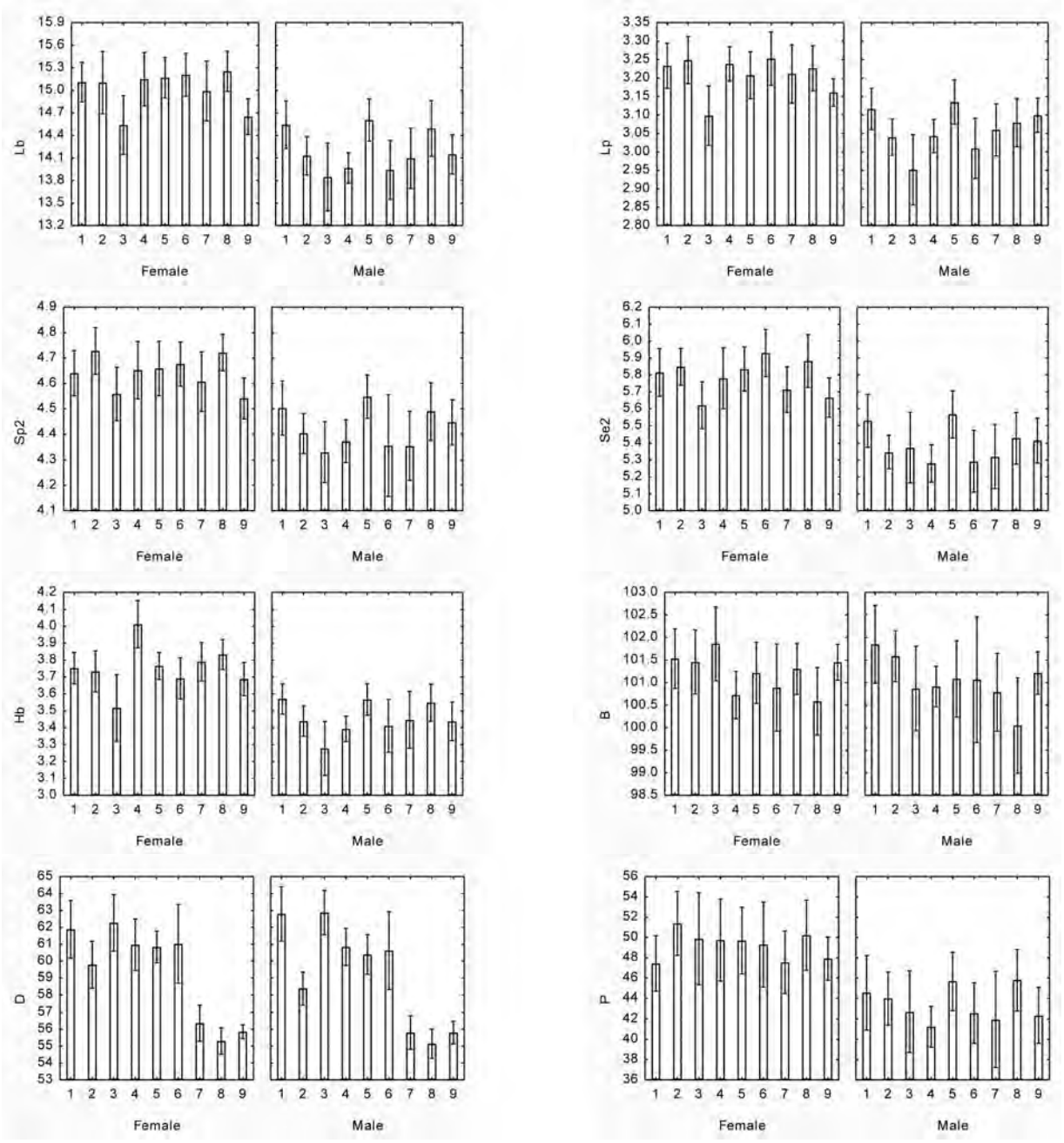

Fig. 7. Mean value and standard deviation of the main morphometric characteristics in *H. rufipes*: Lb – body length (mm), Lp – prothorax length (mm), Se2 – maximum elytra width (mm), Hb – body height (mm), B – prothorax back angle (degrees), D – vertex angle of elytra (degrees) and P – quantity of pores on the elytron area (pc./0.15 mm^2); on the right – males (n = 183), on the left – females (n = 209); on X-axis from 1 to 9 population numbers are indicated (see Table 1), on Y-axis the index value is indicated.

With regard to length (Lp, Fig. 7), sexual dimorphism is also significant ($P = 9.5 \cdot 10^{-21}$, $F = 97.94$, $F_{0.05(1,\ 390)} = 3.87$). Length of prothorax in females is 4.5% greater than in males (3.204 ± 0.143 and 3.064 ± 0.137 mm, accordingly). The shortest prothorax in females and males is recorded for sampling plot 3 (elm-ash forest). Fluctuations of the prothorax length in males are larger than those in females.

Length of elytra in males (8.30 ± 0.47 mm) is also significantly ($P = 9.6 \cdot 10^{-20}$, $F = 92.25$, $F_{0.05(1, 390)} = 3.87$) less (by 5.8%) than the length of elytra in females (8.78 ± 0.52 mm). Minimum distance between the inner mar-

gin of eyes in males (2.383 ± 0.135 mm) and females (2.527 ± 0.133 mm) significantly ($P = 2.2 \cdot 10^{-23}$, $F = 113.20$, $F_{0.05(1, 390)} = 3.87$) differs by 6.0% as well.

Maximum width of prothorax (Sp, Fig. 7) in females (4.63 ± 0.24 mm) is greater by 4.7% ($P = 7.5 \cdot 10^{-17}$, $F = 76.24$, $F_{0.05(1, 390)} = 3.87$) than in males (4.43 ± 0.23 mm). Minimum length and width of prothorax is typical for males and females of population 3 (elm-ash forest); variations within sex groups between the populations are not significant.

Distance between the humeral angles (Se1) in females (4.66 ± 0.26 mm) is significantly (by 5.7%, $P = 1.3 \cdot 10^{-19}$, $F = 91.46$, $F_{0.05(1, 390)} = 3.87$) greater than in males (4.41 ± 0.26 mm). No significant inter-population differences are recorded.

Maximum width of elytra (Se2, Fig. 7) differs significantly (by 7.0%, $P = 2.1 \cdot 10^{-25}$, $F = 125.24$, $F_{0.05(1, 390)} = 3.87$) between males (5.39 ± 0.32 mm) and females (5.77 ± 0.35 mm). The most pronounced sexual dimorphism on this character is for the populations 6, 4, 2 and 7 (in males of these populations the narrowest elytra are recorded). No significant inter-population differences in the elytra width of females are recorded.

Body height (Hb, Fig. 7) also significantly differs (by 8.9%, $P = 2.0 \cdot 10^{-24}$, $F = 119.23$, $F_{0.05(1, 390)} = 3.87$) between males (3.45 ± 0.26 mm) and females (3.76 ± 0.30 mm). Maximum body height is observed in females of population 4 (acacia forest belt), while minimum body height is recorded in females of population 3 (elm-ash forest).

There are no significant differences recorded on the value of the front (A) and back angle of prothorax (B, Fig. 7) between males and females (mean values differ for A – by 0.5º, for B – by 0.1º). Besides, there are no significant population differences. Most identification guides also state as a diagnostic species characteristic of *H. rufipes*, apart from body size, that the back angles of the prothorax should be right angles (Lindroth, 1985; Hůrka, 1996; Freude et al., 2004). In the populations studied the value of prothorax back angle (on average, 101.1º) is actually unchanged (fluctuations do not exceed 1–2º).

The vertex angle of elytra (D, Fig. 7) in the populations 7, 8 and 9 (Pavlograd, Dnipropetrovsk and Petrikovka districts) significantly differs from that of populations 1–6 (Novomoskovsk district). These differences require additional analysis taking into account the likely variability of the vertex angle of elytra in other populations. Because of considerable inter-population differences in the elytra vertex angle, sexual dimorphism on the given character is not significant ($P = 0.76$, $F = 0.09$, $F_{0.05(1, 390)} = 3.87$).

Density of hairs on the elytra (P, Fig. 7) in males is 13.3% lower than in females: 289 ± 46 pc./mm^2 in males and 327 ± 50 pc./mm^2 in females ($P = 3.4 \cdot 10^{-14}$, $F = 62.00$, $F_{0.05(1, 390)} = 3.87$). No significant inter-population differences in males ($P = 0.31$, $F = 1.19$, $F_{0.05(8, 174)} = 1.99$) and in females ($P = 0.65$, $F = 0.74$, $F_{0.05(8, 200)} = 1.98$) are recorded.

Values of morphometric indices on the different sampling plots

The ratio of body length to its height (Lb/Hb, Fig. 8) reflects the degree of "convexity" of beetles. More flattened specimens can more easily squeeze into the narrow slots of dry soil, which is solid in its mechanical composition. The mean values of the index show significant sexual dimorphism ($P = 4.2 \cdot 10^{-9}$, $F = 34.72$, $F_{0.05(1, 390)} = 3.87$): they are minimal for females (3.999 ± 0.215), and maximal for males (4.123 ± 0.200). In population 4 (acacia forest belt) the values of this index in females are significantly lower than in other populations. For males and females of other populations no significant values are found.

The ratio of the arithmetic mean of width of head, prothorax and elytra to body length ((Sc + Sp + Se)/3Lb, Fig. 8) reflects the relative "broadness" of the beetles. Sex differences between males (0.308 ± 0.006) and females (0.309 ± 0.007) on this index are not significant ($P = 0.339$, $F = 0.91$, $F_{0.05(1, 390)} = 3.87$). This index is minimal for males and females of populations 1 and 7, females of population 4 and males of population 8 (Fig. 8). The maximum value of the index is recorded in females of populations 2 and 3 and males of the population 3.

The ratio of length and maximum width of prothorax (Lp/Sp2) is one of the most stable characteristics of this species. No significant variations between males and females of population 1 (lucerne field), 2 (clover field), 6 (disturbed steppe area), 7 (oak plantation with the traces of cattle grazing) and 8 (corn field) on the given characteristic are found (Table 3). Therefore, on the most anthropogenically transformed plots no sexual dimorphism in the ratio of length and maximum width of prothorax is manifested.

In males (Fig. 8, Table 3) the mean values of the index Lp/Sp2 do not differ significantly ($P = 0.829$, $F = 0.05$, $F_{0.05(1, 390)} = 3.87$) from females (0.693 ± 0.030 and 0.692 ± 0.028 respectively). Distribution of the ratio of length to width of prothorax in females and males of populations 2 and 6, as well as males of populations 5 and 8 is normal (asymmetry and excess are not significant, Table 3). Significant negative asymmetry (specimens with high values of the index prevail in the population) is recorded in males and females of population 3 and females of population 8. In males and females of populations 1, 4, 7, 9, as well as in females of population 5, significant positive excess on the ratio of length and width of prothorax (a larger number of specimens with lower values of the index) is recorded.

The ratio of elytra length to prothorax length (Le/Lp, Fig. 8) significantly differs ($P = 0.009$, $F = 6.85$, $F_{0.05(1, 390)} = 3.87$) between males and females (2.709

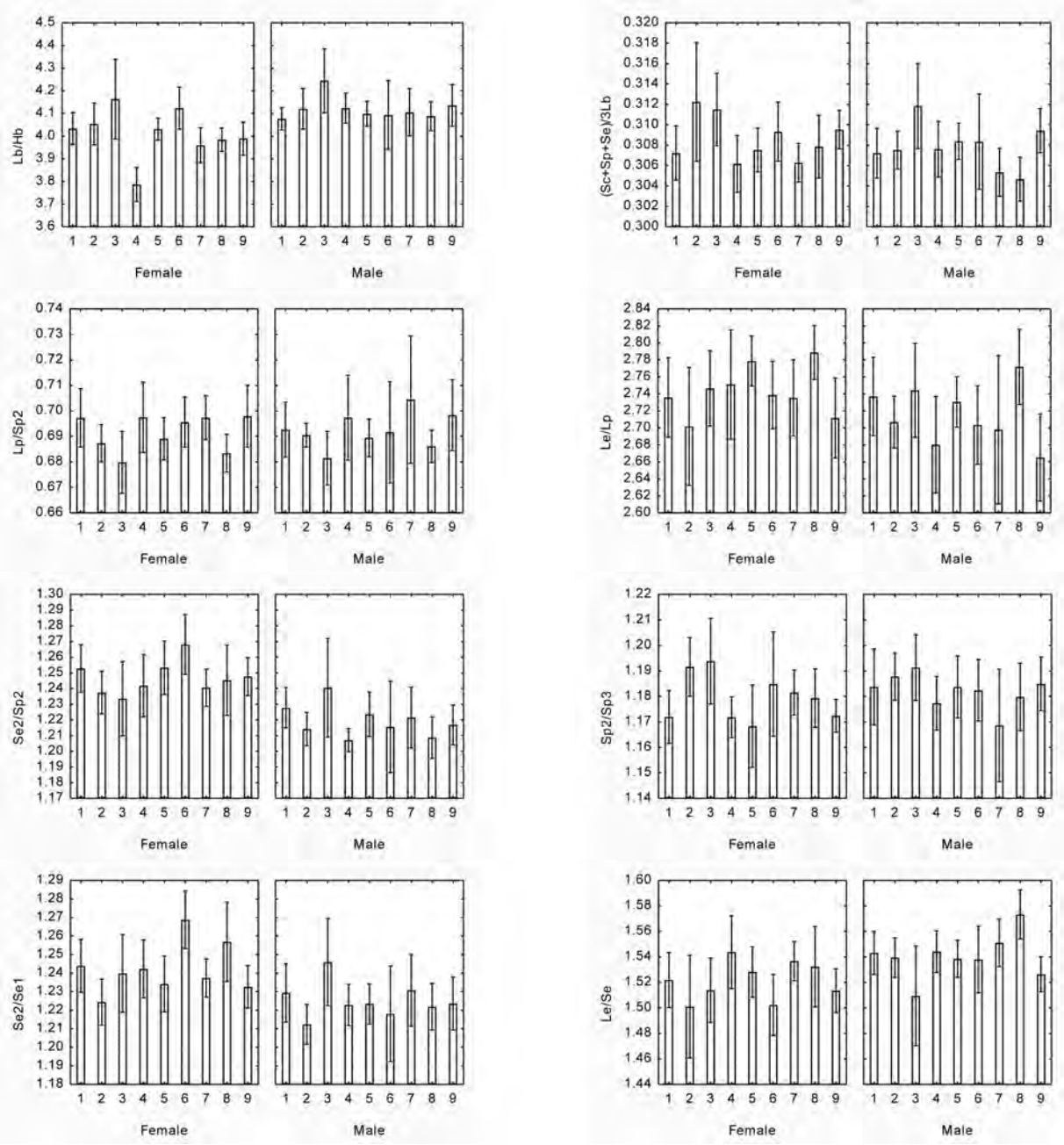

Fig. 8. Mean value and standard deviation of morphometric indices of *H. rufipes*: Lb/Hb – ratio of body length to body height, (Sc + Sp + Se)/3Lb – ratio of arithmetic mean value of width of the head, prothorax and elytra to body length, Lp/Sp2 – ratio of length and maximum width of prothorax, Le/Lp – ratio of elytra length to prothorax length, Se2/Sp2 – ratio of maximum elytra width to maximum prothorax width, Sp2/Sp3 – ratio of maximum width of prothorax to width between its back angles, Se2/Se1 – ratio of maximum elytra width to the distance between their humeral angles, Le/Se – ratio of elytra length to their width; on the right – males (n = 183), on the left – females (n = 209); on X-axis from 1 to 9 population numbers are indicated (see Table 1), on Y-axis the index value is indicated.

± 0.118 and 2.741 ± 0.122 accordingly). This index shows no significant differences between populations. Maximum values of this index for males and females are recorded in population 8 (corn field).

For the ratio of maximum elytra width to maximum prothorax width (Se2/Sp2, Table 4, Fig. 8) between males (1.218 ± 0.034) and females (1.246 ± 0.040) significant sexual dimorphism ($P = 1.3 \cdot 10^{-12}$, $F = 53.84$, $F_{0.05(1, 390)} = 3.87$) is recorded. On the driest areas (1 – lucerne field, 5 and 6 – steppe areas) the maximum ratio of elytra width to prothorax width is recorded in females.

Table 3. Variability of the ratio of prothorax length to its maximum width (Lp/Sp2) in the studied populations of *H. rufipes* (*n* = 392)

Ecosystem	Sex	n	x ± SD	Min – Max	D	Ex ± SD	As ± SD	F	$F_{0.05}$ (df1, df2)	P
1	m	18	0.693 ± 0.022	0.657–0.756	0.099	3.45 ± 1.02**	1.19 ± 0.53*	0.356	4.09 (1, 39)	0.554
	f	23	0.697 ± 0.026	0.657–0.782	0.124	3.71 ± 0.93***	1.27 ± 0.48*			
2	m	29	0.691 ± 0.012	0.667–0.715	0.048	–0.24 ± 0.84	–0.02 ± 0.43	0.639	4.04 (1, 48)	0.428
	f	21	0.687 ± 0.016	0.647–0.720	0.073	1.21 ± 0.96	–0.24 ± 0.50			
3	m	17	0.682 ± 0.020	0.621–0.705	0.084	4.10 ± 1.04**	–1.78 ± 0.55**	0.049	4.16 (1, 31)	0.827
	f	16	0.680 ± 0.023	0.615–0.710	0.095	3.51 ± 1.07**	–1.56 ± 0.56*			
4	m	29	0.697 ± 0.044	0.615–0.876	0.260	10.41 ± 0.84***	2.68 ± 0.43***	0.001	4.03 (1, 51)	0.990
	f	24	0.697 ± 0.033	0.667–0.799	0.133	5.10 ± 0.91***	2.21 ± 0.47***			
5	m	19	0.689 ± 0.015	0.659–0.722	0.063	0.37 ± 1.00	–0.09 ± 0.52	0.005	4.07 (1, 43)	0.946
	f	26	0.689 ± 0.021	0.648–0.760	0.112	5.03 ± 0.88***	1.49 ± 0.45**			
6	m	7	0.692 ± 0.021	0.663–0.726	0.063	–0.42 ± 1.41	0.40 ± 0.77	0.215	4.54 (1, 15)	0.649
	f	10	0.696 ± 0.014	0.678–0.719	0.040	–1.11 ± 1.26	0.40 ± 0.68			
7	m	16	0.704 ± 0.047	0.673–0.850	0.177	6.56 ± 1.07***	2.59 ± 0.56***	0.389	4.13 (1, 34)	0.537
	f	20	0.697 ± 0.018	0.677–0.760	0.083	6.71 ± 0.98***	2.13 ± 0.51***			
8	m	16	0.686 ± 0.012	0.667–0.760	0.039	–0.99 ± 1.07	–0.05 ± 0.56	0.304	4.11 (1, 37)	0.585
	f	23	0.683 ± 0.017	0.633–0.708	0.075	2.08 ± 0.93*	–1.17 ± 0.48*			
9	m	32	0.698 ± 0.039	0.645–0.808	0.163	2.78 ± 0.81**	1.76 ± 0.41***	0.002	3.97 (1, 76)	0.960
	f	46	0.698 ± 0.041	0.655–0.859	0.204	9.42 ± 0.69***	3.05 ± 0.35***			
Total	m	183	0.693 ± 0.030	0.615–0.876	0.260	12.99 ± 0.36***	2.87 ± 0.18***	0.047	3.87 (1, 390)	0.829
	f	209	0.692 ± 0.028	0.615–0.859	0.244	13.29 ± 0.33***	2.80 ± 0.17***			
Differences between males								0.948	1.99 (8, 174)	0.479
Differences between females								1.360	1.98 (8, 200)	0.216

Numbers of ecosystems and their brief characteristic see in Table 1; sex m – male, f – female; Min–Max – minimum and maximum value of the characteristic; D – variation range equal to Max – Min; As – asymmetry; Ex – excess; *, ** and *** – significance of asymmetry and excess $P < 0.05$, 0.01 and 0.001, respectively.

Table 4. Variability of the ratio of maximum elytra width to maximum prothorax width (Se2/Sp2) in the studied populations of *H. rufipes* (*n* = 392)

Ecosystem	Sex	n	x ± SD	Min – Max	D	Ex ± SD	As ± SD	F	$F_{0.05}$ (df1, df2)	P
1	m	18	1.228 ± 0.026	1.188–1.280	0.092	–0.50 ± 1.02	0.38 ± 0.53	6.427	4.09 (1, 39)	0.015
	f	23	1.253 ± 0.035	1.189–1.335	0.146	0.24 ± 0.93	0.05 ± 0.48			
2	m	29	1.214 ± 0.028	1.149–1.266	0.117	–0.03 ± 0.84	–0.14 ± 0.43	7.998	4.04 (1, 48)	0.007
	f	21	1.237 ± 0.030	1.195–1.291	0.096	–0.96 ± 0.96	0.59 ± 0.50			
3	m	17	1.240 ± 0.061	1.143–1.377	0.233	1.15 ± 1.04	0.97 ± 0.55	0.137	4.16 (1, 31)	0.714
	f	16	1.234 ± 0.044	1.140–1.309	0.169	–0.13 ± 1.07	–0.32 ± 0.56			
4	m	29	1.207 ± 0.019	1.167–1.256	0.089	0.40 ± 0.84	0.38 ± 0.43	13.23	4.03 (1, 51)	$6.4*10^{-4}$
	f	24	1.242 ± 0.047	1.147–1.326	0.178	–0.45 ± 0.91	–0.13 ± 0.47			
5	m	19	1.224 ± 0.029	1.166–1.271	0.104	–0.29 ± 1.00	0.09 ± 0.52	6.985	4.07 (1, 43)	0.011
	f	26	1.253 ± 0.042	1.210–1.388	0.179	3.56 ± 0.88	1.78 ± 0.45			
6	m	7	1.216 ± 0.031	1.181–1.269	0.088	–0.41 ± 1.41	0.70 ± 0.77	13.77	4.54 (1, 15)	0.002
	f	10	1.268 ± 0.027	1.235–1.325	0.090	1.19 ± 1.26	1.18 ± 0.68			
7	m	16	1.221 ± 0.036	1.150–1.286	0.136	–0.29 ± 1.07	0.01 ± 0.56	3.424	4.13 (1, 34)	0.073
	f	20	1.241 ± 0.025	1.193–1.281	0.088	–0.79 ± 0.98	–0.29 ± 0.51			
8	m	16	1.209 ± 0.025	1.166–1.246	0.080	–0.94 ± 1.07	–0.44 ± 0.56	6.890	4.11 (1, 37)	0.013
	f	23	1.245 ± 0.052	1.161–1.349	0.188	–0.47 ± 0.93	–0.01 ± 0.48			
9	m	32	1.217 ± 0.035	1.131–1.284	0.153	–0.06 ± 0.81	–0.23 ± 0.41	12.10	3.97 (1, 76)	$8.4*10^{-4}$
	f	46	1.248 ± 0.041	1.048–1.311	0.263	12.21 ± 0.69	–2.59 ± 0.35			
Total	m	183	1.218 ± 0.034	1.131–1.377	0.246	3.62 ± 0.36	0.96 ± 0.18	53.84	3.87 (1, 390)	$1.3*10^{-12}$
	f	209	1.246 ± 0.040	1.048–1.388	0.340	3.02 ± 0.33	–0.38 ± 0.17			
Differences between males								1.859	1.99 (8, 174)	0.069
Differences between females								0.970	1.98 (8, 200)	0.461

Numbers of ecosystems and their brief characteristic see in Table 1; sex m – male, f – female; Min–Max – minimum and maximum value of the characteristic; D – variation range equal to Max–Min; As – asymmetry; Ex – excess; *, ** and *** – significance of asymmetry and excess $P < 0.05$, 0.01 and 0.001, respectively.

The ratio of maximum width of prothorax to width between its back angles (Sp2/Sp3, Fig. 8) features no significant difference ($P = 0.075$, $F = 3.17$, $F_{0.05(1, 390)} = 3.87$) between males (1.183 ± 0.028) and females (1.178 ± 0.027). Inter-population differences are also not significant.

The ratio of maximum elytra width to distance between their humeral angles (Se2/Se1, Fig. 8) has a pronounced sexual dimorphism ($P = 2.7 \cdot 10^{-5}$, $F = 18.02$, $F_{0.05(1, 390)} = 3.87$) between males (1.225 ± 0.033) and females (1.240 ± 0.037). It shows significant difference between individual populations of females only. The index Se2/Se1 is maximal for females of populations 6 (steppe area) and 8 (corn field).

The ratio of elytra length to width (Le/Se, Fig. 8) shows significant sexual dimorphism ($P = 1.6 \cdot 10^{-3}$, $F = 10.05$, $F_{0.05(1, 390)} = 3.87$) between males (1.539 ± 0.044) and females (1.522 ± 0.060). Inter-population differences for females of various populations are not significant on the given index. For males maximum values of the index are recorded in population 8 (corn field), and minimum values in populations 3 (elm-ash forest) and 9 (maple-ash forest).

General variability parameters

Analysis of joint variability of 19 characteristics (14 linear measurements, 4 angular characteristics and density of elytra downiness – see Material and methods) for 391 specimens of beetles showed a complex pattern of interdependencies among the studied characteristics. In view of results of the PCA (Fig. 9) more than 60% of the effect on the sample variability is created by Factor 1, which determines the joint variability of all linear characteristics except for length of eyes (La). Angular characteristics (A, B, C and D) are also not affected by factor 1 (Fig. 10a). Since in the previous parts of this paper we thoroughly established that for most of the characteristics the significant differences were based on sex, we consider that factor 1 can be identified as the sex of the ground beetles. This is confirmed by the distribution of specimens in the factor space of factors 1 and 2 (Fig. 11).

Factor 2 determining 7.3% of the dispersion was interpreted by us as the geographic location of the ecosystem. Most specimens collected outside Novomoskovsk district (sampling plots 7, 8 and 9) have maximum values on the given factor (Fig. 11). Factors 2, 3, 4 and 5 are determined by the values of predominantly angular characteristics (Fig. 10a, b): value of humeral angles of elytra (C), vertex angle of elytra (D), front (A) and back angles or prothorax (B). Factor 6 determining only 3.6% of total sample dispersion (Fig. 10c), correlates to the density of elytra downiness. Factor 7 (3.2% of total dispersion, Fig. 10d) correlates to eye length (La), while factor 8 (2.6% of total dispersion, Fig. 10d) – to clypeus length (Lcl).

Therefore, PCA results showed that the most of linear characteristics were connected with the sex of the beetle, while angular characteristics and the degree of elytra downiness varied regardless of the sex of the *H. rufipes* specimens.

Discussion

Identification guides state that the body length of *H. rufipes* varies from 11 to 16 mm (Lindroth, 1985; Hůrka, 1996; Freude et al., 2004). On the basis of the results of our study, the size of this species of beetle fluctuates within the limits of 11.6 to 16.4 mm. Our data are shifted towards the literature data, which fact may prove not only a simple rounding up to the nearest whole number (in mm), but a slight increase in beetle body length as well.

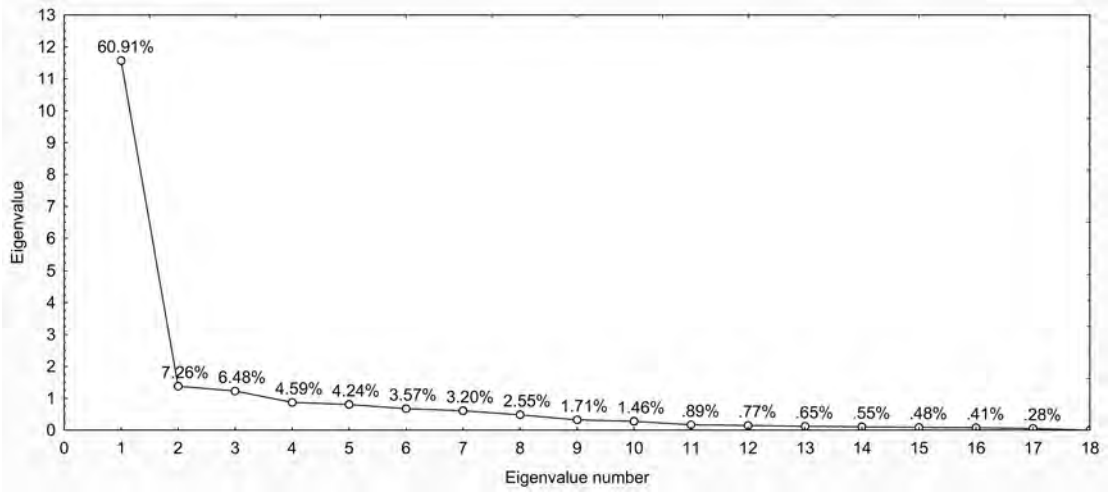

Fig. 9. Eigenvalues of correlation matrix of PCA of studied *H. rufipes* populations.

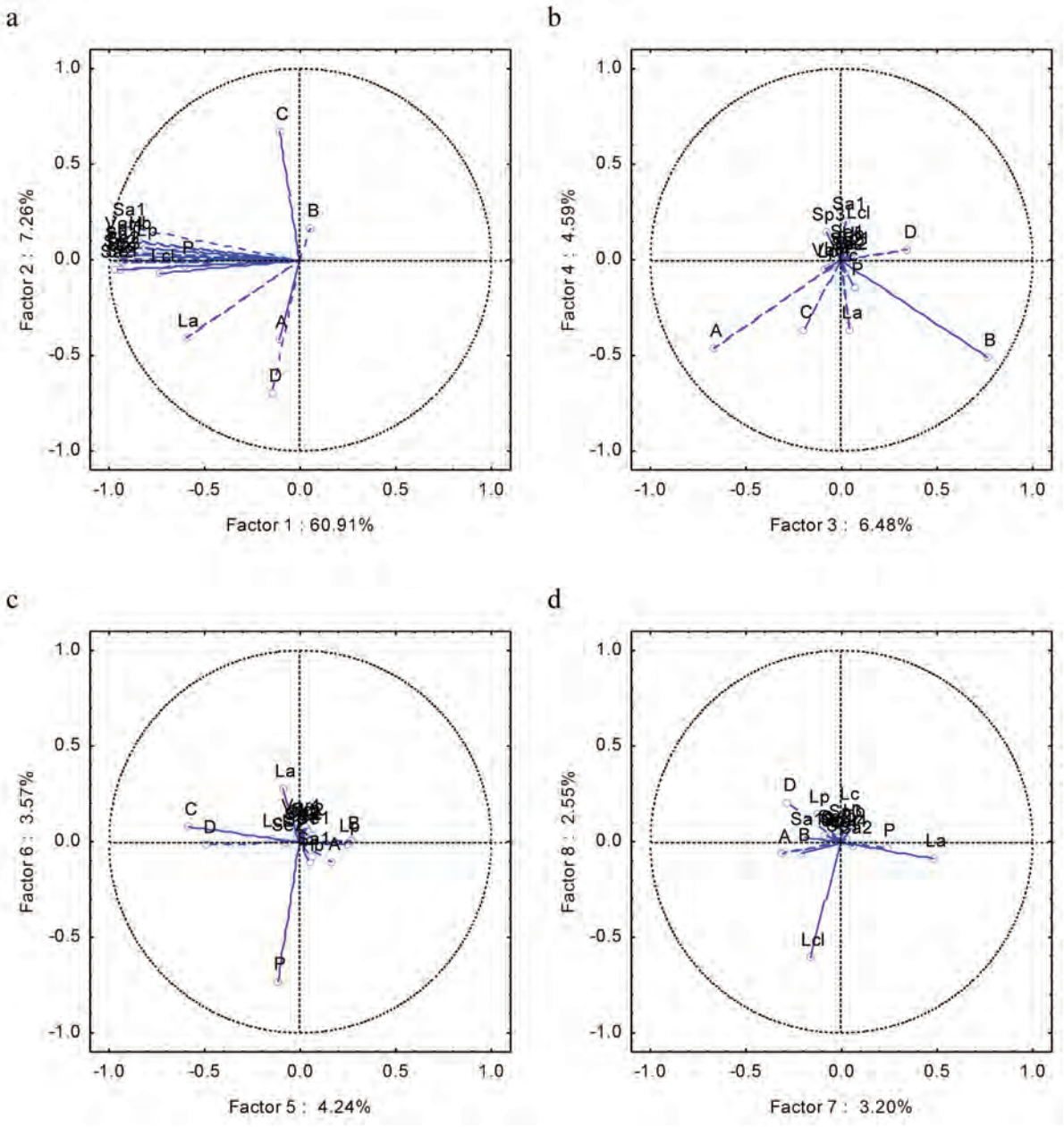

Fig. 10. Results of PCA analysis of studied *H. rufipes* populations in the factor space (a–d) of 8 most significant factors.

As a result of assessment of the body length of *H. rufipes* it was found that for the forest ecosystems (forests with closed canopy and heavy layer of litter) a minimum size of both males and females is typical. At the same time in agrocenoses the body length reaches its maximum in males. In our opinion, this fact can be explained by two reasons. Firstly, in the examined agrocenoses *H. rufipes* is the largest of the dominant species of ground beetles (on the fields there are numerous species of the geni *Poecilus*, *Calathus*, *Amara*, *Bembidion*, *Microlestes*, and other species of the genus *Harpalus*). In contrast to the other types of ecosystem studied, larger ground beetles of the geni *Pterostichus*, *Carabus* and *Calosoma*, capable of feeding on specimens of *H. rufipes* on the fields examined, were virtually absent. Therefore, in the agrocenoses of the area under study *H. rufipes* is one of the top links of the trophic chain among invertebrate animals (Brygadyrenko, 2003; Korolev and Brygadyrenko, 2012, 2014). Secondly, in spite of the fact that the studied species is a ubiquitous habitat generalist, living in a wide variety of moisture conditions from ultra-hygrophilous meadow ecosystems

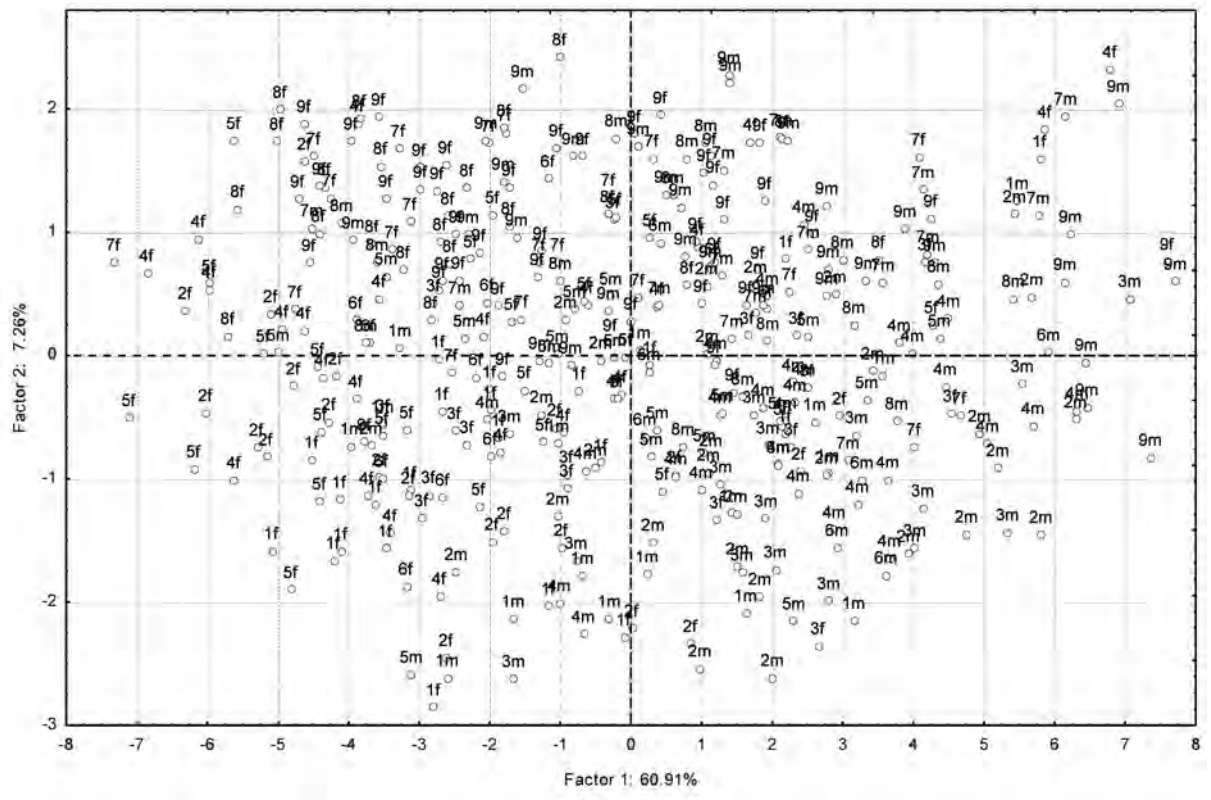

Fig. 11. Distribution of specimens of *H. rufipes* in the factor space of factor 1 (sex: positive values – males, negative values – females) and 2 (ecosystem: positive values – predominantly, ecosystems 7, 8, 9, located in Pavlograd, Dnipropetrovsk, and Petrikovka districts, negative values – all the other ecosystems, located in Novomoskovsk district).

and reed beds on banks of water bodies to xerophilous sand ecosystems on secondary river terraces (Kryzhanovsky et al., 1995; Hůrka, 1996; Brygadyrenko, 2003), *H. rufipes* finds its optimal living environment in the mesoxerophilous and xeromesophilous conditions of agrocenoses (Luff, 1980; Wallin, 1988; Shearin et al., 2007, 2008; Reshetniak and Brygadyrenko, 2013). Here the species reaches its highest numbers, since these habitats offer *H. rufipes* the most varied trophic resources of both vegetable and animal origin (Briggs, 1965; Birthisel, 2013; Birthisel et al., 2014; Brygadyrenko and Reshetniak, 2014).

The presence of sexual dimorphism (males tend to be smaller than females) revealed for most of the linear characteristics of the species may have various ecological interpretations (Slatkin, 1984). In accordance with the evolutionary theory of sex (Geodakyan, 1983) we may suggest the reduction of the absolute size of the body in the process of phylogenesis. However, the significant ($P < 0.001$) negative asymmetry in females and its absence ($P > 0.05$) in males found in our study is evidence, on the contrary, of increase in female size and maintenance of constant body size in males. *H. rufipes* is one of the largest representatives of the genus *Harpalus*. It is probable that the ancestral form of this species had a considerably smaller body than that observed in modern populations of *H. rufipes*. In its size, it should be closer to the modern species *H. griseus* (Panzer, 1797). In our opinion, it would be rewarding to assess the manifestation of sexual dimorphism in populations living in environments under varying degrees of anthropogenic pressure or in proximity to varying numbers of competitor species (Niemelä, 1993; Blake et al., 1994; Roy et al., 2013).

The degree of manifestation of sexual dimorphism can be evidence not only of presence of sexual selection in the population but also of extreme microclimatic conditions affecting the beetles (high temperature and low humidity in summer period) which limit the survival of certain groups of specimens in the population (Lande, 1980; Andersson, 1994; Daly et al., 1998; Bolnick and Doebeli, 2003; Fairbairn, 2007; Cooper et al., 2011; Bobyliov et al., 2014). Fluctuations of numbers and survival rate of *H. rufipes* in the ecosystems where litter horizon is absent (agrocenoses, steppe areas with over-grazing by cattle, areas with regular burning of crop residues) may lead to disappearance of smaller ground beetle species from the macrofauna, as well

as elimination of smaller specimens from the populations of *H. rufipes* (Den Boer, 1985; Holland, 2002; Denno et al., 2005; Davis and Raghu, 2010). On the other hand, in the given ecosystems the trophic pressure of predators may grow considerably; in the agrocenoses such predators on *H. rufipes* are first of all rodents and birds, and in areas with sufficient moisture also reptiles and amphibians (Parmenter and Macmahon, 1988; Churchfield et al., 1991; Denno et al., 2005; Guerrero et al., 2010). The diet of the species under study varies both in different periods of the season and in different habitats, their ability to reach a wide variety of food sources being promoted by their characteristic flight migrations and well developed wing musculature (Thiele, 1977; Hamon et al., 1990; Lovei, 1996; Kromp, 1999; Midtgaard, 1999; Collins et al., 2002; Honek et al., 2003; Irmler, 2003; Matalin, 2003; Harrison and Gallant, 2012). Possibly, the morphology of imagoes of *H. rufipes* is also affected by the type of life cycle: Matalin (2007) has found that for part of the population reproduction was typical during the first year of life, and for another part – during the later period of ontogenesis. Specimens with a one-year and two-year lifecycle may coexist in the studied ecosystems (Matalin, 2007).

For many species of plant-eating invertebrates dependence of mandible shape on the diet (Patterson, 1984; Bernays, 1991) has been traced. For *H. rufipes* such dependence has not been traced so far, whereas for other dominant species of ground beetles, *Pterostichus melanarius* (Illiger, 1798), we have found differences in mandible shape (unpublished data).

In our opinion, the most valuable diagnostic character of the state of a population studied in the course of environmental research is not the mean value of any characteristic, but the type of its distribution. Absence of the normal distribution (significant excess or asymmetry of the sample) can be evidence of directional selection in the populations of *H. rufipes* (Schluter, 2000; Rueffler et al., 2006). Over time, this selection may lead to the fixation of certain characteristic values at the genetic level.

Our findings will form the basis for environmental monitoring of the population status of this species in anthropogenically disturbed ecosystems. Possibly, comparison with the reference values of the characteristics given in this paper will provide an opportunity for further identification of indicator characteristics and wider use of this dominant species in bio-indicator studies (Rainio and Niemelä, 2003).

Conclusions

The result of this study is the finding of significant ($P < 0.001$) negative asymmetry in females and its absence ($P > 0.05$) in males for body length, head length, elytra length, distance between eyes, head width, prothorax width between the front and back angles, elytra width between humeral angles, and maximum width of elytra. For all these characteristics the excess in males is not significant ($P > 0.05$), while in females in most cases it is significantly positive ($P < 0.05$), which is evidence of the large number of females with greater body length, and greater width of head, prothorax and elytra. Thus, in the populations of *H. rufipes* females gradually increase their size, while males retain a constant size.

Absence of the significant asymmetry ($P > 0.05$) for males and females suggests the absence of directional selection in the populations of *H. rufipes* on the density of elytra downiness and value of the prothorax back angle. Significant negative asymmetry is recorded in males and females on maximum prothorax width ($P < 0.001$) and body height ($P < 0.05$), i.e. unidirectional increase in these characteristics occurs in specimens of both sexes.

Analysis of body proportions shows that for all 8 considered morphometric indices the values of excess are significantly positive ($P < 0.001$) in males and in females. This suggests a much higher constancy of body proportions than of full size.

For most of linear characteristics the significant ($P < 0.001$) sexual dimorphism is recorded.

In the areas where annual burning of crop residues and litter is observed, differences between males and females in length are two times higher than differences between males and females for the ecosystems with no such burning.

In the most anthropogenically disturbed areas, as distinct from natural areas, sexual dimorphism in the ratio of length and maximum width of prothorax is not manifested.

In the driest areas, maximum ratio of elytra width to prothorax width is recorded in females.

Vertex angle of elytra significantly differs in the populations of various administrative districts.

Average density of elytra downiness in males is 13.3% lower than in females.

The results of PCA have shown that most of the linear characteristics are connected with the sex of the beetle while the angular characteristics and degree of elytra downiness change regardless of the sex of *H. rufipes* specimens.

The hypotheses formulated in the introduction to this paper were not confirmed as a whole: (1) distribution of a considerable part of the morphometric parameters was not normal in the ecosystems under study, (2) significant changes in mean values typically did not occur with the increase of anthropogenic transformation of ecosystems; in some cases asymmetry and excess of morphometric characteristics and indices grew, and (3) in the conditions of anthropogenically transformed ecosystems differences between males and females of *H. rufipes* did indeed show an increase for most characteristics.

The results of this paper have shown that in bio-indication studies during assessment of the anthropogenic load or variability of populations in natural ecosystems of various types the mean values of morphometric characteristics and indices could have the less diagnostic value, than the type of their distribution in the population.

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Birch necrotic leaf spots caused by fungal pathogens

Helena Ivanová

Branch for Woody Plant Biology, Institute of Forest Ecology of the Slovak Academy of Sciences, Akademická 2, 949 01 Nitra, Slovak Republic, e-mail: helena.ivanova@savzv.sk

Abstract

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Microscopic fungi associated with lesions on the leaves of *Betula pendula* Roth. species growing in different greenery types of Nitra town are causal factors weakening the health state and vitality of these trees. Many of them caused anthracnose and formed small, irregular, circular brown spots with dark brown margins or broad necrotic blotches. Disease symptoms begin as a large spots or blotches, which rapidly widen and join, resulting in large areas of dead tissue. The fungi overwinter on infected fallen leaves. Sporulation begins when spring conditions become warm and wet. During the study of the mycoflora of birch leaves ten fungal species were recorded: *Asteroma microspermum, Cryptocline betularum, Discula betulae*, *Marssonina betulae*, *Melanconium betulinum, Phoma* sp.*, Phomopsis* sp.*, Phyllosticta betulina, Pyrenopeziza betulicola, Stemphylium* sp. In this work were described disease symptoms and some distinctive morphological features.

Keywords

Betula pendula Roth.*,* fungal pathogens, greenery types, necrosis of leaves, Nitra town

Introduction

The common birch (*Betula pendula* Roth) belongs among most frequent tree species in urban greenery. Birches grow in the squares, parks and streets of the cities. Urban birches lose resistance to several species of pathogenic fungi which are associated with lesions on leaves. Many fungal species which overwinter on leaf litter have been described as the causal agents of necrotic leaf spots of the genus *Betula* that can lead to premature defoliation. The development of symptoms depends on the weather conditions and may take a week before the symptoms of diseases become clearly visible.

The aim of this study was to identify the microscopic fungi associated with the leaves of *Betula pendula* species in different greenery types of Nitra town and to describe the distinctive morphological features for these ten fungi as causal factors involved in the decline of health state and vitality weakening of *B. pendula* species.

Material and methods

The samples of birch leaves exhibiting necrotic lesions were collected from 25 trees at 5 sites (SP, HE, NRP, PP, SPG) in Nitra town during the growing season in 2013. Altogether 125 birch trees grown in these five different greenery types were evaluated. The age of evaluated trees was between 10–40 years. The number of fungal taxa found in different categories of urban vegetation in individual localities of town is in Table 1. Pathogenic fungi have been isolated *in vitro* from infected ulcerous places of birch. Cultures of the fungi were obtained from tissues of leaf spots, and grown on potato-dextrose agar (PDA). The holotypes of the species were deposited in the Herbarium of the Branch for Woody Plants Biology in Nitra of the Institute of Forest Ecology of the Slovak Academy of Sciences in Zvolen.

Classical phytopathological approaches were used to isolate and obtain pure cultures. The leaf parts cut from the diseased plants were surface-sterilized by immersion in sodium hypochlorite solution (1% available

Table 1. The numbers of fungal taxa found in Nitra town

Location Categories of planting Taxon	Nitra / Chrenová SP, HE, NRP, PP, SPG	Nitra / Zobor SP, NRP	Nitra / Centrum SP, PP	Nitra / Čermáň SP, NRP	Nitra / Kalvária SP, NRP, SPG	Nitra / Klokočina SP, HE, SPG, NRP
Discula betulae		+	+	+		
Marssonina betulae	+					+
Asteroma microspermum	+	+	+	+		
Pyrenopeziza betulicola				+		+
Phoma sp.	+	+	+	+	+	+
Phomopsis sp.	+	+	+	+	+	+
Stemphylium sp.		+	+	+	+	+
Cryptocline betularum	+	+		+	+	+
Melanconium betulinum	+		+			
Phyllosticta betulina		+			+	+

SP, plantings along streets; HE, housing estate; NRP, main roadside plantings; PP, park plantings; SPG, special purpose greenery.

chlorine) for 20 minutes, rinsed twice or three times in sterile distilled water and then dried carefully with filter paper. The plant samples were subsequently cut to 3–5 mm large fragments which were placed on potato-dextrose agar (PDA) in plastic Petri dishes. This was followed by cultivation at 24 ± 1 °C and 45% humidity in dark conditions in a versatile environmental test chamber MLR-351H (Sanyo) and subsequent isolation on the culture medium. Pure fungal cultures were obtained by using multiple purifications. The obtained isolates were transferred on PDA medium to induce sporulation. Study of fungal structures was performed with a clinical microscope BX41 (Olympus) in water-based mounting medium under 400× and 1,000× magnification. Software Quick Photo Micro Version 2.3 was used for measurements of the size of microscopic objects. Fungal species were identified and the morphometric values were ascertained by routine mycological methods using morphological keys (Pei et al., 2010) and data of morphological studies (Ellis and Ellis, 1997; Paavolainen et al., 2000; Watanabe, 2002; Eriksson, 2006a, 2006b; Farr et al., 2007 and Gomes et al., 2013).

Results and discussion

As a result of the extremely wet spring this year, we found a lot of common diseases, as well as uncommon diseases. Occurrence of these fungi is common in years with wet rainy spring weather. This disease appears as a spotting on the leaf tissue and may cause some defoliation. Under optimum disease conditions run together to form larger blotches. The first lesions which produce conidia usually appear in the beginning of July at the earliest.

During the study of the mycoflora of birch leaves fungal species: *Asteroma microspermum, Cryptocline betularum, Discula betulae, Marssonina betulae, Melanconium betulinum, Phoma* sp., *Phomopsis* sp., *Phyllosticta betulina, Pyrenopeziza betulicola* and *Stemphylium* sp. were recorded as a causal factors involved in the decline of health state and vitality weakening of *B. pendula* species.

Phoma Sacc. represents a complicated asexual genus of fungi generally considered to be a taxonomically difficult group of mitosporic fungi with a wide geographical range, more than 2,000 described infrageneric taxa (Montel et al., 1991) and a limited range of useful morphological characters for distinguishing species. Species are found in numerous ecological niches, include as opportunistic or primary plant pathogens, saprobic soil or water organisms, or parasites of other fungi, lichens, insects and vertebrates (Crous et al., 2009; Aveskamp et al., 2010) occurring on leaves. Pycnidia were scattered, erumpent, globose, black, smooth with a subrimose ostiole. In our experiments the conidia were 7–9 × 2.5–3 µm (Fig. 1a, b). Chlamydospores were absent. This data, which are obtained by cultivation on PDA medium are in scope of variability on this species. According to literature data the dimensions of conidiogenous cells were 4–6 × 3.5–5 µm, globose to bottle shape. Conidia are ellipsoidal, occasionally curved, with dimensions of 3.5–4.5 × 1.5–1.75 µm, on average 3.9 × 1.48 µm, with 2 or 3 guttules, often fusoid, biguttulate, 7–10 µm long or hyaline, ellipsoidal, one-celled, with size 6 (7)–8 (9) × 3 (2.5) µm, (on avg. 7.5 × 2.58 µm). According to Hečková et al. (2013) ellipsoid, hyaline and single-celled conidia occurring on fresh leaves of *Betula pendula* were 11–6 × 6–3 µm, (mean 9 × 4 µm) large.

***Phomopsis* sp.**, asexual states of *Diaporthe* Nitschke, includes important taxa occurring as plant pathogens, endophytes and saprobes on a wide range of hosts (Rossman and Palm-Hernández 2008; Udayanga et al., 2011, 2012). This fungus is widely distributed and some species can incite cankers, rots, wilts, and diebacks in some economically important plants, includ-

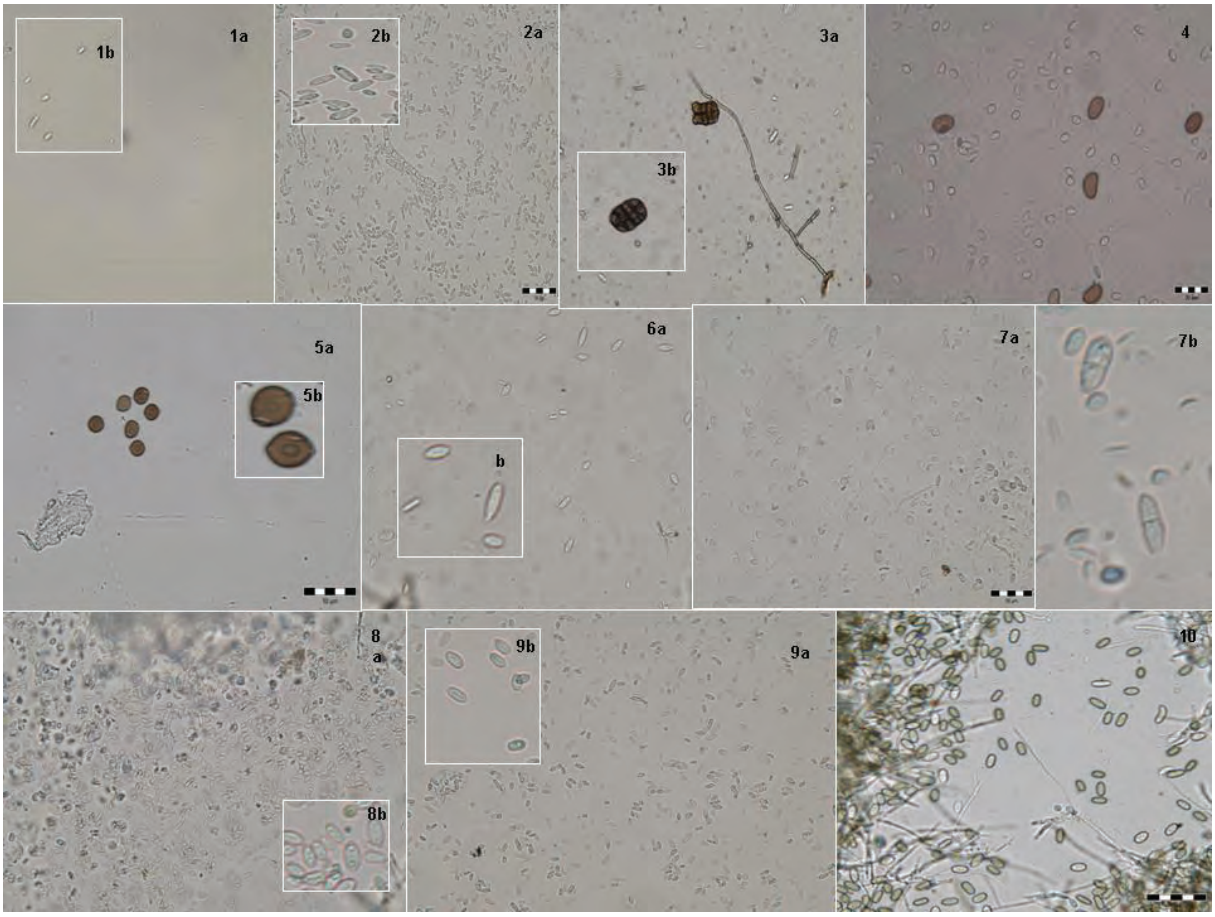

Fig. 1–10. Fungal pathogens caused birch necrotic leaf spots: 1a, b, *Phoma* sp.; 2a, b, *Phomopsis* sp.; 3a, b, *Stemphylium* sp.; 4, *Melanconium betulinum*; 5a, b, *Cryptocline betularum*; 6a, b, *Discula betulae*; 7a, b, *Marssonina betulae;* 8a, b, *Asteroma microspermum*; 9a, b, *Pyrenopeziza betulicola*; 10, *Phyllosticta betulina*.

ing birch, causing severe damage, dieback, leaf spots, blights, decay, wilt and significant losses (Santos et al., 2011; Thompson et al., 2011; Gomes et al., 2013).

The major morphological characters of *Phomopsis* are conidiomata ostiolate, conidiophores phialidic, conidia dimorphic, with usually fusiform and biguttulate alpha conidia and usually filiform, hamate, and non-guttulate beta conidia (Rehner and Uecker 1994; Udayanga et al., 2011). According to Gao et al. (2013) alpha conidia are unicellular, hyaline, aseptate, oval or fusiform, usually with one guttule at each end, rounded at both ends, present more frequently than beta conidia. This fact was confirmed also in our experiments: colonies were woolly to cottony, white, later light brown. Conidiomata were pycnidial, stromatic, dark brown to black, single or aggregated in culture. Alpha conidia were unicellular, hyaline, straight, biguttulate and rounded at both ends, with dimensions of 4–8 (10) × 2–3 (4) µm, beta conidia were not seen (Figs. 2a, b). This data are in scope of variability on this species. Dimensions of spores of *Phomopsis* sp. on *Betula* reported from examined material are 11 (12)–15 × 5 (6) 8 µm (on avg. 13 × 5.87 µm). According to Arnold (1967) on *Betula papyrifera* and *Betula alleghaniensis* (*Betula lenta*) were dimensions of conidiomata 200–250 × 100–170 µm, conidiophore 3–26 × (1–) 1.5–2 (–2.5) µm, alpha conidia (4–) 5–8 × (1–) 1.5–2 (–2.5) µm and beta conidia 14–21 (–25) × 0.7 (–1) µm. Author expressed the view that the fungus could be a potentially serious pathogen for seedlings subjected to low light intensity and high humidity in their natural habitat.

Most species of ***Stemphylium*** are plant pathogens, and are less commonly isolated from soil and decaying plant material. *Stemphylium* sp. in our experiments was isolated from necrotic leaf spots on *Betula pendula*. Colonies on PDA were pale brown, cottony. Mycelium was superficial, hyphae branched, septate, pale brown, smooth, 4.5–5 µm wide. The spores of this fungus are fairly large, 25 (30)–35 ×15 (18)–20 µm, (on avg. 29.8 × 18.6 µm), dark, and have internal cross walls running crosswise and lengthwise, dividing the spore into a number of segments and are typically constricted at the central septum (Figs. 3a, b). Conidia developing singly at the apex of each conidiophore are oblong, ovoid or broadly ellipsoidal, conical at the apex and rounded or subtruncate at the base, with 2–3 transverse septa and 1

(–3) longitudinal or oblique septa. Data are in scope of variability on this species. According to Szabó (2001) colonies of *Stemphylium* are fast growing, suede-like to cottony in texture, and gray, brown, or brownish-black in colour. It grows well on general cellulose surfaces but spores may take longer than normal to develop or may be completely absent. Simmons (1967) delineated this genus, which shares several characters with *Alternaria* and *Ulocladium*, including muriform, usually pigmented conidia. *Stemphylium* is separated from *Alternaria* and *Ulocladium* by the principal morphological characteristic of currently proliferating conidiophores. Taxonomy of *Stemphylium* species was based primarily on conidial morphology, including variations in conidial shape, size, length/width ratio, colour, septation and ornamentation (Simmons, 2004). According to Leach and Aragaki (1970) and Hosen et al. (2009) morphological characteristics in *Stemphylium* sp. may vary depending on environmental conditions such as temperature and substrate. Pei et al. (2010) isolated from diseased leaves of *Lycium chinense* the *Stemphylium lycii* and this species was described by morphological and molecular phylogenetic analyses.

Melanconium betulinum (Kunze et Schm) is considered a weak parasite. This fungus invades weakened or dead tissue and causes what is referred to as *Melanconis* dieback. *Melanconium bicolor* is known as a frequent colonizer of damaged or declining birch shoots (Peace, 1962; Bennell and Millar, 1984) and attacks the tree when it has been weakened by drought conditions, winter kill or phenoxy-acetic acid herbicide exposure causing progressive dieback. First the foliage becomes thin, with chlorotic or curled leaves at the shoot tips. Twigs then become bare because of lack of sufficient vigour to re-foliate. Then the branches and parts of the crown die, below which a bunching of the foliage develops that tends to be confined to the lower part of crown. Death usually takes place within three to five years after the onset of symptoms. *Melanconium* produces black acervuli and ovoid to ellipsoid, 1-celled, dark brown conidiospores that average 13–18 (19) × 5–7 (9) µm. In our experiments conidia measured 10 (13)–16 × 6–9 µm were one-celled, ovoid, dark brown (Fig. 4). All data are in scope of variability on this species.

Anthracnose of birch leaves exhibits brown spots with dark brown to black margins and is caused by fungus ***Cryptocline betularum*** (Ellis & G. Martin) Arx. Johnson et al. (2001) evaluated eight cultivars of birch (*Betula* spp.) for resistance to that birch leaf-spot disease. *Betula pendula* Roth. together with *B. nigra* L. and *B. nigra* L. cv. Heritage were most susceptible to defoliation caused by birch leaf spot disease. Infected leaves fall from tree even if still partly green. Leaves on lower branches are most affected. Leaf spots may be 3–10 mm wide brown spots to large brown blotches surrounded by yellow tissue. This fungus produces acervuli which are intra- or subepidermal (von Arx, 1970). The conidia are aseptate and measure 10 (13)–15 (17) × 7 (8)–9 µm (on avg. 13.5 × 8 µm). In our experiments the conidia measured 12 (13)–16 (17) × 10 (11) µm and were aseptate, dark brown, oblong and tapered on one end (Figs. 5a, b). This data, which are obtained by cultivation on PDA medium are in scope of variability on this species.

Discula betulae (Westend.), (syn. *Discula betulina* (J. Kickx f.) Arx., syn. *Gloeosporium betulinum* (J. Kickx f.) is a common foliar pathogen on birch in whole Europe causing characteristic leaf spots that can lead to premature defoliation (Adamska, 2005; Green and Castlebury, 2007; Green and MacAskill, 2007). Dieback of shoots is caused only when it is combined with other stress factors initiated by unsuitable climatic or site conditions. This fungus, which is generally regarded as a leaf disease, forms brown lesions with dark margins on both sides of the leaves. Leaf spots may be 3–4 mm wide brown rings with a light centre surrounded by yellow tissue. Fungus is producing fast-growing colonies on PDA agar. The hyaline conidia are ovoid, aseptate, large 4–10 × 2.5–3 µm (mean 7 × 2.5 µm). Spores in our experiments were large 4 (5)–8 × 2.5 (3)–4 µm (Figs. 6a, b). This data are in scope of variability on this species. According to Green and Castlebury (2007) diseased shoots and leaves of birch after 4–5 weeks incubation on 2% malt agar producing fast-growing colonies of white aerial mycelium and aseptate hyaline conidia large 4–8 × 1.5–2.5 µm (mean 6 × 2 µm). Hečková et al. (2013) reported, that dimensions of *D. betulae* spores isolated from fresh silver birch leaves were 5–9 × 3–2 µm (mean 7 × 2 µm) and according to Szabó (2001) the conidia were large 6–16 × 2.5–4 µm.

Marssonina betulae (Lib.) Sacc. (syn. *Gloeosporium betulae* (Lib.) Mont) is a primary pathogen on shoots and stems of birch, causing cankers and dieback**.** This fungus belong to foliar pathogen on birch, where causes characteristic leaf spots (Peace, 1962; Bennell and Millar, 1984). Together with other factors, such as unsuitable provenance of birch, incorrect site selection, poor silvicultural management and climatic damage, may also cause birch dieback or predispose trees to disease. *M. betulae* caused dieback of shoots without requiring prior wounding, which indicated that this fungus is a more aggressive pathogen than suggested in the current literature (Green, 2004; Green and MacAskill, 2007). The sporulation of this fungus made leaf lesions on birch. Primary infections caused by fungus *Marssonina* spp. tend to occur in spring shortly after the leaves emerge on the host, and are initiated by conidia from acervuli overwintering in lesions on shoots and fallen leaves. *Marssonina* was able to form acervuli on inoculation lesions, with the potential to release conidia

which are probably spread via rain splash (Sinclair and Lyon, 2005). Conidia, which occurred in our experiment were two celled, hyaline. Dimensions of conidia were 19 (22) × 5 (7) µm (Figs. 7a, b) and are in scope of variability on this species. According to Szabó (2001) conidia were large 17–22 × 8–10 µm and in experiments of Green (2004), Green and MacAskill (2007) conidia measured 10–12 (14)–18 (20) × 5 (6)–7 (8) µm, (on avg. 14.1×7 µm).

Asteroma microspermum (Peck) Sutton (syn. *Gloeosporium betulicola* Sacc. & Dearn.) occurred on affected leaves and formed roundish, irregular spots, several mm in size. Spore bearing structures in the spots are formed in summer and in early autumn. In our experiments spores, which were observed on the surface of agar medium (PDA) were hyaline, 1-celled with thick cell wall and measured 10 (15)–20 × 5–7 µm (Figs. 8a, b). *Asteroma* conidia with dimensions 10 (14)–18 (24) × 4 (5)–7 µm (on avg. 16.7 × 5.28 µm) are produced on the same kind of lesions as of *Gloeosporium* (*Discula* sp.) conidia, but they are smaller than *Gloeosporium* conidia (Kurkela, 1995) and have not been induced to germinate on agar. It is possible that *Asteroma* is the microconidial form of *Gloeosporium* (Lilja et al., 1997). Its presence on *Betula pendula* host was confirmed by Chlebicki and Mułenko (1992) and Mułenko (1996).

Pyrenopeziza betulicola (Fuckel), (anamorpha: *Cylindrosporium concentricum* Grev.) has recently been identified to be the major causative agent of leaf spot disease on *Betula pendula* and *Betula pubescens* (Pappinum et al., 2002). Fungus *Pyrenopeziza betulicola* forms the small circular brown spots without definite borders in the leaves. The fungus is the causative agent of the symptoms occurring in form of spots on the birch leaves (Paavolainen et al., 2000). This pathogen causes premature yellowing and falling of leaves, but not complete defoliation has been observed so far. The economic loss resulting from this disease involves premature leaf-fall, reduction of photosynthesis and may cause problems in frost hardening. Conidia 10–15 × 3 µm long (Figs. 9a, b) are in scope of variability on this species.

Phyllosticta betulina Sacc. fungus spreads easily and causes unsightly blemishes on the leaves**.** The leaves may eventually die and drop. The first signs of a *Phyllosticta* infection appear as elongated tiny purplish or black spots along the veins of the leaves. These lesions gradually grow in size and eventually take on the characteristic eyespot appearance. It is most common in warmer climates, but can be found throughout the world on a number of different types of trees. Adamska (2005) confirms the occurrence of 9 species of parasitic fungi of plants of the genera *Betula* in Poland. Between them *Phyllosticta betulina* is rare. This fact was confirmed by Chlebicki and Mułenko (1992) and Mułenko (1996) on *Betula pendula* host. Two types of spores of this fungus germinate when moisture is present: ascospores and conidia. The ascospores are discharged into the air and can travel between plants on a breeze or current. If they land on a moist leaf, ascospores germinate. Conidia can quickly be carried from diseased plants to healthy ones by splashing rainwater, sprinklers or watering. Conidia which formed in great amount on PDA medium are in scope of variability on this species. In our experiments they were elliptical, 1-celled, pale brown, with thick wall, 13–14 (16) × 6 (7) 8 µm long (Fig. 10).

Conclusions

Parasitic mycoflora of *Betula pendula* growing in different greenery types of Nitra town had considerable diversity. The microscopic parasitical fungi caused premature drying of assimilative organs, branches and also individual trees and these causal factors were weakening the health state and vitality of these trees. The destruction effects of these fungi resulted in various large spots or broad necrotic blotches forming large areas of dead tissue. We have confirmed necessity of the causal agent diagnosis of these trees.

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Space-time patterns of soil pH and conductivity in submountain beech ecosystems in the West Carpathians

Rastislav Janík[1], Eduard Bublinec[1, 2], Margita Dubová[1]

[1]Institute of Forest Ecology of the Slovak Academy of Sciences, Štúrova 2, 960 53 Zvolen, Slovak Republic, e-mail: janik@savzv.sk
[2]Institute of Biology and Ecology, Faculty of Education, Catholic University at Ružomberok, Hrabovská 1/A, 0340 01 Ružomberok, Slovak Republic

Abstract
Janík, R., Bublinec, E., Dubová, M. 2014. Space-time patterns of soil pH and conductivity in submountain beech ecosystems in the West Carpathians. *Folia oecol*., 41: 141–145.

In this work are summarised the results of a monitoring of pH values and conductivity which we have performed for 13 years in the localities of Kremnické vrchy Mts. The study locality, situated in the Western Carpathians Mts, was previously exposed to a moderate pollution only, and the pH values we obtained on a deforested plot in this locality were: 6.08 for precipitation water, 6.05 for the surface humus and 6.36 at a soil depth of 0.1 m. In a forest stand in the same locality we recorded 6.11 in the cover humus and then the values decreased down to 5.99 at 0.25m. The electric conductivity values showed a similar trend.

Keywords
pH, conductivity, throughfall, submountain beech forest

Introduction

The principal and the easiest at hand indicator of soil acidity is its pH and conductivity.

Acidification and eutrofication of forest soils belong to the main factors responsible for significant changes to forest ecosystem diversity in a long term prospect (Bobbink et al., 2010; Dupre et al., 2010; Maskell et al., 2010). This results in the depletion of cations providing basic nutrients necessary for production and vitality of tree layer (Fenn et al., 2006), drop in pH values and lowering the humus quality (Borůvka et al., 2005; Bonneau, 2005).

The soil vulnerability is firstly implied by the parent rock material, altitude and other basic soil forming factors (Manderscheid et al., 2000; Katutis et al., 2008). The main anthropogenic agents affecting soil acidification are acid deposition and forestry. To understand and control the factors underlying forest stand development means a very complex process, the individual constituents of which may interact in a positive or negative way. The effects of each of these factors can be multiple and simultaneous. Despite the dramatic pan-European decrease in sulphur emissions recorded in the recent decades (Kunca, 2008; Pichler et al., 2006; Jandl et al., 2012; Zapletal, 2006) the soil acidification is still remaining a serious forestry-related and environmental problem.

The drop in soil acidity and the nitrogen deposition in context of the contemporary climate change will limit the silvicultural function of forest communities. Trapping carbon and nitrogen, these communities act as an effective filter and a cleaning layer for water flowing through them. Storing and accumulation of nitrogen in soil can mitigate negative impacts of the climate change.

The aim of this paper was to show the trends in pH and conductivity values in the beech stand and open plot in the Kremnické vrchy Mts (the West Carpathians Mts).

Methods

The research plots (RP) are situated in the Kremnické vrchy Mts (48°38´ N, 19º04´ E), in the central part of the West Carpathians Mts. The leading, high dominant forest woody plant is beech, the stand age is 90–100 years, the average tree height is 28 m. The site is at 450–510 m asl, on a slope with an inclination of 17–20° (Barna, 2008; Kellerová, 2006). The site climate is moderate warm, hilly district B5 with an average annual temperature $t_{1951–1980}$ of 6.8 °C and an average annual precipitation total of 778 mm (Schieber, 2006).

The mean annual precipitation total in the growing season is 395 mm, the growing season length is 115–165 days.

The prevailing soil forming substrate consists of andesite tuff agglomerates from which there has been formed a saturated variant of cambisol andosolic with skeleton content increasing with the depth. The soil body is layered, composed of the main and the basal layer system (Pichler, 2006; Pichler et al., 2009; Kukla, 2002).

The lysimetric water was sampled into plastic collectors (1,000 cm^2) in three different soil horizons. The first set is installed in the layer H_{oo}, i.e. the surface horizon (organic layer), the second at a depth of 0.10 m below the soil surface (upper mineral layer) and the third at 0.25 m below the surface (lower mineral layer) (Kukla, 2002).

Atmospheric precipitation and throughfall water were collected into funnels issuing in closed collectors (660 cm^2). The collectors were spaced regularly throughout the beech stand and the adjacent open plot (by 10 pcs on each). The representative samples were obtained by mixing the water from all collectors in the forest stands and open plots. In both cases, the samples were collected at regular time intervals (per month for 13 years) and after each relevant precipitation episode (Lindberg and Turner, 1988).

Results and discussion

The mean annual pH value obtained over the study period on the forested plot was 6.1. The maximum representing 6.49 was recorded in 2001, the minimum of 5.37 was in 2003. The variability was very low and the overall trend was decreasing.

The mean pH value of soil solution at 0.1 m was 6.18, with a maximum of 6.55 recorded in 2012, and a minimum of 5.81 recorded in 2009. The coefficient of variation was only 1.12% (Table 1). The pH value of water percolated through surface humus may be lower (reported from the localities Želivka and Rájec in the Czech Republic, Lochman, 1997) or somewhat higher (documented in south England, Gower et al., 1995), than the original pH value on the surface. As far we know, the highest pH increase in the water percolated

Table 1. Descriptive statistics of pH and conductivity in the Kremnické vrchy (Western Carpathians Mts.) in years 2000–2012

Lysimeter	K_{00}	K_{10}	K_{25}	Forest throughfall	H_{00}	H_{10}	H_{25}	Open plot wet deposition
				pH				
Mean	6.11	6.18	5.99	5.77	6.05	6.36	6.33	6.08
Min	5.37	5.81	4.89	5.40	5.72	5.92	5.66	5.59
Max	6.49	6.55	6.80	6.32	6.35	6.81	6.77	6.61
Range	1.12	0.73	1.91	0.92	0.63	0.89	1.11	1.02
v_x %	1.30	1.12	2.50	2.96	3.62	4.38	4.58	4.90
Std. Dev.	0.31	0.25	0.57	0.32	0.22	0.28	0.29	0.31
Std. Error	0.09	0.07	0.15	0.11	0.06	0.07	0.08	0.08
				Conductivity [µS]				
Mean	101.61	64.53	78.51	76.51	88.42	80.96	72.58	78.96
Min	24.61	33.22	37.66	28.56	40.97	30.14	25.09	34.64
Max	243.37	126.64	149.93	123.11	156.95	186.28	159.96	150.34
Range	218.76	93.42	112.26	94.55	115.98	156.14	134.78	115.70
v_x %	15.52	13.06	12.52	14.23	10.65	16.19	12.97	13.13
Std. Dev.	56.88	30.39	35.47	32.66	33.95	47.27	33.93	37.96
Std. Error	15.77	8.43	9.83	10.88	9.41	13.11	9.41	10.37

v_x – coefficient of variation

K_{00}–K_{25}, lysimeter in the forest; H_{00}–H_{25}, lysimeter in the open plot.

under surface humus was observed in the locality Hukavský grúň, Poľana Mts, Slovakia (Minďáš, 2005).

The mean pH value at 0.25 m was 5.99. So, the spatial pattern of pH values looks different from the Štiavnické vrchy Mts in which pH decreased with increasing depth, and in overall the values reached were lower (according to our measurements, by 1.2 pH on average).

The mean pH value in the throughfall was 5.77, which represented the lowest value in this locality (Table 2).

The pH values and their course on the deforested plot were very similar to the ones in the forest stand – in the surface humus, and also at 0.1 m and 0.25 m below the surface (Table 1, 2). The pH values decreased with increasing depth. The opposite has been reported for lysimetric water sampled on a clear-cut plot at the locality Biely Váh (High Tatras Mts) for which Kukla (1994) obtained a pH value of 6.67 in the surface humus and 7.97 at 0.1 m. Mihálik et al. (1993) compared pH values between free precipitation and beech throughfall in the locality Mláčik in the Kremnické vrchy Mts, and they obtained (in 1990): 4.92 on open plot moderately increased to 5.29 in beech throughfall in case of old trees (130 years) and to 5.26 in case of young trees.

The pH conditions in the free precipitation in open area were found very similar to the throughfall. There has only been recorded a minute drop in pH values (by

Table 2. pH and conductivity in the Kremnické vrchy (Western Carpathians Mts) in years 2000–2012

Lysimeter Year	K_{00}	K_{10}	K_{25}	Forest throughfall	H_{00}	H_{10}	H_{25}	Open plot wet deposition
				pH				
2000	6.43	6.38	5.82	6.18	6.18	6.02	6.41	6.12
2001	6.49	6.54	6.80	6.32	6.24	6.60	6.52	5.82
2002	6.42	6.26	6.07	5.42	6.27	6.69	6.58	6.25
2003	5.37	5.95	4.89	5.58	6.05	6.12	–	5.92
2004	6.26	6.36	5.23	5.40	5.90	6.18	6.26	5.82
2005	5.92	6.16	5.28	5.81	5.74	6.09	6.22	6.38
2006	5.88	5.88	5.91	5.56	5.76	5.92	5.92	5.59
2007	6.27	5.96	6.44	–	6.20	6.81	6.77	6.61
2008	6.23	5.93	6.10	–	6.06	6.46	6.36	6.34
2009	5.87	5.81	6.01	–	5.72	6.32	6.66	6.37
2010	6.19	6.21	6.58	–	5.89	6.29	6.41	6.22
2011	5.94	6.36	6.29	5.74	6.23	6.59	6.52	5.65
2012	6.27	6.55	6.52	5.99	6.35	6.59	6.43	6.02
				Conductivity [μS]				
2000	133.79	80.92	91.38	123.11	156.95	165.13	69.60	135.67
2001	131.32	70.86	73.60	119.34	138.77	88.50	94.28	113.49
2002	97.01	66.48	87.73	82.66	91.48	79.19	64.48	79.10
2003	243.37	67.62	149.93	99.33	110.75	65.55	60.58	80.07
2004	136.53	113.53	119.83	46.05	105.30	93.05	74.59	40.88
2005	74.78	33.22	40.91	28.56	65.84	30.14	25.09	34.64
2006	72.84	42.06	53.95	60.38	61.33	51.70	40.80	46.42
2007	67.47	81.59	120.40	–	102.57	186.28	159.96	150.34
2008	125.55	126.64	90.16	–	86.53	95.08	101.05	107.73
2009	33.52	48.35	53.83	–	63.27	47.92	51.19	76.68
2010	24.61	36.77	62.27	74.65	40.97	34.69	55.22	56.46
2011	116.94	33.37	37.67	54.58	72.33	68.84	91.46	58.15
2012	63.15	37.54	38.94	32.14	53.43	46.51	55.26	46.83

K_{00}–K_{25}, lysimeter in the forest; H_{00}–H_{25}, lysimeter in the open plot.

0.03) in the forest stand. This implies that the forest had a little impact on precipitation falling through the crowns, by reducing its pH value only slightly. Minďáš (2006) speaks about a distinct change after percolation through the cover humus were the original precipitation pH values were mostly shifted from the acid or strong acid range (throughfall) to moderately acid range (statistically significant at $\alpha = 0.01$).

In comparison with the Štiavnické vrchy Mts, formerly exposed to a heavy airborne pollution load, the mean conductivity values were lower on both plots studied, and the values of this variable decreased with increasing depth: on the plot covered with forest stand from 101.61 in the surface humus to 78.51 at 0.25 m, and on the open plot from 88.42 µS in the surface humus to 72.58 µS at 0.25 m (Table 1). The two localities also show similar variability, maximum and minimum values, with lower values recorded in the Kremnické vrchy Mts in overall. Minďáš (2005) reports only statistically insignificant differences in electric conductivity between canopy gaps and throughfall in beech stands. According to the same author, spruce throughfall manifested statistically significant ($\alpha = 0.01$) changes and also more amplitude in electric conductivity – an indicator of overall mineralization. Even more amplitudes can occur after percolation through the surface humus layer. In this case the difference against the free precipitation above the stand was statistically significant ($\alpha = 0.01$), analogically as in the case of lysimetric water in the mineral soil layer.

Conclusion

The accumulative trend of acidifying components of forest soils downwards the soil depth has been confirmed in two beech ecosystem differing in their pollution history. The corresponding values in the Kremnické vrchy Mts, relatively pollution-free, were 6.25 in open area and 6.06 in beech forest stand.

In the Kremnické vrchy Mts, the pH values in throughfall and in lysimetric water were higher than the pH values in free precipitation. In both study localities, the forest stand's influence on the throughfall pH was statistically insignificant.

The pH values can also depend on the total precipitation sum, as abundant rainfall can cause leaching of base elements and shifting pH values towards the acid range.

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Analysis of ambient ozone in a foothill area in the Western Carpathians

Daniela Kellerová, Rastislav Janík

Institute of Forest Ecology of the Slovak Academy of Sciences, Štúrova 2, 960 53 Zvolen, Slovak Republic,
e-mails: kellerova@savzv.sk, janik@savzv.sk

Abstract

Kellerová, D., Janík, R. 2014. Analysis of ambient ozone in a foothill area in the Western Carpathians. *Folia oecol*., 41: 146–152.

This work analyses the ambient ozone concentrations measured during 1999–2008, parallel on two research plots differing in their vegetation cover (beech forest stand and open plot), situated in a rural area. There was detected a difference in the ozone concentration values between the two plots due to their spatial arrangement, but this difference was not significant. On the other hand, a noticeable statistically significant difference in ozone concentrations and differences in selected climatic variables were observed. The influence of average temperature and of rainfall sum was very significant, at $p < 0.0001$. The variability of the measured ambient ozone values ranged from 50.01% in the stand to 57.36% on the open plot. Ozone maxima occurred frequently, mainly after the year 2004. The increase in ozone concentrations, primarily in form of extreme events, means a serious risk for the environment.

Keywords

ambient ozone, forest beech, open plot, rural area, statistical analysis, the Western Carpathians

Introduction

Several factors, both anthropogenic and natural, influence forest ecosystems in central Europe and cause important changes to these ecosystems, their climate included. The former status quo in the emitted and airborne pollution has been changed considerably. Comparing to the past, the effects of acid substances have been mitigated (Fleischer et al., 2009). On the other hand, the trees, both broadleaved and coniferous, became affected negatively or even destructively by ambient ozone present in high concentrations (Muzika et al., 2004; Karlsson et al., 2007; Sanz et al. 2007; EEA, 2010). Generation, formation and accumulation of ambient ozone is a process comprising several levels, and it is determined by various conditions and interactions. The dominant mechanism of pollution transport to Slovakia is transboundary. The ozone precursors are transported in the free troposphere, in horizontal direction. They then descend vertically to the lower layers and mix with nitrogen oxides (NO_x) and volatile organic compounds (VOCs) coming from local anthropogenic and natural sources. The main local source of nitrogen oxides is car transport, expanding dynamically. Another important source is NO_x emitted from soils, in the Slovak Republic estimated up to 15% of the total amount emitted in Europe (SHMI, 2008). Not only in Slovakia, but also in Europe, the trend of concentration of nitrogen oxides does not decrease comparatively to the sulfur oxides (Smidt et al., 2010; SHMI, 2010).

Forests, the dominant source of biogenic volatile organic compounds, represent 41% of the territory of Slovakia. Accordingly, they can produce VOCs (primarily terpenes and isoprenes) in amounts sufficient, under certain circumstances, to promote the local development of ground level ozone.

In the central Europe, the role of nitrogen oxides interacting with anthropogenic and biogenic organic substances and with solar radiation is much more important than formerly supposed. Further significant factors are: the daily wind course, thermal circulation in rugged terrain, deposition onto the surface and interaction among all ozone layers (SHMI, 2006; Šec et al., 2007; Castell et al., 2008).

In this context, there is an urgent need to investigate the relation between the prominent pollutant – ambient ozone, and the ecosystem and its surroundings influenced by this pollutant (Paoletti et al., 2010; Serengil, et al., 2011; Matyssek et al., 2012).

The aim of the experiment was to assess the actual condition and the differences in the ozone distribution patterns for two different research areas in rural environment. We analysed the extent to which the ozone concentrations were dependent on the local temperature and precipitation.

Material and methods

The results of the experiment demonstrate the differences in the ground level ozone concentrations measured simultaneously on two different plots situated at the same altitude. The first plot was covered with a connected beech forest stand; the second was an open area, without forest cover. We analysed the differences related to the actual local situation concerning the airborne pollution, microclimate and meteorology. Our question was whether a forest stand could affect ground level ozone concentrations as it is known for other common pollutants for which forests act as effective filters capturing undesirable harmful substances. We investigated spatial and temporal distribution, differences in seasonal and annual concentrations, and exceeding-limits events. This research's duration was 10 years, so the data obtained could be used to found out if the concentration development showed some trends and dynamics. The parameters were explored and characteristics were processed statistically.

Study site

The experimental plots are situated in the Kremnické vrchy Mts (φ = 48°38´10´´ N, λ = 19° 04´08´´ E), Slovakia, Central Europe. The plot with dominant beech (94.7%) is located at 480–510 m a.s.l. (Barna and Dobrovič, 2010). The slope is west oriented, with an inclination of 30 to 36% (Schieber, 2007). The stand age at the beginning of the experiment was 100–110 years. The open plot is on the foothills of a beech-covered slope, at ca 480 m a.s.l. The distance between the two plots is ca 100–150 m.

The climate in the Kremnické vrchy Mts is moderate warm and moderate humid. The long-term mean annual air temperature is 8.2 °C, in the growing season 14.9 °C; the annual rainfall total ranges from 510 mm to 1,040 mm, in the growing season from 160 to 530 mm (Kellerová and Dubová, 2002; Janík, 2006).

As for the airborne pollution, the research plots investigated are outside the direct impact of polluting substances; neither are they severely affected by the transboundary pollution transport. Three stationary energy production units in the Zvolenská basin close (ca 10 km) to the research site can influence the plots in case of "assistant" meteorological conditions. We were doing our research in the spring and summer when the meteorological, conditions in the basin were mostly favourable for ozone generation (SHMI, 2012). It is supposed that the ozone generation in the locality Kremnické vrchy may be elevated due to precursors (isoprenes and terpenes) coming from the natural sources – forests. Input of further airborne pollutants into the Kremnické vrchy Mts is discussed in Dubová and Bublinec (2006) and Janík et al. (2011).

The temperature data were taken from the SHMI station Sliač (φ = 48°38´33´ N, λ = 19°08´31´´ E), located at 313 m a.s.l. The climatic characteristics are reported for the period 1999 to 2008.

Monitoring of O_3 concentration

The possibilities for monitoring of airborne pollutans in submountain conditions are limited, so the data are supplemented with figures obtained by statistical processing or with information assembled by using passive samplers. The passive samplers are easy to operate. They only demand low costs, work without an energy source, and enable precise discrimination of risk territories with regards to the potential damage to the ecosystems. The methods are progressively improving, simplified, and their results can be compared with the results obtained by using continual analysers (Gerosa et al., 2001; Cox, 2003; De Vries et al., 2003; Bytnerowicz et al., 2004; Šrámek and Novotný; 2009). The drawback of these methods is that they are not suitable for 24-hour monitoring of the dynamics of the ozone concentrations.

The Werner's method (1991) for ozone quantification is based on the selective reaction between a layer of indigo applied on a filter paper and the atmospheric ozone. Such papers are exposed in the field for 7–10 days, during growing season (April–September). The passive ozone samplers are placed on each plot, in pairs, at 1.5 m above the ground. After the exposition, the papers are extracted with ethanol in the laboratory. The reaction between indigo and ozone results in the production of isatin, which is indicated by yellowing of the test papers. The content of isatin is determined by spectrophotometry at a wave-length of 408 nm. The final value of the extinction is proportional to the isatin content and, consequently, also to the ozone content. The measured extinction values reflect the ozone sums according to the calibration curve. The ozone concentrations are given in standard units of ppb per a day or in μg m^{-3} per a day. From the daily values, we calculated the monthly and annual characteristics. The indigo papers were exposed in an equipment consisting of a roofed stand and a perforated cylinder (passive sampler) into which the papers prepared in the labora-

tory were fixed. The detail description is in Kellerová (2002).

The precipitation was captured with funnels (each 660 cm^2 in area) into closed collectors. The collectors were spaced regularly across the stand (by 10 ts) and across the adjacent open area. The samples were taken at regular intervals and after each precipitation event. They were then subjected to chemical analysis and processed in the laboratory. The representative samples were defined as mixture from all the collectors in the stand and in the open plot.

Statistical characteristics of measure and position were calculated and the tests were performed with using the software package Statistica v 7. The normalness of distribution was tested with the Shapiro-Wilk W test. The significance of differences of basic sets between the monitoring plots was evaluated with the Student's t-test for independent variables. The results were also verified with using the software package Statistica.

Results and discussion

Our research purpose was to examine the differences in the ambient ozone concentrations between two researches plots situated in the Kremnické vrchy Mts, Slovakia, Central Europe: one representing a beech forest stand (*Fagus sylvatica L.*) and the other an open area, outside the forest. We analysed the extent to which the ozone concentrations were dependent on the local temperature and rainfall. The measurements were carried out during the growing season, from April to September, when the ozone concentrations are generally higher than in colder months.

The first comparison of the temporal and spatial patterns of ozone concentration between the plots resulted in finding that in the first half of the research period 1999–2003, the ozone concentrations in the beech forested plot were higher (38 μg m^{-3}) than on the open plot (32 μg m^{-3}). In the second half of the experiment, 2004–2008, this trend was only observed in 2005 and 2007 (Fig. 1). Nevertheless, in any case, the differences in ozone concentrations between the two plots were not statistically significant.

To sum up, after 2004, the ozone concentrations increased from 39 to 55 μg m^{-3}. The difference in ozone concentrations between 1999–2003 and 2004–2008 was tested statistically, and it was found very significant (Table 1). The cause may be due to the high air temperature and low rainfall, mainly in years 2006–2008. The air temperature mean we obtained in the growing season was 16.0 °C which is evidently higher than the corresponding long-term mean of 14.5 °C reported from Sliač for the period 1951–1980.

The clearly highest average values of the ozone concentrations on the two experimental plots in the Kremnické vrchy Mts were recorded in 2007 (beech stand 88, open plot 75 μg m^{-3}). In this year, there was also measured the highest average air temperature in the growing season (16.2 °C). According to the evaluation by SHMI (2008), the photochemical activity in year 2007 was above average, and the ozone concentrations in rural areas were by 30–40 μg m^{-3} higher than the values corresponding to the same altitudes in years with lower photochemical activity.

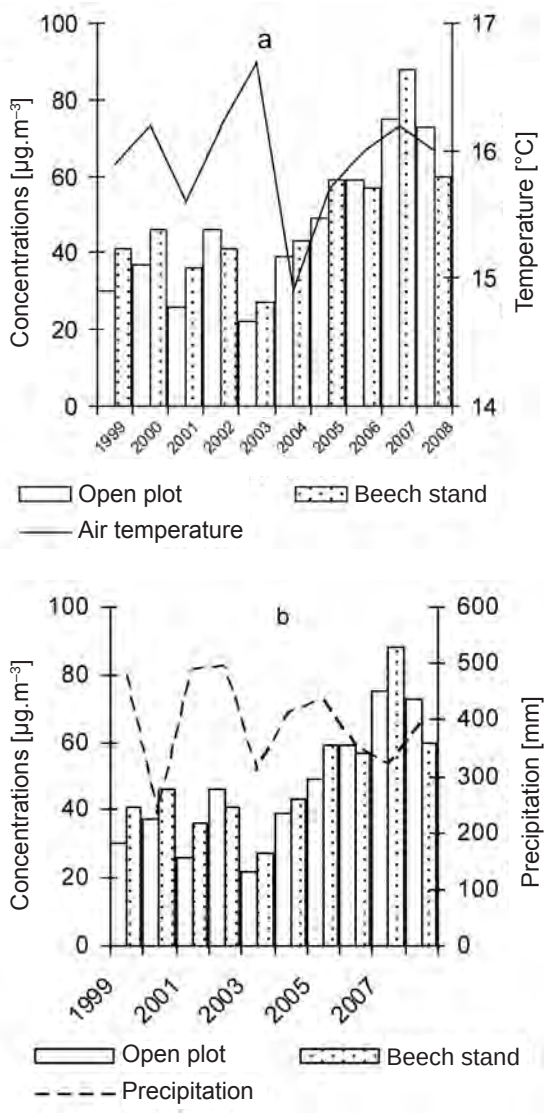

Fig. 1. Average annual ambient ozone concentrations on two-sample plots (open area and beech forest), compared to air temperature (a) and rainfall (b).

In summary, the variability of our ambient ozone concentrations ranged from 50.01% in the stand to 57.36% on the open plot (Table 2). Our research results do not allow us to judge about time trends, as our interannual variability in ozone concentrations was high,

Table 1. T-test and p values for open plot, beech stand, air temperature and precipitation total in the Kremnické vrchy Mts in years 1999–2008

	Open plot	Beech stand	Air temperature	Precipitation total	Open plot	Beech stand	Air temperature	Precipitation depth
	t-values				p<			
Open plot	–	–0.526	5.045***	–12.255***	–	–	–	–
Beech stand	0.526	–	6.267***	–12.147***	–	–	–	–
Air temperature	–5.045***	–6.267***	–	–13.669***	–	–	–	–
Precipitation depth	12.255***	12.147***	13.609***	–	–	–	–	–
Open plot	–	–	–	–	–	0.605	0.00001	0.00000
Beech stand	–	–	–	–	0.605	–	0.00001	0.00000
Air temperature	–	–	–	–	–0.0008	–0.00001	–	0.00000
Precipitation total	–	–	–	–	0.00000	–0.00000	0.00000	–

Marked differences are significant at $p < 0.05$.

Table 2. Descriptive statistics of ambient ozone in the Kremnické vrchy Mts in years 1999–2008

Statistical parameters	Open plot	Beech stand
Valid	48.00	54.00
Mean	48.11	51.39
Standard error	3.98	3.49
Standard deviation	27.59	25.72
Minimum	8.00	10.00
Maximum	144.00	140.00
Range	136.00	130.00
Variance	761.46	661.29
Lower Quartile	25.50	32.00
Upper Quartile	64.00	64.00
Coefficient of Variation	57.35	50.05
Sum	2,309.00	2,775.00

and the trend evaluation in this area requires much longer time series than the series we have recorded till now (JONSON, et al., 2006).

Figure 2 illustrates that until 2005, the measured maxima had exceeded the limit of 65 µg m^{-3} only in three cases, whereas later all the maxima were above this limit value.

Fig. 2. Maximum ozone concentrations on two plots in the Kremnické vrchy Mts, representing open area and beech forest in summer period (April–September) on 1999–2008. Maximum concentrations compared with ecological limit for vegetation (65 µg m^{-3}).

The ground level ozone concentrations and short-term limit values of airborne pollutants were compared with the value of 65 µg m^{-3} (32.5 ppb day^{-1}) approved as ecological limit for the 24 h average concentration

of this substance by the EU in 1992 (EEC 1992). The events of exceeding this limit in the stand were by 10% more frequent than on the open plot. In total, the highest ground level ozone concentrations were measured in 2007 (140 µg m^{-3}) in the stand and in 2008 (144 µg m^{-3}) on the open plot, the lowest in 2005 (8 µg m^{-3}) on the open plot and (10 µg m^{-3}) in the stand.

From the viewpoint of seasonal dynamics, in the late summer (July, August), higher ambient ozone concentrations were recorded on the beech-forested plot; in the spring and early summer (April, June), on the open plot. The year 2004 seemed to change the former trend, probably owing to the meteorological situation during the measurement. This can be concluded from the influence of selected climatic variables (average temperature and rainfall sum) on the ozone concentrations. The changes in meteorological parameters, especially the increase in air temperature linked with the global climate change, become evident also according to recent research (Bičárová and Fleischer, 2007). Another cause may be the tree harvest in close proximity of our beech stand, which could influence the microclimate and site conditions in this stand. The rise in ozone concentrations and in air temperature recorded recently in this submountain area may be warning, despite its values are not as extreme as those reported from other regions of Slovakia and from Central Europe (Hůnová et al., 2012; Šrámek et al., 2007; Melkonyan and Wagner; 2013). The conditions promoting ozone formation depend on the local situation in airborne pollutants and on the local microclimate and meteorology during anti-cyclones expanding over big areas. High ground level ozone concentrations developed mainly during episodes with abundant photo-chemical smog linked typically with stagnant air, sunny summer heat and low air humidity.

The number of episodes in exceeding the limit was somewhat higher in the stand than on the open plot. This suggests the presence of further agents acting synergically and influencing ozone concentration in forest stands. Biogenic emissions of volatile organic compounds and nitrogen oxides from natural sources can play an important role in the ozone formation over Europe. This role may be much more important than supposed so far. Such ozone precursors as terpenes and isoprenes are forest products. The major part of isoprenes and more than one half of terpenes are emitted from May to August, and the emission of these substances rises exponentially with raising temperature (SHMI, 2008). The beech forests contribute to the total biogenic VOC emissions by ca 10% (Bičárová, 2008). The primary local sources are supposed: anthropogenic emissions from car traffic, recently built industrial park and local combustion of solid fuels. The diverse terrain topography enables, under specific conditions, mixing of ozone and its precursors with the valley circulation, which also increases the local ozone concentrations (SHMI, 2006).

Given that research lasted 10 years, we may outline certain trends in ozone concentrations. Figure 3 shows that the trends on both plots were rising significantly.

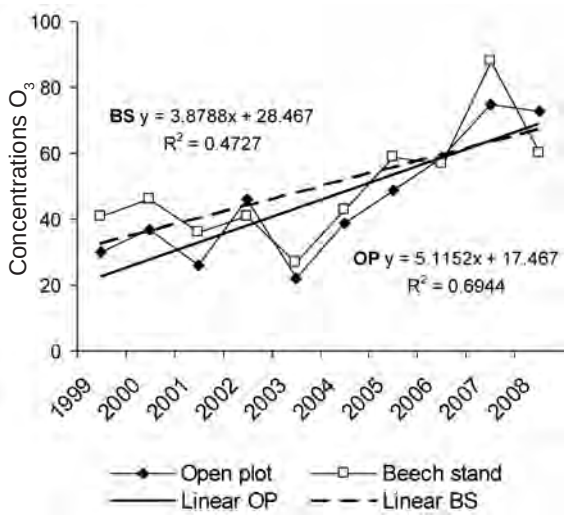

Fig. 3. Linear trend and temporal distribution of ozone concentrations on research plots in the Kremnické vrchy Mts.

Conclusion

We analysed and evaluated ambient ozone concentrations measured over the period 1999–2008 on two research rural plots situated at the same altitude but differing in their vegetation cover.

The statistical analysis revealed that the differences in the ozone concentration values between the two plots were not significant. In the case of ambient ozone, the forest did not act as a filter for trapping the pollutants. Contrarily, the forest contributed to the pollution, due to the precursors produced by the forest itself. On the other hand, the pollution manifested distinct temporal variability, because the average monthly ozone concentration values were more often high, even above limits, and the inter-annual differences were impressive just after the year 2004. It is not easy to draw conclusions for the temporal trends, because the inter-annual variability of the ozone concentration was relatively high and because the judgment about the trends needs temporal series much longer than the series we dispose with.

Statistically very significant was found the influence of average temperature, rainfall sum and clearly also tree felling in the surrounding stands. In terms of seasonal dynamics, higher ambient ozone concentration values in the forest stand were recorded in July and August, on the open plot in April and June. The rise in ozone concentration, primarily in the form of extreme events, seems to be a serious risk for the environment.

Acknowledgement

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Fungi and slime molds of alder and willow alluvial forests of the upper part of the Muránka river (central Slovakia)

Ivan Mihál[1], Drahoš Blanár[2]

[1]Institute of Forest Ecology of the Slovak Academy of Sciences, Štúrova 2, 960 53 Zvolen, Slovak Republic, e-mail: mihal@savzv.sk
[2]Administration of the Muránska planina National Park, J. Kráľa 12, 050 01 Revúca, Slovak Republic, e-mail: drahos.blanar@sopsr.sk

Abstract
Mihál, I., Blanár, D. 2014. Fungi and slime molds of alder and willow alluvial forests of the upper part of the Muránka river (central Slovakia). *Folia oecol.*, 41: 153–172.

Mycological and floristic research was carried out in alluvial forests (alliances *Alnion incanae* Pawłowski in Pawłowski et al. 1928 and *Salicion albae* Soó 1930) in the alluvium of the Muránka river in the north-western part of the Gemer region in central Slovakia during 2009–2012. In the studied forest stands the authors identified altogether 236 macromycetes and 13 slime molds (in total 249 taxa). As the first records for Slovakia following specimens were found out: *Diderma globosum* var. *europaeum*, *Fuligo laeviderma* (Myxomycota), *Entomophthora coleopterorum* (Zygomycota), *Acrospermum compressum, Belonopsis filispora, Echnoa infernalis, Xylaria digitata* (Ascomycota) and *Hohenbuehelia angustata, Melampsora amygdalinae* (Basidiomycota). The highest number of taxa belong among lignicolous saprotrophes (158 species) and terrestric saprotrophes (51 species), this might be because of enough dead wood substrate and rich humus litter layer on alluvial soils in the habitats. On the other hand, the number of lignicolous parasites (13 taxa) and ectomycorrhizal symbionts (11 taxa) were rather low.

Keywords
alluvial forests, *Alnion incanae*, macromycetes, *Salicion albae*, slime molds, the Western Carpathians

Introduction

The upper and central Gemer region with the upper part of the Muránka river is a very interesting territory thanks to its botanical and mycological values. From Muráň to Jelšava southwards, the Muránka watercourse is bordered with a wide alluvial floodplain – a natural artery and biocorridor of the whole river valley of Muránka. This segment of the river, together with its tributaries, is rich in large tree stands with dominant *Alnus glutinosa* and/or *Salix fragilis*, in lower parts also with *S. alba* (alliances *Alnion incanae* Pawłowski in Pawłowski et al. 1928 and *Salicion albae* Soó 1930). However, only a few of these habitats (relatively well preserved and developed) may be considered the remnants of the original alluvial forests. In most cases, they are fragments of the successional alluvial forests which have been developing on the abandonned alluvial meadows since the first half of the 20th century. It is also necessary to note that the concerned territory has been exposed to the strong influence of various human activities, such as regulation of water streams, controlling of river beds, traffic, agriculture, illegal tree cutting, invasions of alien plant species and similar factors.

The first floristic data from the alluvium of the Muránka river were published by Gustáv Maurícius Reuss, a medical man and an important Slovak botanist and polymath (Reuss, 1853–1854). Later and recently published data can be found in Urvichiarová (1967) and in Blanár and Mihál (2002). From the mycological view, the macromycetes in the alluvial forests of the Muránka river have been studied only in one locality near Revúca city (Blanár and Mihál, 2002). On the other hand, the diverse forest communities in the sur-

roundings – the Muránska planina Mts, the Stolické vrchy Mts and the Revúcka vrchovina Hills were subject to several mycological studies. For example, fragmental but comprehensive data from the Muránska planina, Stolické vrchy and Revúcka vrchovina were reported by Kotlaba et al. (1991), Glejdura (2013), Kučera and Kautmanová (2011), Mihál et al. (2011), Mihál and Blanár (1999, 2007, 2011) and Ripková and Blanár (2002, 2004). Across the wider neighbourhood of the investigated area, mycological research was carried out on selected localities in alluvial floodplain forests, gravel pits and marshes in the Cerová vrchovina highlands (Mihál, 1995, 2006), in Ostrôžky Mts (Mihál, 2001) and along the Ipeľ river (Mihál, 1997a, 1997b).

The aim of this work is to present the results of our mycological, floristic and phytocoenological research of alluvial floodplain forests in selected localities along the upper part of the Muránka river, between the village Muráň and Jelšava city.

Material and methods

The research was carried out on selected 7 localities in the alluvium of the upper part of the Muránka river and of its righ-side tributary Lehotský potok creek. Field excursions were realised at irregular time intervals, from 2009 to 2012. The time schedule of the excursions is in the appendix taxa. Several additional samplings for verification and taxonomical revision were also performed in 2013. In this study, we also present the mycological data adopted from Blanár and Mihál (2002), Ripková and Blanár (2004), Mihál and Blanár (2011), Mihál et al. (2011).

The nomenclature and authors´ abbreviations have been adopted from Lizoň and Bacigálová (1998), Škubla (2003), some also from the database by Cooper and Kirk (2013). The nomenclature of vascular plants follows the Checklist of non-vascular and vascular plants of Slovakia (Marhold, 1998). The phytocoenological relevés were prepared according to the methods designed by the Zürich-Montpellier school (Braun-Blanquet, 1964), with an extended scale for the species cover. This abundance and dominance scale consists of nine degrees, the degree 2 diversified into 2a, 2b and 2m (Barkman et al., 1964). The nomenclature of syntaxa is according to the List of the syntaxa of Slovakia (Jarolímek et al., 2008). Mentioned in the text for the first time, the names of syntaxa are given together with the name of their authors or also with the year of their description. The habitats are classified as in the Catalogue of habitats of Slovakia (Stanová and Valachovič, 2002).

The several abbreviations are used in the text – abbreviations of the collectors and/or determinators of studied material: DB (Drahoš Blanár), IM (Ivan Mihál), SJ (Soňa Jančovičová), SG (Stanislav Glejdura), VK (Viktor Kučera), VaK (Václav Kautman); abbreviations of the herbaria: BRA (h. Slovak National Museum in Bratislava), SLO (h. Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany), herb. IM (personal herbarium of I. Mihál), herb. DB (of D. Blanár), PVK (of V. Kautman), PSG (of S. Glejdura); marking of biotop is following: Ls1.1 (Natura 2000: 91E0* Mixed ash-alder alluvial forests of temperate and Boreal Europe), Ls1.3 (Natura 2000: 91E0* Ash-alder submountain alluvial forests); abbreviation of syntaxons: assoc. – association; another abbreviations/marks: agg. (aggregate taxon), juv. – juveniles (plant aged less than 1 year), ca – circa, ø – average, N – north, S – south, E – east, W – west, E_C – total vegetation cover, E_3 – cover/diversity of tree layer, E_2 – cover/diversity of shrub layer, E_1 – cover/diversity of herb layer, E_0 – cover/diversity of mosses, h_{E3} – average height of E_3 layer (height estimation), h_{E2} – average height of E_1 layer, h_{E1} – average height of E_1 layer, NP – National Park.

Not deposited material in herbaria is abbreviated as not**.** sensu Kotlaba (1999). The elevation marks for the mountains have been adopted from the tourist map Stolické vrchy – Revúca with a scale of 1 : 50,000 (Kordováner, 2006).

The area of research

The investigated area covers the alluvium of the upper part of the Muránka river together with its tributary Lehotský potok creek, partly also the alluvium of Zdychavka stream (its inflow into the Muránka), namely between the village of Muránska Lehota and the town of Jelšava. The individual studied localities are marked in the map (Fig. 1).

The watercourse Muránka is situated in the river basin of the Slaná river (cf. Turbek, 1980). According to the phytogeographic classification, studied localities belong to the West-Carpathian flora (*Carpaticum occidentale*), pre-Carpathian flora division (*Praecarpaticum*), district 15 – Slovenské rudohorie; one locality (Lehotský potok) is on the borderline between district 16 – Muránska planina and district 15. In terms of geomorphology (Mazúr and Lukniš, 1980), the studied alluvium is situated in the province West Carpathians, sub-province inner West Carpathians, region Slovenské rudohorie, geomorphological units Stolické vrchy and Revúcka vrchovina. The localities are situated on Pleistocene proluvial clay-silicate to gravel sediments of the Muráň (Muránka) river and Lehotský potok creek. As for the climate (Tarábek, 1980), the studied area belongs to the sub-type of moderately warm mountain climate (localities north of Revúca) and warm mountain climate (localities south of Revúca).

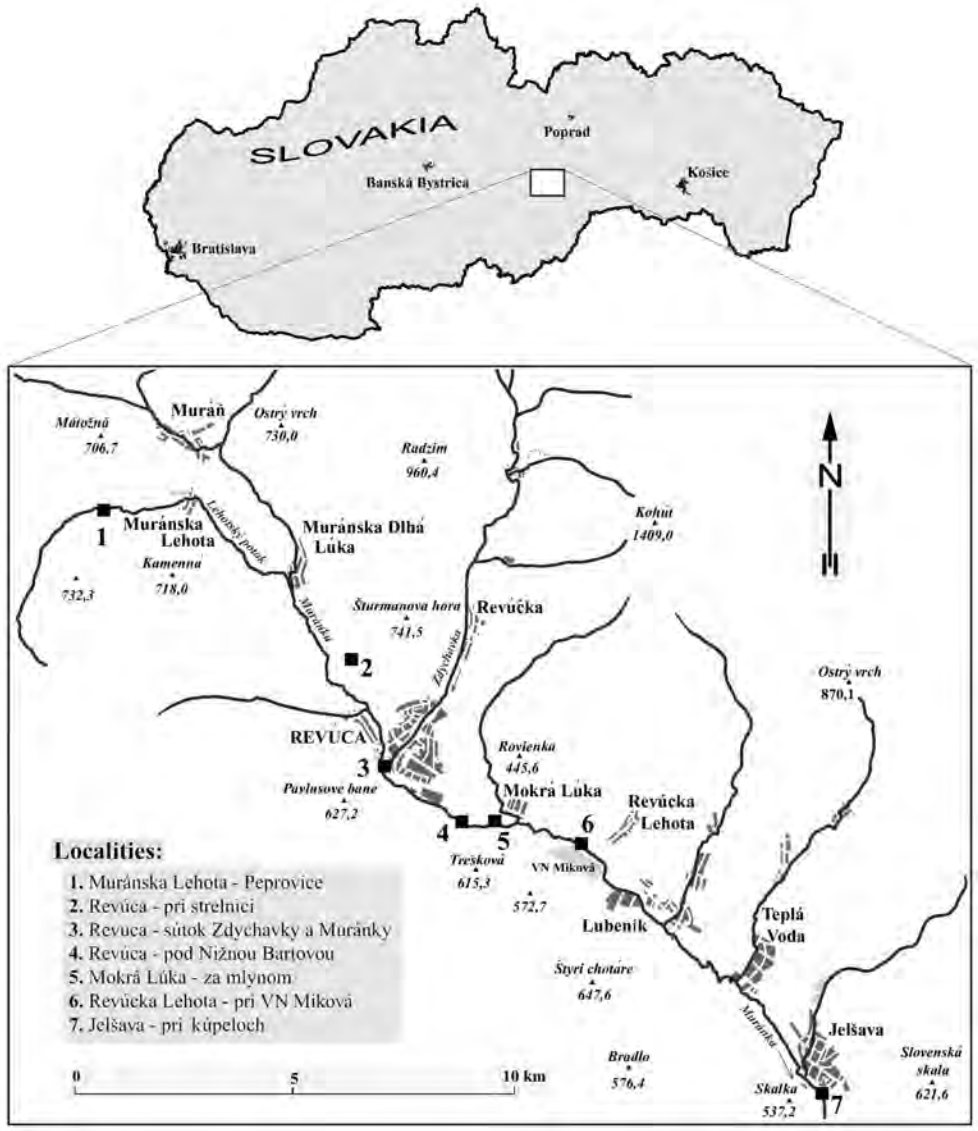

Fig. 1. The map of investigated area of alluvial forests of the Muránka river (Author D. Blanár).

We have recorded species diversity of macromycetes in 7 localities in the alluvium of the Muránka river (and also Lehotský potok creek and at the inflow of Zdychavka creek). The localities were selected to cover the wide range of types of alder and willow floodplain stands with *Salix alba* and *S. fragilis*, together with fragments and successional stages of such stands in the upper part of the Muránka river. Each locality is supplemented by the following informations: geomorphological unit; cadastral area, locality (closer description), altitude; central European mapping grid quadrant DFS (cf. Jasičová and Zahradníková, 1976); habitat (biotope, syntaxon – alliance or association); substrate; approx. area of the habitat (in ha^{-1}). We have also provided a short description of the vegetation (list of some dominant and characteristic species of vascular plants and mosses-bryophytes).

Characteristics of localities

Locality No. 1: Muránska planina Mts/Stolické vrchy Mts; Muráň; Peprovice (W of the Muránska Lehota village), SSW of the beginning of Javorníkova dolina valley, alluvium of Lehotský potok creek; 405–400 m a.s.l.; DFS 7286c; alder stand (Ls1.3; *Alnion incanae*); sandy and sandy-loam soil; ca 1.5 ha^{-1}.

The alder stand (assoc. *Stellario-Alnetum glutinosae* Lohmeyer 1957) is described by the following phytocoenological record:

Record No. 1: Muránska Lehota, W of the village, alder stand in alluvium of Lehotský stream; 48.725900° N –20.023266° E ± 7 m; 400 m a.s.l., exp. NNE, slope 2°, area of record: 15 × 20 m, 2. 7. 2010, D. Blanár [original releve DB-9/2010].

Cover E_c: 98%, E_3: 95%, E_2: 35%, E_1: 95%, E_0: 3%; h_{E3} = 18–20 m (*Alnus glutinosa, Fraxinus excelsior*)/8–12 m (*Acer pseudoplatanus*), h_{E2} = 3–4 m, h_{E1} = 15–20 cm (*Aegopodium podograria, Galeobdolon montanum*)/50–80 cm (*Rubus caesius*)

E_3: *Alnus glutinosa* 5, *Fraxinus excelsior* 1, *Alnus incana* +, *Acer pseudoplatanus* +, *Tilia cordata* +, *Salix fragilis* +
E_2: *Corylus avellana* 2b, *Carpinus betulus* 1, *Fraxinus excelsior* 1, *Acer pseudoplatanus* +, *Alnus glutinosa* +, *Fagus sylvatica* +, *Humulus lupulus* +, *Sambucus nigra* +, *Swida sanguinea* agg. +, *Tilia cordata* +
E_1: *Crepis paludosa* 2b, *Galeobdolon montanum* 2b, *Aegopodium podograria* 2a, *Fraxinus excelsior* 2a, *Rubus caesius* 1, *R. fruticosus* 2a, *Athyrium filix-femina* 1, *Brachypodium sylvaticum* 1, *Caltha laeta* 1, *Carex sylvatica* 1, *Deschampsia caespitosa* 1, *Rubus idaeus* 1, *Viburnum opulus* +, *Asarum europaeum* +–1, *Impatiens parviflora* +–1, *Acer pseudoplatanus* +, *Alnus incana* +, *Cerasus avium* +, *Circaea lutetiana* +, *Cirsium oleraceum* +, *Corylus avellana* +, *Crataegus monogyna* +, *Dentaria bulbifera* +, *Dryopteris carthusiana* +, *D. filix-mas* +, *Festuca gigantea* +, *Euonymus europaeus* +, *Filipendula ulmaria* +, *Geum urbanum* +, *Humulus lupulus* +, *Lycopus europaeus* +, *Myosotis scorpioides* agg. +, *Lysimachia vulgaris* 1, *Malus sylvestris* +, *Paris quadrifolia* +, *Primula elatior* +, *Ranunculus auricomus* agg. +, *Ribes uva-crispa* subsp. *grossularia* +, *Sambucus nigra* +, *Stachys sylvatica* +, *Stellaria nemorum* +, *Solanum dulcamara* +, *Tilia cordata* +, *Juglans regia* r, *Lychnis flos-cuculi* +, *Quercus petraea* agg. r
E_0: *Atrichum undulatum* +, *Brachytecium* sp. 1, *Plagiomnium* sp. +.

Locality No. 2: Stolické vrchy Mts; Revúca; near target range, SW–SWW of the Šturmanova hora Mt (741.5 m a.s.l.), along to the railway line; 325–330 m a.s.l.; DFS 7286d/7386b; willow stand (Ls1.3; *Alnion incanae*); sandy-loam soil; ca 1 ha^{-1}.

The vegetation on this locality (assoc. *Stellario-Alnetum glutinosae*) is described by the following record:

Record No. 2: Revúca; SW–SWW of Šturmanova hora Mt (741.5 m a.s.l.); alder-willow stand (30–50 years forest); 48.700944° N – 20.095527° E ± 9 m; 330 m a.s.l., exp. SW, slope 1°, area of record: 15 × 20 m, 2. 10. 2009, D. Blanár [original releve DB-2.10.2009].
Cover E_c: 98%, E_3: 97%, E_2: 45%, E_1: 40%, E_0: 3%; h_{E3} = 15–20 m, h_{E2} = 2–4 m, h_{E1} = 15–20/40 cm
E_3: *Alnus glutinosa* 3, *Salix fragilis* 3, *Padus racemosa* 1, *Carpinus betulus* +
E_2: *Swida sanguinea* agg. 3, *Sambucus nigra* 1, *Viburnum opulus* 1, *Carpinus betulus* +, *Euonymus europaeus* +, *Hummulus lupulus* +, *Tilia cordata* +, *Ulmus glabra* +
E_1: *Chrysosplenium alternifolium* 2b, *Glechoma hirsuta* 2a, *Urtica dioica* 2a, *Galium aparine* (juv.) 2m, *Swida sanguinea* agg. 1, *Brachypodium sylvaticum* +, *Circaea lutetiana* +, *Deschampsia caespitosa* +, *Euonymus europaeus* +, *Filipendula ulmaria* +, *Geum urbanum* +, *Lamium maculatum* +, *Lysimachia nummularia* +, *Padus racemosa* +, *Rubus caesius* +, *Stachys sylvatica* +, *Ulmus glabra* +, *Athyrium filix-femina* r, *Fagus sylvatica* r
E_0: *Brachythecium rivulare* 1, *Eurhynchium hians* 1.

Locality No. 3: Revúcka vrchovina Hills; Revúca; conflux of the Zdychavka creek and Muránka river, alluvium; ca 310 m a.s.l.; DFS 7386b; willow and alder-willow stand (Ls1.1, Ls1.3; *Salicion albae*, *Alnion incanae*); sandy-loam soil; ca 0.8 ha^{-1}.

3a. willow-alder stand – in part near the Zdychavka river (*Alnion incanae*).

The vegetation of assoc. *Stellario-Alnetum glutinosae* is characterised by the phytocoenological record according to Blanár and Mihál (2002). The woody species *Alnus glutinosa*, *A. incana* and *Salix fragilis* are typical for this locality as well as the bushes species *Salix caprea*, *S. cinerea*, *Sambucus nigra* and *Padus racemosa* (in Blanár and Mihál, 2002 the *S. cinerea* is wrongly presented as *Salix incana*!).

3b. willow stand – in part further to the Zdychavka river (*Salicion albae*).

Vegetation-forming woody species is *Salix fragilis*. The herba layer is mainly formed by *Urtica dioica*, *Lamium maculatum* and *Chrysosplenium alternifolium*. The characteristic vernal species are *Anemone ranunculoides*, *Corydalis solida*, *Dentaria glandulosa* and *Ficaria verna*.

Locality No. 4: Revúcka vrchovina Hills; Revúca; below the Nižná Bartová, (alluvium of the Muránka river); 290–294 m a.s.l.; DFS 7386b; willow mixed forest on distributary delta of Muránka river (Ls1.1, Ls1.3; *Salicion albae*, *Alnion incanae*); sandy-loam soil; ca 1.5 ha^{-1}.

Salix fragilis, *Ulmus glabra*, *Padus racemosa*, *Alnus glutinosa*, *A. incana* species are dominant, *Salix alba* and *Populus nigra* are subdominant woody species. The liane *Parthenocissus quinquefolia* occurrs on the woody species. The species *Humulus lupulus*, *Euonymus europaeus*, *Sambucus nigra*, *Salix cinerea*, *S. purpurea* have been found out in the shrub layer. The herb layer is developed by *Urtica dioica*, *Rubus caesius*, *Lamium maculatum*, *Glechoma hirsuta*, *Aegopodium podograria*, *Galium aparine*, *Myosoton aquaticum* and *Chrysosplenium alternifolium*. The species *Parietaria officinalis*, *Lunaria rediviva* and *Virga pilosa* are rare. As the vernal species, *Anemone nemorosa*, *Dentaria glandulosa* and *Gagea lutea* have been found out. The protected *Matteuccia struthiopteris* species also occurrs rarely. As the invasive species are *Fallopia japonica*, *Helianthus tuberosus* and *Impatiens glandulifera.* The stand was dominated by *Salix fragilis* (alliance *Salicion albae*) which is characterised by the following record:

Record No. 3: Revúca; fragment of willow alluvial forest; 20.133472° E – 48.669888° N ± 9 m; 290 m a.s.l.; exp. SSE, slope 1°, area of the record: 15 × 20 m, 15. 8. 2009, D. Blanár [original releve DB-15.8.2009].
Cover E_c: 100%, E_3: 75%, E_2: 45%, E_1: 96%, E_0: do 1%; h_{E3} = 25 m, h_{E2} = 4–6 m, h_{E1} = 190/70/25 cm
E_3: *Salix fragilis* 4, *Alnus glutinosa* 2a, *Ulmus glabra* + 1, *Parthenocissus quinquefolia* 1, *Alnus incana* (+)
E_2: *Sambucus nigra* 3, *Parthenocissus quinquefolia* 2a, *Corylus avellana* 1, *Aesculus hippocastanum* +, *Alnus incana* +, *Euonymus europaeus* +, *Padus racemosa* +, *Hummulus lupulus* +, *Swida hungarica* +, *Ulmus laevis* +
E_1: *Lamium maculatum* 3, *Matteuccia struthiopteris* 3, *Urtica dioica* 3, *Chrysosplenium alternifolium* 2a, *Aegopodium podograria* 1, *Impatiens noli-tangere* 1, *Lunaria rediviva* 1, *Parthenocissus quinquefolia* 1, *Stellaria nemorum* 1, *Anthriscus nitida* +, *Asarum europaeum* +, *Brachypodium sylvaticum* +, *Chaerophyllum hirsutum* +, *Circaea intermedia* +, *Cirsium oleraceum* +, *Cucubalus baccifer* +, *Euonymus europaeus* +, *Festuca gigantea* +, *Galium aparine* +, *Geum urbanum* +, *Glechoma hederacea* +, *Impatiens glandulifera* +, *I. parviflora* +, *Lapsana communis* +, *Padus racemosa* +, *Poa* sp. +, *Roegneria canina* +, *Rubus caesius* +, *Sambucus nigra* +, *Swida hungarica* +, *Ulmus glabra* +, *Aesculus hippocastanum* r, *Echinocystis lobata* r, *Persicaria dubia* r, *Stachys sylvatica* r
E_0: *Brachythecium rivulare* +, *B. salebrosum* +, *Eurhynchium hians* +, *Plagiomnium cuspidatum*, *Leskea polycarpa* +.

Locality No. 5: Revúcka vrchovina Hills; Mokrá Lúka; beyond the mill (alluvium on the right bank of the Muránka river); ca 288 m a.s.l.; DFS 7386b; willow stand (Ls1.1; *Salicion albae*); sandy-loam soil; ca 0.8 ha^{-1}.

Salix alba and *S. fragilis* are dominant woody species, with admixed *Alnus glutinosa*. *Sambucus nigra* species dominates in the shrub layer. *Urtica dioica*, *Aegopodium podograria*, *Chrysosplenium alternifolium*, *Lamium maculatum* are predominant species in the herba layer. The invasive species *Fallopia japonica* and *Helianthus tuberosus* are expressively representated. The protected *Matteuccia struthiopteris* species occurrs rarely. The species *Eurynchium hians*, *Brachythecium oedipodium* and *B. rivulare* were dominant in layer of bryophytes. The willow stand is negativelly influenced by invasive plants and by the illegal cutting interventions.

Locality No. 6: Revúcka vrchovina Hills; Revúcka Lehota; near water reservoir Miková, (between state road and railway dam); ca 282 m a.s.l.; DFS 7387a; alluvial willow stand (Ls1.1, Ls1.3; *Salicion albae*, *Alnion incanae*); sandy-loam soil; ca 1 ha^{-1}.

The *Salix fragilis* is dominant woody species, *Padus racemosa* and *Alnus glutinosa* are admixed. The species *Humulus lupulus*, *Sambucus nigra*, *Salix cinerea* and *Viburnum opulus* are characteristic for busher layer. The herb layer is primarily occupied by *Urtica dioica*, *Rubus caesius*, *Galium palustre*, *Impatiens noli-tangere*, *Aegopodium podograria*, *Lamium maculatum*, *Phalaroides arundinacea* var. *arundinacea* and others. The sedge species *Carex paniculata* and *C. pseudocyperus* are sporadic, *C. vesicaria* is more frequent. The species *Brachythecium rivulare* occurs in the bryophytes layer. The stand with dominant species *Padus racemosa* (association on locality No. 6 is more similar to the assoc. *Pruno-Fraxinetum* Oberd. 1953 of the alliance *Alnion incanae*) is described by the following phytocoenological record:

Record No. 4: Revúcka Lehota, SWW of the village, stand of *Padus racemosa*, *Salix fragilis*, *Alnus glutinosa* between state road and railway dam; 48.66799° N – 20.16687° E ± 9 m; 282 m a.s.l., slope 0°, area of the record: 15 × 20 m, 3. 9. 2009, D. Blanár [original releve DB-3/3.9.2009].
Cover E_c: 98%, E_3: 95%, E_2: 15%, E_1: 60%, E_0: 20%; h_{E3} = 20–25 m, h_{E2} = 2–5 m, h_{E1} = 30 cm
E_3: *Padus racemosa* 5, *Salix fragilis* 2b, *Alnus glutinosa* 2a
E_2: *Padus racemosa* 2a, *Sambucus nigra* 1, *Viburnum opulus* 1, *Humulus lupulus* +
E_1: *Aegopodium podograria* 3, *Urtica dioica* 2a, *Euonymus europaeus* 1, *Brachypodium sylvaticum* +, *Circaea lutetiana* +, *Fraxinus excelsior* +, *Geum urbanum* +, *Geranium phaeum* +, *Impatiens parviflora* +, *Padus racemosa* +, *Rubus caesius* +, *Sambucus nigra* +, *Viburnum opulus* +, *Anthriscus nitida* r
E_0: *Brachythecium rivulare* 2b.

Locality No. 7. Revúcka vrchovina Hills; Jelšava; near bathhouse, (between Muránka river and railway station); ca 245 m a.s.l.; DFS 7387c; alluvial willow stand (Ls1.1; *Salicion albae*); sandy-loam soil; ca 2 ha^{-1}.

Salix alba and *S. fragilis* are dominant woody species, *Alnus glutinosa* and *Ulmus laevis* are admixed. The occurrence of lianes *Parthenocissus quinquefolia* and *Humulus lupulus* is characteristic for this locality. *Sambucus nigra* dominates the shrub layer. The herb layer is primarily formed by *Aegopodium podograria*, *Lamium maculatum*, *Rubus caesius*, *Urtica dioica*, *Impatiens noli-tanger*. The invasive species *Aster lanceolata*, *Fallopia japonica*, *Echinocystis lobata*, *Helianthus tuberosus* have been recorded sporadically. On the other hand the invasive species occurr massively on the stand edges. Invasive woody species *Negundo aceroides* has been found out sporadically. The species *Brachythecium rivulare* is characteristic bryophyte species. This forest stand is described by the following phytocoenological record:

Record No. 5: Jelšava, S of the town, alluvial forest; 48.622694° N – 20.242138° E ± 8 m; 240 m a.s.l, slope 0°, area of the record: 20 × 20 m, 3. 9. 2009, D. Blanár [original releve DB-2/10.8.2009].
Cover E_c: 98%, E_3: 80%, E_2: 45%, E_1: 90%, E_0: do 1%; h_{E3} = 25 m, h_{E2} = 5 m, h_{E1} = 150/30–40/15 cm
E_3: *Salix alba* 4, *Salix fragilis* 2b
E_2: *Sambucus nigra* 3, *Hummulus lupulus* 2a, *Salix fragilis* 1, *Acer campestre* +, *Euonymus europaeus* +, *Fraxinus excelsior* +, *Swida hungarica* +
E_1: *Urtica dioica* 4, *Lamium maculatum* 3, *Aegopodium podograria* 2b, *Euonymus europaeus* 2a, *Chelidonium majus* 1, *Hedera helix* 1, *Hummulus lupulus* 1, *Sambucus nigra* 1, *Angelica sylvestris* +, *Chaerophyllum aromaticum* +, *Carduus personata* +, *Juglans regia* +, *Parthenocissus quinquefolia* r
E_0: *Amblystegium serpens* +, *Brachythecium rivulare* +.

Notes to the localities: The locality No.1 and localities No. 3–6 are listed to the European Signification Area SKUEV0285 Alluvium of Muráň river (proposed Protected Area Alúvium Muráňa – cf. Blanár et al., in prep.), hereby the locality No.1 is a component part of Protected zone of the Muránska planina National Park.

Results and discussion

Based on our research in the willow and alder floodplain stands and their fragments in the alluvium of the waterstreams Muránka river and Lehotský potok creek in 2009–2013 (together with the data published in 2000 and 2001 – see Blanár and Mihál, 2002), we report 236 macromycetes species and 13 slime molds (249 taxa) in this paper. All the identified taxa are presented in the Appendix. The species have been arranged alphabetically, classified into Myxomycota, Zygomycota, Ascomycota and Basidiomycota. Table 1 shows the numbers of recorded taxa according to the localities, ecotrophical groups and individual divisions. Table 1 shows that the highest species diversity was found out in the locality No. 4 with 84 taxa, the poorest one was the locality No. 5 with 29 taxa, most of the species (185) are of Basidiomycota, 50 of Ascomycota, relatively high number Myxomycota (13) and only one of Zygomycota. The higher number of taxa belongs among lignicolous saprotrophes (158 species) and terrrestric saprotrophes (51) – they are profiting from abundant dead wood substrate and rich humus layer on alluvial soils in the investigated habitats. On the other hand, the relatively low number of lignicolous parasites (13 species) and ectomycorrhizal symbionts (11 ones) were found out.

Table 1. The number of recorded taxa of the individual localities, ecotrophic groups and divisions

Locality No.	LP	HP	MP	IP	LS	TS	HS	MS	Total	Myxo	Zygo	Asco	Basi
1	2	2			47	13	3	7	74	4		8	62
2	1	2			29	6	2	1	41	2		4	35
3	2	3	2		61	9	1		78	4		17	57
4	6	1	2	1	57	13	2	2	84	3	1	22	58
5	2		1		25	1			29	2		4	23
6	5	1			22	13	2	1	44	2		4	38
7	5		1		37	9	1		53	6		6	41

LP, lignoparasites; HP, herboparasites; MP, mycoparasites; IP, insectoparasites; LS, lignicolous saprotroph; TS, terrestrical saprotroph; HS, herbosaprotroph; MS, ectomycorrhizal symbionts; Myxo, Myxomycota; Zygo, Zygomycota; Asco, Ascomycota; Basi, Basidiomycota.

From the rare or otherwise attractive findings we present:

M y x o m y c o t a

Diderma globosum* var. *europaeum Buyck
Škubla (2003) reports probably the oldest finding of a relative species *Diderma floriforme* (Bull.) Pers. for Slovakia; it was recorded as early as in 1900 by the eminent botanist and polymath Andrej Kmeť on Sitno in the Štiavnické vrchy Mts. Records of other relative *Diderma* species from Slovakia, such as *D. alpinum* Meyl., *D. effusum* (Schwein.) Morgan, *D. floriforme*, *D. niveum* (Rostaf.) T. Macbr., *D. spumarioides* (Fr.) Fr., *D. subdictyospermum* (Rostaf.) G. Lister and *D. testaceum* (Schrad.) Pers. were summarised by Mereďa (2002).

We have observed *D. globosum* var. *europaeum* in the alder stand in the alluvium of Lehotský potok creek. Sporocarps grew in litterfall on leaves of *Alnus glutinosa* and *Corylus avellana* and on grass leaves (Poaceae). This finding may be considered the first for this species in Slovakia.

Fuligo laeviderma H. Neubert, Nowotny & K. Baumann
An interesting slime mold species, similar to the well-known and common relative species *Fuligo septica* (L.) F. H. Wigg. from which it differs in peridium structure. Besides *F. septica*, also *Fuligo rufa* Pers. occurs in Slovakia, recorded by the Ružínska water dam on 24th July 2006 by Kešeľák (2013a). Moreover, there have been reported also findings of *Fuligo cinerea* (Schwein.) Morgan and *F. gyrosa* (Rostaf.) E. Jahn (Škubla, 2003).

We have found this rare slime mold growing in locality No. 4 on decomposed wood of *Salix* spp. and its occurrence in Slovakia is very rare, our finding may be considered the first in Slovakia. *Fuligo laeviderma* reported as relatively rare, is known e.g. in East Ukraine (Dudka et al., 2009; Leontiev, 2006).

Other rare and interesting slime molds that we have recorded are: *Arcyria ferruginea* and *Metatrichia vesparium* – 3rd finding for Slovakia (cf. Škubla, 2003), *Badhamia macrocarpa* – 4th finding (cf. Blanár and Mihál, 2002; Škubla, 2003) and morphologically notable species *Lycogala flavofuscum* (Fig. 2).

Fig. 2. *Lycogala flavofuscum* – interesting decorative slime molds, locality Jelšava, near bathhouse (Photo D. Blanár).

Z y g o m y c o t a

Entomophthora coleopterorum Petch.
An entomopathogenic species belonging to Entomophthorales, Zygomycota. It parasitizes on adult beetles (Coleoptera). The conidia of this fungus germinate in the affected beetles, later conidiophores branch over the body surface, and finely they envelope the whole body of the attacked individuals. In our case, we have observed this parasitic fungus on an adult of *Polydrusus* sp. (Curculionidae) in leaf litter.

In Slovakia also occurs the species *Entomophthora muscae* (Cohn) Fresen, attacking flies (Diptera), reported as the first published finding for Slovakia by Mihál et al. (2012). In the same way, *E. coleopterorum* may be classified as the first finding for Slovakia.

Ascomycota

Acrospermum compressum Tode
An interesting species that prefers dead stalks of *Urtica dioica*. This substrate and tiny ascomata are probably the reasons why the species is not commonly recorded during ordinary mycological researches. It is obviously the most frequent in floodplain forests and also in degraded ruderal stands overgrown with *Urtica dioica*. Schmid-Heckel (1988) reported *A. compressum* as a fairly rare saprotrophic fungus occurring on stalks of plants of genera *Adenostyles* sp. and *Aruncus* sp. In our case, we have observed this fungus growing on dead stalks of *U. dioica* in locality No. 2. Another finding of *A. compressum* on the same substrate was reported by J. Kuriplach. This author observed it in Limbach in the Malé Karpaty Mts on 25th May 2013 (Kuriplach, 2013). Our finding of *A. compressum* is the first published and the second documented one of this species in Slovakia.

Belonopsis filispora (Cooke) Nannf.

A rare fungus, forming tiny light-grey apothecia on dead plant stalks. As such, it probably escapes attention during ordinary mycological observations. In floodplain forests, it can reach high local abundance on decomposing litter and plant stalks. The relative species *Belonopsis obscura* (Rehm) Aebi was presented by Schmid-Heckel (1988) as a lignicolous saprotrophic species on *Calluna vulgaris* in mountain forests. Moser (1963) reported several relative species growing on other substrates: *Belonopsis excelsior* (Karts.) Rehm on *Phragmites* sp., and *B. pallens* (Sacc.) Kreissl. on *Brachypodium* sp. Our finding about dry stalks of *Phragmites australis* is the first finding of this species in Slovakia.

Echnoa infernalis (Kunze) Fuckel

A very interesting and rare species growing saprotrophically on broadleaved woody plants. Its tiny dark-brown hairy perithecia break through out the bark in massive amounts. We have observed this rare ascomycetous species on decomposing branches of *Salix fragilis*. Červenka et al. (1972) reported oak (*Quercus* spp.) branches as other substrate for this fungus. We have considered our finding of this species the first for Slovakia.

Ophiostoma ulmi (Buisman) Nannf.

A very interesting tiny fungus originating an asexual structure (anamorpha) as a part of their life cycle. We have found out *O. ulmi* growing on dead wood of *Alnus incana*. It formed tiny standing sporocarps (coremia) with widened heads consisting of branched conidiophores. In general the species of the genus *Ophiostoma* are well-known as the causal organisms of tracheomycotic diseases of various woody plants, especially the species *O. ulmi* provokes the tracheomycotic disease of elm. In Slovakia (except our finding) only one published record for *Ophiostoma ulmi* is known (Adamčík et al., 1998 in Škubla, 2003).

Xylaria digitata (L. ex Fr.) Grev. (Fig. 3)

Fig. 3. *Xylaria digitata* – rare ascomycetous species, locality Revúca, below the Nižná Bartová
(Photo D. Blanár).

The occurrence of *Xylaria digitata* in Slovakia has not been reported yet. During our research we have recorded this species in a floodplain mixed forest stand near Revúca city in locality No. 4, as rarely growing on bark of a fallen rotting stem of *Tilia cordata*. Besides this locality, we have observed it also in one locality in the Revúcka vrchovina Hills near Revúca city (NWW from Revúca, locality Keslo, in the depression under a garden, ca 360 m a.s.l, on fallen rotting stem of *Populus tremula*, 6. 12. 2009, leg. DB, det. IM, herb DB).

Other rare or in another way attractive ascomycetous species we have recorded and represented their second to fourth record in Slovakia by today. These species are: *Anthostoma turgidum* – 3rd finding for Slovakia (cf. Mihál and Blanár, 2011), *Cyathicula coronata* (Fig. 4) – 4th finding for Slovakia (cf. Škubla, 2003), *Heyderia sclerotiorus* – 2nd published finding (cf. Glejdura, 1997), *Pezizella gemmarum* – 3rd finding for Slovakia (cf. Glejdura, 1997; Verkin, 2013) and *Pezizella alniella* – 3rd finding (cf. Glejdura, 1997; Škubla, 2003). *Pezizella alniella* was relatively frequent in alder stands or in mixed floodplain forest stands with alder. In these localities, the ascomata of this fungus were growing on wet leaf litter and on old last year´s cones of *Alnus glutinosa.*

Fig. 4. *Cyathicula coronata* – infrequent ascomycetous species, locality Jelšava, near bathhouse (Original drawing V. Blanár).

B a s i d i o m y c o t a

Clitocybe truncicola (Peck) Sacc.
An interesting basidiomycetous fungus, belonging to the wide-ranged and taxonomically instable *Clitocybe* genus. This genus comprises only a few species growing saprotrophically on wood substrate, such as *C. truncicola* that we have observed on decomposed wood of *Salix* cf. *fragilis* in locality No. 7. This species is related to *Ossicaulis lignatilis* (Pers.) Redhead & Ginns, growing on similar wood substrates. The differring trait for *C. truncicola* is a distinct earthy scent.

In Slovakia, this rare species was observed growing on beech wood in the National Park Poloniny in the Bukovské vrchy Mts (Adamčík et al., 2007). Our finding of *C. truncicola* is the second one for Slovakia.

Gloeocystidiellum porosum (Berk. et M. A. Curtis) Donk
A rare species belonging to Aphyllophorales s.l. This fungus forms thiny to membranous, white to creamy basidiomata on wood of broadleaved trees and shrubs (Hagara et al., 1999). In our case, we have observed it on branches of *Salix fragilis* in localities No. 2 and 3. From Slovakia, only one and relatively older-dated finding from the Poľana Mts was reported (Pilát, 1954 in Škubla, 2003). The most recent findings reports Kešeľák (2013b) from the Slovenský raj Mts recorded in 2006 and from the Šarišská vrchovina Mts in 2008. Our finding is probably the fourth finding of this species in Slovakia.

Hohenbuehelia angustata (Berk.) Singer
A rare species, taxonomically relative to the fungi of the genus *Pleurotus*. This fungus forms yellowish to ochroid, spatulate petales on woody substrate. It is very rare, growing solitary or in groups on dead wood of broadleaved species, primarily poplars, elms and ashes. In Europe, *H. angustata* has been observed in floodplain forests in Southern Moravia and in Lower Austria (Hagara et al., 1999). Our finding of this species in the floodplain forest in locality No. 4 was recorded on a decomposed stem of *Padus racemosa*. It represents the first finding of *H. angustata* in Slovakia.

Melampsora amygdalinae Kleb.
An interesting species from the order Uredinales. It grows parasitically on leaves of woody plants from the family Saliciaceae. We have found *M. amygdalinae* growing on fallen leaves of *Alnus glutinosa* in three of our study localities and the collection from the alluvium of the Muránka river we consider the first published one for Slovakia. However, due to the large area of broadleaved floodplain forests in Slovakia, we suppose that its occurrence should be much higher.

Mycoacia nothofagi (G. Cunn.) Ryvarden
A rare species belonging to Aphyllophorales s.l. It forms wooly basidiomata spreading onto surface of broadleaved woody plants, which are characteristic by yellow-creamy to brown spines, and their scent is strongly sugary. In the locality No. 3, this species grew on the dead stem of *Salix fragilis*. In Slovakia, the first collection of *M. nothofagi* was reported from the Bukovské vrchy Mts (Kuthan et al., 1999 in Škubla, 2003). Some recent findings of this fungus were reported by P. Kešelák from the Šarišská vrchovina Mts recorded in 2007, and V. Kunca from the Kremnické vrchy Mts recorded in 2013 (Kunca, 2013). Our collection is probably the fourth finding for Slovakia.

Omphalina discorosea (Pilát) Herink et Kotl.
A very interesting, protected and rare species, which according to Antonín and Bieberová (1995), Hagara et al. (1999) and Kotlaba et al. (1995) belongs to the category of highly endangered macromycetes in both Czech Republic and Slovak Republic. This decorative-

ly coloured species is forming violet to violet-brownish basidiomata on decomposed wood in floodplain forests. The occurrence *O. discorosea* is associated with floodplain forests, and as such, its occurrence is endangered due to the decline of suitable habitats. In the earliest 1990s, the central European area of this fungus was only limited to the floodplain forests of Southern Moravia (Procházka, 1994). Recently, there have been reported several collection sites of *O. discorosea* restricted to the Podunajská nížina lowland, Western Slovakia (Škubla, 2003). In the alluvium of the Muránka river, we have observed this species growing on decomposed wood in two localies. Our findings extend the occurrence range of this species to central Slovakia, too.

Steccherinum dichroum Pers.
A rare species belonging to Aphyllophorales s.l., forming wooly, onto surface spreading basidiomata with small, protruding hairy caps. The hymenium of this species is formed by short salmon-coloured spikes. The hymenium of relative species *S. ochraceum* (Pers.) Gray, which is relatively abundant and frequent, is formed by yellow-ochraceous spikes. Both species prefer broad-leaved woody plants in warmer regions. We have found *S. dichroum* growing on the dead branch of *Salix cinerea* in the locality No. 3. By now, *S. dichroum* (reported as *Gloeoporus dichrous*) has only been found in the Sedláčkov ostrov island in the Danube river, growing on *Salix alba* (Jančovičová, 1999, 2000a) and in the Bukovské vrchy Mts in the locality Chotinka, growing on a branch of *Quercus* sp. (Kuthan et al, 1999). Our finding in the alluvium of Muránka river is probably the third finding for Slovakia.

We have also recorded several other rare or in another way attractive basidiomycetous species: *Athelia salicum*, *Femsjonia peziziformis*, *Peniophora aurantiaca* and *Subulicystidium longisporum* – 2nd finding for Slovakia (cf. Mihál et al., 2012; Škubla, 2003), or *Hyphoderma medioburiense* and *Laeticorticium roseum* – 3rd finding for Slovakia (cf. Škubla, 2003).

Comparing our data on occurrence of slime molds and macromycetes in floodplain alluvial forests with the data reported by other authors we can see that the Slovak mycological literature comprises relatively a low number of works dealing with mycological research in floodplain forests. Moreover, most of these works concern the floodplain forest mycoflora only in the West Slovakia and in the Podunajsko region (South-Western Slovakia). By now, the mycoflora of the alluvium of the Muránka river has been investigated by Blanár and Mihál (2002). These authors recorded 36 macromycetes species and 3 slime mold species in the assoc. *Stellario-Alnetum glutinosae* surrounding the confluence of the watercourses Zdychavka and Muránka river. Among these species, there were several species characteristic for floodplain forests, such as *Ascocoryne cylichnium, Microstoma protracta, Sarcoscypha austriaca, Flammulina velutipes, Hirneola auricula-judae, Phanerochaete laevis, Pseudoclitocybe cyathiformis, Trametes suaveolens, Typhula erythropus* and other which we have also detected in other localities in the alluvium of Muránka. In broader surroundings of our research locality, in the Muránska planina Mts and in selected localities in the Stolické vrchy Mts and in the Revúcka vrchovina Hills, Ripková and Blanár (2002) studied the occurrence of species of the genus *Sarcoscypha*: they found out *Sarcoscypha austriaca* occurring also in alluvial floodplain forests in the watersheds of Zdychavka and Muránka. Ripková and Blanár (2004) recorded in the same territory and in various localities in the alluvium of the Muránka river and its tributaries also the species of the genus *Crepidotus*, with more species as *Crepidotus applanatus*, *C. calolepis*, *C. cesatii*, *C. lundellii*, *C. mollis*.

In Slovakia, the mycoflora of floodplain forests – alliances *Salicion albae*, *Salicion triandrae* Th. Müller et Görs 1958 and sub-alliance *Ulmenion* Oberd. 1953 – has been investigated also in two localities: Sedláčkov ostrov river island and Sihoť river island, both in the Danube river near Bratislava (Jančovičová, 1999, 2000a, 2000b; Jančovičová and Glejdura, 1999). In the Sedláčkov ostrov, Jančovičová (1999) studied the biodiversity of (Polyporales s.l.) and reported the occurrence of 32 species. Several of them correspond to our collections. In addition, Jančovičová (1999) presented several interesting species such as *Abortiporus biennnis*, *Coriolopsis gallica*, *Polyporus alveolarius*, *Skeletocutis nivea* and *Trametes trogii*. Jančovičová (2000b) carrying her research also on *Pluteus* genus in Sedláčkov ostrov island and in Sihoť island, where she found out altogether 12 species. As rare, she classified *Pluteus cinereofuscus* and *P. leoninus*. Singular finding was *P. aurantiorugosus*, belonging to endangered and rare species of fungi of Slovakia (Kotlaba, 1995).

Localities in the Danube islands Sihoť and Slovanský ostrov near Bratislava city were also studied by Záhorovská et al. (1996). In conditions of Danubian floodplain forests dominated by *Salix* spp., *Alnus* spp., *Populus nigra*, *P. alba*, *Fraxinus excelsior*, *Ulmus laevis* and *Acer negundo*, this author detected altogether 97 fungal species (Sihoť island 58 and Slovanský ostrov island 39 species). Also in this case, the major part of the species was the same as ours: *Auriculariopsis ampla, Daedaleopsis confragosa, Entoloma rhodopolium, Laetiporus sulphureus, Mycena galericulata, Phellinus igniarius, Polyporus squamosus* and similar.

Ascomycetous fungi (Ascomycota) in these Danube islands were studied by Jančovičová and Glejdura (1999) who found out there altogether 26 ascomycetous macromycetes species: 9 of them we have also collected in our studied area: *Ascocoryne sarcoides*, *Bisporella citrina, Daldinia concentrica, Hypoxylon fuscum*, *Morchella esculenta*, *Sarcoscypha austriaca, Verpa bohemica, Xylaria hypoxylon* and *X. polymorpha*.

In hardwood floodplain forests of the sub-alliance *Ulmenion* Oberd. 1953 (in the work reported as *Ulmenion minoris*) in Podunajské Biskupice, macrofungi were investigated by Jančovičová and Zaliberová (2011). From the total number of 68 macromycetes, only 9 ascomycetous species were found by these authors. Among them, there were also the species recorded in our localities: *Clitopilus hobsonii, Coprinus domesticus, Crepidotus cesatii, Pluteus romellii, Radulomyces molaris, Xylaria longipes* and similar.

In conditions of alluvial alder forests in the locality Fenek in the Cerová vrchovina Hills, Mihál (1995, 2006) recorded a total of 94 species of macromycetes. As interesting species may be reported the following ones: *Clavariadelphus ligula*, *Cyathus striatus*, *Hirneola auricula-judae*, *Laetiporus sulphureus*, *Lactarius serifluus*, *Morchella esculenta*, *Pachyella violaceonigra*, *Pluteus salicinus* and other. Similarly, Mihál (1997a, 1997b) investigated alder and willow stands on gravels in the alluvium of the Ipeľ river, where he recorded altogether 30 species in the locality Veľká nad Ipľom and 22 species of macromycetes in the locality Ipeľské Predmostie. Among these species, there were primarily species typical for floodplain forests, such as *Agrocybe dura*, *Auriculariopsis ampla*, *Cyathus olla*, *Macrocystidia cucumis*, *Paxillus involutus*, *Phellinus populicola*, *Sarcoscypha coccinea*, *Tremella mesenterica*, *Xerocomus rubellus* and other.

The knowledge on occurrence and ecology of some new or rare macromycetes such as *Chaetoporellus latitans*, *Hypoxylon ticinense*, *Phlebia ryvardenii*, *Pluteus aurantiorugosus*, *Rhodotus palmatus*, *Scutellina legaliae*, *Spongipellis fractipes* and others, found in floodplain forests in surroundings of Bratislava can be found in Jančovičová (2000b) and Ripková and Hagara (2003).

It follows that the mycoflora of floodplain forests as the azonal forest communities in Slovakia exhibit a high species diversity, comprising both the species regularly occurring and typical for floodplain forest conditions and rare or in another way interesting macrofungi. The reciprocal ecotrophic and ecotopic connections among fungi, herbs and woody plants in floodplain forests are very specific, and, comparing with forest communities situated in the higher altitudinal zone, much more vulnerable due to anthropic influence.

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Appendix

List of macromycetes species recorded in the alluvial forests in the area of Muránka river and Lehotský potok creek

Explanations:
Data on individual species (according to divisions) are given in the following order: taxa, abbreviation of ecotrophical group of species, locality from 1. to 7.; data on records on locality: habitat, date of collection, collector, determinator, herbarium, (references). [Abbreviations of ecotrophical groups are given in the chapter Results and discusions – in Table 1, abbreviations of collectors/determinators are given in the chapter Methods and material].

ZYGOMYCOTA

Entomophthora *coleopterorum* Petch. – IP: **4.** in litter, on imago of the *Polydrusus* sp. (Curculionidae), 17. 11. 2010, leg. DB, det. IM, herb. IM.

MYXOMYCOTA

Arcyria *cinerea* (Bull.) Pers. – LS: **2.** on decaying wood *Salix fragilis*, 2. 7. 2010, leg. DB, det. IM, herb. DB.

A. denudata (L.) Wettst. – LS: **1**. on decaying wood *Salix* sp., 28. 5. 2010, 2. 7. 2010, leg. et det. IM, herb. IM. — **3a.** Blanár and Mihál (2002). — **6.** on decaying wood *Salix* sp., 3. 9. 2009, 28. 5. 2010, leg. et det. IM, herb. IM. — **7.** on decaying wood *Salix* sp., 28. 5. 2010, leg. et det. IM, herb. IM.

A. ferruginea Sauter – LS: **7.** on wood *Salix* sp., **7.** 4. 2010, 28. 5. 2010, leg. et det. IM, herb. IM.

Badhamia *macrocarpa* (Ces.) Rostaf. – LS: **3a.** Blanár and Mihál (2002).

Diderma *globosum* var. *europaeum* Buyck – HS: **1.** in litter, on leaf *Alnus glutinosa* and *Corylus avellana,* on dry grass, 5. 8. 2010, leg. DB, det. IM, herb. DB.

Enteridium *lycoperdon* (Bull.) M. L. Farr – LS: **7.** on decaying wood *Salix fragilis*, 15. 10. 2010, leg et det. IM, not.

Fuligo *leviderma* H. Neubert, Nowotny & K. Baumann – LS: **4.** on decaying wood *Salix* sp., 10. 8. 2011, leg. DB, det. IM, herb, DB, IM.

Hemitrichia *clavata* (Pers.) Rostaf. – LS: **4.** on decaying bark of stem *Tilia cordata*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

H. serpula (Scop.) Rostaf. – LS: **7.** on twig *Salix fragilis*, 6. 6. 2012, leg. DB, det. IM, herb. DB, IM.

Lycogala *epidendrum* (J. C. Buxb. ex L.) Fr. – LS: **1**. on decaying wood *Salix* sp., 2. 7. 2010, 30. 3. 2011, 16. 3. 2011, 26. 3. 2011, leg. DB, IM, det. IM, not. — **2**. on decaying wood *Salix* sp., 28. 5. 2010, 2. 7. 2010, 23. 9. 2010, leg. et det. IM, not. **3b.** on decaying wood *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **4.** on twig *Populus nigra*, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **5.** on decaying wood *Salix alba*, 24. 11. 2010, leg. DB, det. IM, herb. DB. — **6.** on decaying wood *Salix* sp., 2. 7. 2010, 23. 9. 2010, leg. et det. IM, not. — **7.** on decaying wood *Salix* sp., 1. 4. 2011, 6. 7. 2012, leg. IM, det. IM, not.

L. flavofuscum (Ehrenb.) Rostaf. – LS: **7.** on decaying wood *Salix fragilis*, 15. 10. 2010, leg. DB, det. IM, herb. IM, SG.

Metatrichia *vesparium* (Batsch) Nann. – Bremek ex G. W. Martin & Alexop. – LS: **1.** on soil, on decaying broadleaves wood, 15. 10. 2010, leg. DB, det. IM, herb. DB, IM.

Trichia *varia* (Pers.) Pers. – LS: **3a.** Blanár and Mihál (2002). — **5.** on decaying wood *Salix alba*, 24. 11. 2010, leg. DB, det. IM, herb. DB.

ASCOMYCOTA

Acrospermum *compressum* Tode – HS: **2.** on dead stalks *Urtica dioica*, 28. 5. 2010, leg. DB, det. IM, herb. DB, herb. IM.

Anthostoma *turgidum* (Pers.) Nitschke – LS: **2**. on twigs *Salix* sp., 2. 7. 2010, leg. DB, IM, det. IM, herb. IM.

Ascocoryne *cylichnium* (Tul.) Korf. – LS: **3a**. on decaying stump *Salix fragilis*, 13. 10. 2001, leg. DB, det. VK, SAV 6709. — **4.** on broadleaves wood and on stem *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB; on decaying wood *Salix fragilis*, 12. 10. 2012, leg. DB, det. VK, SAV 10746.

A. sarcoides (Jacq.) J. W. Growes et D. E. Wilson – LS: **4.** on decaying wood *Fraxinus excelsior* leg. DB, det. VK, SAV 10280; on decaying wood *Salix fragilis*, 12. 10. 2012, leg. DB, det. VK, SAV 10736.

Belonopsis *filispora* (Cooke) Nannf. – HS: **6**. on dry stalks *Phragmites australis*, 10. 1. 2009, leg. DB, det. IM, herb. IM.

Bisporella *citrina* (Batsch) Korf. & S. E. Carp. – LS: **4.** on roots in water of *Padus racemosa*, 12. 5. 2011, leg. DB, det. IM, herb. DB; on wet roots cf. *Alnus glutinosa*, 10. 8. 2011, leg. DB, det. IM, herb. DB.

Catinella *olivacea* (Batsch) Boud. – LS: **7.** on stem *Salix alba*, 6. 6. 2012, leg. DB, det. et herb. SG, PSG 5007.

Chlorociboria *aeruginascens* (Nyl.) Kanouse ex Ramamurthi et al. – LS: **3a.** Blanár and Mihál (2002).

Ciboria *amentacea* (Balb.) Fuckel – LS: **1.** in litter, on catkin *Corylus avellana*, 30. 3. 2011, leg. DB, det. IM, herb. DB. — **5.** in litter, on catkin *Alnus glutinosa*, 1. 9. 2009, leg. DB, det. IM, herb. DB. — **7.** in litter, on catkin *Alnus glutinosa*, 20. 3. 2012, leg. DB, det. IM, herb. DB.

Cudoniella *clavus* (Alb. et Schwein.) Dennis – LS: **2.** on decaying wood *Salix fragilis*, 28. 5. 2010, leg. DB, det. IM, herb. DB.

Cyathicula *coronata* (Bull.) De Not. ex P. Karst. – LS: **7.** on decaying wood *Salix fragilis*, 15. 10. 2010, leg. DB, det. IM, herb. DB.

Daldinia *concentrica* (Bolton) Ces. et De Not. – LS: **4.** on twig (Ø 4 cm) *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

Diatrype *bullata* (Hoffm.) Fr. – LS: **4.** on *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

Dumontinia *tuberosa* (Hedw.) L. M. Kohn – HP: **3a.** on roots *Anemone nemorosa*, 30. 3. 2012, leg. DB, det. IM, herb. DB.

Echnoa *infernalis* (Kunze) Fuckel – LS: **5.** on twig *Salix fragilis*, 1. 9. 2009, leg. DB, det. IM, herb. DB.

Encoelia *furfuracea* (Roth ex Pers.) P. Karst. – LS: **1.** on twig *Corylus avellana*, 15. 10. 2010, leg. DB et IM, det. IM, herb. DB. — **4.** on twig (Ø 1.5 cm) *Alnus glutinosa*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

Heyderia *sclerotiorus* (Rostr.) D. Benkert – LS: **3a.** in litter, on seed *Alnus glutinosa*, 13. 10. 2001, leg. DB, det. VaK, PVK 617.

Holwaya *mucida* (Schulzer) Korf et Abawi – LS: **4**. on *Tilia cordata*, 17. 11. 2010, leg. DB, det. et herb. SG, PSG 5006.

Hymenoscyphus *fructigenus* (Bull.) Gray – LS: **1.** in litter, inside nutshell of *Corylus avellana,*, 5. 9. 2010, leg. DB, det. IM, herb. DB.

Hypomyces *aurantius* (Pers.) Tul. – MP: **4.** on fruitbodies of *Lenzites betulina* – on twig *Padus racemosa*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

H. chrysospermus Tul. et C. Tul. – MP: **7.** on hymenium *Polyporus melanopus* – on stem *Salix fragilis*, 16. 10. 2010, Mihál and Blanár (2011).

H. lateritius (Fr.) Tul. – MP: **3b.** on hymenium *Hapalopilus nidulans* – on stem *Salix fragilis*, 20. 11. 2010, Mihál and Blanár (2011). — **5.** on fruitbodies *Flammulina velutipes* – on stem *Salix alba*, 24. 11. 2010, Mihál and Blanár (2011).

Hypoxylon *fragiforme* (Pers.) J. Kickx f. – LS: **4.** on twig *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

H. fuscum (Pers.) Fr. – LS: **3b.** on stem *Carpinus betulus*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **4.** on stem *Acer platanoides*, 10. 8. 2011, leg. DB, det. IM, herb. DB. — **5.** on twig (Ø 2 cm) of broadleaves wood species, 24. 11. 2010, leg. DB, det. IM, herb. DB.

H. multiforme (Fr.) Fr. – LS: **3b.** on twig (Ø 3 cm) *Salix fragilis*, 8. 7. 2010, leg. DB, det. IM, herb. DB. — **4.** on stem *Acer pseudoplatanus*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

Humaria *hemisphaerica* (F. H. Wigg.) Fuckel – TS: **3a**. on soil**,** Blanár and Mihál (2002).

Lachnum *virgineum* (Batsch) P. Karst. – LS: **3a.** in litter, on catkin *Alnus glutinosa*, 12. 5. 2011, leg. DB et IM, det. IM, herb. DB.

Lasiosphaeria *spermoides* (Hoffm: Fr.) Ces. et De Not. – LS: **1.** on twig *Populus tremula*, 2. 7. 2010, leg. IM et det. IM, herb. IM.

Melanopsamma *pomiformis* (Pers.) Sacc. – LS: **1.** on bark of stem *Fraxinus excelsior*, 28. 5. 2010, leg. DB, IM, det. IM, herb. DB. — **3b.** on bark of stem *Salix caprea*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

Microstoma *proctracta* (Fr.) Kanouse – LS: **3a.** Blanár and Mihál (2002). — **4.** on decaying twig (Ø 1.5 cm) *Aesculus hippocastanum*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

Mollisia *cinerea* (Batsch) P. Karst. – LS: **5.** on twig (Ø 1 cm) *Padus racemosa*, 2. 7. 2010, leg. DB, det. IM, herb. DB. — **7.** on decaying wood *Salix* cf. *fragilis*, 28. 5. 2010, 15. 10. 2010, leg. DB, det. IM, herb. DB.

Morchella *esculenta* (L.) Pers. – LS: **3a.** on wood in soil, Blanár and Mihál (2002).

Nectria *cinnabarina* (Tode) Fr. – LS: **6.** on twig *Salix fragilis*, 3. 9. 2009, leg. DB, det. IM, herb. DB, Mihál and Blanár (2011).

Neonectria *coccinea* (Pers. : Fr.) Rossman et Samuels – LS: **4.** on decaying wood *Tilia cordata* (Ø 20 cm), twig *Alnus glutinosa* (ø 2,5 cm), 17. 11. 2010, leg. DB, det. IM, herb. DB, Mihál and Blanár (2011).

Ophiostoma *ulmi* (Buisman) Nannf. – LP: **4.** on decaying wood *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

Orbilia *xanthostigma* (Fr.) Fr. – LS: **4.** on decaying wood , 17. 11. 2010, leg. et det. IM, herb. IM.

Pezizella *alniella* (Nyl.) Dennis – LS: **1.** in litter, on catkin *Alnus glutinosa*, 15. 10. 2010, leg. DB, det. IM, herb. DB, herb. IM. — **2.** in litter, on catkin *Alnus glutinosa*, 15. 10. 2010, leg. DB, det. IM, herb. DB, herb. IM. — **6.** in litter, on catkin *Alnus glutinosa*, 15. 10. 2010, leg. DB, det. IM, herb. DB.

Pezizella *gemmarum* (Boud.) Dennis – HP: **4.** in litter, on decaying buds *Populus nigra*, 30. 3. 2012, leg. DB, det. SG, herb. DB, SG.

Rhytisma *acerinum* (Pers.) Fr. – HP: **1.** on leaves *Acer* sp., 5. 9. 2010, leg. et det. IM, not.

Sarcoscypha *austriaca* (Beck) Boud. – LS: **1.** on twig *Alnus glutinosa*, 30. 3. 2011, leg. DB, det. SJ, SLO 978. — **3a.** (Blanár & Mihál 2002, Ripková & Blanár 2004).

Sepultaria *arenosa* (Fuckel) Rehm – TS: **4.** on sandy soil, 12. 10. 2012, leg. DB, det. IM, herb. IM.

Scutellinia *crinita* (Bull.) Lambotte – LS: **3a.** on decaying wood, 13. 10. 2001, leg. DB, det. et herb. SG.

S. umbrorum (Fr.) Lambotte – LS: **4.** on sandy soil, 15. 8. 2009, leg. DB, det. SG, herb. SG.

Tarzetta *cupularis* (L.) Lambotte – TS: **3a.** on soil, 13. 10. 2001, leg. DB, det. IM, herb. IM.

Trichophaea *woolhopeia* (Cooke et W. Phillips) L. Arnauld – LS: **6.** on decaying wood *Salix fragilis*, 2. 7. 2011, leg. DB, det. IM, herb. DB, herb. IM.

Verpa *bohemica* (Krombh.) J. Schröt. – TS: **7.** on soil, 7. 4. 2010, leg. DB, det. IM, herb. DB.

Xylaria *digitata* (L. ex Fr.) Grev. – LS: **4.** on bark of stem *Tilia cordata*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

X. hypoxylon (L.) Grev. – LS: **3b.** on decaying wood *Salix caprea*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **4.** on decaying wood *Acer platanoides*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

X. longipes Nitschke – LS: **4.** on twig *Alnus glutinosa*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

X. polymorpha (Pers. ex Mérat) Grev. – LS: **3a.** on decaying stump, Blanár and Mihál (2002).

BASIDIOMYCOTA

Alnicola *melinoides* (Bull.) Kühner – LS: **1.** on bark of stem *Alnus glutinosa*, 5. 8. 2010, leg. DB, det. IM, herb. DB. — **2.** on decaying wood *Salix fragilis*, 2. 10. 2009, leg. DB, det. IM, herb. DB.

Antrodia *macra* (Sommerf.) Niemelä – LS: **1.** on decaying wood cf. *Salix fragilis*, 2. 7. 2010, leg. DB, det. IM, herb. DB.

Antrodiella *fragrans* (A. David et Tortić) A. David et Tortić – LS: **4.** on decaying twig *Salix fragilis*, 17. 11. 2010, leg. DB, det. IM, herb. DB. — **5.** on decaying stem *Salix alba*, 24. 11. 2010, leg. DB, det. IM, herb. DB.

Armillaria *lutea* Gillet – LS: **4.** on decaying wood *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

A. mellea agg. – LS: **3a.** Blanár and Mihál (2002).

A. socialis (DC.) Herink – LS: **4.** on decaying stem *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

Athelia *epiphylla* Pers. – LS: **1.** on decaying twig *Corylus avellana*, 16. 3. 2011, leg. DB, det. IM, herb. DB; bark of broadleaves tree, 2. 7. 2010, leg. IM et DB, det. IM, herb. DB.

A. salicum Pers. – LS: **4.** on decaying stem *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB, herb. IM.

Auricularia *mesenterica* (Dicks.) Pers. – LS: **1.** on decaying stem *Alnus glutinosa*, 30. 3. 2011, leg. DB, det. IM, herb. DB. — **3b.** on decaying stem *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **5.** on decaying stem *Salix alba*, 1. 9. 2009, leg. DB, det. IM, herb. DB.

Auriculariopsis *ampla* (Lév.) Maire – LS: **4.** on decaying twig *Salix fragilis*, 22. 8. 2009, leg. DB, det. IM, herb. DB; decaying stem *Acer platanoides*, 10. 8. 2011, leg. DB, det. IM, herb. DB. — **7.** on decaying twig (Ø 0.5–1 cm) *Salix alba*, 25. 11. 2003, leg. DB, det. IM, herb. DB; decaying twig (Ø 1cm) *Salix alba* and *S. fragilis*, 10. 8. 2009, leg. DB, det. IM, herb. DB.

Basidioradulum *radula* (Fr.) Nobles – LS: **5.** on decaying twig (Ø 2 cm) *Salix alba*, 24. 11. 2010, leg. DB, det. IM, herb. DB.

Bjerkandera *adusta* (Willd.) P. Karst. – LS: **3b.** on decaying stem *Carpinus betulus*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **4.** on decaying stem *Alnus incana* and *Tilia cordata*, 17. 11. 2010, leg. DB, det. IM, herb. DB; on stem *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **5.** on decaying stem *Salix alba*, 24. 11. 2010, leg. DB, det. IM, herb. DB. — **7.** on decaying stem *Salix fragilis*, 15. 10. 2010, leg. DB, det. IM, herb. DB.

B. fumosa (Pers.) P. Karst. – LS: **5.** on twig *Salix* sp., 24. 11. 2010, leg. et det. IM, not. — **7.** on decaying stem *Salix fragilis*, 15. 10. 2010, leg. et det. IM, not.

Botryobasidium *conspersum* J. Erikss. – LS: **7.** on twig *Sambucus nigra*, 6. 6. 2012, leg. DB, det. IM, herb. DB.

Cerrena *unicolor* (Bull.) Murrill – LS: **3a.** Blanár and Mihál (2002). — **4.** on decaying stem (Ø 15 cm), bark *Salix alba*, 17. 11. 2010, leg. DB, det. IM, herb. DB. — **5.** on decaying stem *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

Chondrostereum *purpureum* (Pers.) Pouzar – LS: **1.** on twig *Acer* sp., 30. 3. 2011, leg. et det. IM, not.; on twig *Salix* sp., 5. 8. 2010, leg. et det. IM, not. — **3a.** Blanár and Mihál (2002) — **7.** on twig *Salix alba*, 25. 11. 2003, leg. DB, det. IM, herb. DB.

Clavaria *fragilis* Holmsk. – TS: **1.** on soil, 15. 10. 2010, leg. et det. IM, herb. IM.

Clavicorona *pyxidata* (Pers.) Donk – LS: **2.** on decaying wood *Salix fragilis*, 2. 10. 2009, leg. DB, det. IM, herb. DB. — **4.** in litter, on decaying wood, 10. 8. 2011, leg. DB, det. IM, herb. DB.

Clavulina *cinerea* (Bull.) J. Schröt. – TS: **6.** on soil, 3. 9. 2009, leg. DB, det. IM, herb. DB.

Clitocybe *brumalis* (Fr.) P. Kumm. – TS: **1.** on soil, 15. 10. 2010, leg. et det. IM, not. — **2.** on soil, 6. 8. 2010, leg. et det. IM, not. — **6.** on soil, 23. 9. 2010, leg. et det. IM, not. — **7.** on soil, 15. 10. 2010, leg. et det. IM, not.

C. candicans (Pers.) P. Kumm. – TS: **1.** on soil, 5. 9. 2010, leg. et det. IM, not.

C. clavipes (Pers.) P. Kumm. – TS: **4.** on decaying wood *Salix fragilis*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

C. nebularis (Batsch.) P. Kumm. – TS: **1.** on soil, 23. 9. 2010, leg. et det. IM, not. — **6.** on soil, 23. 9. 2010, leg. et det. IM, not.

C. phyllophila (Pers.) P. Kumm. – TS: **4.** on soil, 12. 10. 2012, leg. DB, det. IM, herb. DB.

C. truncicola (Peck) Sacc. – LS: **6.** on decaying wood *Salix fragilis*, 15. 10. 2010, leg. DB, det. IM, herb. IM — **7.** on decaying wood *Salix fragilis*, 19. 8. 2010, leg. DB, det. IM, herb. DB.

***Clitopilus** hobsonii* (Berk. et Broome) P. D. Orton – LS: **2.** on decaying wood *Salix* sp., 2. 7. 2010, leg. et det. IM, herb. IM. — **6.** on twig *Salix* sp., 2. 7. 2010, leg. et det. IM, herb. IM.

C. prunulus (Scop.) P. Kumm. – TS: **1.** on soil, 15. 10. 2010, leg. et det. IM, not.

***Conocybe** digitalina* (Velen.) Svrček – TS: **7.** on soil, 7. 4. 2010, 19. 8. 2010, leg. DB, det. IM, herb. DB, herb. IM.

C. rickeniana P. D. Orton – TS: **4.** on decaying wood *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

***Coprinus** atramentarius* (Bull.) Fr. – TS: **3a.** on soil, Blanár and Mihál (2002).

C. comatus (O. F. Müll.) Gray – TS: **2.** on soil, 28. 5. 2010, leg. et det. IM, not. – **7.** on soil, 18. 8. 2010, leg. et det. IM, not.

C. disseminatus (Pers.) Gray – LS: **1.** on decaying wood *Alnus glutinosa*, 16. 3. 2011, leg. et det. IM, not. — **6.** on decaying stem *Salix* sp., 23. 9. 2010, leg. et det. IM, not. — **7.** on twig *Salix* sp., 6. 7. 2012, leg. et det. IM, not.

C. domesticus (Bolton) Gray – TS: **7.** on soil, leg. et det. IM, not.

C. micaceus (Bull.) Fr. – TS: **3a.** Blanár and Mihál (2002). — **4.** on decaying wood of broadleaves tree, 10. 8. 2011, leg. DB, det. IM, herb. DB.

C. plicatilis (M. A. Curtis) Fr. – TS: **3a.** Blanár and Mihál (2002). — **6.** on soil, 3. 9. 2009, leg. et det. IM, not. — **7.** soil, 19. 8. 2010, leg. et det. IM, not.

***Crepidotus** applanatus* (Pers.) P. Kumm – LS: **1.** on decaying stem *Salix fragilis*, 5. 9. 2010, leg. DB, det. SJ, SLO.

C. caspari Velen. – LS: **1.** on decaying stem *Salix fragilis*, 5. 9. 2010, leg. DB, det. SJ, SLO 977; on decaying twig *Swida sanguinea* agg., 5. 8. 2010, leg. DB, det. SJ, SLO 991. — **2.** on decaying twig (Ø 2–6 cm) *Salix fragilis*, 16. 10. 2010, leg. DB, det. SJ, SLO 981, SLO 992. — **4.** on decaying twig *Salix fragilis*, on dry stalk *Lunaria rediviva*, 15. 8. 2009, leg. DB, det. SJ, SLO. — **5.** on decaying twig (Ø 2 cm) *Salix alba*, 24. 11. 2010, leg. DB, det. SJ, SLO 972.

C. cesatii Rabenh. – LS: **2.** on twig (Ø 2.5 cm) *Salix fragilis*, 15. 10. 2010, leg. DB, det. SJ, SLO 990. — **4.** on decaying twig *Populus nigra*, 10. 8. 2011, leg. DB, det. SJ, SLO 990.

C. lundellii Pilát – LS: **3a.** Blanár and Mihál (002).

C. luteolus (Lambotte) Sacc. – LS: **3b.** on decaying twig (Ø 0.1–0.3 cm) *Salix fragilis* and dry stalk *Urtica dioica*, 20. 11. 2010, leg. DB, det. SJ, SLO 980, SLO 989.

C. mollis (Schaeff.) Staude – LS: **1.** on decaying stem *Salix caprea*, 5. 9. 2009, leg. DB, det. SJ, SLO 979. — **4.** on decaying wood *Populus nigra*, 10. 8. 2011, leg. DB, det. SJ, SLO 985.

***Cystolepiota** seminuda* (Lasch) Bon – TS: **6.** on soil, 23. 9. 2010, leg. et det. IM, herb. IM.

***Dacrymyces** minor* Peck – LS: **1.** on twig *Salix* sp., 14. 7. 2010, leg. et det. IM, not.

***Daedaleopsis** confragosa* (Bolton) J. Schröt. – LS: **1.** on stem *Salix fragilis*, 5. 8. 2010, leg. DB, det. IM, herb. DB. — **2.** on decaying wood *Salix fragilis*, 2. 10. 2009, leg. DB, det. IM, herb. DB. — **4.** on decaying stem *Salix fragilis*, 15. 8. 2009, leg. DB, det. IM, herb. DB. — **5.** on decaying stem *Salix fragilis*, 1. 9. 2009, 12. 10. 2012, leg. DB, det. IM, herb. DB; on bark *Salix alba*, 24. 11. 2010, leg. DB, det. IM, herb. DB. — **6.** on twig *Salix fragilis*, 1. 9. 2009, leg. DB, det. IM, herb. DB; on stem *Salix fragilis*, 3. 9. 2009, leg. DB, det. IM, herb. DB.

***Entoloma** rhodopolium* (Fr.) P. Kumm. – TS: **4.** on soil, 12. 10. 2012, leg. DB, det. IM, herb. DB.

E. sericeum (Bull. ex Mérat) Quél. – TS: **2.** on soil, 6. 8. 2010, leg. DB, det. IM, herb. DB.

***Exidia** glandulosa* (Bull.) Fr. – LS: **1.** on bark of twig *Corylus avellana*, 16. 3. 2011, leg. DB, det. IM, herb. DB. — **2.** on twigs *Salix* sp., 15. 10. 2010, leg. et det. IM, not. — **3b.** on twigs *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **4.** on stem *Salix* sp., 20. 11. 2011, leg. et det. IM, not.

E. recisa (Ditmar) Fr. – LS: **1.** on decaying twigs *Salix fragilis*, 5. 8. 2010, leg. et det. IM, herb. IM. — **7.** on decaying stem *Salix fragilis*, 15. 10. 2010, leg. et det. IM, not.

E. truncata Fr. – LS: **1.** on twigs *Salix fragilis*, 30. 3. 2011, leg. et det. IM, not. — **3b.** on decaying twig *Salix cinerea* and *S. fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

***Femsjonia** pezizaeformis* (Léveille) P. Karst. – LS: **3b.** on decaying twig (Ø 1 cm) *Salix fragilis*, 12. 5. 2011, leg. DB, IM, det. IM, herb. DB, herb. IM.

***Flammulina** velutipes* complex – LS: **3a.** Blanár and Mihál (2002) — **3b.** on decaying stem *Salix fragilis*, 20. 11. 2010, leg. DB, det. SJ, SLO 993. — **4.** on stem *Ulmus laevis*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

***Fomes** fomentarius* (L.) J. Kickx – LP: **7.** on stem *Salix fragilis*, 6. 6. 2012, leg. DB, det. IM, herb. DB.

***Fomitopsis** pinicola* (Sw.) P. Karst. – LP: **4.** on decaying stem *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB. — **5.** on stem *Salix alba*, 24. 11. 2010, leg. DB, det. IM, herb. DB.

***Ganoderma** australe* (Fr.) Pat. – LP: **4.** on stem *Salix* sp., 12. 5. 2011, leg. et det. IM, not. — **7.** on stem *Salix fragilis*, 6. 7. 2012, leg. et det. IM, not.

G. lipsiense (Batsch) G. F. Atk. – LP: **3a.** Blanár and Mihál (2002). — **7.** on stem *Salix fragilis*, 10. 8. 2009, 6. 6. 2012, leg. DB, det. IM, herb. DB.

Gymnopilus *junonius* (Fr.) P. D. Orton – LS: **1.** on twigs *Corylus avellana*, 23. 9. 2010, leg. et det. IM, not. — **4.** on decaying stem *Salix* sp., 17. 11. 2011, leg. et det. IM, not.

G. penetrans (Fr.) Murrill – LS: **6.** on twigs *Salix* sp., 3. 9. 2009, leg. et det. IM, not.

Gymnopus *peronatus* (Bolton) Antonín et al. – TS: **1.** on soil, 5. 8. 2010, leg. DB, det. IM, herb. DB.

Gloeocystidiellum *porosum* (Berk. et M. A. Curtis) Donk – LS: **2.** on decaying twig (Ø 1 cm) *Salix fragilis*, 2. 10. 2009, leg. DB, det. IM, herb. DB. — **3b.** on decaying twig *Salix* sp., 12. 5. 2011, leg. DB, IM, det. IM, herb. DB, herb. IM.

Gloeoporus *dichrous* (Fr.) Bres. – LS: **3b.** on bark of stem *Salix caprea*, 20. 11. 2010, leg. DB, det. IM, herb. DB, herb. IM.

Hapalopilus *nidulans* (Fr.) P. Karst. – LS: **3b.** on stem *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

Haplotrichum *rubiginosum* (Fr.) Hol.-Jech. – LS: **4.** on decaying stem *Tilia cordata*, 17. 11. 2010, leg. DB, det. IM, herb. DB, herb. IM. — **7.** on decaying wood *Salix alba*, 6. 6. 2012, leg. DB, det. IM, herb. DB.

Hirneola *auricula-judae* (Bull.) Berk. – LS: **3a.** Blanár and Mihál (2002). — **3b.** on decaying wood *Euonymus europaeus*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **7.** on dry twigs (Ø 1.5 cm) *Sambucus nigra*, 25. 11. 2003, 10. 8. 2009, leg. DB, det. IM, herb. DB.

Hohenbuehelia *angustata* (Berk.) Singer – LS: **4.** on stem *Padus racemosa*, 30. 12. 2011, leg. DB, det. IM, herb. DB, herb. IM.

Hydropus *subalpinus* (Höhn.) Singer – LS: **7.** on decaying stem *Salix alba* and *S. fragilis*, 19. 8. 2010, leg. DB, det. IM, herb. DB.

Hymenochaete *tabacina* (Sowerby) Lév. – LS: **4.** on stem *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB. — **6.** on stem *Alnus glutinosa*, 15. 1. 2010, leg. DB, IM, det. IM, herb. DB.

Hyphoderma *medioburiense* (Burt) Donk – LS: **7.** on twig *Salix fragilis* and *Salix alba*, 6. 6. 2012, leg. DB, det. IM, herb. DB, herb. IM.

Hypholoma *capnoides* (Fr.) P. Kumm. – LS: **2.** on twig *Salix* sp., 23. 9. 2010, leg. et det. IM, not.

H. fasiculare (Huds.) P. Kumm. – LS: **1.** on twigs *Corylus avellana*, 15. 10. 2010, leg. et det. IM, not. — **2.** on decaying wood *Salix fragilis*, 2. 10. 2009, leg. DB, det. IM, herb. DB. — **3a.** Blanár and Mihál (2002). — **4.** on decaying wood *Salix fragilis*, 15. 8. 2009, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **6.** on decaying wood *Salix* sp., 23. 9. 2010, leg. et det. IM, not.

H. subviride (Berk. et M. A. Curtis) Dennis – LS: **4.** on stem *Alnus glutinosa*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

H. sublateritium (Schaeff.) Quél. – LS: **3a.** on decaying wood *Salix* sp., Blanár and Mihál (2002).

Inocybe *geophylla* (Fr.) P. Kumm. – MS: **4.** in litter, on soil, 10. 8. 2011, leg. DB, det. IM, herb. DB.

I. nitidiuscula (Britzelm.) Sacc. – MS: **4.** in litter, on soil, 10. 8. 2011, leg. DB, det. IM, herb. DB.

Kuehneromyces *mutabilis* (Schaeff.) Singer et A. H. Sm. – LS: **2.** on decaying stem *Salix* sp., 6. 8. 2010, leg. et det. IM, not.

Laccaria *laccata* agg. – MS: **1.** on soil, 14. 7. 2010, 23. 9. 2010, leg. et det. IM, not.

Lacrymaria *lacrymabunda* (Bull.) Pat. – LS: **1.** on soil, 5. 8. 2010, leg. DB, det. IM, herb. DB.

Lactarius *chrysorrheus* Fr. – MS: **1.** on soil, 14. 7. 2010, leg. et det. IM, not.

L. lilacinus (Lasch) Fr. – MS: **1.** on soil, 14. 7. 2010, leg. et det. IM, not.

L. serifluus (DC.) Fr. – MS: **1.** on soil, 23. 9. 2010, leg. et det. IM, not.

Laeticorticium *roseum* (Pers.) Donk – LS: **1.** on decaying stem *Salix caprea*, 5. 8. 2010, 15. 10. 2010, leg. DB, det. IM, herb. DB, herb. IM. — **3b.** on decaying stem *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB, herb. IM.

Laetiporus *sulphureus* (Bull.) Bondartsev et Singer – LP: **7.** on stem *Salix* sp., 7. 4. 2010, leg. et det. IM, not.

Lenzites *betulina* (L.) Fr. – LS: **4.** on decaying twig *Padus racemosa*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

Lepiota *clypeolaria* (Bull.) P. Kumm. – TS: **5.** on soil, 1. 9. 2009, leg. DB, det. IM, herb. DB.

L. cristata (Alb. et Schwein.) P. Kumm. – TS: **4.** on soil, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **6.** on soil, 3. 9. 2009, leg. DB, det. IM, herb. DB.

L. felina (Pers.) P. Karst. – TS: **6.** on soil, 23. 9. 2010, leg. et det. IM, herb. IM.

L. pseudohelveola Kühner ex Hora – TS: **6.** on soil, 3. 9. 2009, leg. DB, det. IM, herb. DB.

Lepista *nuda* (Bull.) Cooke – TS: **3a.** Blanár and Mihál (2002).

Lycoperdon *lividum* Pers. – TS: **2.** on soil, 15. 10. 2010, leg. IM et DB, det. IM, herb. DB.

L. pyriforme Schaeff. – LS: **2.** on decaying wood, 6. 8. 2010, leg. et det. IM, not. — **3a.** Blanár and Mihál (2002).

Macrocystidia *cucumis* (Pers.) Joss. – TS: **1.** on soil, 5. 8. 2010, leg. DB, det. IM, herb. DB.

Macrotyphula *juncea* (Fr.) Berthier – TS: **4.** in litter, on leaves *Alnus glutinosa*, *Salix fragilis*, *Ulmus laevis*, 17. 11. 2010, leg. DB, det. IM, herb. DB.

Marasmiellus *foetidus* (Sowerby) Antonín et al. – LS: **3a.** on twigs, Blanár and Mihál (2002). — **6.** on twigs *Salix* sp., 2. 7. 2010, leg. et det. IM, not.

M. ramealis (Bull.) Singer – LS: **1.** on decaying wood in soil, 2. 7. 2010, leg. IM, det. IM, herb. DB. — **2.** on decaying wood *Salix* sp., leg. et det. IM, not.

Marasmius *bulliardii* Quél. – LS: **4.** on decaying twig *Ribes uva-crispa*, 10. 8. 2010, leg. DB, det. IM, herb DB. — **5.** on stem *Salix* cf. *alba*, 1. 9. 2009, leg. DB, det. IM, herb. DB.

M. rotula (Scop.) Fr. – LS: **1.** on decaying wood, 15. 10. 2010, leg. et det. IM, not.

M. wynnei Berk. et Broome – TS: **3b.** in litter, on leaves *Salix cinerea*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

Megacollybia *platyphylla* (Pers.) Kotl. et Pouz. – LS: **1.** on decaying wood, 15. 10. 2010, leg. et det. IM, not. — **5.** on decaying wood *Salix alba*, 1. 9. 2009, leg. DB, det. IM, herb. DB. — **6.** on decaying wood *Salix* sp., 2. 7. 2010, leg. et det. IM, not.

Melampsora *amygdalinae* Kleb. – HP: **2.** in litter, on leaves *Alnus glutinosa*, 15. 10. 2010, leg. DB, det. IM, herb. DB. — **3a.** in litter, on leaves *Alnus glutinosa*, 20. 11. 2010, leg. et det. IM, herb. IM. — **6.** in litter, on leaves *Alnus glutinosa*, 15. 10. 2010, leg. IM et DB, det. IM, herb. DB.

Melampsoridium *carpini* (Nees) Dietel **–** HP: **3a.** Blanár and Mihál (2002).

Melanophyllum *haematospermum* (Bull.) Kreisel – TS: **4.** on soil, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **7.** on soil, 19. 8. 2010, leg. DB, det. IM, herb. DB.

Mycena *acicula* (Schaeff.) P. Kumm. – TS: **7.** on decaying stem *Salix fragilis*, 28. 5. 2010, leg. DB, det. IM, herb. DB.

M. capillaris (Schumach.) P. Kumm. – HS: **1.** on decaying leaves *Salix* sp., 5. 8. 2010, leg. et det. IM, not. — **2.** on decaying leaves *Salix* sp., 23. 9. 2010, leg. et det. IM, not. — **4.** on decaying leaves *Salix* sp., 25. 8. 2009, leg. et det. IM, not.

M. cinerella (P. Karst.) P. Karst. – TS: **6.** on soil, 15. 10. 2010, leg. et det. IM, not. — **7.** on soil, 2. 7. 2010, leg. et det. IM, not.

M. crocata (Schrad.) P. Kumm. – TS: **6.** on soil, 15. 10. 2010, leg. et det. IM, not.

M. filopes (Bull.) P. Kumm. – LS: **3a.** Blanár and Mihál (2002). — **7.** on decaying stem *Salix fragilis*, 28. 5. 2010, leg. DB, det. IM, herb. DB.

M. galericulata (Scop.) Gray – LS: **1.** on decaying wood *Alnus glutinosa*, 5. 9. 2010, leg. et det. IM, not. — **2.** on decaying wood *Salix sp.*, 23. 9. 2010, leg. et det. IM, not. — **6.** on decaying wood, 23. 9. 2010, leg. et det. IM, not.

M. haematopus (Pers.) P. Kumm. – TS: **1.** on decaying wood in soil, 15. 10. 2010, leg. et det. IM, not.

M. polygramma (Bull.) Gray – TS: **1.** on decaying wood in soil, 15. 10. 2010, leg. et det. IM, not.

M. pura (Pers.) P. Kumm. – TS: **6.** on soil, 3. 9. 2009, leg. DB, det. IM, herb. DB.

M. renati Quél. – LS: **6.** on decaying wood, 3. 9. 2009, 15. 10. 2010, leg. et det. IM, not.

M. sanguinolenta (Alb. et Schwein.) P. Kumm. – TS: **4.** on soil, 12. 10. 2012, leg. DB, det. IM, herb. DB.

M. tintinnabulum (Batsch) Quél. – LS: **7.** on decaying stem *Salix alba* or *S. fragilis*, 19. 8. 2010, leg. DB, det. IM, herb. DB.

Mycoacia *nothofagi* (G. Cunn.) Ryvarden – LS: **3b.** on decaying stem *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB, herb. IM.

Oligoporus *stipticus* (Pers.) Gilb. et Ryvarden – LS: **6.** on decaying stem *Salix* sp., 2. 9. 2009, leg. et det. IM, not.

O. subcaesius (A. David) Ryvarden et Gilb. – LS: **3b.** on decaying stem *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **6.** on decaying stem *Salix fragilis*, 15. 10. 2010, leg. DB et IM, det. IM, herb. DB.

Omphalina *discorosea* (Pilát) Herink et Kotl. – LS: **1.** on decaying wood in soil, 23. 9. 2010, leg. et det. IM, herb. IM. — **2.** on decaying wood in soil, 23. 9. 2010, leg. et det. IM, herb. IM.

O. oniscus (Fr.) Quél. – LS: **3b.** on decaying stem *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

Ossicaulis *lignatilis* (Pers.) Redhead et Ginns – LS: **7.** on decaying wood *Salix* sp., 15. 10. 2010, leg. et det. IM, herb. IM.

Panaeolina *foenisecii* (Pers.) Maire – TS: **1.** on soil, 2. 7. 2010, leg. DB, det. IM, herb. DB. — **6.** on soil, 3. 9. 2009, leg. DB, det. IM, herb. DB.

Panaeolus *fimicola* (Fr.) Quél – TS: **4.** on soil, 12. 10. 2012, leg. DB, det. IM, herb. DB.

P. retirugis (Fr.) Quél. – TS: **2.** on soil, 28. 5. 2010, leg. et det. IM, herb. IM.

Panellus *serotinus* (Pers.) Kühner – LS: **4.** on decaying stem *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB. — **5.** on decaying wood *Salix* sp., 24. 11. 2010, leg. et det. IM, herb. IM.

P. stipticus (Bull.) P. Karst. – LS: **1.** on decaying wood *Betula pendula*, 5. 8. 2001, leg. et det. DB, not. — **3a.** on decaying stem *Salix fragilis*, 27. 3. 1999, leg. et det. DB, herb. DB. — **3b.** on decaying twig (Ø 0.5 cm), 27. 8. 1999, leg. et det. DB, herb. DB. — **4.** on decaying stem *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB. — **5.** on decaying stump *Alnus glutinosa*, 24. 11. 2010, leg. DB, det. IM, herb. DB.

P. cf. *ringens* (Fr.) Romagn. **–** LS: **3b.** on decaying stem *Salix cinerea*, 20. 11. 2010, leg. DB, det. SJ, SLO 984.

Paxillus *filamentosus* (Scop.) Fr. **–** MS: **1.** on soil, 5. 8. 2010, 23. 9. 2010, leg. et det. IM, not.

Peniophora *aurantiaca* (Bres.) Höhn. et Litsch. – LS: **3b.** on decaying twig (Ø 1 cm) *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB, herb. IM.

Perenniporia *tenuis* (Sacc.) Ryvarden – LS: **2.** on decaying twig *Alnus glutinosa*, 2. 7. 2010, leg. DB, det. IM, herb. DB. — **3b.** on decaying twig (Ø 2 cm) *Salix fragilis*, 8. 8. 2010, leg. DB, det. IM, herb. DB.

Phanerochaete *laevis* (Pers.) J. Erikss. et Ryvarden – LS: **1.** on twig *Salix fragilis*, 15. 10. 2010, leg. et det. IM, not. **— 3a.** Blanár and Mihál (2002). —

5. on decaying twig *Sambucus nigra*, 24. 11. 2010, leg. DB, det. IM, herb. DB.

P. sordida (P. Karst.) J. Erikss. et Ryvarden – LS: **4.** on decaying twig *Sambucus nigra*, 17. 11. 2010, leg. DB, det. IM, herb. DB. – **5.** on twig *Salix* sp., 24. 11. 2010, leg. et det. IM, herb. IM.

***Phellinus** conchatus* (Pers.) Quél. – LP: **6.** on stem *Salix* sp., 3. 9. 2009, leg. et det. IM, herb. IM.

P. igniarius (L.) Quél. – LP: **6.** on stem *Salix fragilis*, 3. 9. 2009, leg. DB, det. IM, herb. DB. — **7.** on stem *Salix fragilis*, 10. 8. 2009, leg. DB, det. IM, herb. DB.

P. populicola Niemelä – LS: **4.** on decaying stem *Populus nigra*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

P. punctatus (Fr.) Pilát – LP: **1.** on stem *Salix fragilis*, 14. 7. 2010, leg. et det. IM, not. — **4.** on stem *Salix caprea*, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **5.** on twig *Salix fragilis*, 1. 9. 2009, leg. DB, det. IM, herb. DB. — **6.** on stem *Salix fragilis*, 3. 9. 2009, leg. DB, det. IM, herb. DB.

***Phlebia** tremellosa* (Schrad.) Nakasone et Burds. – LS: **3a.** Blanár and Mihál (2002).

***Phleogena** faginea* (Fr.) Link – LS: **1.** on decaying stem *Salix caprea*, 5. 9. 2010, leg. DB, det. IM, herb. DB.

***Pholiota** adiposa* (Batsch) P. Kumm. – LP: **1.** on stem *Alnus glutinosa*, 23. 9. 2010, leg. et det. IM, not. — **2.** on stem *Salix fragilis*, 15. 10. 2010, leg. et det. IM, not.

P. alnicola (Fr.) Singer – LP: **4.** on stem *Populus nigra*, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **6.** on stem *Alnus glutinosa*, 23. 9. 2010, leg. et det. IM, not.

P. squarrosa (Weigel) P. Kumm. – LS: **6.** on decaying wood *Salix* sp., 23. 9. 2010, leg. et det. IM, not.

***Pleurotus** dryinus* (Pers.) P. Kumm. – LS: **4.** on decaying wood *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

P. ostreatus (Jacq.) P. Kumm. – LS: **1.** on decaying stem *Corylus avellana*, 5. 9. 2010, leg. et det. IM, not. — **7.** on stem *Ulmus laevis*, 15. 10. 2010, leg. et det. IM, not.

***Plicaturopsis** crispa* (Pers.) D. A. Reid – LS: **1.** on dry twig *Corylus avellana*, 16. 3. 2011, leg. DB, det. IM, herb. DB. — **3b.** on stem *Salix caprea*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

***Pluteus** cervinus* (Schaeff.) P. Kumm. – LS: **2.** on decaying wood, 2. 7. 2010, leg. et det. IM, not. — **4.** on stem *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

P. ephebeus (Fr.) Gillet – LS: **7.** on decaying stem *Salix fragilis*, 15. 10. 2010, leg. IM et DB, det. IM, herb. DB.

P. podospileus Sacc. et Cub. – LS: **7.** on decaying twig *Salix fragilis*, 6. 7. 2012, leg. et det. IM, herb. IM.

P. romellii (Britzelm.) Sacc. – LS: **4.** on decaying wood *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **7.** on decaying twig *Salix* cf. *fragilis*, 15. 10. 2010, leg. DB, det. IM, herb. DB.

P. salicinus (Pers.) P. Kumm. – LS: **2.** on decaying wood *Salix fragilis*, 2. 10. 2009, leg. DB, det. IM, herb. DB. – **3b.** on decaying twig *Salix* sp., 12. 5. 2011, leg. et det. IM, not. — **7.** on decaying wood *Salix alba*, 10. 8. 2009, leg. DB, det. IM, herb. DB.

***Polyporus** badius* (Pers.) Schwein. – LS: **4.** on decaying stem *Salix fragilis*, 15. 8. 2009, leg. DB, det. IM, herb. DB.

P. brumalis (Pers.) Fr. – LS: **1.** on decaying twig *Salix fragilis*, 23. 9. 2010, leg. et det. IM, not. — **3a.** Blanár and Mihál (2002). — **4.** on stem *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

P. ciliatus Fr. – LS: **4.** on stem *Populus nigra*, 12. 5. 2012, leg. DB, det. IM, herb. DB.

P. melanopus (Sw.) Fr. – LS: **2.** on decaying twig *Salix fragilis*, 6. 8. 2010, leg. et det. IM, not. – **6.** on stem *Alnus glutinosa*, 23. 9. 2010, leg. et det. IM, not. — **7.** on decaying stem *Salix alba*, 15. 10. 2010, leg. et det. IM, not.

P. squamosus (Huds.) Fr. – LP: **7.** on stem *Salix fragilis*, 10. 8. 2009, leg. DB, det. IM, herb. DB.

***Protomerulius** caryae* (Schwein.) Ryvarden – MP: **4.** on old fruitbodies *Ganoderma australe* (stem *Salix fragilis*), 30. 12. 2011, leg. DB, det. IM, herb. DB, herb. IM.

***Psathyrella** candolleana* (Fr.) Maire – LS: **1.** on decaying stem *Alnus glutinosa*, 5. 9. 2010, leg. DB, det. IM, herb. DB. — **7.** on decaying wood *Salix fragilis* or *S. alba*, 15. 10. 2010, leg. DB, det. IM, herb. DB.

P. piluliformis (Bull.) P. D. Orton – TS: **1.** on soil, 5. 8. 2010, leg. et det. IM, not. — **4.** on soil, 12. 5. 2011, leg. et det. IM, not.

***Pseudoclitocybe** cyathiformis* (Bull.) Singer – LS: **3a.** Blanár and Mihál (2002). — **5.** on decaying wood in soil, 24. 11. 2010, leg. DB, det. IM, herb. DB.

***Psilocybe** inquilina* var. *crobula* (Fr.) Høil. – TS: **1.** on soil, 23. 9. 2010, leg. et det. IM, not.

P. cyanescens (Maire) Wakef – TS: **2.** on soil, 28. 5. 2010, leg. et det. IM, not.

***Puccinia** sp.* – HP: **1.** on leaves *Alnus glutinosa*, 15. 10. 2010, leg. et det. IM, herb. IM. — **2.** on leaves *Alnus glutinosa*, 15. 10. 2010, leg. et det. IM, herb. IM.

***Radulomyces** molaris* (Chaillet ex Fr.) M. P. Christ. – LS: **3b.** on decaying twig *Salix cinerea*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

***Rhodocollybia** butyracea* (Bull.) Lennox – TS: **3a.** Blanár and Mihál (2002).

***Rhodocybe** gemina* (Fr.) Kuyper et Noordel. – TS: **4.** on soil, 10. 8. 2011, leg. DB, det. IM, herb. DB.

***Rickenella** fibula* (Bull.) Raithelh. – LS: **1.** on decaying wood *Alnus glutinosa*, 14. 7. 2010, leg. DB, det. IM, herb. DB. — **2.** on decaying stump *Alnus glutinosa* and *Salix fragilis*, 6. 8. 2010, leg. DB, det. IM, herb. DB. — **7.** on decaying stem *Salix* cf. *fragilis*, 19. 8.

Schizophyllum *commune* Fr. – LS: **1.** on twigs *Salix fragilis*, 14. 7. 2010, leg. et det. IM, not. — **5.** on decaying stem *Salix alba*, 24. 11. 2010, leg. DB, det. IM, herb. DB. — **6.** on decaying stem *Salix sp*., 2. 7. 2010, leg. et det. IM, not. — **7.** on decaying twig *Salix fragilis*, 19. 8. 2010, leg. DB, det. IM, herb. DB.

Schizopora *flavipora* (Berk et M. A. Curtis ex Cooke) Ryvarden – LS: **3b.** on decaying twig *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

Scleroderma *verrucosum* (Bull.) Pers. – MS: **4.** on sandy soil, 10. 8. 2011, leg. DB, det. IM, herb. DB.

Scytinostroma *portentosum* (Berk. et M. A. Curtis) Donk – LS: **3b.** on decaying twig *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

Setulipes *androsaceus* (L.) Antonín – LS: **2.** on decaying twig *Salix fragilis*, 2. 7. 2010, leg. DB, det. IM, herb. DB. — **3b.** on decaying twig *Salix fragilis*, 8. 7. 2010, leg. DB, det. IM, herb. DB.

Steccherinum *dichroum* Pers. – LS: **3b.** on twig *Salix cinerea*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

Stereum *gausapatum* (Fr.) Fr. – LP: **3a.** Blanár and Mihál (2002). — **4.** on stem *Populus nigra*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

S. hirsutum (Willd.) Gray – LS: **2.** on decaying twig *Salix* sp., 6. 8. 2010, leg. et det. IM, not. — **5.** on decaying stem *Salix alba*, 1. 9. 2009, 24. 11. 2010, leg. DB, det. IM, herb. DB.

S. rugosum (Pers.) Fr. – LS: **2.** on decaying stem *Alnus glutinosa*, 6. 8. 2010, leg. et det. IM, not.

S. subtomentosum Pouzar – LS: **4.** on twig *Salix fragilis*, 12. 10. 2012, leg. DB, det. IM, herb. DB.

Stropharia *caerulea* Kreisel – TS: **3a.** Blanár and Mihál (2002).

Subulicystidium *longisporum* (Pat.) Parmasto – LS: **4.** on dry twigs *Sambucus nigra*, 15. 8. 2009, leg. DB, det. IM, herb. DB. — **5.** on twig *Salix fragilis*, 1. 9. 2009, leg. DB, det. IM, herb. DB.

Thelephora *penicillata* Pers. – MS: **6.** on soil, 3. 9. 2009, leg. DB, det. IM, herb. DB.

Trametes *gibbosa* (Pers.) Fr. – LS: **7.** on stem *Salix fragilis*, 10. 8. 2009, leg. DB, det. IM, herb. DB.

T. suaveolens (L.) Fr. – LS: **1.** on stem *Salix fragilis*, 14. 7. 2010, leg. et det. IM, not. — **3a.** Blanár and Mihál (2002). — **3b.** on stem *Salix fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB. — **5.** on decaying stem *Salix alba* and twig *Alnus glutinosa*, 24. 11. 2010, leg. DB, det. IM, herb. DB. — **7.** on stem *Salix alba*, 1. 4. 2011, leg. DB, det. IM, herb. DB; on decaying stem *Salix fragilis*, 19. 8. 2010, leg. DB, det. IM, herb. DB.

T. velutina (Planer) G. Cunn. – LS: **1.** twig *Salix fragilis*, 15. 10. 2011, leg. et det. IM, not. — **4.** on decaying stem *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB. — **7.** on stem *Alnus glutinosa*, 19. 8. 2010, leg. et det. IM, not.

T. versicolor (L.) Pilát – LS: **1.** on twig *Alnus glutinosa*, 15. 10. 2011, leg. et det. IM, not. — **3a.** Blanár and Mihál (2002). — **4.** on decaying stem *Salix fragilis* and *Padus racemosa*, 15. 8. 2009, 12. 10. 2012, leg. DB, det. IM, herb. DB; on decaying wood *Alnus incana*, 17. 11. 2010, leg. DB, det. IM, herb. DB. — **5.** on stem *Salix fragilis*, 1. 9. 2009, leg. DB, det. IM, herb. DB. — **6.** on decaying stem *Salix fragilis*, 28. 5. 2010, leg. et det. IM, not.

Tremella *mesenterica* Retz. – LS: **1.** on dry twig *Corylus avellana*, 16. 3. 2011, leg. DB, det. IM, herb. DB. — **2.** on twig *Sambucus nigra*, 2. 7. 2010, leg. et det. IM, not. — **3b.** on twigs *Salix cinerea* and *S. fragilis*, 20. 11. 2010, leg. DB, det. IM, herb. DB.

Tricholoma *saponaceum* (Fr.) P. Kumm. – MS: **2.** on soil, 15. 10. 2010, leg. et det. IM, not.

Tubaria *conspersa* (Pers.) Fayod. – TS: **1.** on soil, 23. 9. 2010, leg. et det. IM, not.

T. romagnesiana Arnolds – TS: **6.** on soil, 3. 9. 2009, leg. DB, det. IM, herb. DB.

Typhula *erythropus* (Pers.) Fr. – HS: **1.** in litter, on leaves *Alnus glutinosa*, 15. 10. 2010, leg. DB, det. IM, herb. DB. — **3a.** Blanár and Mihál (2002); in litter, on twig *Populus canescens*, 12. 10. 2012, leg. DB, det. IM, herb. DB. — **4.** in litter, on leaves *A. glutinosa* and *Salix fragilis*, 15. 10. 2010, leg. DB, det. IM, herb. DB. — **6.** in litter, on leaves *A. glutinosa* and *Salix fragilis*, 15. 10. 2010, leg. DB, det. IM, herb. DB. — **7.** in litter, on leaves *Salix alba*, 25. 11. 2011, leg. DB, det. IM, herb. DB.

Xerocomus *rubellus* (Krombh.) Quél. – MS: **4.** on soil, 10. 8. 2011, leg. DB, det. IM, herb. DB.

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Temporal variability of spring phenological phases and diameter increment of Norway spruce (*Picea abies* /L./ Karst.) provenances

Alena Pástorová, Jana Škvareninová, Katarína Střelcová, Adriana Leštianska

Faculty of Forestry, Technical University in Zvolen, T. G. Masaryka 24, 960 53 Zvolen, Slovak Republic, e-mail: xpastorova@tuzvo.sk

Abstract

Pástorová, A., Škvareninová, J., Střelcová, K., Leštianska, A. 2014. Temporal variability of spring phenological phases and diameter increment of Norway spruce (*Picea abies* /L./ Karst.) provenances. *Folia oecol*., 41: 173–183.

The presented paper analyses temporal variability of the beginning, end, and the length of growth, and of the selected spring phenological phases of Norway spruce. The experiment was performed on three provenances of Norway spruce (*Picea abies* /L./ Karst.) originating from the orographical unit of Volovské vrchy, and growing in Borová hora arboretum, during the three years 2010, 2011 and 2012. The examined provenances were selected on the base of the elevation gradient from three elevations: 500 m a.s.l., 750 m a.s.l. and 1,100 m a.s.l. Tree stem circumference changes were continuously measured, and spring phenological phases were assessed. Our results proved that a significant temporal shift in the onset of the phenophases to a later period occurs in provenances originating from higher elevation. We found that during the period from 2010 to 2012, the sum of the effective air and soil temperatures needed for the onset of the phenological phases decreased from one year to another. The highest effective sums of air and soil temperatures were required for the provenance originating from the highest elevation (1,100 m). The analysis of diameter increment showed that the lowest increment value equal to 4.35% was recorded in April 2010, while the increment in April 2011 was 13.11% and in 2012 it was 10.09% of the overall increment of the stem diameter in growing season. This was caused by the later onset of spring phenophases in April 2010 caused by lower air temperatures in this month.

Keywords

increment, *Picea abies*, provenances, spring phenological phases, temperature sums

Introduction

In the last years, phenology (Leith, 1974) has become an indicator of global climate changes, mainly during spring months (Crick and Sparks, 1999; Parmesan et al. 1999; Thomas and Lennon, 1999).

Nowadays, spring phenological phases occur earlier in the northern hemisphere than in previous decades (Menzel and Fabian, 1999). Higher temperatures in late summer postpone spring phenophases in the next year (Sparks et al., 2000; Heide, 2003; Chmielewski et al., 2005). Thus, in order to predict the bud reaction in spring it is important to know the climatic conditions during the onset of bud dormancy in previous autumn (Gordo and Sanz, 2010). According to the results of Gordo and Sanz (2010), significant increase of autumn temperatures could cause postponing of spring phenological phases in the next year.

Climate changes cause changes of temperatures mainly in winter and early spring. Flowering time of early flowering species is expected to change most (Gordo and Sanz, 2010). On the base of the models, the flowering date is expected to shift to a later time at a rate of 6.2 days per 1 °C, if GCM temperatures increase by 4.5 °C between 1990 and 2099 and greenhouse gases increase by 1% per year (Osborne et al., 2000).

Spruce is the most important coniferous tree species in Slovakia. It creates pure stands exclusively in the zone of high-elevation spruce forests under the upper timber line. However, in the past centuries spruce was artificially planted at lower elevations, where it

nowadays represents a significant part of stands and frequently forms single-species communities. Due to climate changes, spruce at lower elevations begins to suffer from drought, which also affects the survival of Norway spruce seedlings growing under unconstrained moisture conditions (Pichlerová et al., 2013). Nowadays, phenological events are considered one of the most suitable indicators of climate changes and of their impact on tree species.

In this paper we analyse the temporal variability of the onset, the end, and the duration of selected spring phenological phases between the provenances of Norway spruce originating from different elevations during the three years from 2010 to 2012 in connection with the spring growth in stem circumference.

Material and methods

The changes in stem circumference, spring phenological phases and microclimatic characteristics were assessed in Borová hora arboretum in Zvolen, during the growing season of 2010, 2011, 2012. Borová hora arboretum is situated in the watershed of the middle Hron River, approximately 3 km from Zvolen town centre, between 48°35´42´´ and 48°36´06´´ of northern latitude, and between 19°07´58´´ and 19°10´00´´ of eastern longitude. The research plots are situated at an elevation of 350 m above sea level on a hillside with prevailing northwestern aspect and 5–10% slope.

Geological parent rock consists of andesite tuff and travertines, the main soil types are pararendzinas, cambisols and fluvial soils. Arboretum belongs to 2nd beech-oak forest vegetation tier. From the point of climate, the site belongs to a warm, slightly moist region with cold winter.

In the experiment we used three provenances of Norway spruce (*Picea abies* /L./ Karst.), each represented with six 35-year-old sample trees (in the year 2010 only four sample trees), originating from different elevations from the Volovské vrchy mountain range:

- 1st provenance – 500 m a.s.l.
- 2nd provenance – 750 m a.s.l.
- 3rd provenance – 1,100 m a.s.l.

On each sample tree, we installed DRL 26 dendrometer (EMS Brno, CZ) with automated data storage into a built-in datalogger. The changes in tree circumference were recorded continuously in one hour intervals. From the data we derived the beginning, the end and the length of the growth.

For the measurements of soil water potential (SWP) we installed gypsum blocks near each provenance at depths of 15 cm, 30 cm and 50 cm. The blocks were equipped with a data logger for automatic data storage at one hour intervals.

Close to the selected sample trees, we also measured meteorological characteristics: global radiation [W m^{-2}], air temperature [°C], relative air humidity [%], precipitation totals [mm] and soil temperature [°C]. Air temperature and humidity were measured at 2 m height above ground with Minikin TH tool (EMS Brno, CZ), and soil temperature was measured at 10 cm depth. The measurement of global radiation was performed with Minikin RT (EMS Brno, CZ). All data were stored in a datalogger at 10 minute-interval. Since the values of meteorological characteristics were recorded at different intervals, we re-calculated the measured values into hourly sums.

During the spring of 2010, 2011 and 2012, we observed the development of the following phenological phases of vegetative organs of Norway spruce on four sample trees of each provenance:

- leaf bud swelling (LBS) – 10%, 50%, 100%
- sprouting of leaves (SL) – 10%, 50%, 100%
- leaf unfolding (LU) – 10%, 50%, 100%.

We used these data for the determination of the onset, end, and the length of the phenological phases for each provenance.

According to Bednářova et al. (2012) the onset of spring phenological phases is mainly affected by the air temperature above 5 °C and soil temperature of 1 °C. Thus, we calculated the means of sums of effective temperatures for these 1 °C and 5 °C temperature limits from 1st January. The earliest date (10%), when the phenological stage was noticed, was considered as the beginning of the onset of phenological phases.

To determine the differences in the onset, end, and the length of the phenological phases and increment between the provenances, we performed multifactorial analysis of variance using STATISTICA 10 and Microsoft Excel. Significant differences were revealed for the significance level $p = 0.05$.

Results and discussion

Diameter growth

In 2010, diameter growth started on 121 DOY and finished on 279 DOY. In 2011, diameter growth started on 112 DOY and finished on 229 DOY. The third provenance originating from the highest elevation finished its diameter growth earlier, on 220 DOY. In 2012, diameter growth started on 111 DOY and finished on 328 DOY. In August, a temporary cessation of diameter growth was observed due to drought and high air temperatures in the 2012 summer (Table 1). This corresponds with the findings of Nielsen and Jørgensen (2003), who found that the cessation of growth can also be significantly influenced by soil water. In September, increment increased again and growth continued until the end of November. A detailed microclimatic characteristic of the three years in comparison with the long-term average (1961–1990) and the overview of the diameter increment in the years 2010–2012 can be seen in Table 1.

Table 1. Climate assessment of years 2010, 2011 and 2012 compared with the long-term average from 1961 to 1990 and overview of the increment of the stem diameter in these growing seasons; N %, percentage of the long-term average precipitation; AT, air temperature; ΔAT, deviation of the long-term average; GS, growing season; VOP %, percentage of the overall increment of the stem diameter in growing season.

Month	P [mm]	N %	Description	AT [°C]	Δ AT	Description	Increment [mm]	VOP [%]
April	63.0	137.0	Moist / above normal	9.8	1.3	Warm / above normal	1.66	4.35
May	123.2	186.7	Very moist / strongly above normal	14.3	0.9	Normal / normal	9.82	25.71
June	168.8	201.0	Very moist / strongly above normal	18.3	2.0	Warm / above normal	11.90	31.17
July	64.8	101.3	Normal / normal	21.2	3.3	xtremely warm / extremely above normal	10.22	26.76
August	34.0	54.0	Arid / subnormal	18.5	1.4	Warm / above normal	3.79	9.92
September	103.6	191.9	Very moist / strongly above normal	12.8	–0.6	Normal / normal	0.89	2.32
October	32.6	72.4	Normal / normal	6.8	–1.6	Cold / subnormal	–0.23	–0.60
November	89.2	146.2	Moist / above normal	7.0	3.9	Extremely warm / extremely above normal	0.14	0.38
GS 2010	679.2	140.6		13.6	1.3		38.18	100.00

Month	P [mm]	N %	Description	AT [°C]	Δ AT	Description	Increment [mm]	VOP [%]
April	20.6	44.8	Arid / subnormal	11.7	3.2	Very warm / strongly above normal	4.37	12.89
May	39.6	60.0	Arid / subnormal	14.6	1.2	Normal / normal	9.84	29.01
June	106.2	126.4	Normal / normal	18.1	1.8	Warm / above normal	12.48	36.80
July	150.8	235.6	Extremely moist / extremely above normal	18.6	0.7	Normal / normal	5.74	16.91
August	15.0	23.8	Very arid / strongly subnormal	20.2	3.1	Extremely warm / extremely above normal	3.22	9.49
September	3.6	6.7	Very arid / strongly subnormal	16.7	3.3	Very warm / strongly above normal	–0.69	–2.03
October	16.6	36.9	Arid / subnormal	8.3	–0.1	Normal / normal	–0.91	–2.68
November	1.8	3.0	Extremely moist / extremely above normal	1.2	–1.9	Cold / subnormal	–0.13	–0.39
GS 2011	354.2	73.3		13.7	1.4		33.93	100.00

Month	P [mm]	N %	Description	AT [°C]	Δ AT	Description	Increment [mm]	VOP [%]
April	46.6	101.3	Normal / normal	10.5	2.0	Warm / above normal	2.94	10.00
May	17.0	25.8	Extremely arid / extremely subnormal	15.6	2.2	Warm / above normal	7.38	25.12
June	93.2	111.0	Normal / normal	18.8	2.5	Very warm / strongly above normal	10.44	35.50
July	126.0	196.9	Very moist / strongly above normal	20.7	2.8	Extremely warm / extremely above normal	7.73	26.30
August	6.8	10.8	Extremely arid / extremely subnormal	20.2	3.1	Extremely warm / extremely above normal	–1.97	–6.71
September	30.6	56.7	Normal / normal	16.0	2.6	Very warm / strongly above normal	1.23	4.18
October	120.0	266.7	Very moist / strongly above normal	9.4	1.0	Normal / normal	1.65	5.60
November	40.6	66.6	Normal / normal	6.3	3.2	Very warm / strongly above normal	0.00	0.01
GS 2012	480.8	99.5		14.7	2.4		29.40	100.00

In Figure 1 we can see that after the phenological phase of leaf-unfolding, the increment begins to increase significantly as a result of maximum photosynthetical activity of physiologically mature leaves. Multifactorial analysis of variance revealed that there is a significant difference at the beginning of diameter

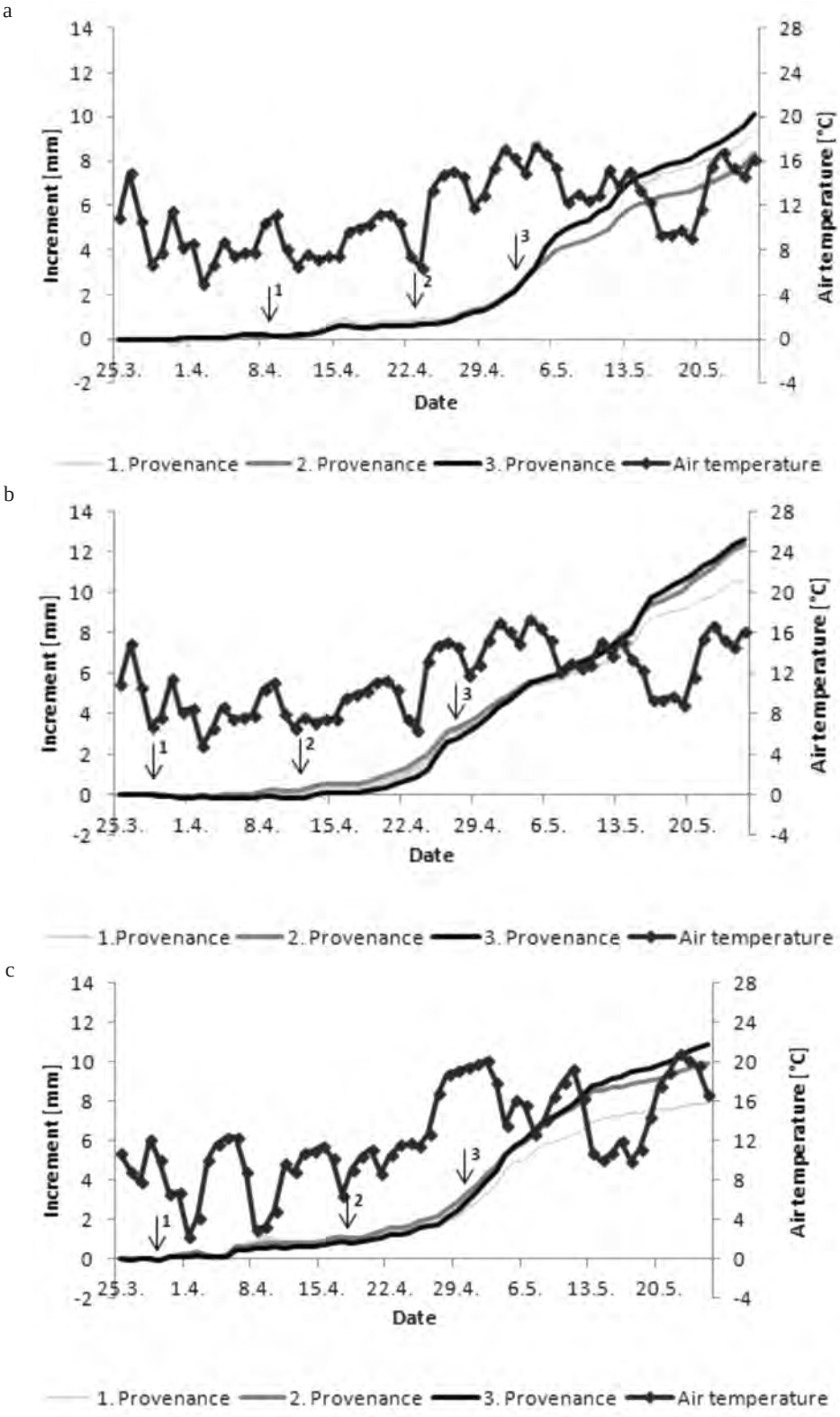

Fig. 1. Influence of spring phenological phases on the increment of stem diameter in years a) 2010, b) 2011, c) 2012; (1, leaf bud swelling; 2, sprouting of leaves; 3, leaf unfolding).

growth between the provenances and the years 2010, 2011 and 2012 (Fig. 2a). This confirms the knowledge that provenances from higher elevations begin their diameter growth later than provenances from lower elevations.

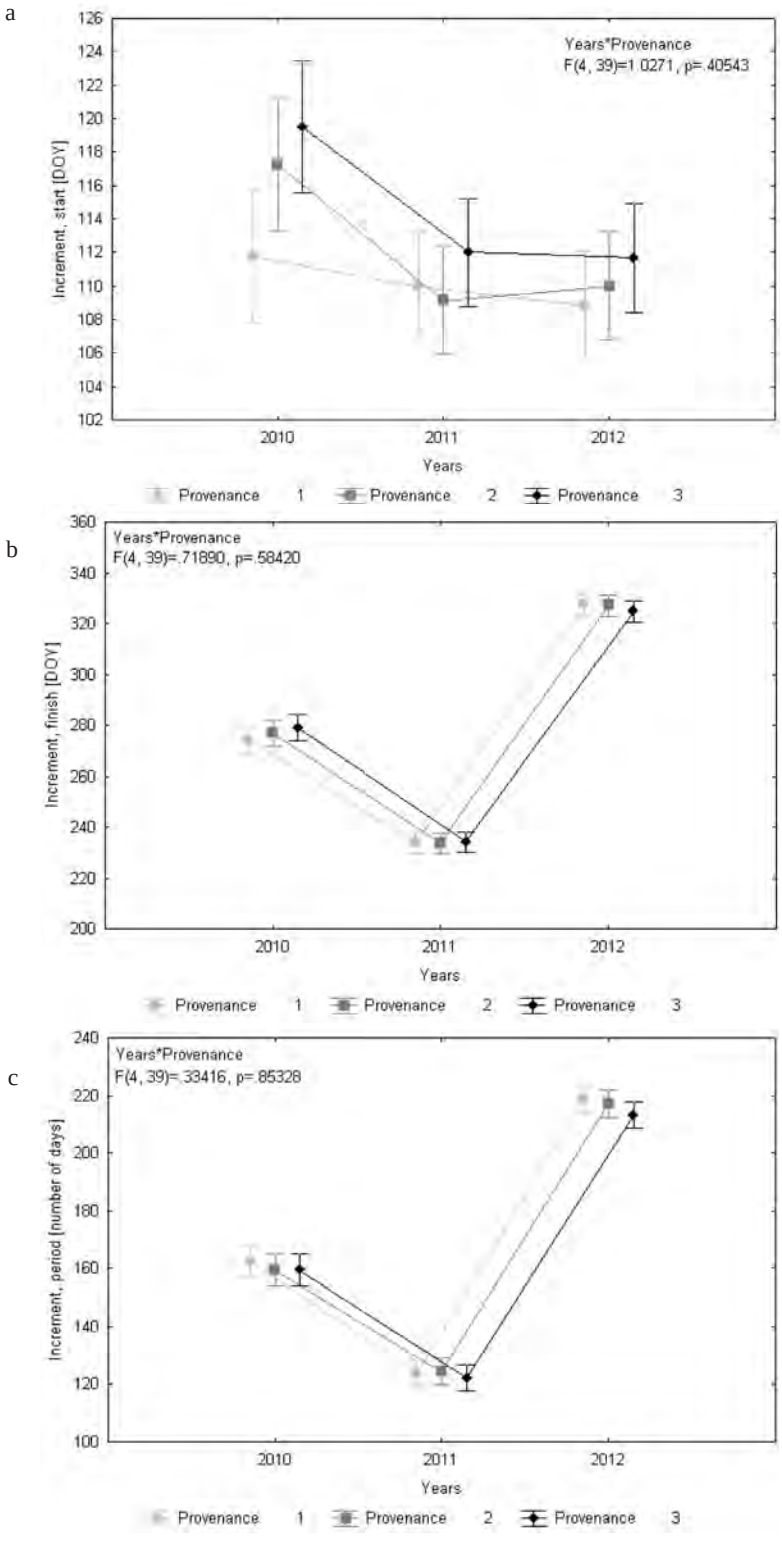

Fig. 2. Multifactorial analysis of variance of the beginning (a), end (b) and the length of growth period (c) between the provenances and years.

The end date of the growth and the growth length significantly differed only between the years (Fig. 2b, c). In the year 2012, the average growth period was by about 53 days longer than in the year 2010, and by 94 days longer than in the year 2011.

According to the findings of several authors, populations from high latitudes or higher elevations seem to start their growth earlier (Ekberg et al., 1985; Skroppa and Magnussen 1993; Hannerz, 1998), finish their growth earlier, have a shorter period of sprout lengthening (Modrzyński, 1995; Hannerz and Westin, 2000).

The analysis of diameter increment revealed significant differences in the monthly increments during April and May (Table 1) between individual years. In the year 2010, the average difference between monthly increments in April and May was 8.16 mm, while in 2011 the difference was 5.47 mm and in 2012 it was only 4.44 mm. This difference can be explained by the fact that in April, new foliage of spruce trees was not sufficiently developed (in relation to phenological phases), nor the optimum monthly mean air temperature for the sufficient tree activity was achieved. In the years 2011 and 2012, the onset of spring phenophases was faster and hence, the differences in the increment between April and May gradually decreased.

Phenological phases

The sums of effective air temperatures above 5 °C and soil temperatures above 1 °C that are needed for the onset of spring phenophases are given in Tables 2 and 3. For example, in the year 2010 the first phenophase leaf bud swelling (LBS) required the sum of effective air temperatures to be equal to 208 °C, 230 °C, and 275 °C for the first, second, and third provenance from elevations 500, 750 and 1,100 m a.s.l., respectively. From these results we can see that the sum of effective air temperatures required for the onset of the phenological phase increases with the increasing elevation. In the year 2011, lower sums of effective air temperatures were required, and in the year 2012 they further decreased (Table 2). Similar trend can also be observed in the effective sums of soil temperatures in Table 3.

Hence, we can state that the required sum of effective air and soil temperatures decreases from year to year. We also found that the third provenance, which originates from the highest elevation, requires the greatest sums of effective air and soil temperatures. Similar findings were presented by Magová (2011).

The differences in the onset of vegetative phenological phases between provenances and years we can see in Figure 3. The results of the onset of vegetative phenological phases of spruce in Slovakia showed the time shift between elevation groups (Škvareninová, 2013). Our results showed that the ability of gradual time delay of the onset of other vegetative phenophases of spruce with the increasing elevation remained also

Table 2. Sums of effective air temperature above 5 °C for the onset spring phenological phases, LBS, leaf bud swelling; SL, sprouting of leaves; LU, leaf unfolding

Year	2010					2011					2012				
Provenance/Phenol. phase	LBS	Date	SL	Date	LU	LBS	Date	SL	Date	LU	LBS	Date	SL	Date	LU	Date
1. 500 m a.s.l.	208.7	11.4.	315.6	23.4.	468.6	125.3	28.3.	298.9	14.4.	466.6	181.4	30.3.	318.7	18.4.	471.2	30.4.
2. 750 m a.s.l.	230.3	14.4.	365.4	27.4.	468.6	202.7	5.4.	358.0	20.4.	496.1	205.0	4.4.	392.1	25.4.	578.4	6.5.
3. 1,100 m a.s.l.	275.2	19.4.	420.3	1.5.	584.0	213.2	6.4.	400.8	23.4.	576.5	241.4	7.4.	416.3	27.4.	594.1	7.5.

Table 3. Sums of effective soil temperature above 1 °C for the onset spring phenological phases, LBS, leaf bud swelling, SL, sprouting of leaves; LU, leaf unfolding

Year	2010					2011					2012				
Provenance/Phenol. phase	LBS	Date	SL	Date	LU	LBS	Date	SL	Date	LU	LBS	Date	SL	Date	LU	Date
1. 500 m a.s.l.	178.6	11.4.	275.4	23.4.	399.9	85.4	28.3.	221.9	14.4.	361.5	53.6	30.3.	174.4	18.4.	296.3	30.4.
2. 750 m a.s.l.	194.0	14.4.	315.8	27.4.	399.9	142.3	5.4.	273.8	20.4.	385.5	72.4	4.4.	239.4	25.4.	379.0	6.5.
3. 1,100 m a.s.l.	236.2	19.4.	361.7	1.5.	510.5	151.1	6.4.	306.0	23.4.	475.4	92.9	7.4.	260.3	27.4.	393.1	7.5.

after its plantations in new uniform conditions of Zvolenská valley.

The differences at the end of vegetative phenological phases between provenances and years we can see in Figure 4. The differences in the length of vegetative phenological phases between provenances and years we can see in Figure 5. The average length of LBS phenophase was 10–13 days, 11–17 days, and 9–17 days,

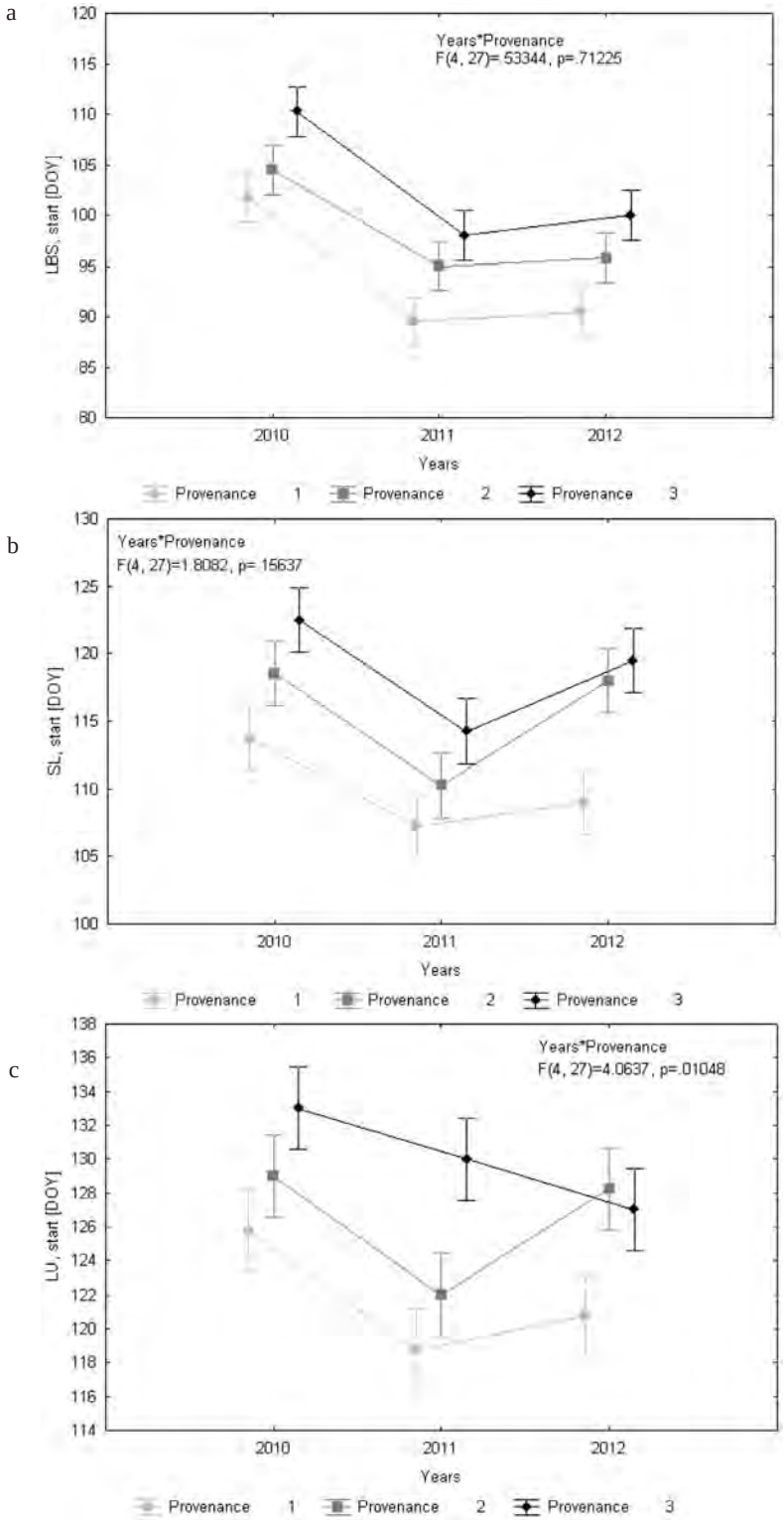

Fig. 3. Multifactorial analysis of variance of the onset of spring phenological phases between the provenances and years, a) LBS, b) SL, c) LU.

in the case of the first, second, and third provenance, respectively. The average length of SL phenophase for the first, second and third provenance was 7–10 days, 8–10 days, and 9–13 days, respectively.

According to the results of several authors, time shift of spring phenophases to earlier dates was more profound than the shift of summer and autumn phenophases; and also early spring phenophases seem to have

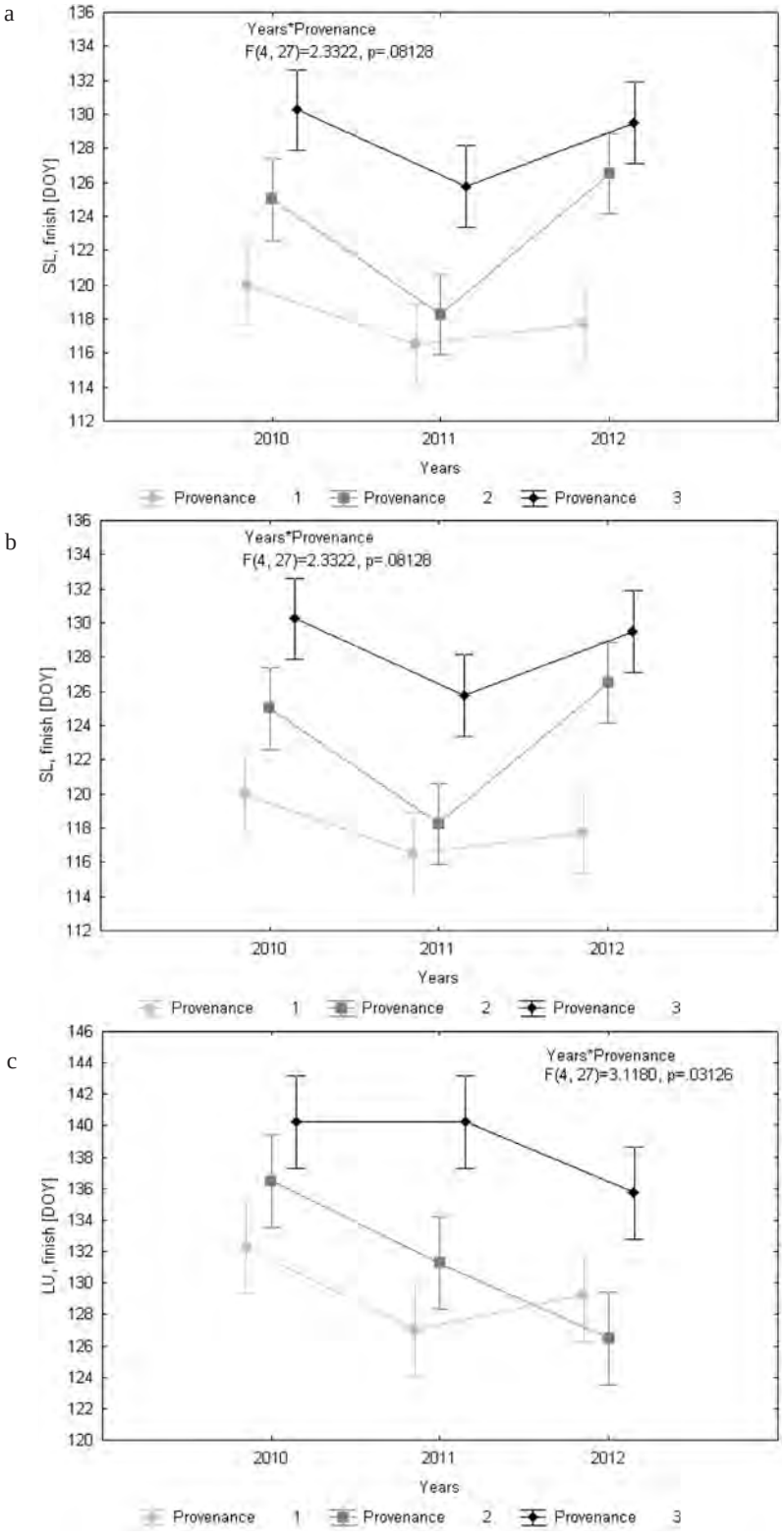

Fig. 4. Multifactorial analysis of variance of the end date of spring phenological phases between the provenances and years, a) LBS, b) SL, c) LU.

larger time shifts to earlier dates than late spring and early summer phenophases (MENZEL, 2004; GORDO and SANZ, 2005; WOLFE et al., 2005; MILLER-RUSHING et al., 2007).

Conclusion

The research was performed on the sample trees of Norway spruce (*Picea abies* /L./ Karst.) originating

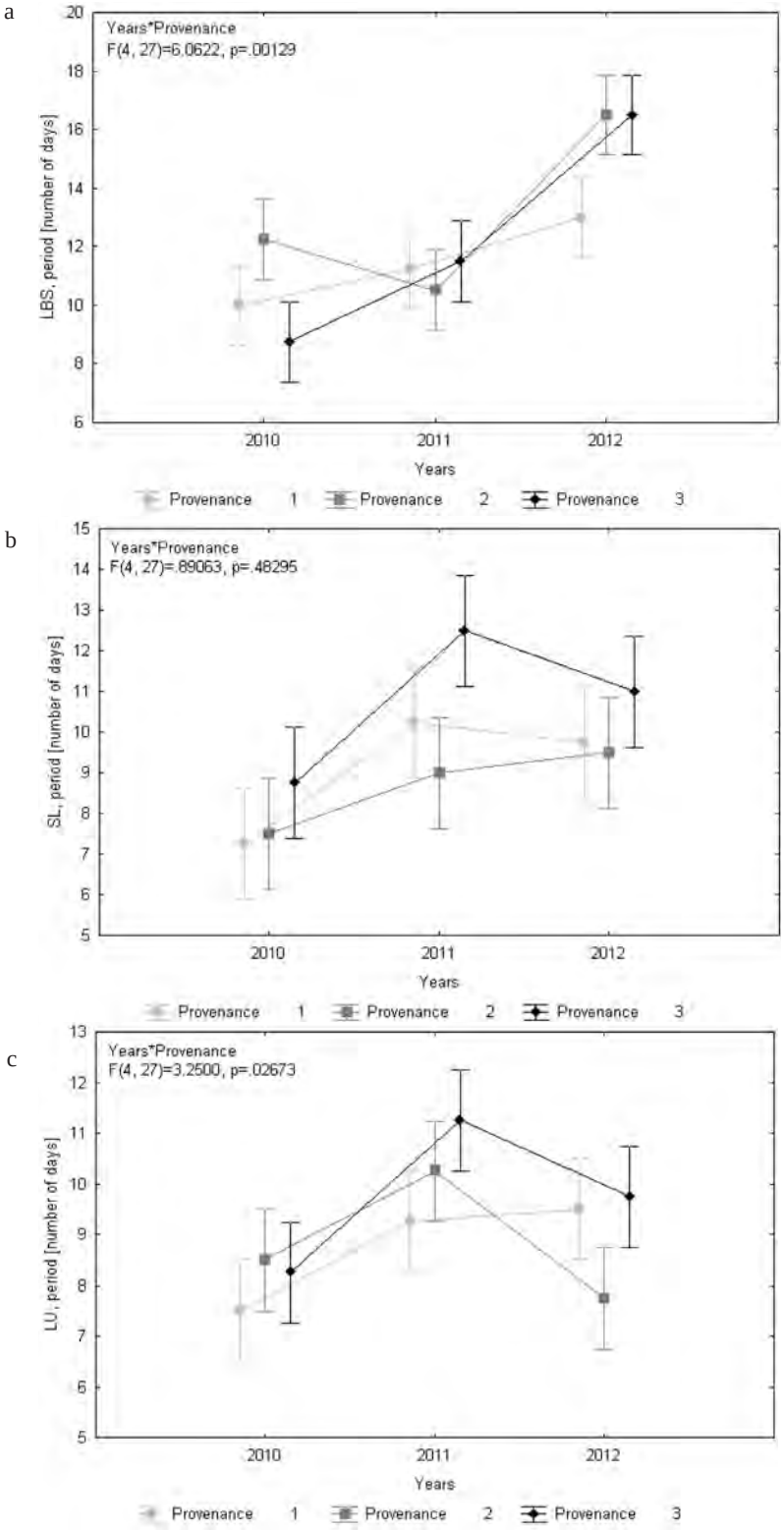

Fig. 5. Multifactorial analysis of variance of the duration of spring phenological phases between the provenances and years, a) LBS, b) SL, c) LU.

from three different elevations (500, 750 and 1,100 m n. m.) from the Volovské vrchy Mts and growing in Borová hora arboretum, in the years 2010, 2011 and 2012. The analysis of variance revealed significant differences at the beginning, end, and length of growth between the years. We also found significant differences between the provenances at p = 0.05 significance level, but they did not have a clear positive or negative trend during the three years. The differences changed every year. Multifactorial analysis of variance did not show any clear positive or negative trend in significant differences between the provenances and years during the investigated period at p = 0.05 significance level.

The research confirmed the knowledge that the provenances from higher elevations begin their diameter growth later than the provenances from lower elevations. Diameter growth of the third provenance coming from the highest elevation started last, on average by 3–8 days later than in the case of the first and second provenance. In 2010, the first provenance finished its growth on average by 3 and 5 days earlier than the second and the third provenance, respectively. In the year 2011, all provenances finished their growth at the same time, and in 2012, the third provenance finished its growth by 3 and 2 days earlier than the first and the second provenance, respectively. In the year 2012, the growth period was by 53 and 94 days longer than in the years 2010 and 2011, respectively. The analysis of diameter increment revealed the lowest increment values equal to 1.66 mm in April 2010, while the increment in 2011 was 4.37 mm and in 2012 it was 2.94 mm. This was caused by the later onset of spring phenophases in April 2010 caused by lower air temperatures in this month.

We can conclude that during the years 2010 and 2012, each year a lower sum of effective air and soil temperatures required for the onset of phenological phases was needed. The highest effective sums of air and soil temperatures are needed for the third provenance originating from the highest elevation. Our results confirmed that the origin of the provenances causes significant time shifts of phenophases, the phenophases of the provenances originating from higher elevations are shifted to a later time. This could be in future efficiently utilised for planting provenances from higher elevations at lower elevations with the aim to eliminate frost damage of spring sprouts.

Acknowledgement

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Using noninvasive DNA sampling to estimate abundance and some genetic properties of the Brown bear (*Ursus arctos*) in the Western Carpathians

Pavol Pepich[1, 2], Diana Krajmerová[3], Miroslav Saniga[4]

[1]Institute of High Mountain Biology, University of Žilina, Tatranská Javorina 7, SK-059 56 Tatranská Javorina, Slovak Republic, e-mail: pavol.pepich@ucj.uniza.sk,
[2]Institute of Foreign Languages, University of Žilina, Univerzitná 8215/1, SK-010 26 Žilina, Slovak Republic, e-mail: pavol.pepich@ucj.uniza.sk,
[3]Department of Phytology, Faculty of Forestry, Technical University in Zvolen, T. G. Masaryka 24, SK-960 53 Zvolen, Slovak Republic, e-mail: diana.krajmerova@tuzvo.sk,
[4]Institute of Forest Ecology of the Slovak Academy of Sciences, Research Station, SK-976 02 Staré Hory, Slovak Republic, e-mail: miro.saniga@gmail.com

Abstract

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In Slovakia, there is a constant need for scientifically based information to manage its bear population after it has been allowed to increase in size and range. In this study we assessed population size, sex structure and genetic variability of a local brown bear population in Strážovské vrchy Mts (North-western Slovakia). This goal has been achieved by using noninvasive method of genetic sampling in 2011–2012. Brown bear DNA for analysis was obtained from 94 out of 232 samples (41%), among which 24 unique genotypes were identified. Average observed heterozygosity was 0.56 in 2011 and 0.63 in 2012. Minimum population size was determined from the number of unique genotypes and population size estimates were calculated via Lincoln-Peterson CMR method (n = 38) and Rarefaction models according Kohn method (n = 36), Eggert method (n = 25) and Chessel's equation (n = 19). Additionally, relative spatial activity and movement pattern of some individuals have been inferred from the distribution of typed samples.

Keywords

noninvasive genetic tracking, population size, rub trees, sex ratio, spatial activity pattern

Introduction

The brown bear (*Ursus arctos* Linnaeus, 1758) is the most widespread ursid in the world with a distribution in Europe, Asia and North America traversing from northern arctic tundra to dry desert habitats (Swenson et al., 2000). In Europe, almost all European bears belong to large populations occurring in Eastern and Northern European countries, whereas less than 1% of all European bears are found in western and south-western Europe (Zedrosser et al., 2001).

In Slovakia, the brown bear (*Ursus arctos*) population is increasing and expanding after that successful conservation measures were employed during the 20th century (Hell and Slamečka, 1999). Currently, the Western Carpathian bear population extends across all mountain ranges of central, northern, northwest and northeast Slovakia but shows only a fragmented, discontinuous habitat pattern due to topographic characteristics of the country's landscape resulting in mountain ranges with prime bear habitat separated by areas of denser human settlement and activity in broad river valleys (Rigg and Adamec, 2007). As a consequence, for Slovak society, there is a constant need for scientifically based information to successfully manage its bear population. Two important issues in conservation and management are of pressing concern: 1. to understand how bears use their habitat, at different spatial-temporal scales, and 2.

to estimate size, structure and trend of the population from regional to national scales.

However, such data are often difficult to obtain, especially for rare, elusive or species with large home ranges that overlap to certain degree (Bellemain et al., 2005). To overcome many of these obstacles several methods have been developed over a time to address the needs of conservation biologist and researchers. Particularly, the development of noninvasive DNA-based methods have brought several advantages over conventional methods as genetic samples (i.e., hairs or faeces) are easily collected without the need to see, disturb or trap the animal (Taberlet et al., 1999). Because each individual is characterized by a unique multilocus genotype, it is possible to determine the number of animals sampled and, through the use of statistical models, also to estimate population size (Kohn and Wayne, 1997; Mowat and Strobeck, 2000; Bellemain et al., 2005). Additionally, important population data on behavioural ecology like home range, habitat use and spatial activity pattern can be partially inferred from distribution of typed samples (Kohn et al., 1999).

Brown bears had been apparently absent from 1930s until the mid1960s (Hell and Slamečka, 1999; Rigg and Adamec, 2007) but re-colonised Strážovské vrchy Mts during the period 1967–1984, when the recovering Western Carpathian bear population expanded its range 40 km north-westwards (Janík et al., 1986). According to Turček (1949) and Feriancova (1955) bears still did not occur in this area until 1960 and the first reference to 4 migrating bears comes from range-wide bear census in 1966 and 1968 (Škultéty, 1970; Randík, 1971). Currently, Brown bears in Strážovské vrchy Mts form a stable sub-population in the westernmost portion of their Carpathian range.

Before the beginning of this study, bear numbers were estimated up to 30 individuals based on snow and mad tracking, direct observations and camera trapping (Pepich and Pepich, 2012; Pepich and Pepich, 2013). We, however assume that some of them might have home ranges extending beyond the study area (data from telemetry or DNA studies are not yet available from nearby mountain ranges). The main aim of our study was to examine the degree of genetic variability and several population parameters such as minimum population size, population size estimates and social structure. Relative spatial activity and movement pattern of some individuals have been consequently inferred from distribution of typed samples as described by Kohn et al. (1999).We also tried to evaluate whether it is feasible to use hairs from rub trees to produce population estimates.

Material and methods

Study area

Fieldwork was conducted in the Protected landscape area (PLA) Strážovské vrchy (included in the NATURA 2000), which extends over an area of 300 km^2 in northwest Slovakia, forming part of the Inner Western Carpathian Mountains. To obtain more credible data, some additional areas of 200 km^2 that lie immediately to the PLA in the east, west and south were also included in the fieldwork because all these parts form relatively compact bear habitat and there is an assumption that this area is shared by one sub-population unit. Total size of the study area (Fig. 1) was 500 km^2. Elevations range from c. 200 m in walleyes to 1,213 m in the central part of Strážovské vrchy Mts and 1,352 m in the south of Lučianska Malá Fatra Mts. Most of the area is covered with intensively managed deciduous and mixed forest (78%), dominated by European beech (*Fagus syl-*

Fig. 1. Location of the study area in Slovakia and its total size (500 km^2).

vatica L.) followed by Norway spruce (*Pices abies* L.) and Sycamore maple (*Acer pseudoplatanus* L.), 19% accounts for mountain meadows and farmland and 3% for built-up and water areas (Pepich and Pepich, 2012). The study area covered fully 8 and partially 20 hunting grounds.

Sample collection

The noninvasive sampling was conducted in two consecutive years 2011 and 2012. Each year, there were two study periods (spring-early summer and autumn). The study area was divided into 22 parts (transects), where regular searches for bear hair and faeces were conducted in 2–3 weeks' time periods. Samples for genotyping were detected only in the southern parts (200 km^2) of the study area (Fig. 2).

A total 232 samples were collected of which 191 were hair samples and 41 were faeces samples. Hair samples were collected regularly from 48 out of 90 known bear rub trees (n = 181), several day beds (n = 4) and four dens (n = 6), whereas all 41 faeces samples were collected opportunistically throughout the whole year on forest roads and animal trails. Individual hairs or clumps of hair stuck in bark were scanned visually and those that showed probability of the dry cells were placed in an envelope with cautious approach to prevent contamination by human DNA. Consequently, all remaining hairs were removed mechanically (using a brash) from the bark to prevent recollection of the same hair in next sampling collection. No hair plucking devises (barbed wire) were used for hair collection at rub trees, only hair naturally stuck in bark by bear rubbing against a tree was used. Searches for bear faeces were conducted throughout the study area, even in parts, where bears are usually rare or non-existent (Pepich and Pepich, 2012). When a bear scat was discovered, sample of 1–2 cm^3 was picked up with a stick of wood and put in a labelled plastic bag. A different stick and plastic bag were used for each sample. Only samples from relatively fresh faeces were taken (subjective opinion). For each sample, a sampling date, a geographical location (WGS84) and the collector's name were recorded.

Sample preservation and DNA extraction

To limit the degradation of DNA before extraction, we preserved hair dry or with silica gel. Each hair sample (5–50 hairs) was placed in a different labelled envelope, whereas all faeces samples were stored dry in a freezer (–20 °C) as described by Walsh et al. (1991), Taberlet et al. (1997).

All extractions were carried out in a room dedicated to processing noninvasive samples like hair and faeces.

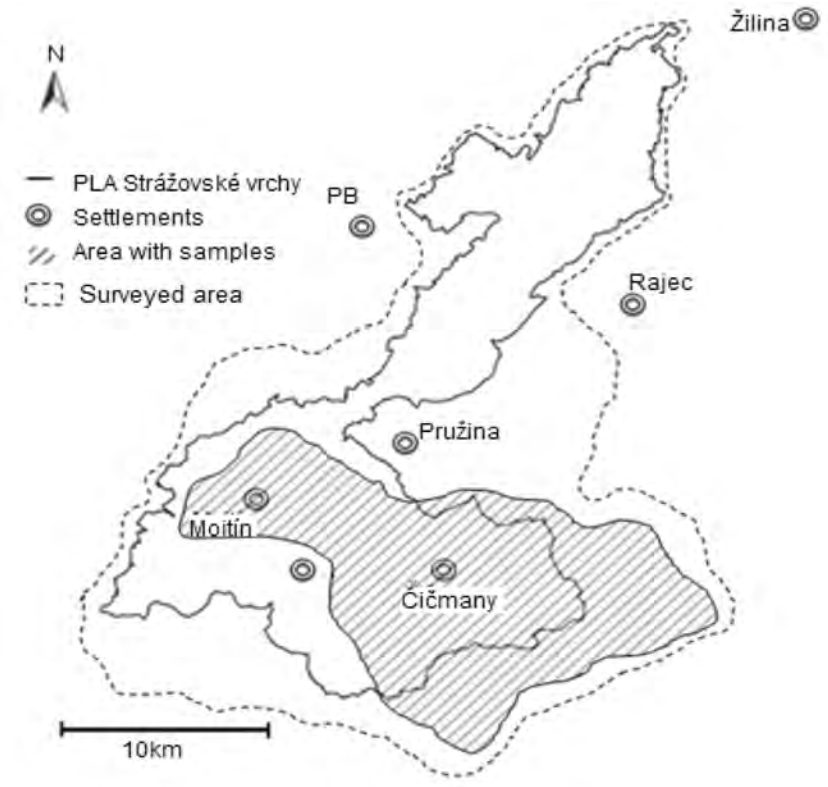

Fig. 2. Distribution of samples within the study area in 2011–2012.

For all collected hair samples, only one extraction was carried out per sample and only one hair was used per extraction. The suitable hairs were chosen by binocular magnifier loupe according to the presence of hair bulbs (dry cells). The root part (5 mm) of the hair was cut and added to 50 µl of extraction buffer (10 mM Tris-HCL pH 8.0, 50 mM KCL, 0.5% Tween20) with addition of 1 µl of the Proteinase K (20 mg ml^{-1}). For every collected faeces sample, DNA extractions were performed using the QIAmp DNA Stool mini Kit (Qiagen 51504) according the manufacturer's instructions. This kit has been developed especially for this type of material. To detect cross-contamination, tubes without bear faeces or without hair root were treated identically as samples through both the extraction procedure and the subsequent amplification (Walsh et al., 1991).

Genetic typing and sex determination

For individual genotyping, ten polymorphic microsatellite loci Mu10, Mu50, Mu23, G10L, Mu15, G10C, Mu59, G10P, Mu09, G1D, SRY (Table 1) were amplified using PCR. These markers were chosen for their discriminatory power (loci with the lowest PI) based on previous brown bear noninvsive data set (Skrbinšek at al., 2010).

Bear sex was determined by amplification of SRY fragment on the Y chromosome together with other microsatellites (Skrbinšek at al., 2010). Primers were fluorescently labelled. All markers were amplified in one reaction and products were amplified using Qiagen multiplex Mix (Qiagen), the final mixture volume for PCR reaction was 6 µl, DNA volume was 2 µl. Preliminary analyses were carried out and only samples that could be typed in some loci were kept for subsequent analysis. Consequently, PCR reactions and electrophoresis were carried out three times. PCR products (fragment lengths) were analysed using automatic sequencer ABI 3130 (Applied Biosystems). The final mixture volume consisted of 9 µl formamide; 0.2 µl size standard and 0.8 µl PCR product. Genotypes were determined by using GeneMapper 4.0.

Table 1. Characteristics of used markers

Primer	Microsatellite sequence	Flourescence	Allele size range (bp)	Reference
Mu10F	ATTCAGATTTCATCAGTTTGACA	FAM	121–127	Bellemain et al., 2004
Mu10R	TCAGCATAGTTACACAAATCTCC			
Mu50F	GTCTCTGTCATTTCCCCATC	FAM	94–100	Bellemain et al., 2004
Mu50R	AACCTGGAACAAAAATTAACAC			
Mu23F	TAGACCACCAAGGCATCAG	NED	143–157	Bellemain et al., 2004
Mu23R	TAGACCACCAAGGCATCAG			
G10LF	ACTGATTTTATTCACATTTCCC	PET	141–161	Bellemain et al., 2004
G10LR	GATACAGAAACCTACCCATGCG			
Mu15F	CTGAATTATGCAATTAAACAGC	PET	117–129	Taberlet et al., 1997
Mu15R	AAATAAGGGAGGCTTGGGT			
G10CF	AAAGCAGAAGGCCTTGATTTCCTG	VIC	122–138	Paetkau et al., 1995
G10CR	GGGACATAAACACCGAGACAGC			
Mu59F	GCTCCTTTGGGACATTGTAA	NED	98–118	Bellemain et al., 2004
Mu59R	TGACTGTCACCAGCAGGAG			
G10PF	TACATAGGAGGAAGAAAGATGG	VIC	141–173	Taberlet et al., 1997
G10PR	AAAAGGCCTAAGCTACATCG			
Mu09F	AGCCACTTTGTAAGGAGTAGT	VIC	190–196	Taberlet et al., 1997
Mu09R	ATATAGCAGCATATTTTTGGCT			
G1DF	CTACTCTTCCTACTCTTTAAGAG	FAM	171–178	Paetkau et al., 1995
G1DR	ATCTGTGGGTTTATAGGTTACA			
SRYF	GAACGCATTCTTGGTGTGGTC	PET	75	Taberlet et al., 1997
SRYR	TGATCTCTGAGTTTTGCATTTG			

Reliability of the DNA results

To distinguish individual samples among themselves with confidence we used sufficient number of polymorphic markers with low probability of identity – PI (Paetkau and Strobeck, 1994; Waits et al., 2001). Using five markers with lowest PI, the probability of finding identical non-kin genotypes would be 1 : 3.5 million what highly exceeds total world brown bear population and it is therefore very little probable that in the study area occur two bears with the identical genotype (Table 2). Reliability of genotypes was assessed with the program RELIOTYPE with default settings.

Table 2. Probability of identical genotypes

	PI (biased)	PI (unbiased)	PI (sibs)
Mu59	$9.131\ 10^{-2}$	$5.316\ 10^{-2}$	$3.891\ 10^{-1}$
Mu23	$8.826\ 10^{-3}$	$2.956\ 10^{-3}$	$1.539\ 10^{-1}$
G1D	$1.242\ 10^{-3}$	$2.859\ 10^{-4}$	$6.688\ 10^{-2}$
G10P	$2.054\ 10^{-4}$	$3.143\ 10^{-5}$	$3.086\ 10^{-2}$
Mu15	$3.471\ 10^{-5}$	$3.594\ 10^{-6}$	$1.431\ 10^{-2}$
G10C	$7.286\ 10^{-6}$	$5.801\ 10^{-7}$	$7.039\ 10^{-3}$
Mu09	$1.916\ 10^{-6}$	$1.154\ 10^{-7}$	$3.692\ 10^{-3}$
Mu10	$5.052\ 10^{-7}$	$2.631\ 10^{-8}$	$2.002\ 10^{-3}$
Mu50	$1.475\ 10^{-7}$	$6.487\ 10^{-9}$	$1.105\ 10^{-3}$
G10L	$8.064\ 10^{-8}$	$2.994\ 10^{-9}$	$8.296\ 10^{-4}$

Genetic variability

Observed (H_o) and expected (H_e) heterozygosity were calculated using Cervus 3.0 software (Field Genetics). Observed descriptive statistics for each locus (mean number of alleles per locus, heterozygosities and polymorphic information content) were calculated from genotypes.

Population size estimates

Minimum population size was determined by number of unique genotypes successfully typed from collected samples in 2011 and 2012 (Taberlet et al., 1997; Bellemain et al., 2005). Total population size estimates were subsequently calculated from faecal and hair genotypes by using Lincoln-Peterson CMR estimator and Rarefaction statistical models.

CMR estimator

The CMR (Capture-Mark-Recapture) estimator was based on grouping identical multilocus genotypes and compiling a "capture" and "recapture" history for each individual by dividing the data set into 20 weekly sampling period for 2011 and 16 weekly period for 2012 or by dividing the data set into spring – early summer sampling period and autumn sampling period in 2011 and 2012 respectively. Only the weeks with typed samples were considered for estimating population size. If an individual's hair or faeces were captured two or more times within the same capture period, only one capture was considered. Consequently, the following Lincoln-Peterson CMR estimator was applied to estimate the population size (Seber, 1982):

$$N = \frac{(C+1)(M+1)-1}{R+1},$$

where N = estimate of total population size, M = number of unique genotypes typed in 2011 or in autumn sampling period, C = number of unique genotypes typed in 2012 or in spring early summer sampling period, R = number of unique genotypes that were typed in both years 2011 and 2012 or were retyped in both sampling periods within the same year.

Rarefaction indices

Following the method described in Kohn et al. (1999), we compared the multilocus genotype of each sample with all those drawn previously and calculated the population size as the asymptote of the relationship between the cumulative number of unique genotypes and the number of samples typed. This curve is defined by the equation: $y = (ax) / (b + x)$, where a is the asymptote, x is the number faeces sampled, y is the number unique genotype, and b is the rate of decline in the value of slope. Chessel proposed to use equation (**y = a – a(1 – 1/a)^x**) which in the case of heterogeneity of capture probability seems to underestimate the population size. Eggert et al. (2003) derived another estimator with a similar equation: $y = a(1 - e^{bx})$.

Results

We managed to collect 232 samples (191 hair and 41 faeces samples) in two consecutive years 2011 and 2012 (Table 3). In 2011, 20 faecal samples and 150 hair samples were collected and 68 (41%) were successfully amplified for ten loci (including the sex locus). From these 68 samples, 16 unique genotypes were obtained (50% males and 50% females). Each multilocus genotype was found from 1 to 29 times. In 2012, 21 faecal samples and 41 hair samples were collected and 26 (42%) provided enough DNA for a complete genetic typing at all ten polymorphic loci (including the sex locus). From these 26 samples, 13 unique genotypes were obtained (46% males and 54% females). Each multilocus genotype was found from 1 to 6 times. In 2012 we sampled 5 individuals (32%) identified also in 2011. In total, sufficient brown bear DNA for analysis was obtained from 94 (41%) out of 232 samples.

Table 3. Sampling for DNA analysis in 2011 and 2012

	Hair		Faeces		Total		Unique genotypes
	Samples	Typed	Samples	Typed	Samples	Typed	
2011	150	54	20	14	170	68 (41%)	16
2012	41	15	21	11	62	26 (42%)	13
Total	191	69 (37%)	41	25 (63%)	232	94 (41%)	24

Using programme Gimlet (Valiére, 2002), a total of 31 different genotypes were identified, but only 24 were identified with confidence among these 94 samples. Other seven genotypes were considered not to be reliable due to the missing alleles. If only one allele was missing and other alleles matched with other genotype such samples were believed to belong to the same individual. Three samples (3.1%) that showed no available data on more than two alleles were not considered for further analysis and in some samples (5%) sex of individuals could not be determined.

Minimum population size was determined by number of unique genotypes successfully typed from collected samples in 2011 (16 individuals) and 2012 (13 individuals) and 24 individuals in 2011–2012. Total population size estimates were calculated via Lincoln–Petersen CMR estimator. The CMR estimate was calculated using the number of unique faeces and hair genotypes typed in 2012 (C = 13), number of unique genotypes typed in 2011 (M = 16) and number of unique genotypes that were typed in 2011 and then retyped in 2012 (R = 5). Using the Lincoln–Petersen CMR model the total population size has been estimated to 38.6 individuals. The Lincoln-Petersen CMR model was also used to estimate population size in 2011 and 2012 respectively, where number of unique genotypes typed in autumn 2011 (C = 14) and autumn 2012 (C = 8), number of unique genotypes typed in spring-early summer 2011 (M = 7) and spring–early summer 2012 (M = 7), number of unique genotypes that were typed in spring-early summer 2011 and then retyped in autumn 2011 (R = 5) and number of unique genotypes that were typed in spring-early summer 2012 and then retyped in autumn 2012 (R = 2). Total population size was calculated by statistical models Lincoln-Petersen CMR and was estimated at 19 individuals in 2011 and 23 individuals in 2012 and 38 individuals in 2011–2012. The population size was also estimated by programme GIMLET to be 36 according to Kohn's method (Kohn et al., 1999), 25 according Eggert's method (Eggert et al., 2003), 19 according to Chessel's equation for joint analysis 2011 and 2012. Kohn method in 2011 accounted for 23 individuals and 53 in 2012. According to Eggert method there were 15 bears in 2011 and 26 in 2012, whereas according to Chessel's equation there were 11 bears in 2011 and 17 in 2012 (Fig. 3).

It seems that the best results were provided by Kohn method (n = 36) and Eggert (n = 25) method, which are relatively consistent with population size (n = 32) obtained in range-wide census conducted in 2012 (Pepich and Pepich, 2012). Chessel's equation provided underestimation of total population size as it was lower than obtained minimum population size (n = 24). Our findings confirm that Chessel's method has tendency to underestimate population size, especially in cases of heterogeneity of capture probability like is also our situation. Chessels method gave even lower estimates than minimum number of unique genotypes found.

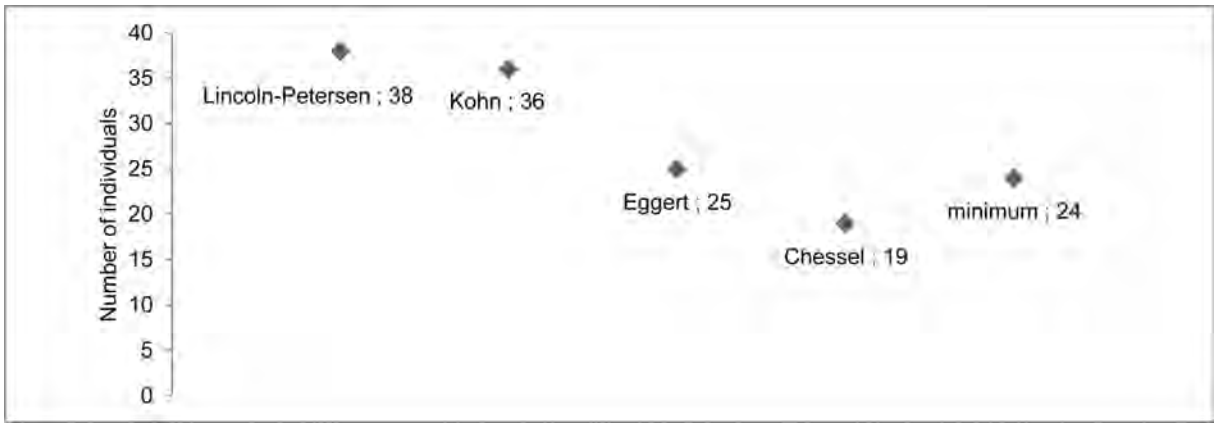

Fig. 3. Population size estimates based on statistical models and minimum population size.

The number of alleles per locus ranged from three to six, with a mean observed heterozygosity of 0.55 in 2011. The number of alleles per locus ranged from four to seven, with a mean observed heterozygosity of 0.63 in 2012 (Table 4).

Five individuals (21%) out of 24 were typed in both years 2011 and 2012. Genotypes of ten individuals (42%) were sampled more times (2–29), whereas fourteen (58%) unique genotypes were sampled only once. All typed samples were found in an area of c. 200 km^2, in the central and south-eastern part of study area, which represents 40% out of the total study area (Fig. 2). Although, the presence of bears is occasionally reported even in other areas (south-western and northern) during our study we did not detect any hair or faeces samples in these parts of the study area. The sampling location of each genotype was plotted on a map to see their distribution (Fig. 4).

Table 4. Observed and expected heterozigosity and effective number of alleles in 2011–2012

	H_{exp}		H_{obs}		n_a		n_e	
Locus	2011	2012	2011	2012	2011	2012	2011	2012
G10C	0.62	0.6524	0.62	0.7419	5	5	2.6406	2.8772
G10L	0.27	0.1920	0.23	0.1724	3	4	1.3740	1.2377
G10P	0.66	0.6383	0.77	0.7333	4	4	2.9391	2.7650
G1D	0.70	0.6778	0.46	0.5862	4	4	3.3465	3.1033
Mu09	0.58	0.6296	0.62	0.6207	4	4	2.3960	2.6998
Mu10	0.55	0.5541	0.77	0.7097	3	4	2.2092	2.2427
Mu15	0.66	0.7598	0.54	0.7241	4	5	2.9157	4.1634
Mu23	0.76	0.7320	0.54	0.6129	6	7	4.1220	3.7320
Mu50	0.54	0.6165	0.31	0.5862	3	4	2.1818	2.6078
Mu59	0.77	0.7384	0.69	0.7931	5	7	4.2985	3.8227
Mean	0.61	0.6191	0.55	0.6281	5	4.8	2.6406	2.9252

H_{exp}, expected heterozygozity; H_{obs}, observed heterozygosity; n_a, observed number of alleles 59 n_e, effective number of alleles.

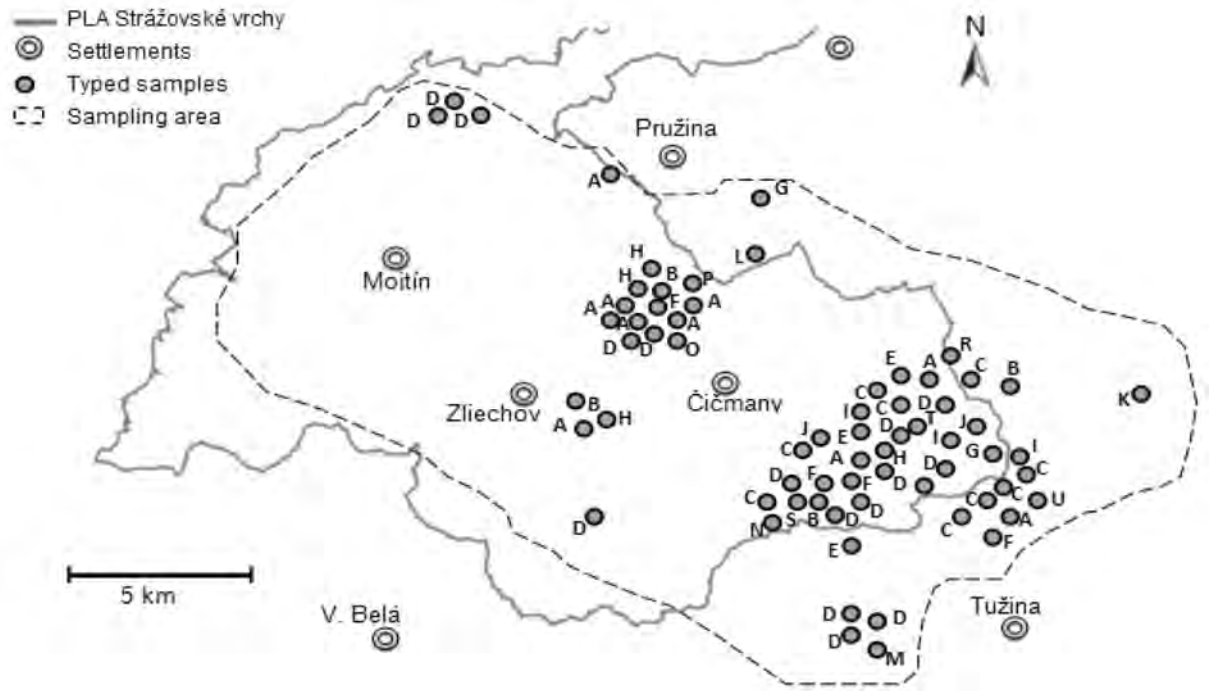

Fig. 4. Location of typed samples in the study area. The sampling area (250 km^2) indicates where hair and faeces samples were collected. Dark-grey circles represent unique genotypes. Sex structure of genotypes is as follows: A♂, B♀, C♂, D♂, E♀, F♂, G♀, H♂, I♀, J♂, K♂, L♂, M♀, N♂, O♀, P♂, R♂, S♀, T♀, U♀, V♀, X♀, Y♂, Z♀.

The spatial activity pattern was consequently estimated from distribution of typed samples. This was particularly possible for 10 unique genotypes whose samples had been typed more times and their subsequent polygons (MCP) could have been portrayed from the distribution of typed samples. The best results were obtained for one male (D) with 29 typed samples covering an area of 175 km^2 (Fig. 5) within seven different hunting grounds and ten cadastres in 30 days. All individuals with more typed samples showed wide spatial activity from spring to autumn but their late autumn / early winter and early spring samples were predominantly detected in one area, which indicates the main den site in Strážovské vrchy Mts.

Discussion and conclusion

This study demonstrated that it is feasible to obtain several population parameters like minimum population size, population size estimate and sex structure from noninvasively collected samples without capturing or even seeing the animals.

To facilitate this genetic study, we utilized data from a 10 year long fieldwork, where positions of all known bear rub trees, day beds and den sites were recorded (GPS). Data on bear distribution and spatial activity pattern obtained by annual ground tracking survey (2010–2012) and range-wide census (2010, 2012) were also taken into consideration. Very useful data were provided by camera trapping method on the feasibility of rub trees for genetic study because these trees proved to be visited not only by dominant males but also by other categories of bears such as females with cubs and young independent bears. Average number of bears visiting a particular rub tree (n = 10) accounted for 4.4 bears in 2011 and for 4.8 bears in 2012 (data supported by camera trapping).

Minimum population size was obtained by unique genotypes typed in each year. Population size estimate was calculated by statistical models: Lincoln-Petersen

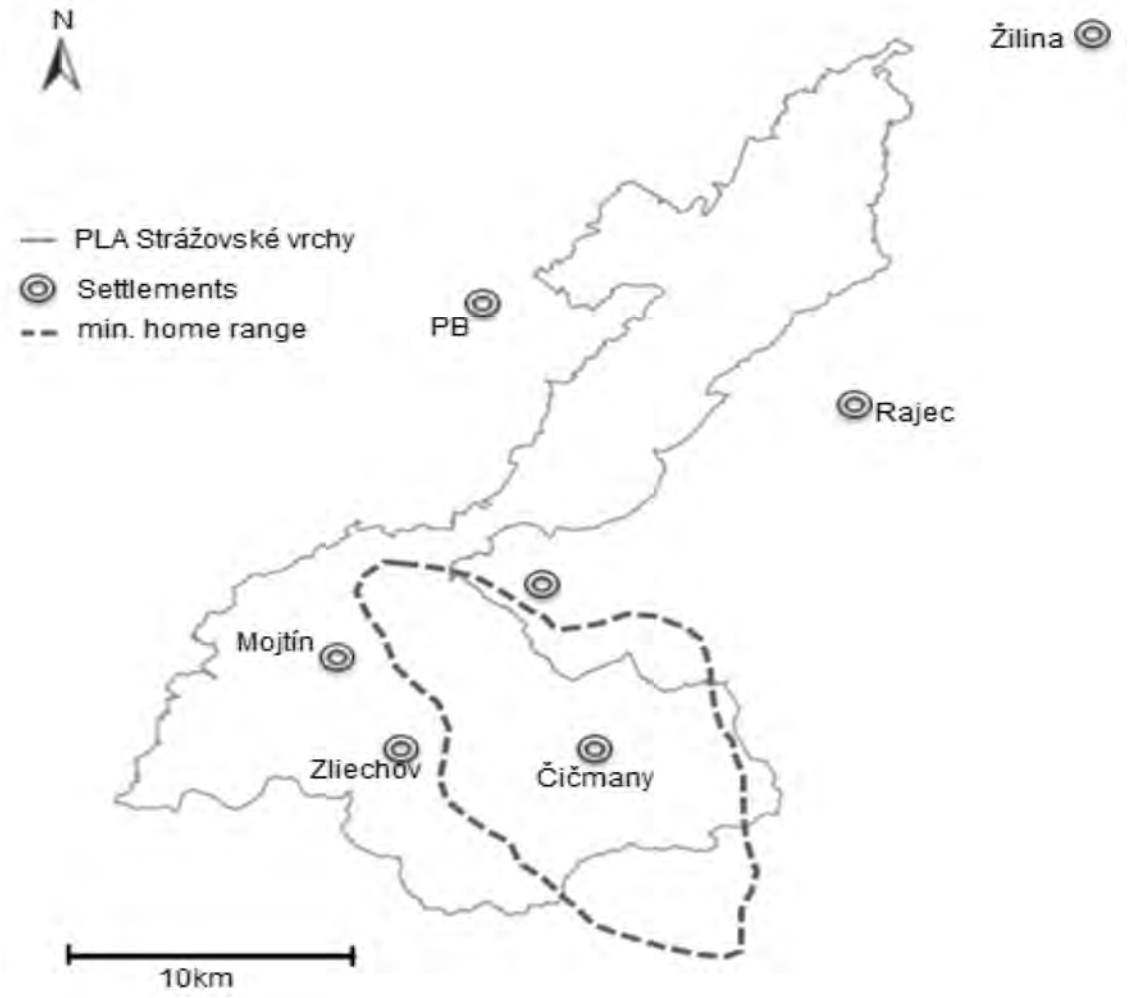

Fig. 5. Spatial activity pattern of a male bear (D) with MCP of 175 km^2.

CMR (n = 38) and rarefaction models according Kohn method (n = 36), Eggert's method (n = 25) and Chessel's equation (n = 19) for joint analysis 2011 and 2012. These population estimates were subsequently compared with reliable census results (excellent time and weather conditions) from October 2012 (32 individuals). Results of all statistical models ranged between 19–38 individuals. We can thus conclude that in all conducted research methods and statistical models in the study period (2011–2012), population size of brown bear in PLA Strážovské vrchy Mts (c. 300 km^2) and surroundings areas (c. 200 km^2) does not exceed 40 individuals.

Not only fundamental population parameters like size and structure but also some important genetic properties have been detected from typed samples. The relatively high level of heterozygosity and low degree of inbreeding detected in this study or in the study conducted by Graban et al. (2013) imply that the sub-population of bears in Strážovské vrchy is not geographically isolated and gene exchange with other segments of the Western Carpathian population is maintained to certain degree. Although the results of the present study shows relatively high variability, microsatellite analysis of brown bears in Malá Fatra National Park (Janiga et al., 2006) found higher numbers of alleles per locus. Moreover, the difference between observed and expected heterozygosity was greater in Malá Fatra than in Strážovské vrchy and low values of FIS for each locus demonstrated a higher occurrence of heterozygotes (Graban at al., 2013). Higher variability has also been found in central and northern Slovakia (Straka et al., 2012). Lower genetic variability of bear population in the study area might be a result of its smaller size and lower migratory level when compared with populations in other mountain ranges in Slovakia.

Our sampling efforts have been biased by uneven distribution of collected samples because there are considerable variations in bear distribution, spatial activity pattern, and density of bears within the study area (Pepich and Pepich, 2013). The study area is also characterised by uneven distribution of rub trees, from which hair samples were systematically collected for genotyping. Large amount of such trees can be found only in the central and south-eastern parts of the study area, where high densities of bears can be found. No bear rub trees had been found before or throughout the study period in south-western and northern parts. Considerable variations in bear densities but mainly a fact that some parts of the study area are inhabited by bears only seasonally (transient individuals) biased also our faecal sampling. Bear faeces for genotyping were not detected in northern and south-western parts of the study area as a consequence that bears inhabit these parts only seasonally when they migrate in search for food or mating opportunities.

Low number of successfully typed hair samples (37%) might be results of low quality and quantity of DNA but is mainly due to the financial limits for DNA analysis. However, proportion of typed faeces samples (63%) in this study is relatively consistent with other studies conducted in Slovakia or elsewhere in the world. In other studies, the successful portion of typed samples was for faeces 70% (Bellemain et al., 2005) in Scandinavia and in the DNA study conducted the Pyrenees only 57 samples (36 hair and 21 faeces) out of 352 samples provided enough DNA for a complete genetic typing at all polymorphic loci (Taberlet et al., 1997). In Slovakia, the successful portion of typed samples was for faeces 48% in Veporské vrchy and 65% in Poloniny (Straka et al., 2009). In the study conducted by Graban et al. (2013), 39% of samples provided enough DNA for a genetic typing.

To gain better picture of the studied population we matched four unique genotypes (17%) with corresponding track sizes (faeces or hair samples were found when tracking bears in snow) and nine unique genotypes (38%) were matched with remote sensing camera photos (10 rub trees were fitted with trail cameras). Three genotypes were supplemented with both track sizes and remote sensing camera photos, one genotype was matched only with track size and six genotypes were matched only with remote sensing camera photos. No track data of dominant male (width of front paw at least 15 cm) were matched with genotypes and only one dominant male captured by remote sensing camera photos was matched with typed samples.

Our results represent a comprehensive study of a subpopulation which has hitherto received little attention from researchers. We demonstrated that noninvasive genetic methods have become an efficient tool and are especially appropriate for use with elusive species in small populations. We believe that obtained data on population size, population structure, spatial activity pattern, movement pattern, and genetic variability will be used for further work building on our study to contribute to comprehensive knowledge of this charismatic species and that knowledge will be finally employed for proper conservation and management of bears within and between protected areas.

We also hope that our results might confute spreading disinformation about bears in this part of Slovakia (e.g.: large number of bears in every cadastre, total population size exceeding 100 individuals, large number of dominant males) but mainly will be used as a means of providing credible and reliable data to inform both experts and the public in Slovakia.

Acknowledgements

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The influence of stand density on the structure of centipede (Chilopoda) and millipede (Diplopoda) communities in the submountain beech forest

Slavomír Stašiov, Marek Svitok

Department of Biology and General Ecology, Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, T. G. Masaryka 24, 960 53 Zvolen, Slovak Republic, e-mail: stasiov@tuzvo.sk

Abstract

Stašiov, S., Svitok, M. 2014. The influence of stand density on the structure of centipede (Chilopoda) and millipede (Diplopoda) communities in the submountain beech forest. *Folia oecol*., 41: 195–201.

The paper deals with the effect of stand density on the community composition of centipede (Chilopoda) and millipede (Diplopoda) communities. The study was conducted in Kováčovská dolina valley (Kremnické vrchy Mts, Central Slovakia) in 1997 and 1998 by pitfall trapping. In total, 17 species of centipedes from 4 families and 7 species of millipedes from 6 families were recorded. The intensity of previous thinning influenced the species structure of both studied communities. Species richness increased with increasing intensity of past thinning, especially for centipede communities. The effect of stand density was apparent for all but eurythopic species, namely: *Lithobius forficatus*, *Lithobius mutabilis* (Chilopoda) and *Unciger foetidus*, *Polydesmus complanatus* (Diplopoda).

Key words

beech forest, centipedes, Chilopoda, Diplopoda, Kováčovská dolina valley, Kremnické vrchy Mts, millipedes, stand density, Slovakia

Introduction

Centipedes and millipedes belong to the soil invertebrates, which respond quickly to environmental changes. Temperature and humidity of soil are considered the main abiotic factors influencing the structure of millipede communities (Branquart et al., 1995; Meyer et al., 1999; Gava, 2004). Besides that, the character of their communities is markedly influenced by vegetation. For example, the species structure of communities of both these groups is markedly influenced by stand density, which determine microclimate, the amount and quality of leaf litter and other environmental variables related to centipede and millipede distribution.

The impact of vegetation on community structure of Myriapoda was studied by Bokor (1993), Dangerfield (1992), David et al. (1999), Korsós (1997), Madari et al. (1996a, 1996b), Meyer and Singer (1997), Rahmani and Mayvan (2003), Stašiov and Maršalek (1998), Stašiov et al. (2012b), Tuf and Ožanová (1998), etc.

The work brings results of two-year research, dealing with the influence of stand density on the structure of centipede and millipede communities in the submountain beech forest.

Material and methods

The research was carried out on 4 research plots (marked as S1 to S4) situated in the Kováčovská dolina valley (Kremnické vrchy Mts) (48°38′ N, 19°4′ E). The altitude of experimental sites is 450–475 m a.s.l. All experimental sites were placed on the same slope with 30% gradient and western exposure. The soil on the experimental sites is a loamy-clay cambisol with the depth of 51–70 cm.

The forest stand consists of *Fagus sylvatica* (62% dominance), *Abies alba* (22%), *Quercus dalechampii* (7%), *Carpinus betulus* (6%) and *Tilia cordata* (3%). Its age is on average 90 years.

The stand density was modified on the experimental sites S1 (0.3 – heavy thinning), S2 (0.5 – moderate thinning) and S3 (0.7 – light thinning) in February, 1989. The last stationary (S4) remained without changes.

The research was carried out in 1997 and 1998 by pitfall trapping. The invertebrates were trapped on each of the experimental sites in 5 pitfall traps, placed on a contour line in a 5 m distance. 0.7l cylindrical jars with an upper diameter of 7.5 cm and a depth of 14 cm were used for this purpose. They were filled with 10% formaldehyde to one-third of their volume.

In both years of the investigations, the traps were exposed from April, 4 to October, 31. The trapped invertebrates were collected at 30-day intervals. The obtained samples were stored in 70% ethyl alcohol and are deposited at the author's workplace.

The Shannon-Weaver index of species diversity (*H'*) using the natural logarithm was used to compare the diversity of centipede and millipede communities on individual plots (Shannon, 1948). The evenness of communities (*E*) was calculated using the Shannon-Weaver index (Begon et al., 1990).

The species similarity of experimental sites was evaluated by means of hierarchical clustering. The cluster analysis was conducted using STATISTICA for Windows 5.1. (StatSoft, Inc., 1999) with Euclidean distance and Ward´s clustering algorithm.

An ordination analysis was used to gain more insight to species composition of sites. Principal coordinate analysis (PCoA) with Bray-Curtis dissimilarity was applied to square-root transformed species data. PCoA was performed in R language (R Development Core Team, 2011).

Results and discussion

In total, 970 individuals of centipedes and 675 individuals of millipedes were obtained. The occurrence of 17 species of centipedes from 4 families and 7 species of millipedes from 6 families was recorded on the studied area (Table 1).

The ascertained species structure of both studied communities of Myriapoda responds to the condition of environment in the submountain beech forests. The high species diversity, especially in centipedes, reflects a relatively low disturbance of the studied area.

The high species richness of centipede communities was also discovered by the other authors. For example, Poser (1988) found 10 centipede species in the beech forests near Göttingen in the North Germany. Stašiov et al. (2012a) revealed the occurrence of 12 centipede species in an enclave of the beech forest in Boky National Nature Reserve (Slovakia). Leśniewska (2000) discovered 19 centipede species at one beech forest site. Leśniewska (1997) found as many as 21 centipede species in the beech forests located in the Buki nad Jeziorem Lutomskim National Nature Reserve (Poland).

The high species richness of centipede communities in beech forests results from the fact that the beech forests offer very favourable habitats for several species. The most species rich in centipede communities can be found in this type of forest stands. Almost one third of all centipede species in Slovakia (42 species) can be found in the beech forests (monocultures or mixed forests).

Diplopodocoenoses of the beech forests are mostly species-poor. Altogether 43 millipede species were found in the beech forests of Slovakia. Communities of these stands are formed by euryvalent species and species of submontane and montane forests. However, some millipede species occurring in the beech forests are not strictly bounded to over-story composition but rather to other environmental conditions and therefore they occur also in other forest stand as well as in open habitats.

Most of the recorded species belong to the native European fauna with a relatively frequent occurrence in Slovakia. They are wide-spread in various forest habitats from lowlands to mountains. Nevertheless, the records of *J. curvicornis* (Diplopoda) are considered especially important. A Carpathian-endemic *J. curvicornis* is hither-to known only from Slovakia and Hungary (Bükk Mts), although its occurrence is also supposed in the Polish part of the High Tatras Mts. In Slovakia, this species is frequent especially in deciduous and coniferous forests with higher altitudes.

In addition to the list of myriapod species presented above, the occurrence of the following further species is known on the same experimental sites from the previous investigations: *Geophilus insculptus* Attems, 1895, *Lithobius borealis* Meinert, 1868 (Stašiov, 1998), *Schendyla nemorensis* (C. L. Koch, 1836), *Clinopodes linearis* (C. L. Koch, 1835), *Lithobius burzenlandicus* Verhoeff, 1931, *Lithobius crassipes* L. Koch, 1862, *Lithobius curtipes* C. L. Koch, 1847, *Lithobius microps* Meinert, 1868, *Lithobius muticus* C. L. Koch, 1847 (Stašiov, 2002) and millipedes *Polyxenus lagurus* (Linnaeus, 1758), *Craspedosoma rawlinsi* Leach, 1814 (Diplopoda) (Stašiov, 1998, 2002). In total, the occurrence of 26 species of centipedes and 9 species of millipedes has been known from Kováčovská dolina valley so far.

The intensity of previous thinning influenced especially the species richness of the studied communities on the observed sites. Species richness increased with increasing intensity of past thinning, especially for centipede communities (Table 1). The elevated species richness was probably caused by the occurrence of species that are able to tolerate more open habitats such as centipedes *S. crassipes*, *L. aeruginosus*, *L. dentatus* and *L. erythrocephalus*. On the other hand, sensitive forest

Table 1. The total epigeic activity of the species found out in 1997–1998 and diversity measures of centipede and millipede communities (*H´* – Shannon index, *E* – evenness)

Family / Species	Experimental site				∑
	S1	S2	S3	S4	
Geophilidae					
Geophilus flavus (De Geer. 1778)	1	1		1	3
Dignathodontidae					
Strigamia acuminata (Leach. 1814)	2		3	3	8
Strigamia crassipes (C. L. Koch. 1835)	1		1		2
Cryptopidae					
Cryptops parisi Brolemann. 1920	1		1	1	3
Lithobiidae					
Lithobius aeruginosus L. Koch. 1862	1				1
Lithobius agilis C. L. Koch. 1847	8				8
Lithobius austriacus (Verhoeff. 1937)		1			1
Lithobius dentatus C. L. Koch. 1844		1	1		2
Lithobius erythrocephalus C. L. Koch. 1847	1		1		2
Lithobius forficatus (Linnaeus. 1758)	44	124	77	111	356
Lithobius lapidicola Meinert. 1872	1	6		4	11
Lithobius macilentus L. Koch. 1862		2			2
Lithobius melanops Newport. 1845	2				2
Lithobius mutabilis L. Koch. 1862	117	160	101	118	496
Lithobius salicis Verhoeff. 1925	1	1			2
Lithobius tenebrosus Meinert. 1872			1		1
Lithobius tricuspis Meinert. 1872		1			1
Lithobius spp. juvenils	26	17	11	10	64
Lithobius spp. damaged	3		1	1	5
Glomeridae					
Glomeris hexasticha Brandt. 1833	44	40	45	33	162
Polyzoniidae					
Polyzonium germanicum Brandt. 1837			2		2
Julidae					
Julus curvicornis Verhoeff. 1899	29	34	41	41	145
Unciger foetidus (C. L. Koch. 1838)	35	8	37	6	86
Mastigophorophyllidae					
Mastigona bosniensis (Verhoeff. 1897)	5	10	9	13	27
Paradoxosomatidae					
Strongylosoma stigmatosum (Eichwald. 1830)	1			1	2
Polydesmidae					
Polydesmus complanatus (Linnaeus. 1761)	59	38	106	38	241
∑ centipede ex.	209	314	198	249	970
∑ centipede spp.	12	9	8	6	17
H´ – centipede communities	1.06	0.90	0.90	0.87	
E – centipede communities	0.43	0.41	0.43	0.49	
∑ millipede ex.	173	130	240	132	675
∑ millipede spp.	6	5	6	6	7
H´ – millipede communities	1.47	1.44	1.43	1.47	
E – millipede communities	0.82	0.89	0.80	0.82	

species may temporary disappear from the sites with lower stand density. The species richness of millipede communities was more or less similar across all stands.

The influence of stand density on the occurrence of saprophagous macroarthropoda (including millipedes) was ascertained by David et al. (1999) on 27 localities near Mas de Cazarils in southern France. They found that the species diversity was the highest on open stands. Both the frequency and the total biomass of saprophagous macroarthropoda were lower on dense stands.

Species richness and Shannon index of species diversity of both groups increased with increasing intensity of past thinning (Table 1). Different pattern was found for evenness where the highest values for centipedes and millipedes were reached at the reference stand S4 and moderate thinning stand S2, respectively.

The similarity of experimental sites was evaluated by cluster analysis using the data on the total epigeic activity of individual species during the entire period of investigations. The results did not demonstrate a significant influence of stand density on the species structure of both investigated communities. Nevertheless, we supposed that the eurythopic species (*L. forficatus, L. mutabilis, P. complanatus* and *U. foetidus*) markedly influenced the results of cluster analysis by their high epigeic activity on all the experimental sites compared (Table 1). Considering this fact, the data of all the eurythopic species were excluded from the further analysis.

The following cluster analysis separates stationary S1 from the others as the most different in terms of the community structure (Fig. 1). The other 3 experimental sites form a separate cluster. The experimental sites S2 and S4 are the most similar, while the stationary S3 is grouped with them as less similar.

The separation of the site S1 from the other experimental sites is apparently due to the different environmental conditions. While the site S1 has the lowest stand density and tree crown canopy, it is the only site where the compact understorey of a new generation of trees develops underneath the main tree layer. In all probability, this thick understorey influenced the microclimatic characteristics of the soil surface layers.

The different environmental conditions on the site S1 in comparison with S2–S4 can also be caused by the ecotone effect, which was the most marked on S1. This site is characterised by the highest difference between the stand density of its forest and the stand density of a forest that encloses it. Bokor (1993) also adverted on the influence of an ecotone effect on centipede communities in her work. This author studied the epigeic mac-

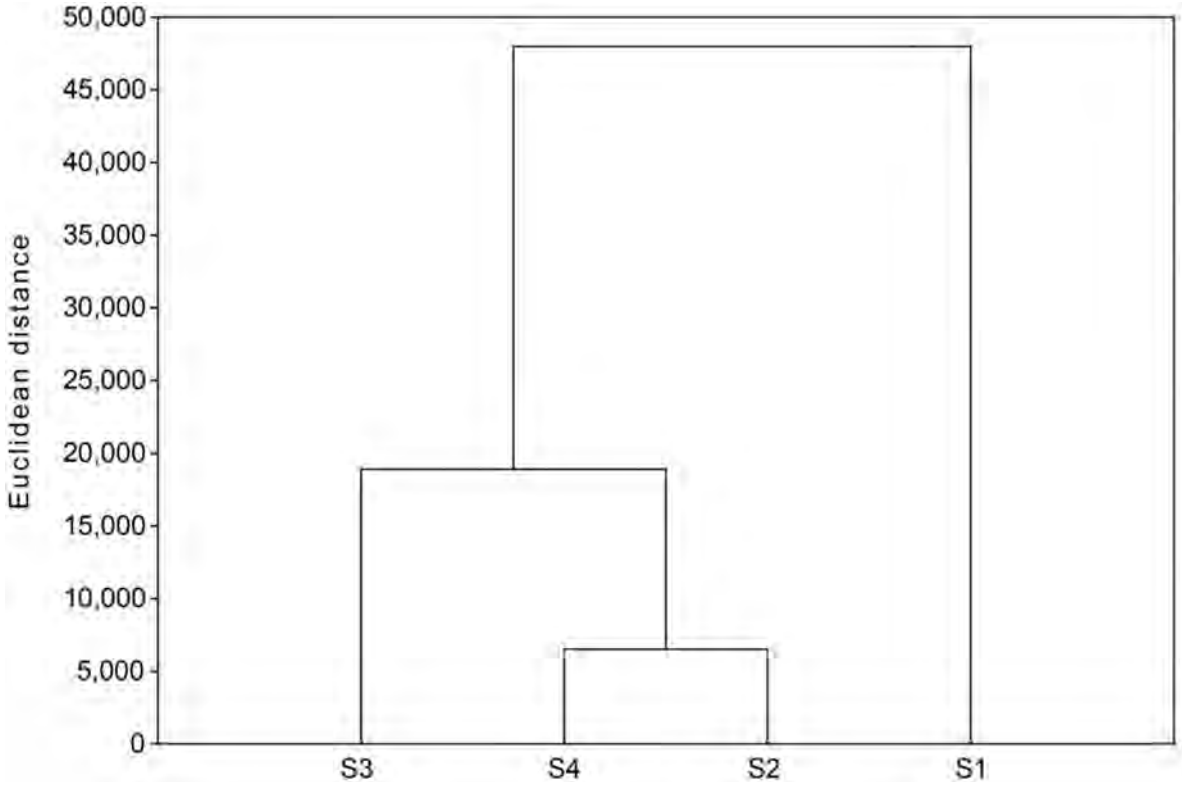

Fig. 1. Community similarity of experimental sites as revealed by the hierarchical cluster analysis using Ward´s method and Euclidean distances.

rofauna in the beech forests of Bükk Mts (Hungary). She found that the frequency of centipedes was twice higher on ecotone sites than on forest sites.

Tuf and Ožanová (1998) demonstrated the influence of ecotone on the epigeic activity of millipedes in chosen localities in Litovské Pomoraví Protected Landscape Area. Using the cluster analysis, they showed a close correlation between the character of habitat and the structure of millipede communities. They found that millipedes had the highest epigeic activity on an ecotone between a floodplain forest and an arable land.

PCoA, like cluster analysis, revealed that stands S2 and S4 were the most similar in term of the total epigeic activity of individual species during the entire period of investigations (Fig. 2). Centipedes *L. forficatus*, *L. lapidicola*, *L. mutabilis* and millipede *M. Bosniensis* were typical for these stands (Fig. 1, Table 1). Millipede *P. complanatus* and centipede *L. agilis* dominated at S3 and S1 stands, respectively.

Summary

Based on the obtained results we can conclude that the changed density of a mature forest in the submountain beech forests by previous thinning may significantly influence the structure of studied communities of epigeic macrofauna only when a very intensive thinning is used and the stand density is reduced to 0.3 or less. The stand density did not influence all the studied species alike. We must therefore analyse the influence of this factor on the structure and the dynamics of individual populations of epigeic fauna separately. The aspiration to generalize the relation of this group to the stand den-

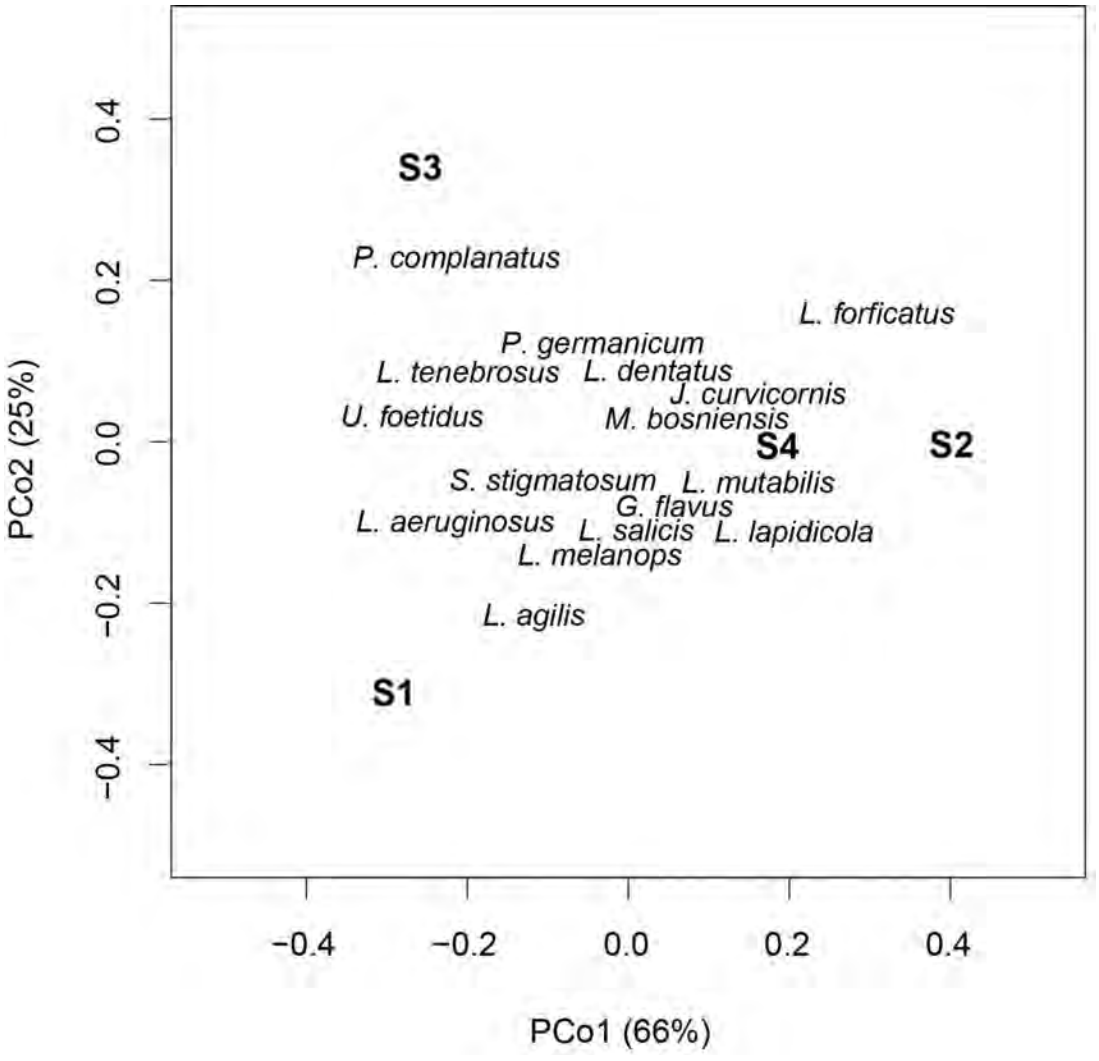

Fig. 2. Results of PCoA on square-root transformed species data. Variation explained by particular axis is given in parentheses. Ordination plot is scaled symmetrically. Only species with higher ordination scores are displayed.

sity is defective in terms of using these invertebrates in biomonitoring of environment.

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Trophic preferences of *Rossiulus kessleri* (Diplopoda, Julidae) for the litter of various tree species

Anastasija O. Svyrydchenko, Viktor V. Brygadyrenko

Department of Zoology and Ecology, Oles' Honchar Dnipropetrovsk National University, pr. Gagarina, 72, 49010 Dnipropetrovsk, Ukraine, e-mail: brigad@ua.fm

Abstract

Svyrydchenko, A. O., Brygadyrenko, V. V. 2014. Trophic preferences of *Rossiulus kessleri* (Diplopoda, Julidae) for the litter of various tree species. *Folia oecol.*, 41: 202–212.

This article analyses the results of a 10-day laboratory experiment investigating the consumption preferences of *Rossiulus kessleri* (Lohm.) when the leaf litter of 16 tree species was offered. During this experiment the rate of microbiological decay of the leaves of each tree species in the litter varied from 0.6 to 4.1% per day. The maximum rate of litter consumption by *R. kessleri* was found for *Acer negundo* L. (0.75 mg/mg of body weight per day), *Quercus robur* L. (0.50), *Malus domestica* Borkh. (0.36) and *Cerasus vulgaris* L. (0.35). For other tree species under investigation it did not exceed 0.11 mg/mg of body weight per day. In the dynamics of acclimation of *R. kessleri* to a new diet there are three main tendencies: 1) sharp increase in body weight on the first day of the experiment and stabilization thereof at a high level on the next day (for *Fraxinus lanceolata* Borkh. there was a 39% gain in weight, for *Salix alba* L. 29%, respectively); 2) decrease during the first three days of the experiment and further stabilization of body weight at a level not significantly different from the initial one (for *Populus alba* L., *Acer platanoides* L., *Cerasus vulgaris* L., *Gleditsia triacanthos* L. and *Aesculus hippocastanum* L.); 3) long-term acclimation of about one-week's duration with return to initial (*Quercus robur* L., *Ulmus laevis* Pall.) or lower body weight (*Populus nigra* L. – 13% lower than initial weight, *Acer negundo* L. – 12% lower, *A. pseudoplatanus* L. – 9% lower, *Pyrus communis* L. – 8% lower, *Pinus sylvestris* L. – 7% lower). In the conditions of absence of food in the container, average body weight for 4 days decreased by 10%, while a 50% death rate of the starved millipedes was recorded on the 6th day. The maximum proportion of daily weight of faeces (dry weight) to live body weight was observed in *R. kessleri* fed on *Pinus sylvestris* L. (0.58%), *Robinia pseudoacacia* L. (0.57%), *Pyrus communis* L. (0.54%) and *Populus alba* L. (0.53%). The minimum average daily formation of faeces was recorded in millipedes fed on *Acer pseudoplatanus* L., *Cerasus vulgaris* L., *Malus domestica* Borkh. and *Aesculus hippocastanum* L. (0.20–0.23% of body weight). A discrepancy in rates of microbiological and zoogenic decomposition of litter was found for various tree species in the conditions of the laboratory experiment.

Key words

Diplopoda, forest litter, Julidae, litter consumption, trophic preferences

Introduction

Litter saprophages of the macrofauna play various roles in the decomposition of plant residues: earthworms intensify the processes of humification (Edwards et al., 1970; Coleman et al., 1983), while Diplopoda and Isopoda, on the contrary, accelerate mineralization of plant residues (Anderson et al., 1983; Dangerfield and Telford, 1989). Peculiarities of the role of individual taxonomic groups in decomposition of plant residues have not been sufficiently studied: the scientific literature deals with feeding behavior of, at most, 15 millipede species (e.g. Telford and Dangerfield, 1993; Hashimoto et al., 2004).

Trophic activity of saprophages is determined by a number of environmental factors: temperature (Striganova, 1972; Fujiyama, 1996), substrate wetting (Baker, 1980; Brygadyrenko, 2006), length of

daylight (Boccardo and Penteado, 1995) and season of observations (Crawford, 1978; Dangerfield and Telford, 1991; Bailey and Kovaliski, 1993; David and Gillon, 2002), set of food substrates, degree of decomposition and depth of their occurrence in soil (Bocock and Heath, 1967), microbial population of intestines and food (Crawford et al., 1983; Márialigeti et al., 1985). Besides, saprophages as well as other animal species are often susceptible to diseases, in particular, in laboratory conditions (Federici, 1984). For this reason studies of the majority of saprophage species should be planned so as to eliminate the possibility of infections arising, which could otherwise influence the results of experiments.

The ratio of food components, i.e. leaf litter of various species of woody and herbaceous plants, has the greatest influence on the intensity of food consumption by saprophages (Bertrand et al., 1987; David et al., 2001; Alhamd et al., 2004; Ashwini and Sridhar, 2005). The issue of food selectivity of saprophages with regard to leaf litter has been insufficiently studied for most tree species (Hunter et al., 2003; Roy and Joy, 2009; Semenyuk and Tiunov, 2011). Species of Diplopoda, when consuming the litter contribute to its mechanical breaking down (Kheirallah, 1990), selectively consume it at the same time, or enrich it with definite groups of microorganisms (Kaneko, 1999; Maraun et al., 2003), digest it or, in contrast, increase the germination ability of plant species with fine seeds (Schowalter, 2011), eliminate helminth eggs and cysts of protozoan parasites (Szlavecz and Pobozsny, 1995). In areas of forest plantations disturbed by industrial forestry millipedes can actively participate in stabilization of the processes of litter decomposition (Topp et al., 2001; Salamon et al., 2008; Kulbachko and Didur, 2012).

Food consumed by saprophages can be assimilated with varying efficiency. The key factor in this case is the age of the animals: young millipedes feed more intensively and assimilate more efficiently the food consumed (Kondeva, 1980; Striganova and Prishutova, 1990; Brygadyrenko, 2004). Intensity of saprophage metabolism is directly evaluated by measuring oxygen consumption or emission of carbon dioxide (Dowdy, 1975; Penteado, 1987; Stamou and Iatrou, 1993; Webb and Telford, 1995; Maraun and Scheu, 1996). Indirectly, the metabolic rate is evaluated by increase in biomass of the animal or intensity of faecal formation (Van der Drift, 1975; Dangerfield, 1993). Addition of certain molecules (secondary compounds of plants) to the diet of saprophages or their exclusion leads to changes in the metabolic rate of saprophages (Sakwa, 1974; Cameron and Lapoint, 1978; Neuhauser and Hartenstein, 1978).

When carrying out laboratory experiments, it is important to evaluate changes in body weight of test animals, since in the course of experiments of several days duration there might be sharp deviations from the general tendency of changes in the animals' body weight (David, 1995). In most experiments, the body weight of each specimen is analyzed at the beginning and at the end of study, and intermediate data are neglected. Investigation of dynamics of substrate consumption processes shows a much more complex pattern in the system of changes in parameters (Couteaux et al., 2002).

Serious difficulties arise with assessment of trophic activity of animals in experiments lasting over one week since the feeding of saprophages at regular intervals and the concurrent uniform microbial decay of the litter necessitate subsequent simplification of experimental procedures and the introduction of corrective coefficients (Gere, 1956; Bertrand et al., 1987; David, 1998).

On the whole, the knowledge of the physiological peculiarities of Diplopoda (Hopkin and Read, 1992; Hertel, 2009) is still far less satisfactory than it is for the ecological and biological peculiarities of, for example, Isopoda (Lardies et al., 2004) and Lumbricidae (Lowe and Butt, 2005).

The ecology of *R. kessleri* has been studied in sufficient detail, compared with other Diplopoda species (Striganova, 1972, 1996; Prishutova, 2001a, 2001b; Brygadyrenko, 2004). However, the food preferences of this species with regard to various tree species have not been explored so far. Until now, no more or less complete analysis of the range of its trophic preferences has been carried out.

The objective of this paper is to evaluate the trophic preferences of *R. kessleri* for the leaf litter of 16 tree species, and assess the potential role of this Diplopoda species in the decomposition of the leaf litter of the most common tree species in its habitat.

Material and methods

Specimens of *R. kessleri* were taken manually on July 28–30, 2013 from the litter and soil surface in an artificial forest plantation of *Fraxinus lanceolata* Borkh. and *Robinia pseudoacacia* L. in the vicinity of Aleksandrovka village (48º45'01"N 34º58'10"E, Magdalynivka district, Dnipropetrovsk region of Ukraine). A total of 204 individuals of *R. kessleri* were used in the study: 17 experiments of 10 days duration were carried out, one for each of 16 plant species plus one control (without food). Before the beginning of the experiments all the millipedes were kept together in a single large container filled with a multi-species mix of leaf litter at optimum moisture. For each experiment there were 12 replicates involving a total of 12 Diplopoda specimens, each in its own separate container. Therefore each millipede was provided with 2 g of dry leaf litter of one species of tree for the duration of the experiment. The litter of the

tree species used in the experiment (see Figs 1–6) was also collected from the forest plantation where the *R. kessleri* were obtained (this contained isolated trees of other species besides ash and acacia) or from nearby fruit orchards. The litter collected was formed from leaves that had fallen the previous autumn.

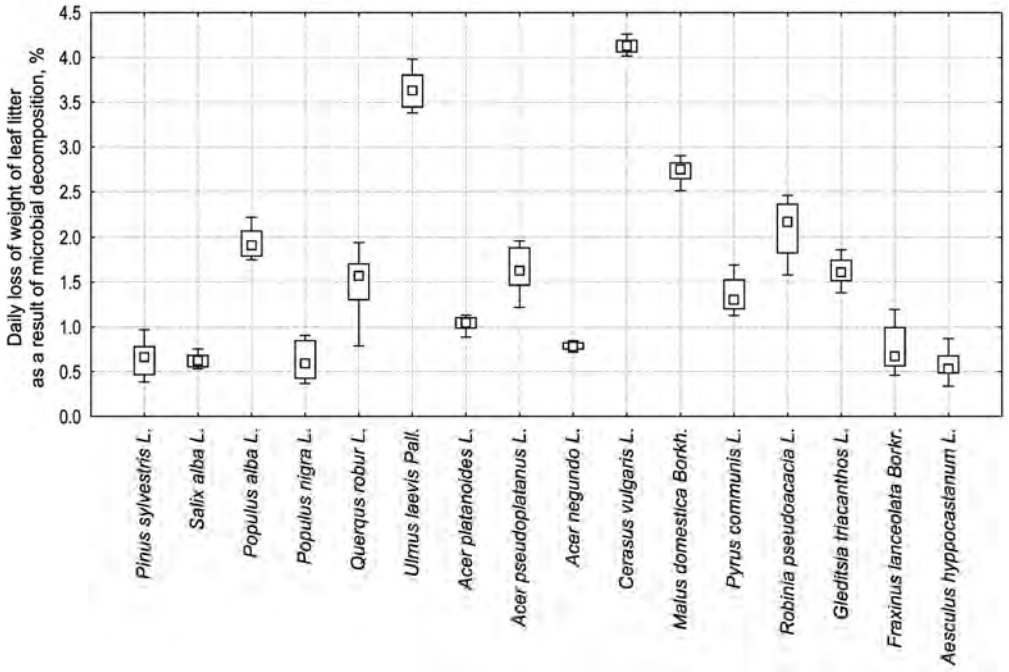

Fig. 1. Rate of microbial decomposition of the litter during experiment in the control (without food activity of *R. kessleri*): abscissa, tree species; ordinate, daily loss of weight of leaf litter as a result of microbial decomposition (%, $n = 12$).

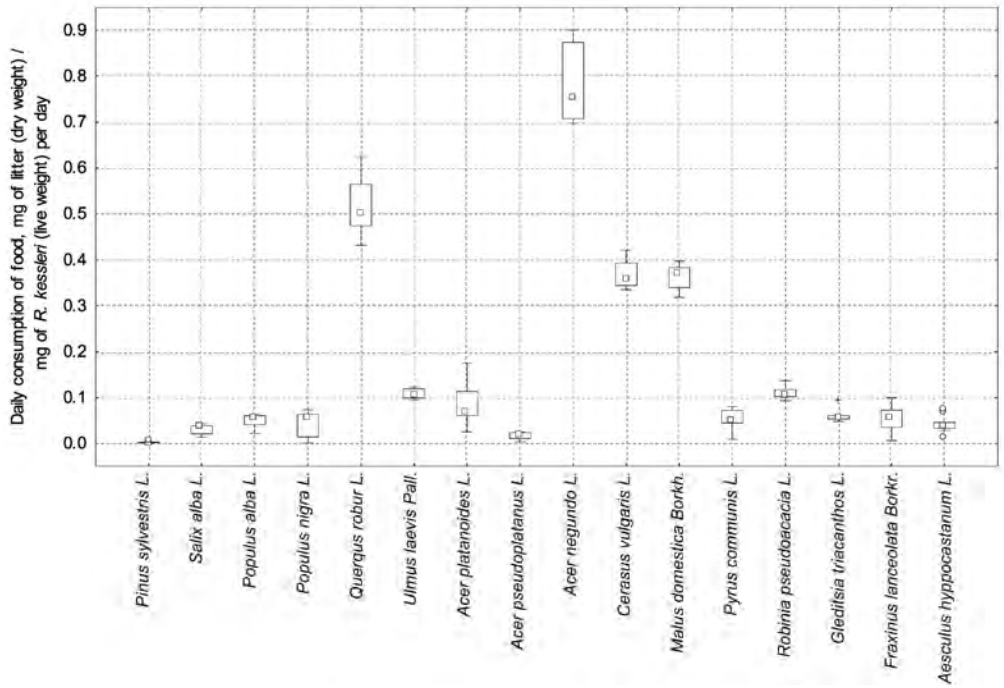

Fig. 2. Rate of consumption of leaf litter of various tree species by *R. kessleri*: abscissa, tree species; ordinate, daily consumption of food (mg of litter (dry weight) / mg of *R. kessleri* (live weight) per day, $n = 12$).

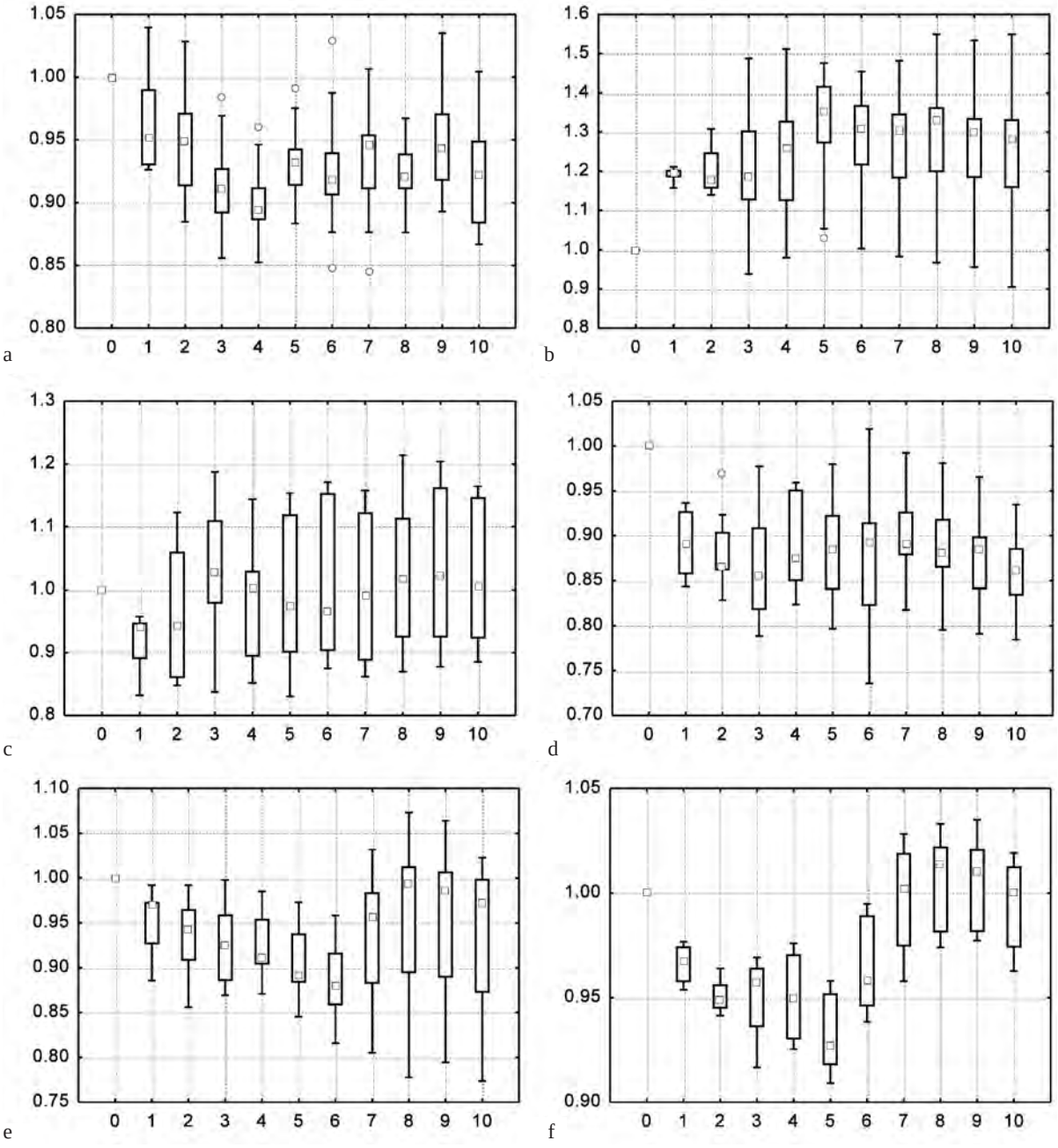

Fig. 3. Dynamics of *R. kessleri* body weight, when it is fed on litter of various tree species: a, *Pinus sylvestris*; b, *Salix alba*; c, *Populus alba*; d, *P. nigra*; *e*, *Quercus robur*; fV, *Ulmus laevis*; abscissa, time (days from the beginning of experiment); ordinate, ratio of *R. kessleri* body weight to its weight before the experiment (in all variants of the experiment $n = 12$).

For the experiments, millipedes (347 ± 86 mg by live weight) were placed in plastic cups of 0.5 l in volume. The weight of the millipdedes, their faeces and the litter was determined with the use of a torsion balance (with accuracy of 0.5 mg). The millipedes were weighed separately before and on each day of the experiment. The faeces of each millipede were weighed only at the end of the experiment after the excrement had been separated from the leaf litter. The leaf litter in each container was weighed in its dry state (prior to sprinkling) before the experiment and, having been dessicated, after the experiment. Throughout the study, a consistent temperature of +26 to +28 ºC and air humidity of 75–90% were maintained in the laboratory. To maintain constant humidity in the containers, the litter was periodically and evenly sprayed with distilled water using spray cans. With a view to reducing moisture evaporation, the cups were covered with paper.

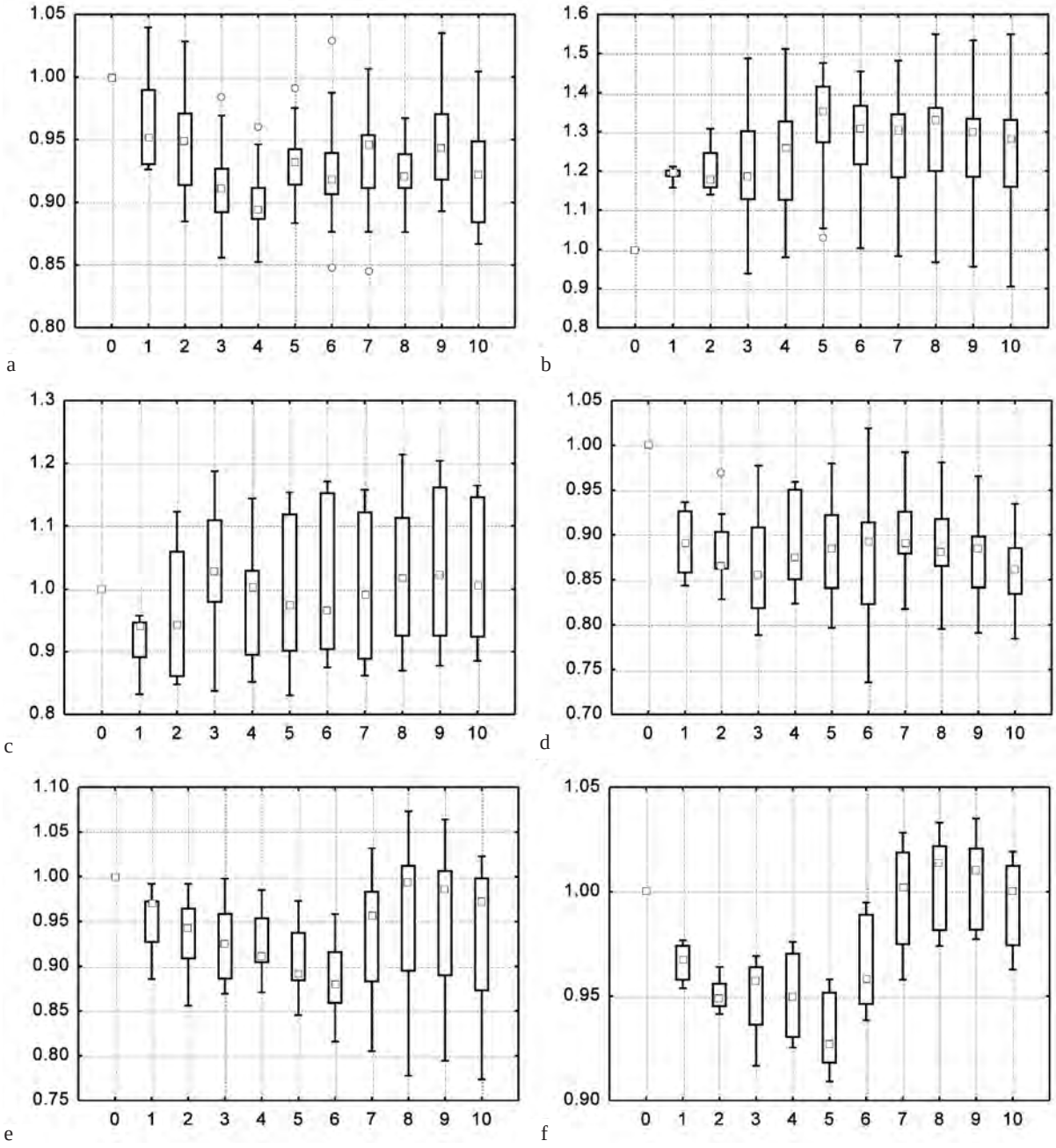

Fig. 4. Dynamics of *R. kessleri* body weight, when it is fed on litter of various tree species: a, *Acer platanoides*; b, *A. pseudoplatanus*; c, *A. negundo*; d, *Cerasus vulgaris*; e, *Malus domestica*; f, *Pyrus communis*; abscissa, time (days from the beginning of experiment); ordinate, ratio of *R. kessleri* body weight to its weight before the experiment (in all variants of the experiment $n = 12$).

Food consumption (C_I^*) was calculated by a modified formula of David (1998):

$$C_I^* = (M_0 - M_0 D - M_n) / (1 - D)^{1/2},$$

where M_0 – initial food weight (dry weight) offered to millipede for consumption, M_n – food weight (dry weight) at the end of the experiment not consumed by the millipede, D – coefficient of reduction of food weight as a result of its microbiological decomposition calculated with the use of control set of experiments ($n = 12$) in identical containers without millipedes ($D = (M'_0 - M'_n) / M'_0$, where M'_0 and M'_n – dry weight of food at the beginning and at the end of control experiment without the presence of millipedes).

Primary processing of measurement results was performed in MS Excell software package. All data (rate of microbial decay, rate of consumption of leaf litter, changes in *R. kessleri* body weight, daily formation

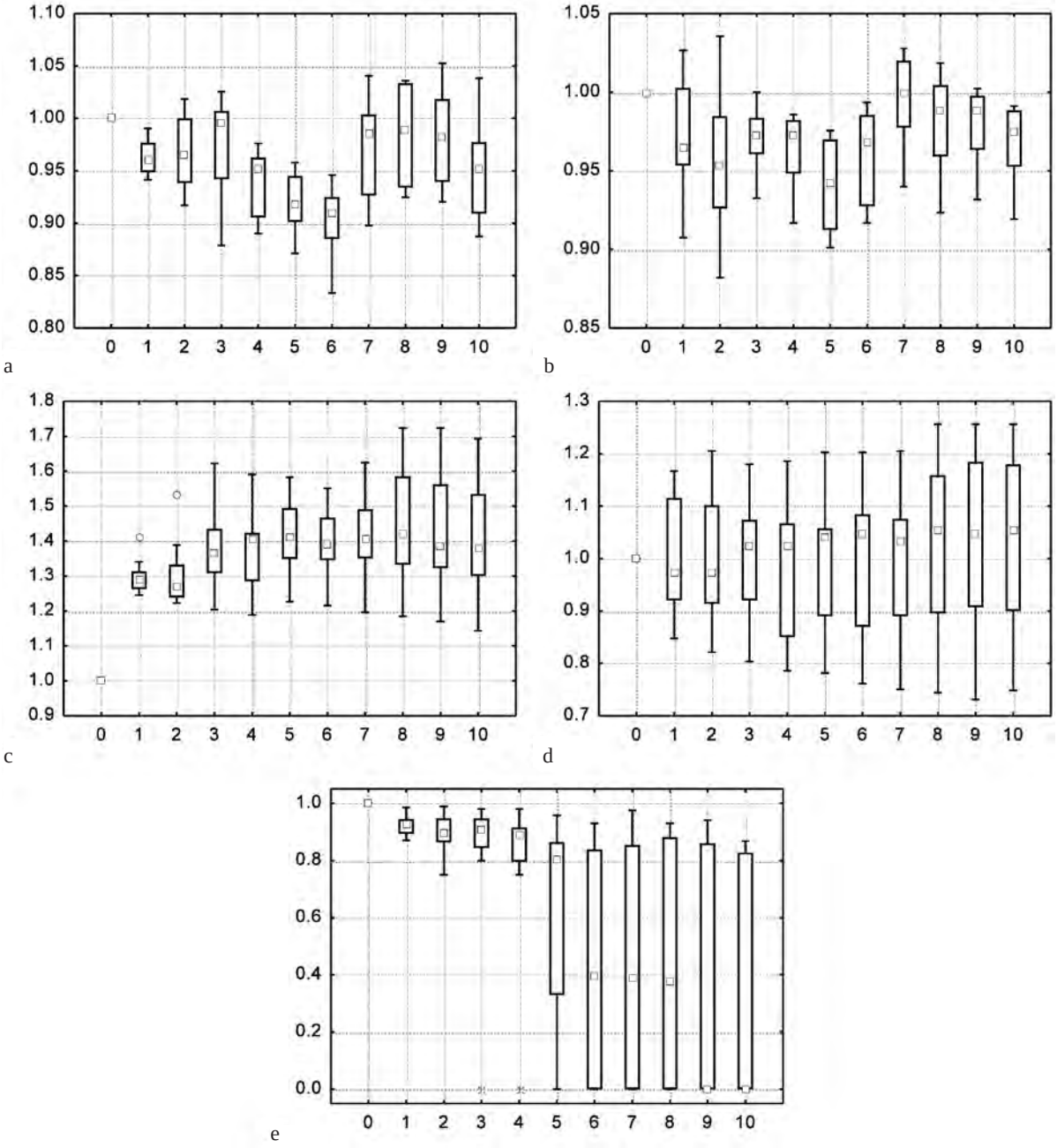

Fig. 5. Dynamics of *R. kessleri* body weight, when it is fed on litter of various tree species: a, *Robinia pseudoacacia*; b, *Gleditsia triacanthos*; c, *Fraxinus lanceolata*; d, *Aesculus hippocastanum*; e, absence of food; abscissa, time (days from the beginning of experiment); ordinate, ratio of *R. kessleri* body weight to its weight before the experiment (in all variants of the experiment $n = 12$).

of faeces) were calculated for individual specimens of millipedes for each specific day of the experiment (Figs 3–5) or for the 10-day study as a whole (Figs 1, 2, 6). Further statistical data analysis was performed in Statistica 8.0 software package. For characteristic of samples, the figures show the median, 25% and 75% quartiles. Differences between samples were considered significant at $P < 0.01$ in one-way analysis of variance.

Results

The rates of litter mass loss in the substrates under study varied by a wide range: from 0.6 to 4.1% per day (Fig. 1). The highest rate of leaf litter mass loss caused by microorganisms was featured by *Cerasus vulgaris* L., *Ulmus laevis* Pall. and *Malus domestica* Borkh. (2.8–4.1% per day). A two-three times lower rate of

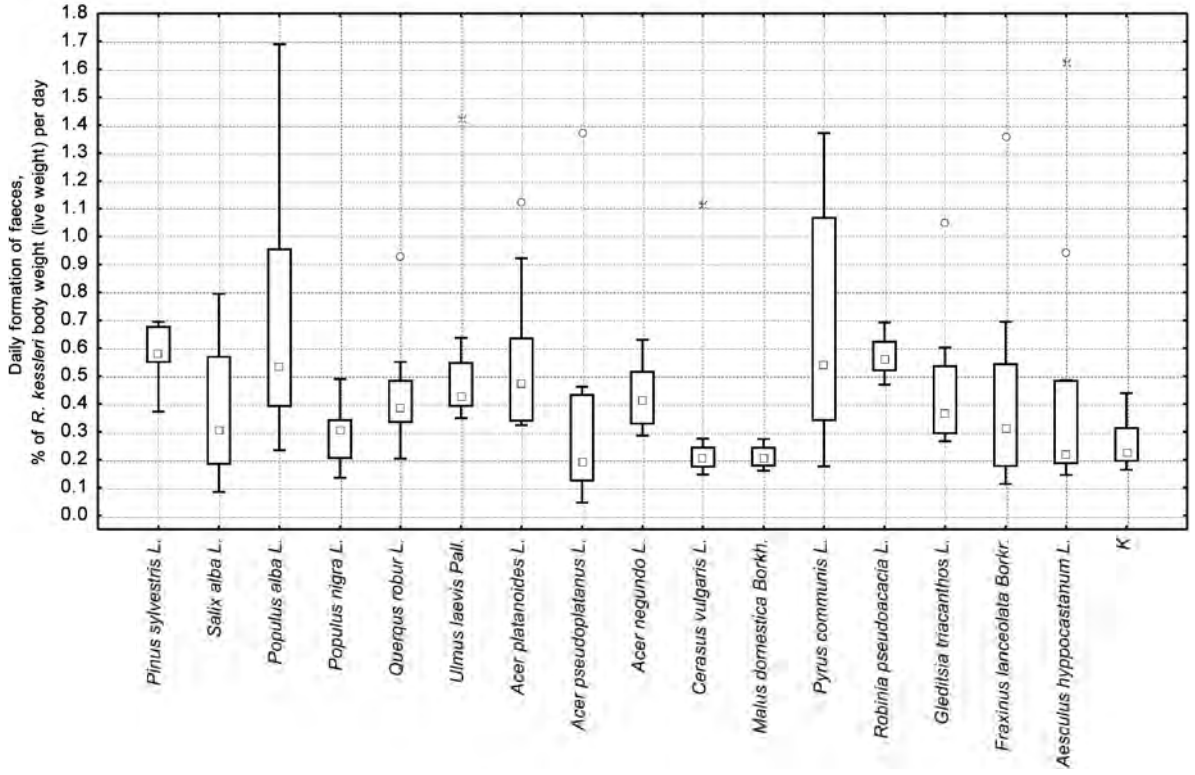

Fig. 6. Daily formation of faeces by *R. kessleri*: abscissa, tree species; *K*, control; ordinate, daily formation of faeces (% of *R. kessleri* body weight (live weight) per day, $n = 12$ for each variant of food).

litter mass loss was observed for *Robinia pseudoacacia* L., *Populus alba* L., *Gleditsia triacanthos* L., *Acer pseudoplatanus* L., *A. platanoides* L., *Quercus robur* L., *Pyrus communis* L. (1.0–2.2% per day). A minimum rate of mass loss through microbial decay was characteristic for *Fraxinus lanceolata* Borkr., *Pinus sylvestris* L., *Salix alba* L., *Populus nigra* L., *Acer negundo* L. and *Aesculus hippocastanum* L. (0.5–0.8% per day). Differences are connected with various periods of formation of the leaf litter, varying conditions of its fermentation in the forest ground litter before commencing the experiment, and peculiarities of the gut microflora of each specimen.

The maximum rate of leaf litter consumption by *R. kessleri* was found for *A. negundo* – 0.75 mg/mg of body weight per day (Fig. 2). The second by the rate of consumption was *Q. robur* (0.50), the third place was shared by *M. domestica* (0.36) and *C. vulgaris* (0.35 mg/mg of body weight per day). For *U. laevis* and *R. pseudoacacia* the rates of consumption ranged around the level of 0.11, and for all the other tree species under study they did not exceed 0.10 mg/mg of body weight per day. The leaves decomposed by microorganisms at the highest rate (Fig. 1) were also those consumed by *R. kessleri* at maximum rates (Fig. 2). The given tendency was not revealed for the leaf litter of *P. alba*, *G. triacanthos*, *A. pseudoplatanus*, *A. platanoides* and *P. communis*, having medium to high rates of microbiological decomposition and very low level of consumption by millipedes.

When millipedes were fed on pine needles (Fig. 3a), their weight decreased during four days and then stabilized at the level of 92–95% of their initial weight. Fluctuations in body weight are, probably, connected with acclimation to consumption of food not typical for the population under study (millipedes for the experiment were collected in an ash-tree-acacia plantation). As all the containers were equally sprinkled with water and then covered by paper to ensure consistent moisture conditions it is unlikely that fluctuations in the millipedes' body weight was due to changes in body water content. When animals were fed on leaves of *S. alba*, on the contrary, their weight for the first day of experiment increased by 20% (Fig. 3b). This continued to increase until the fifth day when it reached a peak of about 36% above the weight on the first day of the experiment, which was followed by a slight decline by the tenth day.

Millipedes feeding on *P. alba* showed no significant changes in body weight during 10 days of the laboratory experiment (Fig. 3c). A diet of leaf litter of *P. nigra*, instead, reduced the millipedes' body weight by 15% by the third day of experiment; however, a certain acclimation occurred subsequently (with the body weight returning to 90% of the initial level).

Oak litter (Fig. 3e) throughout the first week of the experiment caused a practically linear reduction of the animals' body weight (by 12%). Thereafter acclimation took place, and body weight returned to the initial (pre-experiment) level. A similar period of acclimation of *R. kessleri* to leaves of *U. laevis* (Fig. 3f) was one day shorter but the dynamics of body weight change were similar.

The consumption of maple leaves had various effects on the experimental animals (Fig. 4a, b, c). 5–12% gain in body weight after a three-day period of acclimation was observed for *A. platanoides*. Feeding on leaves of *A. pseudoplatanus* and *A. negundo* led to a lengthening of the acclimation period to 6–8 days and a total reduction of body weight by 10–13% by the end of the experiment.

Feeding on leaves of *C. vulgaris* (Fig. 4d) did not significantly change the body weight of *R. kessleri* by the end of the 10-day laboratory experiment. The acclimation period lasted 3 days. A diet of *M. domestica* and *P. communis* (Fig. 4e, f) led to a significant reduction of body weight, down to 92–93% of the initial level by the end of the experiment. The period of acclimation to the diet was 5–6 days.

A diet comprising leaves of *R. pseudoacacia* and *G. triacanthos* (Fig. 5a, b) insignificantly (by 2–5%) reduced the millipedes' body weight by the end of the experiment. The acclimation period was 6–7 days.

The fastest and most stable gain in the body weight of *R. kessleri* (29% of body weight on the first day of experiment reaching, with mild fluctuations, 39% by the end of the experiment) was caused by feeding on the litter of *F. lanceolata* (Fig. 5c).

A diet of leaf litter of *Ae. hippocastanum* led to a gradual increase in body weight of 7% by the end of the experiment (Fig. 5d). The acclimation period was three days.

In the conditions of absence of food in the container the first and the second millipedes died on the third and fourth days of the experiment and the average body weight of the remaining specimens during the first four days decreased by 10% (Fig. 5e). A 50% death rate of the starving animals in the cups was recorded on the sixth day of the experiment.

In the experiment we observed infrequent but rather significant deviations of body weight in individual millipedes from their body weight on the previous day. Compared with the body weight of each individual on the previous day (12 millipedes * 10 days of experiments – 120 measurements of body weight for each diet) sizeable and sharp fluctuations were recorded (70–90 mg increase/decrease in body weight) for *F. lanceolata*, *S. alba*, *A. platanoides*, *A. pseudoplatanus*, *C. vulgaris*, *Ae. hippocastanum* and some other tree species, not attributable to errors in the instruments or records of experimental results.

Average daily formation of faeces by millipedes (Fig. 6) is an indicator of their contribution to mechanical grinding of organic debris on the soil surface, and their role in soil-forming processes as a whole. In terms of the daily mass proportion of faeces to live body weight in *R. kessleri*, the first place among the tree species studied is taken by *P. sylvestris* (0.58%), followed by *R. pseudoacacia* (0.57%), *P. communis* (0.54%) and *P. alba* (0.53%). The minimum average daily formation of faeces is recorded in millipedes fed on *A. pseudoplatanus*, *C. vulgaris*, *M. domestica* and *Ae. hippocastanum* (0.20–0.23% of body weight). It is possible that a diet of leaf litter from these species produces a constipative effect on the millipedes. It is interesting to note that the control group of millipedes which were not fed during the 10-day experiment produced the same faecal mass (0.23% of body weight) as the millipedes fed on the four plant species listed above.

Discussion

Rates of microbial decay of leaves are different for various stages of their decomposition. The results of our unpublished experiments prove that the rate of consumption of ash-tree leaves by *R. kessleri* fluctuates widely by a factor of up to 18.4 over a period of 1–12 months after their falling, due to the varying stages of activity of bacteria and fungi in the process of decomposition of the leaf litter. It is rather difficult to reveal here which factor has the greatest influence on the rate of leaf litter consumption: the degree of leaching and microbiological decomposition of secondary compounds in the leaf, organic nitrogen content, successional changes of microorganisms at various stages of substrate decomposition or other factors (Sakwa, 1974). It is not possible to differentiate these factors at the present stage of development in soil zoology. Therefore, fragments of last-year's leaves served as a substrate for this study. Visually the leaves of each tree species were distinct and so leaves of different species varied considerably in the degree of their decomposition. The data obtained allow us to compare food consumption, gain in body weight and rate of faeces formation of *R. kessleri* when fed on 16 tree species in the second half of the vegetation season (at the beginning of August). In natural conditions in the steppe zone of Ukraine, where millipedes are able to select specific leaves at optimal stages of decomposition from the litter mixture, the trophic load is redistributed in response to seasonal changes in the forest environment, the principal factors being humidity and temperature (Kondeva, 1980; Hopkin and Read, 1992).

It is important to note a sharp divergence of the rates of microbiological and zoogenic decomposition of the litter for various tree species in the conditions

of laboratory experiments (Figs 1, 2). For example, *A. negundo* features the highest rate of food consumption by the millipedes, compared with most other tree species, and at the same time one of the lowest rates of microbial decay of litter. Cherry tree and apple tree leaves were consumed by the millipedes at practically equal rates, while the rate of microbiological decomposition of apple tree leaves was 47% lower than that for those of cherry trees. We have not found similar results in the literature we have searched.

There are three tendencies in the dynamics of acclimation of *R. kessleri* to new diets.

1. Sharp increase in body weight during the first day of the experiment and further stablization thereof at a definite level, for example, for *S. alba* (Fig. 3 b) and *F. lanceolata* (Fig. 5 c).
2. Short-term decrease in body weight during three days of the experiment and further stablization thereof at the level not significantly differing from the initial weight, for example, for *P. alba* (Fig. 3c), *A. platanoides* (Fig. 4a), *C. vulgaris* (Fig. 4d), *G. triacanthos* (Fig. 5b) or *Ae. hippocastanum* (Fig. 5d).
3. Long-term acclimation (for about one week) with return to initial (*Q. robur* (Fig. 3e), *U. laevis* (Fig. 3f)) or lower body weight (*P. sylvestris* (Fig. 3a), *A. pseudoplatanus* (Fig. 4b), *M. domestica* (Fig. 4e), *P. communis* (Fig. 4f)).

As a result of the laboratory experiment, it was found that consumption of leaf litter of *F. lanceolata* significantly increased the body weight of *R. kessleri* by 39% in 10 days, *S. alba* – by 29%. A diet comprising semi-decomposed leaves of *P. nigra* in 10 days significantly decreased the miillipedes' body weight by 13%, *A. negundo* – by 12%, *A. pseudoplatanus* – by 9%, *P. communis* – by 8%, *P. sylvestris* – resulted in a 7% body weight reduction. No statistically significant tendencies in millipede body weight change were found for other tree species.

With equal consumption of food of different types a larger mass of faeces resulting from consumption of a particular food item is evidence of the lower food assimilation (Nicholson et al., 1966). Our experiment has shown that for the four tree species (*A. pseudoplatanus*, *C. vulgaris*, *M. domestica* and *Ae. hippocastanum*) the mass of formed faeces did not significantly differ from the variant with full absence of food. Interesting results were obtained for cherry trees, consumption of which is ranked third among 16 tree species studied (Fig. 2), yet the amount of faeces formed on this diet is the same as in the variant without food (Fig. 6).

R. kessleri is the dominant Diplopoda species in the studied region and the European part of the former USSR (Striganova, 1996; Brygadyrenko, 2006). Food preferences of this species in various tree plantations can be both of theoretical and practical interest. Formation of tree plantations resistant to penetration of steppe species under their canopy is possible only with a stable and strong litter layer (of 30–40 mm thick) required for deceleration of germination of seeds of steppe plants, reduction of moisture evaporation from the soil surface, increase in quantity of litter zoophages which destroy forest pests. The Diplopoda species studied in this paper has great importance in the regulation of the rate of mineralization of plant litter and forest litter.

Conclusions

Our results allow us to detect significant differences in the rates of consumption of leaf litter of various tree species by the Diplopoda species studied. The three types of dynamics of adaptation by *R. kessleri* to diet change documented in the study require further investigations at the physiological (estimation of rate of metabolic processes in the millipede), biochemical (study of the content of secondary compounds and other critical components of the diet in a food substrate) and ecosystem levels (determination of the role of individual groups of microflora in the processes of adaptaion to diet changes). It may be concluded that the results presented here will provide the basis for constructing a simulation model of decomposition of plant litter in forest ecosystems by *R. kessleri* and other species of litter saprophages.

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