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Leaf beetles (Coleoptera, Chrysomelidae) in the pristine peat bog in Belarus: biodiversity and spatial distribution

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Abstract. Pristine peat bogs have a large area in Belarus compared to other Central European countries. Accordingly, ancient and almost intact Belarusian peatlands are valuable for the synecological investigations. The goal of this study was to assess the diversity of leaf beetles in seven main peat bog habitats and to examine environmental factors affecting them. In total, 44 leaf beetles' species were recorded. The results showed a low diversity, evenness and species' richness of Chrysomelidae. The lagg zone and sites covered by scrubs, excluding dome, support higher alpha diversity. Beta diversity analysis revealed a clear separation among the leaf beetles' assemblages of the seven habitats. The primary differences in the assemblages reflect the presence of species trophically associated with sedges (*Plateumaris discolor*) and ericaceous dwarf scrubs (*Lochmaea suturalis, Altica longicollis, Cryptocephalus labiatus*). Moreover, the modelling results (GLM) indicated that a scrub cover strongly influenced leaf beetle species' richness and abundance.

Key words: assemblages, Chrysomelidae, diversity, peat bog, Belarus.

Central European raised bogs are island-like ancient habitats with very specific environmental conditions and biota (Spitzer & Danks 2006). They are stable and slowly changing ecosystems - if natural conditions are preserved (Paavilainen & Päivanen 1995, Bragg et al. 2003). Peat bogs play an important role in the biosphere. Most of the peat bogs receive atmospherically deposited pollutants, including nitrogen, sulphur, and heavy metals, whose local and regional deposition patterns have changed in the past and will continue to change in the coming decades (Paavilainen & Päivanen 1995, Bragg et al. 2003). Nowadays, peat bogs are heavily degraded as a consequence of peat cutting, agricultural activities and drainage. Only five countries of Central Europe, including Belarus, have maintained more than 50% of their peatlands in a relatively natural condition (Bragg et al. 2003, Urák et al. 2010).

Peatland organisms live in an environment which is typically wet and nutrient-poor, partly anoxic, often acid, and in large parts exposed to the wind and the sun. Because of their acidity, low nutrient availability, and wetness, bogs are generally regarded as hostile habitats for many species and some complete taxonomic groups (Rydin & Jeglum 2006). In such environmental conditions plant communities with a poor species' richness and a very specific species' composition, including several species of ericaceous shrubs and sedges, were formed. They comprise characteristic assemblages of species which can exhibit intense patterning of invertebrate communities. Herb-shrub layer provides a range of specific food plants for insects. Specialized tyrphobionts and tyrphophilous species feed obligatorily on cold-adapted peat bog plants (Spitzer & Danks, 2006).

Leaf beetles are important members of food chains. Besides, chrysomelid beetles are one of the most abundant taxa among peatland invertebrates (Maavara 1957, Spungis 2008, Sushko 2017, Gallé et al. 2019, Lehmitz et al. 2019). Over the past fifteen years, predominantly the epigeic invertebrates have been one of the most active areas of entomological research in European peat bogs (Spungis 2008, Dapkus & Tamutis 2008, Urák et al. 2012), yet, the knowledge of leaf beetles' diversity and assemblage composition is still poor (Maavara 1957, Spungis 2008).

The first goal of this study was to assess the diversity of leaf beetles in seven main habitats of an ancient, large pristine peat bog. The second goal was to examine environmental factors affecting chrysomelid species' richness and abundance.

Sampling area. This research was carried out between 2015 and 2017 in a 4602-hectare "Mokh" peat bog in Belarus (55°37' N 28°06' E), which is one of the largest and anthropogenically least modified peat bogs in the country and is currently protected as a hydrological reserve. Their central part is about 3-5 m higher than the peripheral parts. There is a slope, a peak, and a plateau, which is located at the edges of the border zone (lagg zone) and is periodically flooded due to a lower elevation.

Vegetation. Vegetation surveys were conducted according to the method of Brown (1954) in seven of the most typical peat bog habitats: 1) lagg zone (LZ) at the bog margin (plant community: *Eriophorum vaginatum – Sphagnum angustifolium*), 2) pine bogs (PB) on the slope (plant community: *Pinus sylvestris – Eriophorum vaginatum – Ledum palustre – Sphagnum magellanicum*), 3) hollows (HOL) on the slope (plant community: *Rhynchospora alba – Sphagnum cuspidatum*), 4) hummocks (HUM) on the slope (plant community: *Eriophorum vaginatum – Oxycoccus palustris – Andromeda polifolia – Ledum palustre – Sphagnum magellanicum – S. angustifolium*), 5) open bogs (OBS) on the slope (plant community: *Eriophorum vaginatum – Ledum palustre – Chamaedaphne calyculata – Empetrum nigrum – Calluna vulgaris – Oxycoccus palustris – Vaccinium uliginosum – Sphagnum magellanicum*), 6) lake shores (L) on the slope (plant community: *Carex limosa – Sphagnum cuspidatum*), 7) dome (D) (plant community: *Eriophorum vaginatum – Calluna vulgaris – Sphagnum fuscum*) (Fig. 1).

In each habitat 3 sites were investigated. Whereas in each site 3 randomly selected plots (1 x 1 m^2) were sampled. Vegetation was sampled during July 2016. Four vegetation parameters in each plot were recorded: cover (%) of shrubs, cover (%) of herbs, the number of vascular plant species, and the plant height values. Also, the water level was measured (Table 1). Sites were $\geq$100 m from each other.

Leaf beetles' sampling. Leaf beetles were sampled using an entomological sweep-net (diameter 30 cm) along 50 m x 5 m transects (i.e. 1 transect per site, 3 transects per habitat, 27 transects in total). Exactly 50 net sweeps were done on each transect twice a month. For statistical analysis, all data from each site were summed to obtain one value per habitat. Surveys were conducted during the main activity period of adult beetles between May and the end of September.

Table 1. Mean values (SE) of environmental parameters in the seven main peat bog habitats.

Environmental variable	Habitats						
	LZa	PBb	HOLc	HUMd	OBSe	Lf	Dg
shrub cover (%)**	5.9 1SE:1.0 bdefg	45.2 1SE:3.2 acf	5.2 1SE:1.2 bdefg	40.8 1SE:4.6 acdefg	51.3 1SE:2.3 acfg	1.2 1SE:0.2 abcde	47.6 1SE:4.1 acf
herb cover (%)***	47.7 1SE: 3.4 bdeg	9.7 1SE:0.7 acefg	37.1 1SE:2.1 bdefg	14.8 1SE:1.7 acef	25.3 1SE:3.2 abcdf	65.1 1SE:4.9 bcdeg	19.1 1SE:2 abcf
plant height (cm)	36.5 1SE:3.1 cf	40.6 1SE:1.2 cdeg	49.5 1SE:3 bdeg	38.3 1SE:1.6 f	36.8 1SE:3.2 g	44.6 1SE:2.4 a,c,g	37.3 1SE:1.7 C,f
number of vascular plant species**	2 1SE:0.3 bdeg	8.3 1SE:0.3 acdfg	1.8 1SE:0.3 bdeg	7.3 1SE:0.2 abcf	8.1 1SE:0.4 acf	1.8 1SE:0.1 bdeg	7 1SE:0.3 abcf
bog water table (cm) **	2.6 1SE:0.5 bdeg	40.3 1SE:0.6 acdefg	1.5 1SE:0.3 bdeg	16.8 1SE:0.9 abcefg	13.3 1SE:0.4 abcdfg	2.6 1SE:0.4 bdeg	29.5 1SE:2.7 abcdef

Habitat symbols: lagg zone – LZ; pine bog – PB; hollow – HOL; hummock – HUM; open bog – OBS; lake shores – L; dome – D.
Significance (ANOVA or Kruskal–Wallis test): ***p≤ 0.0001, **p≤ 0.001, *p≤ 0.05; a,b,c,d,e,f,g letters indicate significant differences among variables in different habitats (Tukey's or Dunn's post hoc tests)

Data analysis. Statistical estimator Chao 1 was used for calculating and extrapolating leaf beetle species' richness. Chao 1 is based on the abundance and uses the number of rare species in a sample (one and two individuals) to calculate the expected richness (Chao 1987). To examine the leaf beetle alpha diversity, Shannon-Winner (H') and evenness (J') indexes were applied. The indexes were calculated by using the software Past (Hammer et al. 2001). As beta diversity measures, the spatial distribution and heterogeneity in assemblages' composition among sites within a study area were undertaken. The heterogeneity of leaf beetle assemblages among habitats was evaluated by using the analysis of similarity (ANOSIM) and was summarized by using a non-metric multidimensional scaling (NMDS) with the Bray-Curtis similarity index. SIMPER analysis was applied to examine which species contributed to the most of differences in the composition of assemblages. Data were square-root transformed prior to the analyses. Calculations were done by using the software package PAST (Hammer et al. 2001).

Differences of environment parameters, leaf beetle species' richness, abundances and diversity indexes were examined by using the analysis of variance (ANOVA) and Tukey's post hoc test and with the Kruskal–Wallis H test with Dunn's post hoc test with the Bonferroni correction (the level of significance $P < 0.05$). The data were log-transformed. Prior to that, Shapiro–Wilk tests to check for normal distribution of data were applied.

The influence of environmental parameters on leaf beetles' species richness and abundances were tested by applying generalized linear models (GLM) with the Poisson distribution. Collinearity in the environmental variables was assessed by calculating variation inflation factors (VIF), which determined highly correlated variables (VIF > 5) (Zuur et al. 2010). The variables entered in GLM were dwarf shrubs' cover and bog water level. The best-fitting models were selected by using a stepwise backward selection approach based on Akaike information criterion (AIC). If overdispersion was detected, I have had the standard errors corrected by using a quasi-Poisson GLM model and pseudo R^2 (Zuur et al.2009). For the statistical analyses' software, the R 3.4.3, was used (R Development Core Team 2017).

A total of 3,390 specimens of Chrysomelidae belonging to 44 species were found. More than a half (50.00%-70.57%) of species in assemblages were represented by 1-2 individuals. Ten species (22.72% of the total number of species) were singletons (one specimen only) and 5 (11.36%) were doubletons (two specimens).

Non-parametric species' richness estimator Chao1 provided the best average expected species' richness which is close to the actual overall richness recorded in the study area. The estimator indicated that the numbers of chrysomelid species in the study area were 18-50 species suggesting that the observed of 12-27 species represented 63.4-84.4% of the actual richness (Table 2).

Only 4 species, such as *Plateumaris discolor* (6.09%-29.41%), *Lochmaea suturalis* (7.89%-50.31%), *Cryptocephalus labiatus* (5.04%-9.35%) and *Altica longicollis* (4.48%-20.49%) had a high relative abundance (Table 3).

Chrysomelidae species' richness (ANOVA, F = 3.59, P = 0.006) and the abundance (Kruskal–Wallis test, H = 33.77, P = 0.001) significantly differed among the assemblages of seven habitats. The leaf beetle assemblages included 12 to 24 species. The species' richness was at the highest in the pine forests, whereas the lowest mean number of species was recorded upon the lake shores and hollows (Fig. 1a). Mean total chrysomelid abundances were higher in the habitats of the dome, while upon the lake shores abundances were at the lowest (Fig. 1b).

Shannon-Winner index differed significantly among the (Kruskal–Wallis test H=11.37, p = 0.05) habitats. Diversity measures indicate a higher diversity in the lagg zone (H'=1.984) compared to the dome (H'=0.932) and other sites, covered with herbs, such as the lake shores (H'=1.216) and hollows (H'=1.114). On the other hand, chrysomelid evenness (Kruskal–Wallis test H=30.26, p = 0.001) were higher in the lake shores (J'=0.841) and hollows (J'=0.813), as well. It should be noted that increases in Shannon-Winner diversity values and species' richness in the habitats covered with shrubs corresponded to the reduction of the evenness (Table. 2).

A significant difference in leaf beetle assemblages' composition was recorded among sampling sites (ANOSIM, r=0.565, P=0.001) and resulted in the separation of habitats within the NMDS ordination plot (Fig. 2).

Table 2. The main parameters of leaf beetles' assemblages

Parameters	Habitats						
	LZ	PB	HOL	HUM	OBS	L	D
Number of observed species	27	34	13	23	26	12	19
Chao1	42.2	50.2	18.0	34.0	41.6	17.0	22.5
Estimate standard error	10.9	11.0	6.0	8.5	11.7	6.0	3.7
% of the actual species' richness	63.9	67.7	72.2	67.6	63.4	70.5	84.4
Shannon-Wiener index (H')	1.984	1.558	1.114	1.356	1.513	1.216	0.932
H'standard error	0.1	0.2	0.3	0.2	0.2	0.3	0.2
Evenness (J')	0.567	0.347	0.813	0.358	0.392	0.841	0.299
J' standard error	0.05	0.03	0.04	0.04	0.03	0.05	0.01

Habitat symbols: lagg zone (LZ); pine forest (PB); hollow (HOL); hummock (HUM); open bog spaces (OBS), lake shores (L); dome (D).

Table 3. Composition of leaf beetles' assemblages of different habitats of the "Mokh" peat bog

Species	Habitats // relative abundance, %						
	LZ	PB	HOL	HUM	OBS	L	D
Plateumaris discolor (Herbst 1795)	14.93		29.41	6.54	6.09	28.95	4.97
Oulema gallaeciana (Heyden 1870)	1.49	0.72					
Cryptocephalus bipunctatus (L. 1758)		0.72					
C. decemmaculatus (L.1758)	1.49						
C. labiatus (L. 1761)	1.49	5.04		9.35	6.96	5.26	4.35
C. moraei (L. 1758)		0.72					
C. punctiger Paykull 1799	1.49	0.72					
C. sericeus (L. 1758)				0.93	0.87		
Chrysolina sanguinolenta (L. 1758)				0.93	0.87		
Gastrophysa polygoni (L. 1758)					0.87		
Phaedon cochleariae (F. 1792)		0.72		0.93	0.87		
Phratora vulgatissima (L. 1758)		0.72					
Lochmaea caprea (L.1758)		1.44			2.61		
L. suturalis (Thomson 1866)	10.45	43.88	7.84	44.86	43.48	7.89	50.31
Phyllotreta atra (F.1775)		0.72		0.93	0.87		
Ph. nemorum (L. 1758)	1.49	0.72		0.93	0.87	2.63	0.62
Ph. striolata (Illiger 1803)		0.72		0.93	0.87		
Ph. undulata Kutschera 1860	1.49	0.72		0.93	0.87		
Ph. vittula (Redtenbacher 1849)	2.99	0.72	1.96	1.87	1.74	7.89	1.24
Aphthona erichsoni (Zetterstedt 1838)	2.99	1.44	3.92	1.87	1,74	2,63	1,24
A. euphorbiae (Schrank 1781)	13.43	5.76	9.80	4.67	4.35	10.53	3.11
Longitarsus luridis(Scopuli 1763)		0.72					
L. melanocephalus (De Geer 1775)	1.49						
L. parvulus (Paykull 1799)	4.48	5.04	11.76	1.87	3.48		4.35
L. pratensis (Panzer 1784)	1.49		7.84	0.93	0.87		
Altica longicollis (Allard 1860)	4.48	12.23	7.84	9.35	8.70	13.16	20.50
A. oleracea (Linnaeus 1758)		0.72		0.93	0.87		0.62
Batophila rubi (Paykull 1799)	4.48	2.16					0.62
Asiorestia impressa (F. 1801)	1.49					2.63	
Crepidodera aurata (Marsham 1802)		0.72					
C. aurea (Geoffroy 1785)	1.49						
C. fulvicornis (F. 1792)	2.99	0.72					0.62
Chaetocnema breviuscula (Faldermann 1884)	4.48	2.88	1.96	1.87	3.48		0.62
Ch. hortensis (Geoffroy in Fourcroy 1785)	2.99	0.72	1.96		0.87		0.62
Ch. mannerheimi (Gyllenhal 1827)	2.99	2.16	1.96	1.87	1.74	2.63	1.24
Ch. picipes Stephens 1831		2.16			1.74		1.24
Ch. sahlbergii (Gyllenhal 1827)	7.46	0.72	11.76	4.67	2.61	13.16	3.11
Ch. subcoerulea (Kutschera 1864)	1.49		1.96			2.63	
Ch. tibialis Illiger 1807	1.49	0.72		0.93	0.87		
Psylliodes cucullata (Illiger 1807)		0.72					
Cassida hemisphaerica Herbst 1799	1.49	0.72			0.87		
C. margaritacea Schaller 1783	1.49	0.72		0.93			
C. nebulosa (Linnaeus 1758)		0.72		0.93			0.62

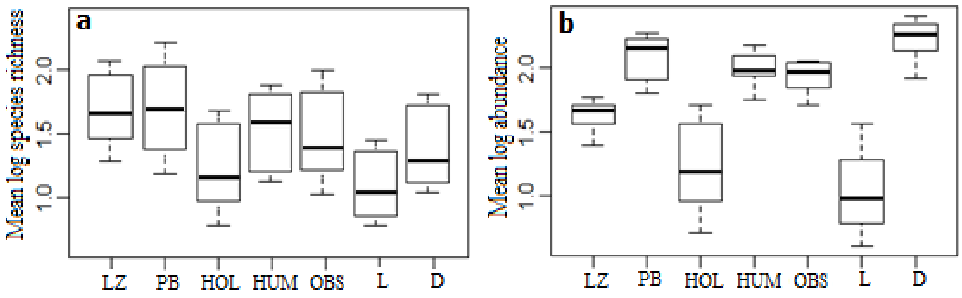

Figure 1. Differences in the mean (±SE) species' richness (a) and abundance (b) in leaf beetles' assemblages. Habitat symbols: lagg zone (LZ); pine forest (PB); hollow (HOL); hummock (HUM); open bog spaces (OBS), lake shores (L); dome (D).

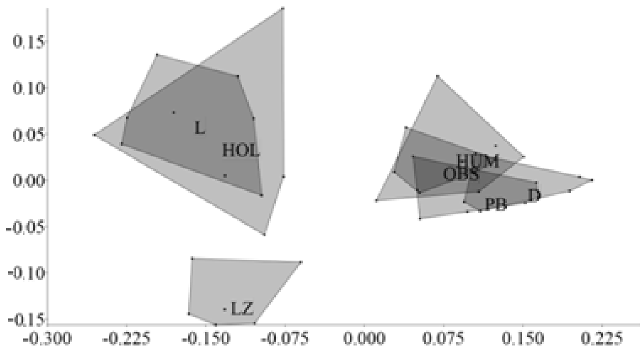

Figure 2. NMDS plot of dissimilarity (Bray-Curtis) of leaf beetles' assemblages in different habitats in the peat bog. Habitat symbols: lagg zone (LZ); pine forest (PB); hollow (HOL); hummock (HUM); open bog spaces (OBS), lake shores (L); dome (D).

Table 4. A relationship between the richness and abundance of leaf beetles' species and environmental factors based on the application of a generalized linear model (GLM) multiple regression equations

Parameters	Estimate	SE	z -value / t -value	P
Species' richness (Poisson GLM)				
intercept	1.961	0.342	5.752	***
shrub cover	0.019	0.002	4.624	***
water level	-0.002	0.007	-0.305	n.s
Abundance (Quasi-Poisson GLM)				
intercept	3.489	0.785	4.439	***
shrub cover	0.253	0.054	4.640	***
water level	0.001	0.017	0.108	n.s

Significance codes: ***p≤ 0.0001, **p≤ 0.001, *p≤ 0.05, n.s – non significance

The sites covered by scrubs (PB, HUM, OBS, D), sites covered by herbs (L, HOL) and the lagg zone (LZ) were widely dispersed, indicating greater assemblages' heterogeneity. SIMPER analysis indicated significant assemblages' heterogeneity and that differences among assemblages were driven by higher abundances of only a few species (*Lochmaea suturalis* — abundance sites covered by scrubs: 83.5, sites covered by herbs: 2.75, lagg zone sites: 2.17 – contributing 42.96% to the dissimilarity, *Plateumaris discolor* – abundance sites covered by scrubs: 2.29, sites covered by herbs: 4.92, lagg zone sites: 17.2 – contributing 7.86% to the dissimilarity, *Altica longicollis* – abundance sites covered by scrubs: 13.4, sites covered by herbs: 2.33, lagg zone sites: 0.5 – contributing 7.04% to the dissimilarity, *Cryptocephalus labiatus* – abundance sites covered by scrubs: 8.63, sites covered by herbs: 0.167, lagg zone sites: 1.5 – contributing 5.81% to the dissimilarity, *Aphtona euphorbiae* – abundance sites covered by scrubs: 5.04, sites covered by herbs: 1.67, lagg zone sites: 5.67 – contributing 3.86% to the dissimilarity).

The generalized linear model has shown that species' richness and abundances of leaf beetles were significantly determined by the shrub cover, which had a positive effect (Table 4).

The study revealed a high dominance in different raised bog habitats of a small number of leaf beetles' species. That is typical of peat bog insects in other European countries as well (Maavara 1957, Spungis 2008, Dapkus & Tamutis 2008). Compared to other investigations, the results of this study are based on a data collection from a wider range of sites that cover the main habitats of natural peatlands. Estimations of the total number of leaf beetles' species confirmed low values of species' richness also.

Of the 44 chrysomelid species, only 4 species were highly abundant. Therefore, they can be regarded as one of the main consumers of peat bog vascular plant species. Probably, other low-abundance species are not playing an important role in peat bog food webs.

The results of the study showed that the lagg zone and sites covered by scrubs, excluding dome, support a higher biodiversity (alpha diversity). The higher diversity of lagg zone leaf beetle assemblages can be explained by the fact that the majority of the species are migrants. Whereas the low diversity in the dome is due to the very high abundance of one specialized species *Lochmaea suturalis,* feeding on the heather, which dominates here in the plant cover.

Beta diversity analysis revealed a clear separation among the leaf beetles' assemblages of the seven main peat bog habitats. Significant spatial heterogeneity was recorded among the bog margin, sites covered by herbs and sites covered by scrubs. The primary differences in the assemblages reflect the presence of species trophically associated with sedges (*Plateumaris discolor*) and ericaceous dwarf scrubs (*Lochmaea suturalis, Altica longicollis, Cryptocephalus labiatus*). However, chrysomelid assemblages among sites covered by herbs were more heterogeneous (more widely dispersed in the NMDS ordination diagram) than the assemblages of sites covered by scrubs. Moreover, the modelling results (GLM) indicated that a scrub cover strongly influenced leaf beetle species' richness and abundance.

Cconservation of biodiversity of ancient pristine peat bogs is a basic priority for insect conservation in Europe. Primarily specialized peat bog leaf beetles, such as *Plateumaris discolor, Cryptocephalus labiatus, Altica longicollis, Aphthona euphorbiae* and *Chaetocnema sahlbergi,* are very sensitive to environmental changes. It is necessary to keep hydrological conditions and, as a result, plant communities in peatlands stable.

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