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Current biodiversity and hotspots in the primeval beech forest – Poloniny National Park, the Eastern Carpathians (Slovakia)

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Abstract. The research was carried out in the territory of the Bukovské vrchy hills, where natural beech forest stands contain an invaluable genetic reservoir of European beech and other species associated and dependent on these forest habitats. Present study is focused on the creation of local biodiversity hotspots. The structure of local biodiversity valuable areas is based on spatial distribution of indicator species of primeval beech forests and species of conservation interest. Field data on rare bryophytes, vascular plants, macrozoobentos and vertebrates were condensed into GIS layers. The new zonation of the Poloniny National Park was suggested. Spatial distribution of relevant species should be included in the new zonation which we believe will better ensure the protection of beech forests.

Key words: indicator species, hotspots, zonation, Bukovské vrchy Mts, Slovakia

Introduction

Despite an increase in conservation efforts, the state of biodiversity continues to decline (CBD 2010). Old-growth forests play a key role in sustaining biodiversity (Gibson *et al.* 2011), but also play an important role in climate change mitigation (Knohl *et al.* 2009). Despite the ecological importance of old-growth forests, globally, they are vanishing at an alarming rate, mainly due to deforestation, unsustainable logging practices, and fire (Achard *et al.* 2009). In central Europe, old-growth forests have survived mainly in remote and inaccessible mountain areas (Frank *et al.* 2009; Schulze *et al.* 2009), where logging of wood has been difficult and unprofitable. These are the areas where the first nature reserves have been established.

Beech forests in the Poloniny Mts represent an outstanding example of undisturbed, complex temperate forest and exhibit complete and comprehensive ecological patterns across a variety of environmental conditions. They contain an invaluable genetic reservoir of European beech and other species associated and dependent on this tree species. A significant component of the ecosystem is decaying wood, which is widely regarded as an important aspect of forest biodiversity forming key habitats for many species. Varried dead-wood features create additional habitat niches which increase habitat diversity (Speight 1989). For example, invertebrates, bryophytes, lichens, birds and mammals depend on or utilise dead wood as a source of food or shelter (Harmon *et al.* 1986; Esseen *et al.* 1997; Siitonen 2001). Birds like woodpeckers, owls, flycatchers and nuthatches dependent on dead wood (Tomiałojć and Wesołowski 2004). The amount of dead wood in the forest reserve provides ideal conditions for occurrence of a high number of wood-inhabiting fungi which are rare and threatened in many parts of Europe (Christensen *et al.* 2005). Dead wood is not only a substrate for vegetation, but its water holding ability contributes significantly to the maintenance of a humid climate in forests (Rambo and Muir 1998). Vast forests including large tracts of old-growth forest provide important habitat for populations of large mammals (*Ursus arctos, Canis lupus, Lynx lynx* and *Bison bonasus*). An effective way to protect a large number of species is to map out biodiversity hotspots (Myers *et al.* 2000). The term 'biodiversity hotspot' was defined by Myers (1988) as an area where exceptional concentrations of endemic species are undergoing exceptional loss of habitat. Many authors consider hotspots to be areas with the highest species diversity, (Samson and Knopf 1993; Williams *et al.* 1996; Kerr 1997; Myers *et al.* 2000; Orme *et al.* 2005; Grenyer *et al.* 2006) or with endemic species (Kerr 1997; Orme *et al.* 2005), rare species (Prendergast *et al.* 1993; Williams *et al.* 1996; Grenyer *et al.* 2006), or threatened species (Dobson *et al.* 1997; Orme *et al.* 2005; Grenyer *et al.* 2006). Carpathians represents one of the major diversity hotspots in Europe (Bálint *et al.* 2011). On a regional scale, each are exhibiting higher species diversity, endemic or threatened species should be mapped. These places should be recognised as local "hotspots", analogical to biodiversity hotspots in the world, and may be very important for the long-term survival of these threatened organisms and conservation of their habitat. Guidelines of the World Conservation Union (IUCN 1994, 2013) served as the basic documents not only for the creation of protected areas, but also for their management and land use in these areas (Bishop *et al.* 2004). The first international system of landscape categorization was created in 1978 (IUCN 1978). This system was replaced in 1994 by the current categorization of protected areas (IUCN 1994), which is the starting point for the definition of six categories of nature and landscape protection and utilization. The subsequent work (Bridgewater *et al.* 1996; IUCN 1998; EUROPARC 2001; Phillips 2002; Bishop *et al.* 2004; IUCN 2004; Dudley 2008) shows how to apply the guidelines in certain specific geographical or other contexts. Zonation is generally regarded as a management tool (Dudley 2008) through which we can, in different parts of the large protected areas, follow different management objectives and restrict potential conflicts associated with the land use of these zones. Therefore, the zonation is the heart of many national parks (Synge 2010). If definitions of zones in protected areas are clear then further success depends on the role of authorities responsible for protected areas. This role usually differs between countries. In Slovakia, administration of national parks (or protected areas) occurs at an advisory level, while an administrator of national parks in Poland may be wholly responsible for management activities within their territory (Fall 2003).

The main motivation for our decision to address this topic is the conflict between nature conservation and economic profit. Slovak environmental organizations require that corporations limit, and in sensitive aresa, completely stop clearcutting.

The fundamental objective of this study was to define the core areas of high biodiversity in the primeval beech forest. For our purposes, we used epiphytic and epilithic bryophytes, herbaceous vascular plants and vertebrates, especially birds of the genus *Ficedula* to map the local biodiversity hotspots. The water quality of the most important streams inside of the forest was evaluated by the measurements of water chemical composition and presence of some important taxa of macrozoobentos.

Material and Methods

Study area

Poloniny National Park is the easternmost large-scall protected area in Slovakis. It is located along the borders of three countries – Slovakia, Poland and Ukraine (Fig. 1). The park was established on October 1st, 1997 (Kramarik 1998). It covers an area of 29,805 hectares, with the specially protected area (buffer zone) amounting to 10,973 hectares. The most valuable parts of the national park are protected in seven national nature reserves (Stužica, Havešová, Riaba skala, Rožok, Pľaša, Stinská, Pod Ruským), twelve natural reserves (Bahno, Borsučiny, Bzaná, Gazdoraň, Hlboké, Hrúnok, Ruské, Stinská Slatina, Stružnická dolina, Šípková, Udava, Uličská Ostrá) and one natural monument (Ulička). In 1993, UNESCO's Man and the Biosphere Programme declared it as the International East Carpathian Biosphere Reserve together with the adjacent Polish (Bieszczadski National Park, Ciśniańsko-Wetliński Landscape Park and Dolina Sanu Landscape Park) and Ukrainian (Užansky Park, Nadsjanskyj Regional Landscape Park) regions, which made it the first trilateral biosphere reserve in the world (Buraľová and Némethová 2009). Poloniny National Park was awarded the prestigious European Council Diploma in 1998 (COE 2015). The National Park is a part of the Bukovské vrchy Mts., which consists mainly of coarse sandy flysh, greenish-grey and red claystone and fine sandstone. It is a moderately warm region, with less than 50 summer days annually on average with a daily maximum air temperature ≥ 25° C and a July mean temperature 16° C or more.

Reserves with strict protection (5th zone), B - Nature Reserves with management (4th zone), C - zone of National park (3rd zone) and D - buffer zone of National Park where are urban areas (2nd zone).

The National Park includes some of the most natural beech forest reserves in Europe. For example, long-term mycological research in Poloniny National Park revealed the occurrence of 1,244 fungal taxa (Kuthan *et al.* 1999). Some proposed indicators and 'species of special interest' apear to be common and abundant, e.g. *Ceriporiopsis gilvescens*, *Dentipellis fragilis*, *Pluteus umbrosus* (Adamčík *et al.* 2007).

In terms of the phytogeographical division of Slovakia, the area of Poloniny National Park is the only are where East Carpathian endemic species occur. They are represented by *Dianthus barbatus* L. subsp. *compactus*, *Campanula abietina, Silene nutans* subsp. *dubia, Festuca saxatilis, Cirsium waldsteinii, Ranunculus carpaticus, Melampyrum herbichii, Scorzonera rosea* or *Viola dacica* (Zemanek 1991; Dostál 1989).

The animal diversity of the region is documented by 5,981 species of invertebrates, including: 91 species of molluscs (Čejka *et al.* 2006); 234 species of mites and 403 species of other arachnids (Mašán and Svatoň 2003); 25 species of opiliones (Mihál *et al.* 2003); 71 species of mayflies and 42 species of stoneflies (Novikmec *et al.* 2007); 1,472 species of beetles (Jászay 2001); 43 species of caddisflies (Novikmec *et al.* 2007); and 819 species of butterflies (Panigaj 2000).

Data collection

The research was carried out in spring, summer and autumn of 2012 as well as during spring and summer 2013. All observed species in tge field were localized by GPS devices. The following information was collected from field research: field lists of herbaceous vascular plants, epiphytic and epilithic bryophytes, vertebrates (including amphibians), indicator species of birds as well as insects and macrozoobenthoites. Emphasis was placed on species of conservation concern, i.e. redlisted, Red book and Natura 2000 species and indicators of primeval beech forests. Indicator species of primeval beech forests were selected through the assumption that old-growth forests are sources of biodiversity and have a high ecological value (Spies and Franklin 1996).

From the available literature, we excerpted species of bryophytes, which the authors considered as indicator species of old-growth forests (or unmanaged forests, or climax forest). In Table 1 is a list of species, which 11 authors (Andersson 1991; Gustafsson and Hallingbäck 1988; Maksimov *et al.* 2003; Ódor and Van Dort 2002; Trass *et al.* 1999;

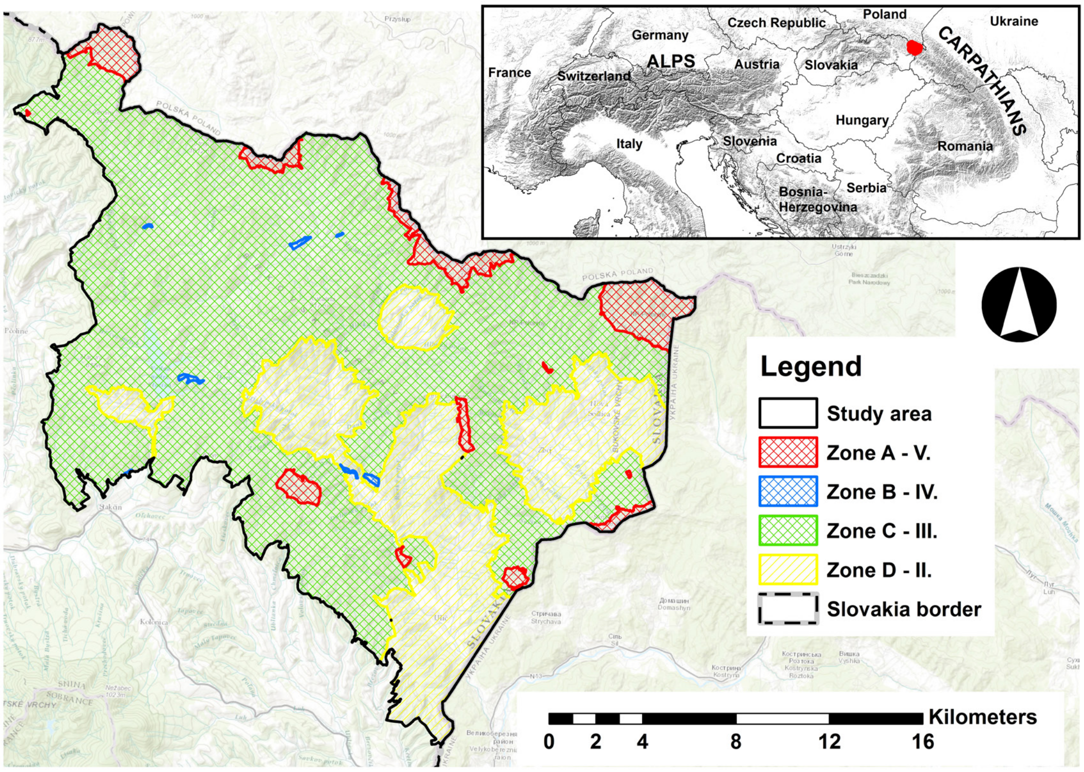

Fig 1. The study area NP Poloniny and current zonation [(N 49° 02' 07.90"; E 22° 19' 39.62") Zones according to Slovak Act. 543/2002 are divided on the basis of the conservation status (five zones of protection): A - National Nature Reserves with strict protection (5th zone), B - Nature Reserves with management (4th zone), C - zone of National park (3rd zone) and D - buffer zone of National Park where are urban areas (2nd zone)].

	F	1	2	3	4	5	6	7	8	9	10	11
Buxbaumia viridis	6	•	•	.	•	•	.	.	.	.	•	•
Calypogeia suecica	6	.	•	•	•	•	.	.	•	.	.	•
Nowellia curvifolia	6	•	•	.	•	•	.	.	•	.	.	•
Anastrophyllum hellerianum	5	•	.	.	•	•	.	.	.	•	.	•
Blepharostoma trichophyllum	5	•	.	.	.	•	.	.	•	•	.	•
Lepidozia reptans	5	.	.	.	.	•	•	.	•	•	.	•
Lophozia ascendens	5	•	.	•	•	.	.	.	.	•	.	•
Lophozia longidens	4	•	.	.	.	•	.	.	.	•	.	•
Lophozia longiflora	4	.	.	•	.	•	.	.	.	•	.	•
Tetraphis pellucida	4	•	.	.	.	.	•	.	.	•	.	•

Table 1. Bryophyte indicator species. F - Frequency, Column 1 - (Andersson 1991) Sweden; Column 2 - (Gustafsson and Hallingbäck 1988) Sweden; Column 3 - (Maksimov *et al.* 2003) Finland; Column 4 - (Ódor and Van Dort 2002) Slovenia; Column 5 - (Trass *et al.* 1999) Estonia; Column 6 - (Vellak and Paal 1999) Estonia; Column 7 - (Hokkanen 2004) Finland; Column 8 - (Ódor *et al.* 2005) Slovenia, Hungary, The Netherlands, Belgium, Denmark; Column 9 - (Ohlson *et al.* 1997) Sweden; Column 10 - (Hodgest 1996) Europe; Column 11 - (Söderström 2006) Sweden.

Vellak and Paal 1999; Hokkanen 2004; Ódor *et al.* 2005; Ohlson *et al.* 1997; Hodgest 1996; Söderström 2006) in Europe consider to be indicator species of old-growth forests (primeval) or natural forests. If less then four authors considered a species to be an indicator, it was excluded from this category.

In unmanaged natural forests the following species of bryophytes occur: *Dicranum fuscescens, Dicranum majus, Harpanthus flotowianus, Herzogiella seligeri, Homalia trichomanoides, Jungermannia leiantha, Lophozia occurr, Mnium stellare, Neckera pennata, Odontoschizma denudatum, Plagiothecium undulatum, Pseudobryum cinclidioides, Rhizomnium punctatum, Riccardia latifrons, Riccardia occurre, Scapania umbrosa, Sphagnum girgensohnii, Sphagnum teres, Trichocolea tomentella, Ulota crispa, Hylocomiastrum umbratum.*

Herbaceous vascular plants were mapped in forest stands following the national classification of habitats (Stanová and Valachovič 2002) for beech and mixed beech forests. We focused on species of conservation interest (IUCN, Red Data Book, SR) using the following categories: EN (endangered), NT (endangered near threatened), VU (vulnerable), CR (critically endangered), east carpathian endemits and occurrence subendemits. Localities were selected predominately within protected areas and the occurrence of species were recorded using GPS. Nomenclature of plant taxa follows Marhold and Hindák (Marhold and Hindák 1998).

In the case of vertebrates, we reviewed the occurrence of some species of mammals, amphibians, reptiles and birds, which are of conservational concern (Natura 2000). We focused particularily on the collared flycatcher (*Ficedula albicollis*) and red-breasted flycatcher (*Ficedula parva*), which are top bio-indicators of natural beech forest in the Poloniny region (Pčola 2012). Special attential was also focused on the yellow-bellied toad (*Bombina variegata*) and carpathian newt (*Triturus montandoni*) because of the ecological requirements of amphibians on their temporary occurrence, they may useful to the design of corridors (from viewpoint of behavioural ecology) between protected sites.

Relative to the corridor design, we analysed the water quality in 17 streams (Stužická rieka, Kamenistý potok, Zbojský potok, Packov potok, Ráztoka, Hrabový potok, Javorník, Hlboký potok, Ulička, Černegov potok, Smolník, Stružnica, Oľchovec, Černinský potok, Udava, Skorský potok, Pčolinka) (Table 2, Fig. 2c), where we collected samples and identified aquatic invertebrates (mayflies, stoneflies and caddisflies). Qualitative samples of macrozoobenthos were collected using a 'kicking' technique

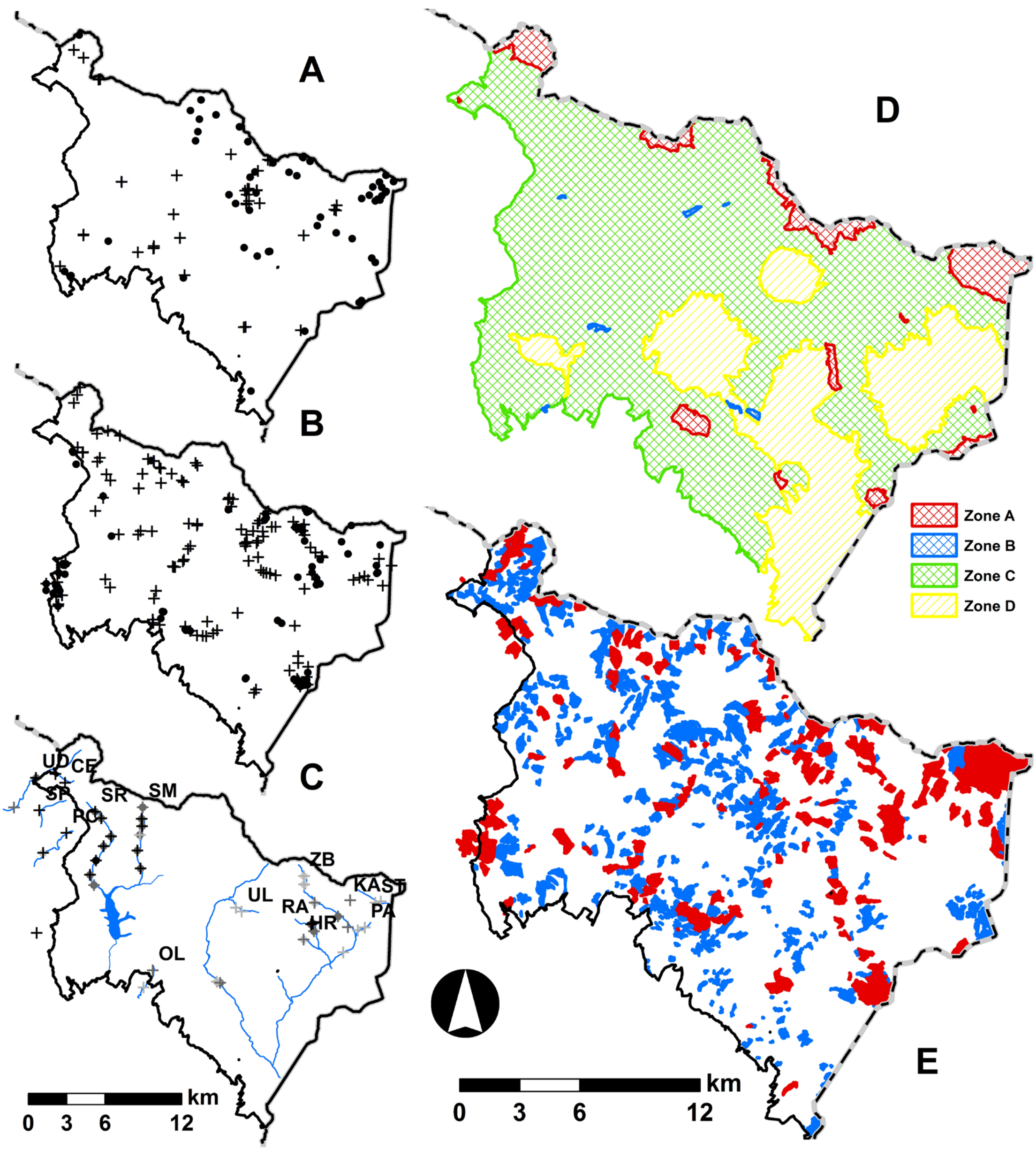

Fig. 2. Landscape matrix; a) – hotspots of Bryophytes (+) and vascular plants (•); b) – hotspots of *Ficedula* ssp. (+) and other vertebrates (•); c) – water quality sampling sites (+), aquatic invertebrates (•) (CE - Černinský brook; HR - Hrabavý brook; KA - Kamenistý brook; OL - Oľchovec brook; PA - Packov brook; PC - Pčolinka brook; RA - Ráztoka brook; SP - Skorský brook; SM - Smolník river; SR - Stružnica brook; ST - Stužická river; UD - Udava brook; UL - Ulička river; ZB - Zbojský brook; see Table 2); d) – current zonation (the most strictly protected zone A represent nature reserves inside of the national park); e) – areas of interest (red) and old grown forest (blue).

(Frost *et al.* 1971). We used a hydrobiology D-net with a 0.25 mm mesh. Samples were supplemented by the individual collection of adult aquatic invertebrates using entomological nets. Collected materials were preserved in 4% formaldehyde (Leuven *et al.* 1985) for transport to the laboratory. In the laboratory, nymphs were separated from detritus andindexed by these determination keys: Bouchard (2004), Krno (1998; 2004), Lillehammer (1988), Malicky (2004), Rozkošný (1980), Soldán and Landa (1999), Szczesny (1978), Waringer and Graf (1997), Waringer *et al.* (2010) and Zwick (2004). Bisel index was counted (Macko *et al.* 2012). At each sampling site physical parameters such as pH, water temperature, conductivity (COND), concentrations of total dissolved solids (TDS), salinity and dissolved oxygen (DO) were measured in "in situ" by a Multi 3430 device (WTW GmbH, Weilheim, Germany). Actual weather, bank characteristics (width and depth) and potential sources of pollution were recorded.

Methodology of hotspot creation

From selected indicator species and species of conservation interest we created layers for hotspots (spatial points of occurrence of the species of interest), which were used to create a basis for new a proposal of zonation for NP Poloniny. Using GIS (method overlaying maps) from the hotspot layers, we mapped the areas of high diversity for relevant species. The spatial units of the forest (spatial division of forest stand units used in forest management in Slovakia) were used as a base layer to plot layers of hotspots. The areas (polygons from base layer of forest stand units) with occurrence of relevant species, were rated as the "areas of interest".

The distribution of all areas of interest was included in the complex of landscape matrix (Fig. 2 a-e), which contains:

a) Spatial distribution of old-beech forests (forests older than 100 years; Fig. 2e). Old-growth forests are sources of biodiversity and have a high ecological value (Spies and Franklin 1996; Brunet *et al.* 2010). We used forest stand maps from the National Forestry Center in Slovakia.

b) Vegetation (Fig. 2a). Some bryophytes of European importance, bryophytes occurring in unmanaged natural forests and more redlisted species were recorded in the comparison to previously published data. Occurence of East Carpathian endemic vascular plants was detected.

c) Vertebrates (Fig. 2b). Temporary occurrence of vertebrates (especially amphibians) may be useful to the corridor's design, the biocorridor's inside and among protected sites.

d) Birds (Fig. 2b). Changes in bird populations are often suspected to be related to environmental modifications caused by man. Variations in the breeding activity of "nest hole" birds are usually endpoints of a series of chained effects at various levels of biological organization. Collared flycatcher (*Ficedula albicollis*) and red-breasted flycatcher (*Ficedula parva*) tend to prefer old and tall trees offering nest holes high enough above the ground (Mitrus and Soćko 2008). The songs of these two bird species are easily recognisable, and the breeding areas are thus extremely suitable for the creation of old natural beech forest maps in the Poloniny region.

e) Water quality (Fig. 2c, Table 2). The network of waterways and riparian vegetation creates the most important biocorridors to ensure connectivity of dispersal protected sites. Presence of important groups of aquatic invertebrates (their quantity and diversity) may indicate the level of water pollution.

Results and Discussion

Vegetation

We have added species of European importance as well as redlisted species (Kubinská *et al.* 2001) to the selected species of bryophytes according to the methodological principles, if they have considered by some authors as indicator species (Fig. 2a). In the territory of the Bukovské vrchy hills there are only two species of Europaean importance - *Buxbaumia viridis* and *Dicranum viride*, which is as indicator species identified by Trass *et al.* (1999) and Hodgest (1996). Within the investigated territory, *Dicranum viride* was collected by Soldán (Šoltés and Buraľ 2012). *Neckera pennata* has been recorded as an indicator species by three authors, and as it is a redlisted bryophyte, it is considered to be an indicator species. The redlisted species *Porella cordaeana*, has been recorded as an indicator by less than three authors, and in the investigated area it was recorded by Peciar (1987). *Orthotrichum gymnostomum*, was also found in the investigated area by Peciar (1987). *Orthotrichum pallens*,was found in the investigated area by Plášek (2007). *Anacamptodon splachnoides*, was found in the investigated area by Soldán (Šoltés and Buraľ 2012), Herben *et al.* (1980). Finally, *Anastrophyllum michauxi*, was recorded in the investigated area by Soldán (Duda and Váňa 1984; Šoltés and Buraľ 2012).

The number of epixylic species correlates with the diameter of lying trunks (Hradílek 1999). Baldwin and Bradfield (2005) examined the relationship between bryophyte diversity and the age of the stands. Bryophyte diversity sharply increased when the age of stands was over 300 years. Similar results have been obtained by Fenton and Bergeron (2008), who investigated correlation between bryophyte diversity and age of *Picea mariana*. The diversity reached its height at an age of 275 years. Gustafsson *et al.* (2004) have elaborated on a list of bryophytes, indicating that habitats of old, uneven aged stands with dead wood result in high diversity. The trunks and stumps have a different ecology. The trunks are more humid, with higher diversity of liverworts. In the managed forests, the stump diversity may even be higher (Rajandu *et al.* 2009).

Bryophyte biodiversity of managed and unmanaged forests has been compared by other authors (Table 1). Vellak and Paal (1999) compared bryophytes in managed and unmanaged forests in Estonia. Up to 50% of the species represented in the virgin forests in Estonia do not occur in younger forests. Thirty percent of the species in old unmanaged forests were liverworts, while in younger managed woods the liverworts only accounted for 17% of bryophytes. Söderström (1988) found that in natural forests liverwort occurs more frequently, while in managed forests lichen of the genus *Cladonia* occurred more frequently. This is because of decreased humidity and the lack of decaying wood in managed forests. Managed forests are younger, approximately evenly aged, and old trees and dead wood are missing; thus, the substrate for epixylic flora is lacking (Söderström 2006). Ódor and Standovár (2001) when compared to the diversity of bryophytes in unmanaged and managed beech stands in Hungary.

Bryophytes in unmanaged stands showed a much greater diversity than in managed forests. In order to conservation biodiversity, it isessential for the bryophytes to protect isolated natural stands, and in particular the availability of dead wood. Gustafsson and Hallingback (1988) compared bryophytes of virgin forests and managed spruce forests in southwestern Sweden. Bryophytes of virgin forests are distinquished from bryophytes of managed forests, in particular, by the presence of liverwort species heralding the presence of thick trunks. Species such as *Calypogeia suecica, Odontoschisma denudatum, Scapania umbrosa* can be found in virgin forests. Andersson and Hytteborn (1991) researched species that occur in both managed forests and virgin forests, followed by species native only to virgin forests, and finally, species found only inmanaged forests. Kushnevskaya *et al.* (2007) compared the bryophytes of managed and semi-natural forests at an advanced stage of succession in the Northwest of Russia. Analysis of bryophytes found different liverworts in managed and semi-natural forests. Trass *et al.* (1999) did not consider hemerophobic species to be indicators in virgin forests, but they meet the ecological conditions in these forests. Ohlson *et al.* (1997) consider epigeic hydromorphic species (*Pseudobryum cinclidioides*, *Rhizomnium pseudopunctatum* and others), as well as other species tied to dead wood, (*Lepidozia reptans, Anastrophyllum hellerianum, Tetraphis pellucida*, among others), to be indicators of natural swampy forests. Hokkanen (2004) defined the group of bryophytes found in unmanaged shady forests. In addition to the lignicole or epiphytic species, a large part of the group is made up of epigeic bryophytes, particularly *Sphagnum* species such as *Sphagnum fimbriatum, S. girgensohnii, S. centrale* and *S. teres*. Lesica *et al.* (1991) compared the abundance of liverworts in the climax forest and managed forests in Canada. They have shown that many species found their optimum in climax forests and are decreasing in managed forests. Hodgest (1996) consider three species of bryophytes, restricted to unmanaged old forests – *Scapania massalongi* (CR), *Buxbaumia viridis* (VU), *Dicranum viride* (EN)- to be endangered species in Europe. These bryophytes are referred to in Annex I of the Berne Convention and in Annex II of Habitats directive, and are important European species occurring in Slovakia.

Regarding vascular plants, an important floristic phenomenon in Poloniny is the presence of elements of East Carpathian flora, including rare and endangered species (Fig. 2a). This was the reason for the classification of Poloniny as an “Important Plant Area “code - IPA EN 262 (Galvánek 2007). In the forest communities, we have recorded species belonging to the Dacian microelement (Hendrych and Hendrychová 1979), including *Helleborus purpurascens* and *Aposeris foetida*. The Dacian migroelements is represented by species *Dentaria glandulosa* and *Symphytum cordatum*. Hadač and Terray (1989) also recorded these species, which are important for the preservation of the gene pool of eastern elements in our flora. *Helleborus purpurascens* is also a species of conservation interest in the EN – endangered category. This species is restricted to the Bukovské vrchy Mts., and has been recorded at 35 sites – Ulič, valley Stužica, Ostrá, etc. (Čeřovský *et al.* 1999). We observed this species in Starina and below Baranec hill. During the evaluated period, we identified the presence of: 5 species considered near threatened (NT); 13 species labelled vulnerable (VU); 3 endangered (EN) species; and 1 critically endangered (CR) species (2 carpathian subendemits – Ks and 2 east carpathian endemits). From the redlisted species list, we recorded *Carex canescens* (NT), *Listera ovata* (VU), *Lycopodium annotinum* (NT), *Molinia caerulea* (VU), *Dactylorhiza fuchsii* subsp. *fuchsii* (VU), *Lilium martagon* supsp. *martagon* (NT), *Lathyrus laevigatus* (EN), *Aconitum moldavicum* (VU), *Centaurium erythraea* subsp. *Erythraea* (NT), *Scutellaria altissima* (CR), *Cephalanthera damasonium* (VU), *Platanthera bifolia* (VU), *Gymnadenia conopsea* (VU), *Menyanthes trifoliata* (EN), *Dactylorhiza majalis* subsp. *majalis* (VU), *Carex lepidocarpa* (NT), *Epipactis palustris* (VU), *Traunsteinera globosa* (VU), *Dianthus barbatus* subsp. *compactus* (VU), *Veratrum album* subsp. *album* (VU), *Gladiolus imbricatus* (VU), *Helleborus purpurascens* (East Carpathian endemit), Aposeris foetida (East Carpathian endemic), *Dentaria glandulosa* (Carpathian subendemic) and *Symphytum cordatum* (Carpathian subendemic).

Aquatic insect and water quality

In total, 53 localities were analysed in 2012 and 2013 in Poloniny National Park and adjacent areas (Fig. 2c). In 2012, 41 species (22 *Ephemeroptera*, 3 *Plecoptera* and 16 *Trichoptera*) of aquatic insect were recorded. In 2013, 18 species of *Plecoptera*, and 17 of *Trichoptera* were recorded. At some sampling sites, *Drusus brunneus*, the Carpathian endemic species of *Trichoptera* was found. Only two known samples exist from Slovakia (Bitušík and Novikmec 1997; Novikmec *et al.* 2007), both from The Eastern Carpathians. From the *Rhitrogena* group, the Carpathian endemic species *Rhitrogena gorganica* was recorded. This species is an indicator of water purity (Mihaljevic *et al.* 1998). As a result of a lack of “reliability” of determination features and due to poor vagility of mayflies and isolation by mountain ridges, the determination of genus is very difficult (Soldán and Landa 1999).

As is seen in Table 2 the water quality in the monitored flow is good. Higher levels of nitrates were occassionaly found in Kamenistý brook, Stužická River and Zbojský brook, Packov brook, Ulička River - probably caused by leaching from subsoil.

Changes in the physical-chemical characterics of water quality are influenced not only by anthropogenic processes (Tymczyna *et al.* 2000; Zielinński *et al.* 2003), but also by natural processes such as hydrological conditions, topography and lithology, climate, precipitation inputs (Walna *et al.* 2003; Polkowska *et al.* 1999), and catchment area (Glinska-Lewczuk 2006), in combination with environmental influence.

Stream	pH	COND [μS/cm]	O_2 [mg/l]	$CaCO_3$ [mg/l]	$N\text{-}NO_3^-$ [mg/l]	amonia [mg/l] N	Cl^- [mg/l]	SO_4^{2-} [mg/l]	Bisel index	Water quality
KA	7.16	47.10	12.29	25	0.955	<<	0.1	1	*	+
ST	7.41	62.93	11.74	35	0.785	<<	0.4	2	*	+
ZB 1	8.29	223.00	10.44	95	>>	0.71	1.1	25	5	+ ●
2	8.35	253.00	10.49	115	>>	<<	0.4	29	5	+ ●
3	8.25	219.00	10.36	120	0.555	0.08	0.8	20	5	+ ●
4	8.23	217.00	10.32	110	0.422	0.67	1	17	7	+ ●
5	6.89	41.53	11.36	20	0.466	<<	0.6	0	*	+
6	8.6	144.46	11.40	85	0.510	0.01	0.2	7	*	+
7	8.37	159.60	10.91	85	0.735	<<	0.5	5	*	+
PA 1	*	148.40	11.4	80	0.705	0.03	0.6	1	*	+
2	7.91	123.00	11.93	75	0.935	<<	0.4	2	*	+
RA 1	7.59	115.13	12.14	70	0.341	0.08	26.5	3	7	+ ●
2	7.60	150.40	11.73	95	0.243	<<	19	0	7	+ ●
3	7.59	131.60	12.53	75	0.477	>>	4.1	0	7	+ ●
HR	7.24	123.10	13.00	65	0.630	<<	0.6	5	6	+ ●
UL 1	8.4	102.57	11.74	60	0.860	0.01	0.6	4	5	+ ●
2	7.80	115.30	11.51	70	>>	<<	0.4	2	5	+ ●
3	7.90	195.80	12.50	115	0.890	<<	2.4	5	5	+ ●
4	8.00	153.17	12.90	70	0.426	0.02	4.5	0	7	+ ●
SM 1	7.88	173.53	9.65	80	0.685	<<	0.8	3	7	+ ●
2	7.86	147.83	9.60	60	0.293	<<	0.7	3	7	+ ●
3	8.00	170.93	9.58	70	0.252	<<	0.4	14	7	+ ●
4	8.3	171.63	9.54	60	>>	<<	0.6	18	7	+ ●
5	8.10	189.90	9.22	80	0.201	<<	0.5	11	7	+ ●
6	8.28	225.00	9.18	95	0.276	0.03	0.3	20	7	+ ●
SR 1	8.29	232.67	9.60	110	0.233	<<	0.1	24	8	+ ●
2	8.36	234.00	9.54	100	0.350	<<	1.0	14	7	+ ●
3	8.43	233.50	9.17	135	0.362	<<	0.3	17	8	+ ●
4	8.51	236.00	9.30	100	0.136	<<	0.0	13	7	+ ●
5	8.36	258.00	9.14	135	0.371	0.07	1.7	25	9	+ ●
6	8.56	256.00	9.45	115	0.381	0.03	0.5	10	7	+ ●
7	8.50	256.00	9.26	105	0.471	<<	1.2	42	7	+ ●
OL 1	8.19	166.80	9.69	95	0.453	0.00	0.9	3	*	+
2	8.19	166.80	9.69	75	0.815	0.02	0.5	7	*	+
CE 1	8.38	302.00	9.63	150	0.235	0.09	1.8	8	*	+
2	8.37	342.67	9.46	155	0.255	0.02	0.8	2	*	+
UD 1	8.37	325.67	10.37	150	0.201	0.04	0.1	3	7	+ ●
2	8.18	350.67	10.3	150	0.473	0.07	1.9	3	5	+ ●
SP	8.8	335.00	9.26	140	0.214	0.21	0.5	5	6	+ ●
PC 1	8.22	342.67	9.34	160	0.265	0.04	0.2	5	*	+
2	8.38	424.00	9.76	195	0.164	0.05	6.9	15	6	+ ●
3	8.13	551.67	9.4	230	0.178	0.05	12	5	7	+ ●

Table 2. The physico-chemical properties of water samples. Legend: CE (Černinský brook), HR (Hrabavý brook), KA (Kamenistý brook), OL (Oľchovec brook), PA (Packov brook), PC (Pčolinka brook), RA (Ráztoka brook), SP (Skorský brook), SM (Smolník river), SR (Stružnica brook), ST (Stužická river), UD (Udava brook), UL (Ulička river), ZB (Zbojský brook), << bellow detection limit, >> above detection limit. Sampling sites are numbered downstream (se Fig. 2 C). Invertebrata evaluation (● without contamination; ● slight contamination; ● medium contamination); + water quality (**+** low level of nitrate; + medium level of nitrate; + high level of nitrate); * Not recorded

Vertebrates

Transferring the data on flycatcher occurrence onto forestry maps shows the essential structure of suitable habitats for birds breeding in nest cavities (Fig. 2b). Moreover, we found nine species of mammals, five species of amphibians, four of reptiles. *Ursus arctos* was recorded at 14 locations, *Bison bonasus* at 6 localities, *Canis lupus* at 5 localities, *Castor fiber* at 2 locations and *Lynx lynx* at 1 locality. Besides mammals and birds we have, for example, recorded 155 individuals of *Bombina variegata* at 61 locations and 34 individuals of *Triturus montandoni* at 29 different sites. Several studies show the difference in bird species richness between managed forests and old-growth forests (Boncina 2000; Müller *et al.* 2007). Dead wood evidently holds a key role for certain bird species like woodpeckers or flycatchers (Swallow *et al.* 1988). Any kind of wood use in beech forests as well as in other forests leads to a significant reduction of a whole series of specialised species, even if the natural tree species composition is not changed (Müller *et al.* 2007). The main difference between managed forests and old-growth forests is in the amount of coarse woody debris. Siitonen (2001) found that amount of dead wood in unmanaged boreal forests varied from 18% to 37% of the total wood volume.

In general, our results concerning the breeding of flycatchers in relation to old beech forest substantiate previous findings for many other European populations of these two species. The selection of the species is very useful to compose hotspot diversity maps in beech forest areas. The information in these maps is long-termed because pairs of flycatchers very often breed in the holes of the previous year or in the nearest one available. The map layer of occurrence of rare or highly-protected vertebrates enable us to design a map of potential ecological corridors (see Corsi *et al.* 2002). Amphibians are vagrants, especially during a nesting period, and their temporary occurrence may be very useful to design the corridors. This approach was very helpful to design the newly suggested zonation map of Poloniny National Park.

Hotspots and zonation

According to the philosophy of global biodiversity hotspots (Myers 2010), we created local biodiversity hotspots in our study area. Grant and Samways (2011) employed a similar definition of 'micro-hotspots' for identification of biodiversity hotspots of dragonflies in the Kogelberg Biosphere Reserve. Our hotspots (Fig. 2a-c) represent points with the presence of relevant species (indicator species and species of conservation interest) which were observed or collected in the field. Here we focus on these species, as the most prominent and readily recognizable form of biodiversity. Therefore, our approach may not be sufficient to fully capture all levels of biodiversity. To solve this issue, some authors (Onaindia *et al.* 2013, Peńa *et al.* 2016) quantified different levels of biodiversity using variables such as plant richness, habitat quality and protection status. We used a simple method (in GIS) of overlaying maps of the hotspots with maps of forest stands (where forests older than 100 years were also included). Old-growth forests are sources of biodiversity and have a high ecological value (Spies and Franklin 1996; Brunet *et al.* 2010). Areas (forest stands) with a presence of relevant species were considered to be the areas of greatest interest in relation to nature conservation.

Hotspots and areas of interest should be one of the sources of suggested zonation. Most of the local hotspots should be included within zone A or zone B (see description of Fig. 1). Zone A represents category 1a (Strict Nature Reserve) according to the IUCN protected areas categories and zone B is category IV (Habitat/Species Management Area). Some hotspots occure outside these zones, e.g. bryophyte *Homalia trichomanoides* is an epiphyte of broadleaved trees. It often occurs in the woods in the residential areas of settlements, surviveing on trees that are remnants of a former continuous forest.

In addition to forestry criteria regarding species composition, spatial and age structure, as well as the presence of indicator species should beincorporated into hotspots to determine suggested zonation. Old, uneven-aged stands with high connectivity and the presence of hotspots should be included in zone A as it includes all the national nature reserves (Stužica, Havešová, Riaba skala, Rožok, Pľaša, Stinská, Pod Ruským) in NP Poloniny.

Compared to the current zonation (Fig. 2d), zone B is quite different. This zone should affect the degree to which a landscape is connected; influencing gene flow, local adaptation, extinction risk, colonization probability, and the potential for organisms to move as they cope with climate change. We think, zone B should be comprised of primarily old beech stands, but also beech stands influenced by clearcuting if situated close to zone A. We consider the successional stage of the forest ecosystem to be part of the virgin forest, as it originated through disturbance of the forest area and it is expected that it will be left to natural development. An important consequence of clearing by human activity is that continuous cover is broken down into isolated patches. If this activity continues, cleared areas may exceed a critical level, which means that landscapes will exhibit two phases; connected and disconnected. This is why we think that zone B should be extended and should link zone A together. Brunet *et al.* (2010), in a review of biodiversity in European beech forests, demonstrates that areas with predominant shelterwood management (in our study this is relevant for zone C and B) only have limited value for conservation of most species groups. Thus, the area of unmanaged reserves of beech forest needs to be larger. Zone C (IUCN category II - National Park), or the buffer zone, is intended to avert the effect of negative environmental or human influences. It links zones A and B, and increases their dynamics or conservation effects. Zone D (IUCN category V - Protected Landscape) consists of residential areas of settlements and cultural landscape. In principle, zonation models of protected sites in Europe are varied (Solár *et al.* 2015). Our zonation model is inspired by zoning in the National Park of Abruzzo, Italy (Singe 2004).

Rare beech stands of this shared Slovakian-Polish-Ukrainian territory have become a subject of protection of World Heritage (Conference of Committee of the UNESCO World Heritage in New Zealand, Christchurch, June 28, 2007). However, at present, increasing economic pressure on timber harvest in beech forests are becoming obvious. Creation of world heritage boundaries has been considered with many small island enclaves, which assume that the phenomenon of fragmentation is adverse.

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