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Distribution and eco-coenotic patterns of the forest orchid *Epipactis pontica* in Slovakia

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Abstract. The present study is aimed at characterising the ecological niche of a typical forest orchid (*Epipactis pontica*) in Slovakia. Vegetation-environmental data were collected across mountain ranges and their foothills in the Western Carpathians in 2011. Numerical classification was performed to delimit the main forest vegetation types and a linear mixed effect model was applied to reveal differences between plots with versus without *E. pontica*. This endangered species of Slovak flora grows in thermophilous turkey oak forests (*Quercion confertae-cerris*), mesophilous broad-leaved mixed oak-hornbeam forests (*Carpinion betuli*), but most stands correspond to the acidic (*Luzulo-Fagion sylvaticae*) and mesotrophic beech forests (*Fagion sylvaticae*). Principal component analysis supported the floristic separation of plant communities and showed some significant vegetation-environmental relationships. *E. pontica* prefers forests with closed canopy (mean canopy openness: 8.5–15.1%) occurring especially on slightly acidic (soil reaction: 4.48–5.65) and nutrient-poor soils (soil conductivity: 43.1–72.6 µS/cm). The proposed Ellenberg indicator values for light (4), temperature (5), continentality (4), moisture (5), soil reaction (6) and nutrients (5) follow species composition pattern of vascular plants in Slovak phytosociological relevés with *E. pontica* occurrence. They are also in accordance with its habitat conditions and ecological requirements in other parts of its range. The linear mixed effect model did not confirm any environmental peculiarity of plots with the presence of *E. pontica* at a microscale level and this result was consistent across the studied sites.
Keywords broad-leaved forests, Central Europe, ecological indicator values, endangered species, habitat conditions, *Orchidaceae*.

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Introduction

The orchid family is one of the world's largest groups of flowering plants with almost 25.000 known taxa (Chase et al. 2003). Its distribution range comprises mainly tropical and subtropical regions, with a decline in species diversity in temperate Europe (Delforge 2005). Variation in number of species reported in Europe has usually been attributed to diverse taxonomic treatment and status of local endemic taxa. The same is also true for the genus *Epipactis* (Tranchida-Lombardo et al. 2011), which consists of a considerably variable group of species in terms of morphology (Hollingsworth et al. 2006).

Epipactis pontica Taubenheim (syn. *E. helleborine* subsp. *pontica* (Taubenheim) Sundermann, *E. persica* subsp. *pontica* (Taubenheim) H. Baumann et R. Lorenz) is a polycarpic and self-pollinating species belonging to the *E. helleborine* group (Delforge 2005). This terrestrial and typical forest species, with a slim stem and lanceolate to narrow lanceolate leaves, can reach a height of 30 cm. The sparse spike is composed of a few small white-green and most often only slightly open flowers. The distinctive floral morphology is characterised by a widely U-shaped mesochile, a flat hypochile and a pronounced but non-functional rostellum (Taubenheim 1975, Batoušek 2010, Batoušek & Kežlínek 2012).

Although *E. pontica* was originally described by Taubenheim (1975) from the Pontic Mountains in the northern part of Turkey, its recent range encompasses predominantly woodlands in the Balkan Peninsula and Central Europe (Delforge 2005, Sulyok & Molnár 2011). Due to the scarcity and vulnerability of its populations, *E. pontica* was declared endangered and included in Central European national red lists (e.g. in Hungary – Király 2007, Czech Republic – Grulich 2012). This species is also considered to be endangered in Slovakia (Feráková et al. 2001), where it was first documented by Vlčko (1995) from 10 scattered sites across the Western Carpathians and their foothills. However, an increased number of new localities have been observed in the last two decades (Mereďa 1997, Vlčko et al. 2003).

Distribution, population dynamics and vitality of the orchid species are closely associated with plant-fungal interactions (Rasmussen 1998, Taylor & Bruns 1999), pollination and seed germination (Rasmussen & Whigham 1993), but environmental factors and human impact also play important roles (Stuckey 1967, Silvertown et al. 1994, Vallius 2001, Janečková et al. 2006). Similar studies devoted to the genus *Epipactis* or specifically to the species *E. pontica* are rare (Ehlers et al. 2002, Jurčák et al. 2005, 2006).

In spite of increasing interest in many aspects of its distribution, morphology and ecology (e.g. Batoušek 1996, Mereďa 1997, Petrova & Venkova 2006), a complex ecological and coenological study has been lacking. Although biological characteristics represent the most-frequently discussed predictors, our attention focused on environmental requirements and coenotic preference of *E. pontica* in Slovakia. The present autecological study could thus provide relevant information necessary for understanding its rarity and effective conservation. We expected that environmental variables affecting large-scale distribution patterns of *E. pontica* would also be predictors of its occurrence at the microscale level. Explanatory variables were selected according to current knowledge of the species`s ecological preferences.

The aims of the present paper were: (i) to summarise all available distribution data of the species in Slovakia, (ii) to characterise the floristic composition of sites with *E. pontica* in Slovakia, (iii) to assess the environmental conditions that are suitable for the occurrence of the species at the microscale level in selected sites in Slovakia.

Methods

Field sampling

The distribution pattern of *E. pontica* in Slovakia was compiled using available literature sources and original unpublished data (Fig. 1, Electronic supporting information). In order to cover a wide variety of environmental conditions, seven sampling sites with sufficiently large *E. pontica* populations were chosen for detailed ecological research in 2011 (Electronic supporting information, Fig. 1). Ten circular sampling plots (0.5 m^2) without *E. pontica* were randomly selected within each sampling site. Subsequently, the closest specimens of *E. pontica* to each of those plots were found and paired plots of the same size were established around those specimens. Pairs of plots with and without *E. pontica* were treated as matched pairs. A total of 140 plots (7 sites × 10 matched-pair plots) were sampled for environmental characteristics that could affect the incidence of *E. pontica*. One phytosociological relevé with a uniform plot size of 400 m^2 (hereinafter called relevé site) was sampled at each site in accordance with the Zürrich-Montpellier approach using the modified nine-degree Braun-Blanquet cover/abundance sampling scale (Barkman et al. 1964).

Geographical coordinates (WGS-84), altitude and aspect were measured using a handheld GPS (Garmin GPSmap 60 CSx) and slope with the Suunto PM-5/1520PC clinometer on relevé sites and plots. Climatic characteristics of sampling sites (Electronic supporting information) were obtained from Miklós (2002). Microrelief shape (concave, convex, flat), per-

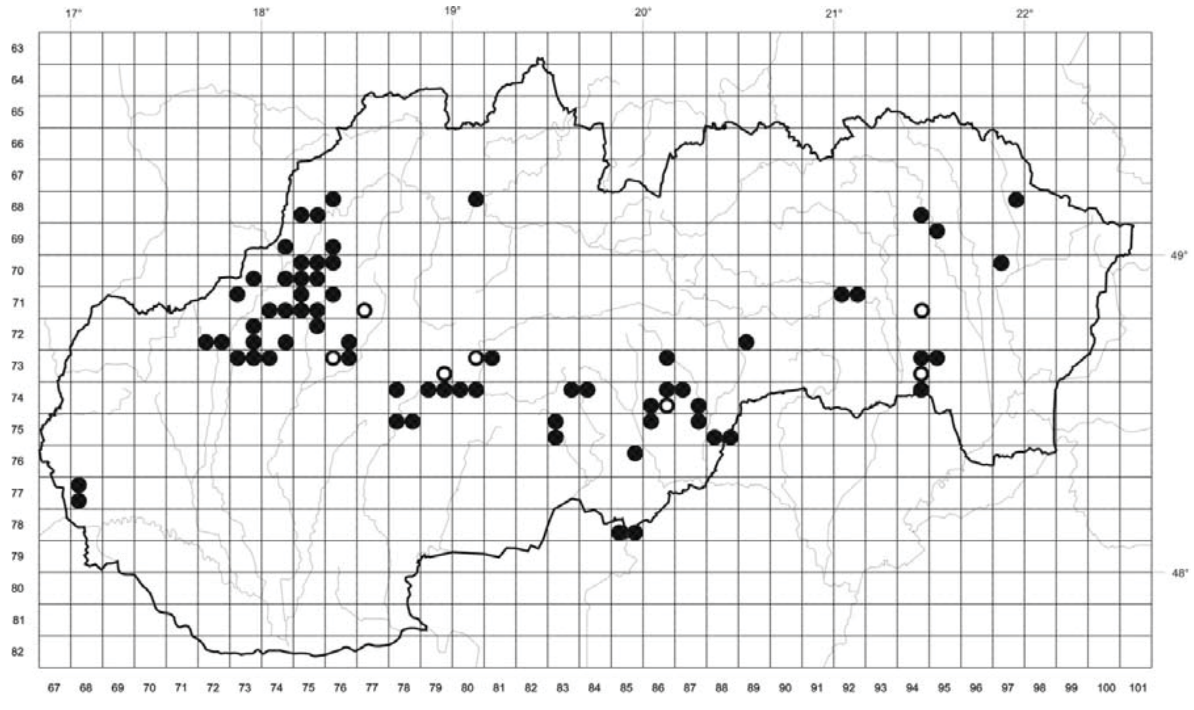

Figure 1 Distribution map of *Epipactis pontica* in Slovakia. Empty circles represent the sampling sites chosen for the present study. From left to right: n. 5 – Skačany; n. 4 – Kanianka; n. 2 – Budča; n. 3 – Vlkanová; n. 1 – Španie Pole; n. 6 – Herľany (upper) and n. 7 – Rákoš (lower)

centage cover of the moss layer (incl. lichens; E_0), herb layer (E_1), stones, bare soil, woody litter and other undecomposed dead biomass were visually estimated in plots. Depth of the litter layer was measured at 10 random places within each plot. Soil samples were taken from three random locations within each plot from the uppermost mineral horizon (8–15 cm, litter removed) in order to reduce soil heterogeneity. Light conditions were assessed from hemispherical photographs taken within the plots. A Nikon Coolpix 5400 digital camera equipped with a fisheye converter FC-E9 was used.

Laboratory and data analysis

Species affinity to the occurrence of *E. pontica* was derived from presence/absence data of all vascular plants, which were recorded in four quadrants within the plots. Relevé sites and plots were stored in the TURBOVEG database software (Hennekens & Schaminée 2001) and exported into the JUICE program (Tichý 2002). Numerical classification of the available phytosociological material (23 relevé sites) was done in the PC-ORD program (McCune & Mefford 1999) using Sørensen's distance as a measure of dissimilarity and the Beta flexible linkage method (β = -0.25) with the logarithmic transformation of species abundance values. The optimal number of clusters was determined using the crispness curve method (Botta-Dukát et al. 2005). Diagnostic species were determined based on the combined concept of fidelity (Φ – phi coefficient; Chytrý et al. 2002) and frequency. Diagnostic species were those with $\Phi > 0.3$ and significant occurrence in a particular cluster (tested by Fisher's exact test at $p < 0.05$), frequency within cluster of > 50% and difference of frequencies among clusters of more than 40%. Before the analysis, several narrowly delimited species or taxonomically complicated ones were united into aggregate taxa or genus level: *Crataegus* sp. (*C. monogyna*, *Crataegus*. sp.), *Galeopsis* sp. (*Galeopsis*. sp., *G. speciosa*, *G. tetrahit*), *Pulmonaria officinalis* agg. (*P. obscura*, *P. officinalis*), *Rosa* sp. (*R. canina* agg., *Rosa* sp.). The species-environment relationship was assessed by principal component analysis (PCA) in the Canoco for Windows package (ter Braak & Šmilauer 2002), as the length of the DCA (detrended correspondence analysis) gradient was 2.69 units. Unweighted Ellenberg's indicator values of vascular plants (EIV; Ellenberg et al. 1992) were used as supplementary environmental variables to explain major variation patterns in the floristic composition. Species abundance values were logarithmically transformed. Correlations between EIVs of individual relevés and relevé scores on the first three ordination axes were assessed by the Spearman's correlation coefficient using STATISTICA software (StatSoft 2001).

Soil samples were air-dried at laboratory temperature, crushed and passed through a 2 mm sieve. Soil pH and conductivity were measured in a distilled-water suspension (soil/water ratio of 1/5) using a pH/conductivity meter (WTW pH/Cond 340i). Canopy openness expressing the percentage of open sky seen from beneath the forest canopy was calculated with the Gap Light Analyser 2.0 program (Frazer et al. 1999). Slope and aspect were used to calculate the xericity index following the equation proposed by Austin et al. (1984). Mean and standard devation (sd) of litter depth were calculated from field measurements. All parameters of sampling sites were derived from data obtained in plots with the presence of *E. pontica*. Environmental data recorded at plot level were treated in a partly nested split-plot design and fitted to a linear mixed effect model of the form:

$$Y = site + matched\text{-}pair\ plot(site) + plot + plot \times site + plot \times matched\text{-}pair\ plot\ (site)$$

where `site`, `matched-pair plot(site)` and interactions are factors with random effects, while `plot` is a factor with a fixed effect testing for differences between plots with and without *E. pontica*. Response variable *Y* is a matrix of 15 environmental variables by 140 plots. Prior to analysis, environmental variables were standardised to minimize the effects of different units of measurement. The model was analysed using permutational multivariate analysis of variance (perMANOVA; Anderson 2001) with Euclidean distance as a dissimilarity measure, an approach analogous to redundancy analysis on a correlation matrix (RDA). The results were visualised as a RDA ordination diagram. Probability values were based on 9999 permutations of the original data. Analyses were performed using DISTLM v.5 (Anderson 2004) and R software (R Development Core Team 2011).

Nomenclature

Nomenclature of vascular plants follows the checklist by Marhold & Hindák (1998), and the names of plant communities and their syntaxonomical position are in accordance with Jarolímek & Šibík (2008).

Results

Distribution in Slovakia

The current known distribution of *E. pontica* in Slovakia comprises 120 sites which are primarily situated in the submontane belt of the central and western part of the Carpathian mountain ranges and the north-eastern edge of the Pannonian Basin (Fig. 1). Its occurrence becomes rarer towards the north regions with some isolated localities in north-eastern Slovakia, while records from the south lowland regions and Inner Carpathians basins are still missing. The largest number of sites (31) are reported from the Strážovské and Súľovské vrchy Mts, followed by the Slovenské rudohorie Mts (15), Biele Karpaty Mts (13) and the Považský Inovec Mts (12). Their altitudinal range is between 200 m and 820 m a.s.l. A detailed overview of sites is presented in the electronic supporting information.

Species composition and vegetation patterns

Sessile oak (*Quercus petraea* agg.) was identified as the most common species and was documented in more than 50% of plots with the presence of *E. pontica* and was also the only species found in all studied sampling sites. Other frequent species in the plots were *Carpinus betulus* (34%), *Fagus sylvatica* (33%) and *Carex pilosa* (29%). The calculation performed on 23 relevé sites provided similar results, where *Fagus sylvatica* (presence in 96% sites), *Quercus petraea* agg. (96%), *Carpinus betulus* (83%) and *Galium odoratum* (70%) were the most frequently co-occurring species.

Differences in species composition within matched-pair plots were found only in sampling site no. 3 (Vlkanová), with only *Carpinus betulus* showing a positive fidelity to *E. pontica* ($\Phi = 0.524$, $p < 0.05$).

Numerical classification divided phytosociological relevés into 4 clusters showing obvious floristic variation (Table 1).

Cluster 1 included rather heterogeneous and species-poor stands (12 species per relevé) with low herb layer cover (8–25%). The monodominant tree layer was composed by *Fagus sylvatica*, whereas species composition of the forest understorey had a conspicuous mosaic structure. This cluster represents a transition between beech-dominated vegetation that corresponded to either mesotrophic canopy-closed beech forests of the association *Dentario bulbiferae-Fagetum* (alliance *Fagion sylvaticae*) or acidophilous beech forests of the association *Luzulo nemorosae-Fagetum* (alliance *Luzulo-Fagion sylvaticae*).

The physiognomy of the forest overstorey in cluster 2 was determined by dominant *Quercus petraea* agg. with a constant admixture of *Carpinus betulus* and *Fagus sylvatica*. This well-differentiated forest vegetation (incl. *Astragalus glycyphyllos*, *Galium schultesii*, *Hi-*

Table 1 Shortened synoptic table of forest vegetation with presence of *Epipactis pontica* showing the frequency and abundance range (as superscripts) of each plant taxa. Only those taxa with occurrence in at least three relevés are shown. They were arranged according to decreasing fidelity to one of the four clusters

Cluster No.	1	2	3	4
No. of relevés	4	4	8	7
Tree layer				
Quercus petraea agg.	75 $^{+}$	100 $^{a-5}$	75 $^{+-3}$	86 $^{a-4}$
Fagus sylvatica	100 $^{b-5}$	50 $^{a-b}$	88 $^{b-5}$	71 $^{+-5}$
Carpinus betulus	75 $^{+-a}$	50 $^{a-3}$	38 $^{b-5}$	86 $^{1-4}$
Populus tremula	25 ^{1}	25 ^{1}	.	14 ^{1}
Pinus sylvestris	.	25 ^{b}	.	14 ^{a}
Picea abies	.	25 ^{1}	12 ^{r}	.
Acer campestre	.	.	.	29 ^{1}
Shrub layer				
Fagus sylvatica	50 $^{a-3}$	25 ^{a}	88 $^{+-1}$	14 ^{a}
Carpinus betulus	75 $^{+-1}$	50 $^{1-b}$	12 $^{+}$	71 $^{+-a}$
Corylus avellana	.	25 ^{1}	.	43 $^{+-1}$
Herb layer: diagnostic species of clusters				
Galium schultesii	.	**75** $^{r-1}$	.	.
Astragalus glycyphyllos	.	**50** $^{+}$	.	.
Poa nemoralis	.	**100** $^{+-1}$	50 $^{+}$	29 $^{+-1}$
Hieracium racemosum	25 ^{1}	**75** $^{+}$	.	29 $^{r-+}$
Carex pilosa	25 $^{+}$	25 ^{1}	**100** $^{+-4}$	14 ^{b}
Lathyrus vernus	25 $^{+}$	.	**75** $^{+-1}$	29 $^{+}$
Pulmonaria officinalis agg.	.	.	12 ^{r}	**100** $^{+-a}$
Polygonatum multiflorum	.	.	.	**71** $^{+}$
Swida sanguinea	.	.	12 $^{+}$	**57** $^{+}$
Rubus hirtus s.lat.	.	.	12 $^{+}$	**57** $^{+}$
Quercus cerris (E_3)	.	.	.	**43** $^{1-3}$
Viburnum opulus	.	.	.	**43** $^{+}$
Ligustrum vulgare	.	.	.	**43** $^{+-a}$
Symphytum tuberosum	25 ^{r}	.	12 $^{+}$	**71** $^{r-+}$
Herb layer: other taxa				
Epipactis pontica	100 $^{+}$	100 $^{+}$	100 $^{+-1}$	100 $^{+}$
Fagus sylvatica	100 $^{1-a}$	100 $^{+-b}$	100 $^{+-a}$	57 $^{+-3}$
Carpinus betulus	25 ^{1}	75 $^{+-b}$	75 $^{r-3}$	86 $^{+-1}$
Galium odoratum	25 ^{1}	50 $^{+-1}$	88 $^{+-3}$	86 $^{+-b}$
Quercus petraea agg.	75 $^{+}$	100 $^{+-1}$	38 $^{r-+}$	86 $^{r-a}$
Luzula luzuloides	75 $^{1-b}$	100 $^{1-a}$	62 $^{+-1}$	29 $^{+}$
Viola reichenbachiana	25 $^{+}$	25 ^{r}	88 $^{r-1}$	71 $^{+}$
Cerasium avium	.	75 $^{r-+}$	50 $^{r-+}$	100 $^{r-1}$
Neottia nidus-avis	50 ^{r}	25 $^{+}$	38 $^{r-+}$	57 $^{r-+}$
Hieracium murorum	75 $^{r-+}$	25 $^{+}$	38 $^{+}$	14 ^{1}
Cephalanthera longifolia	50 ^{r}	50 $^{+-1}$	25 $^{+}$	29 ^{1}
Mycelis muralis	.	75 $^{+-1}$	25 $^{+-1}$	43 $^{+}$
Fraxinus excelsior	.	25 ^{r}	38 $^{+-1}$	57 $^{r-1}$
Crataegus sp.	.	.	38 $^{r-+}$	71 $^{r-+}$
Ajuga reptans	.	.	50 $^{+}$	57 $^{+}$
Cruciata glabra	50 $^{+-1}$	75 $^{+}$	25 $^{+-1}$	.
Dentaria bulbifera	50 $^{+-a}$	25 $^{+}$	25 $^{+}$	29 $^{+}$
Melittis melissophyllum	25 $^{+}$	.	25 $^{+}$	57 $^{r-+}$
Rosa sp.	.	50 $^{r-+}$	25 ^{r}	43 $^{+}$

Table 1 (continued)

Cluster No.	1	2	3	4
No. of relevés	4	4	8	7
Corylus avellana	.	50^{+}	.	71^{+-1}
Melica uniflora	.	25^{+}	25^{+-3}	57^{+-4}
Acer pseudoplatanus	.	25^{+}	38^{r-1}	43^{+}
Asarum europaeum	.	.	38^{r-+}	57^{+-a}
Carex digitata	50^{+}	.	12^{r}	43^{+-a}
Veronica chamaedrys	.	25^{r}	25^{+}	43^{r-+}
Acer campestre	.	25^{+}	12^{r}	57^{+}
Veronica officinalis	.	.	50^{r-+}	29^{+}
Scrophularia nodosa	.	25^{+}	25^{r-+}	29^{r-+}
Tilia cordata	25^{+}	25^{r}	12^{r}	14^{+}
Populus tremula	25^{+}	25^{+}	.	29^{+}
Monotropa hypopitys	25^{r}	25^{+}	12^{+}	14^{+}
Geranium robertianum	.	25^{+}	25^{r-+}	14^{+}
Dryopteris filix-mas	.	25^{+}	12^{1}	29^{+}
Carex sylvatica	.	.	25^{+-1}	29^{+}
Geum urbanum	.	.	12^{r}	43^{r-+}
Melica nutans	.	.	38^{r-+}	14^{+}
Lathyrus niger	.	.	25^{r-+}	29^{+}
Sanicula europaea	.	.	25^{+-a}	29^{+-1}
Athyrium filix-femina	.	.	25^{+-a}	29^{+-a}
Brachypodium sylvaticum	.	.	25^{+}	29^{r-1}
Galeopsis sp.	25^{+}	25^{+}	.	14^{+}
Picea abies	25^{r}	25^{r}	12^{+}	.
Fragaria vesca	.	25^{+}	.	29^{+}
Dactylis glomerata agg.	.	50^{+}	.	14^{+}
Oxalis acetosella	.	25^{+}	25^{+}	.
Campanula persicifolia	.	25^{+}	.	29^{r-+}
Platanthera bifolia	.	.	12^{r}	29^{+}
Prunus spinosa	.	.	12^{r}	29^{r-1}
Hedera helix	.	.	25^{+-1}	14^{r}
Tilia platyphyllos	.	.	25^{+-1}	14^{+}
Maianthemum bifolium	.	.	12^{1}	29^{+}

Note. List of phytosociological relevés in each cluster. Cluster 1: Hrivnák (1997) – 4 relevés; Cluster 2: Author's original (unpublished) – 2 rels., Vlčko (1996) – 2 rels.; Cluster 3: Author's original (unpublished) – 3 rels., Hrivnák (1997) – 1 rel., Mereďa (1997) – 2 rels., Špalková (2000) – 1 rel., Vlčko (1996) – 1 rel., Cluster 4: Author's original (unpublished) – 3 rels., Perný & Mereďa (2000) – 1 rel., Vlčko (1996) – 3 rels.

eracium racemosum, *Poa nemoralis*) showed close syntaxonomical affiliation to the association *Poo nemoralis-Quercetum dalechampii* (alliance *Quercion confertae-cerris*).

While broad-leaved trees (*Fagus sylvatica*, *Carpinus betulus* and *Quercus petraea* agg.) alternated as the dominant species in the tree layer of the stands in cluster 3, the floristic composition of the understorey was driven mainly by mesic species (e.g. *Carex pilosa*, *Lathyrus vernus*). This cluster merged Carpathian sedge oak-hornbeam forests of the *Carici pilosae-Carpinetum* (alliance *Carpinion betuli*) and beech forests of the *Carici pilosae-Fagetum* (alliance *Fagion sylvaticae*).

Cluster 4 included three-layered stands, where dominant *Quercus petraea* agg. in the tree layer was constantly accompanied by *Carpinus betulus*, *Fagus sylvatica* and *Quercus cerris*. The typical grassy aspect of the species-rich herb layer resulted from the prevalence of *Melica uniflora*. An abundant mesic herbs (e.g. *Polygonatum multiflorum*, *Pulmonaria officinalis* agg., *Symphytum tuberosum*) supported

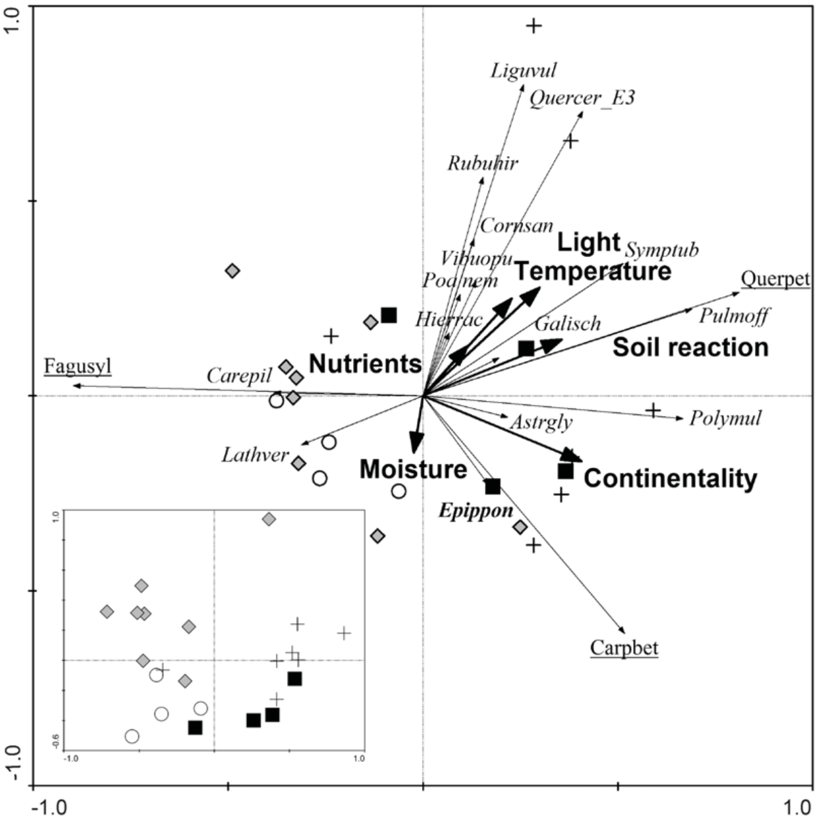

Figure 2 PCA ordination diagram of 23 forest relevé sites with *Epipactis pontica* (empty circles – cluster 1, black squares – cluster 2, shaded diamonds – cluster 3, crosses – cluster 4) and passive projection of Ellenberg indicator values. Position of the relevé sites on the first and the third axes is given in the lower part of the figure. Only diagnostic species for individual clusters (in italics, see Table 1), the dominant tree species (underlined) and *E. pontica* (bold italic) are shown (Astrgly – *Astragalus glycyphyllos*, Carepil – *Carex pilosa*, Carpbet – *Carpinus betulus*, Cornsan – *Swida sanguinea*, Epippon – *Epipactis pontica*, Fagusyl – *Fagus sylvatica*, Galisch – *Galium schultesii*, Hierrac – *Hieracium racemosum*, Lathver – *Lathyrus vernus*, Liguvul – *Ligustrum vulgare*, Poa nem – *Poa nemoralis*, Polymul – *Polygonatum multiflorum*, Pulmoff – *Pulmonaria officinalis* agg., Quercer_E3 – *Quercus cerris* in the tree layer, Querpet – *Quercus petraea* agg., Rubuhir – *Rubus hirtus* s.lat., Symptub – *Symphytum tuberosum*, Vibuopu – *Viburnum opulus*)

its assignment to the *Melico uniflorae-Quercetum petraeae* (alliance *Carpinion betuli*).

PCA ordination showed relatively good floristic differentiation of plant communities, mainly when the first and third axes were displayed (Fig. 2). While the correlations between all EIVs and the first two PCA ordination axes were statistically non-significant ($p > 0.05$; Spearman's correlation coefficients ranged from -0.30 to 0.37), EIV for nutrients ($r = 0.63$) and moisture ($r = 0.55$) were significantly correlated ($p < 0.01$) with the third PCA axis.

Ecological preferences

Analysis of the habitat conditions allowed us partially to characterise the niche of *E. pontica* (Fig. 3). This orchid appears to be a shade-tolerant species preferring canopy-closed forest vegetation on strongly to moderately acidic soils (pH 4.48–5.65). Low content of soluble mineral nutrients in soil solution, indicated by a mean value of soil conductivity of 52.6 μS/cm, pointed towards its affinity to nutrient-poor soil substrates. It was found primarily in habitats with a flat microrelief (71% of

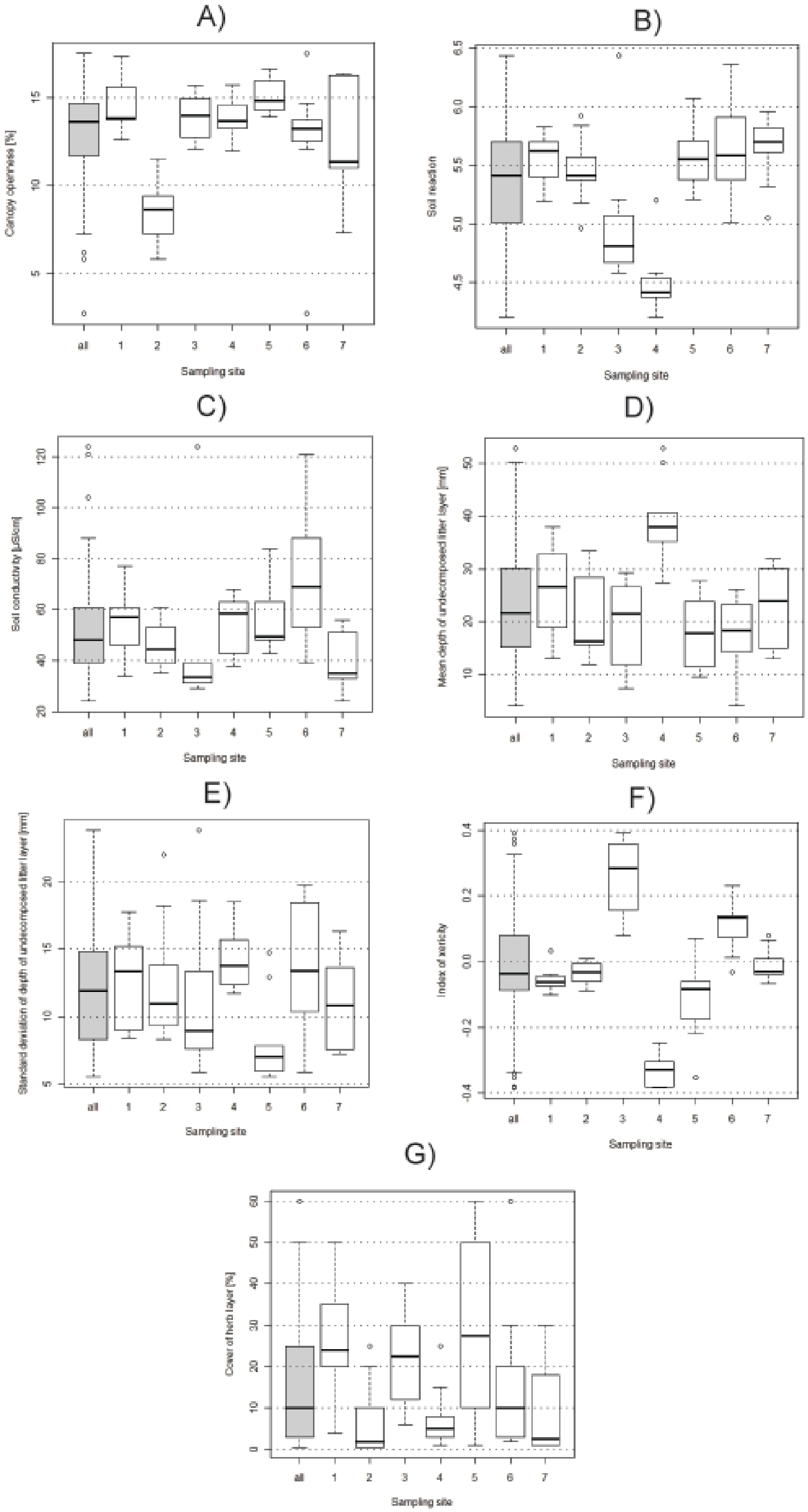

Figure 3 Box-and-whiskers plots of the canopy openness (A), soil reaction (B), soil conductivity (C), depth of undecomposed litter layer (D), standard deviation of depth of undecomposed litter layer (E), index of xericity (F) and cover of herb layer (G) in the sampling sites. The central line of each box refers to the mean value, boxes to the lower (25%) and upper (75%) quartiles, and whiskers to the range of values (minimum and maximum). Legend: all – data from all sampling sites put together; 1 – Španie Pole; 2 – Budča; 3 – Vlkanová; 4 – Kanianka; 5 – Skačany; 6 – Herľany; 7 – Rákoš

all plots) and with relatively uniform (except for the Kanianka sampling site) depth of undecomposed litter layer (23 mm). They showed different slopes and aspects following a high variation in xericity index. The obvious affiliation of *E. pontica* to a less-developed herb layer (with the cover from 8% to 28%) suggests that the species has a low competitive ability. The mean values of the remaining environmental factors were as follows: cover of the moss layer (0.3%), rocks (0.5%), wood litter (3.3%), bare soil (4.8%) and undecomposed phytomass without woody material (94%).

Calculation of EIVs from the Slovak phytosociological relevé sites (Table 1) led to the following mean values of habitats with *E. pontica*: light = 4.46, temperature = 5.44, continentality = 3.53, moisture = 4.95, soil reaction = 6.10 and nutrients = 4.99. To conclude, *E. pontica* is a shade-demanding to shade tolerant species (proposed EIV light 4), occurring mainly in regions with a sub-oceanic climate, mostly in Central Europe, but spreading towards the East (EIV continentality 4). The species is an indicator of moderately acidic to slightly basic fresh soils (EIV soil reaction 6) with a moderate dampness (EIV moisture 5), and sites with an intermediate fertility (EIV nutrients 5) and with moderate average temperatures (EIV temperature 5).

In spite of this relatively well-defined habitat preferences and statistically significant differences among sites, we did not find significant differences in environmental characteristics between plots with versus without *E. pontica* (Table 2). This trend was consistent for all sampling sites (non-significant plot by site interaction) and was also apparent from the RDA ordination which accounted for 29% of variability in the environmental data (Fig. 4).

Discussion

The present study revealed considerable floristic variability of forest understoreys with presence of *Epipactis pontica* in Slovakia. Although the first records of the orchid were reported from mesophilous broad-leaved mixed oak-hornbeam forests of the *Carpinion betuli* alliance and recently from the thermophilous turkey oak forests (*Quercion confertae-cerris*), the species occurred mainly in the herb layer of acidic and mesotrophic beech forests (*Luzulo-Fagion sylvaticae* and *Fagion sylvaticae*). These findings are in agreement with both, the maps of real and potential forest vegetation (Fig. 5A–B) and the main outcomes of some previous studies (e.g. Vlčko et al. 2003).

The overall range of the species is partially disjunct, with some outlying localities in northern Turkey (Delforge 2005). However, the floristic and habitat differences across this broad scale territory did not strongly modify the coenotic preference of *E. pontica* for beech-dominated stands. Such vegetation affinity has already been pointed out in the Central Europe (Presser 2002, Batoušek 2010, Sulyok & Molnár 2011) and the Balkan Peninsula (Petrova & Venkova 2006, Tsiftsis et al. 2008). In addition, this orchid has been also found in the oriental beech (*Fagus orientalis* Lipsky) forests

Table 2 Results of perMANOVA testing for the differences in environmental characteristics between sites, matched-pair plots and plots with presence and absence of *E. pontica* (DF – degrees of freedom; MS – mean square; *pseudo-F* – test criterion; *p* – probability based on 9999 permutations)

Source of variability	DF	MS	*pseudo-F*	*p*
site	6	102.3	8.78	0.0001
matched-pair plot (site)	63	11.6	.	.
plot	1	11.9	0.98	0.4745
plot × site	6	12.1	1.17	0.1768
plot × matched-pair plot (site)	63	10.4	.	.

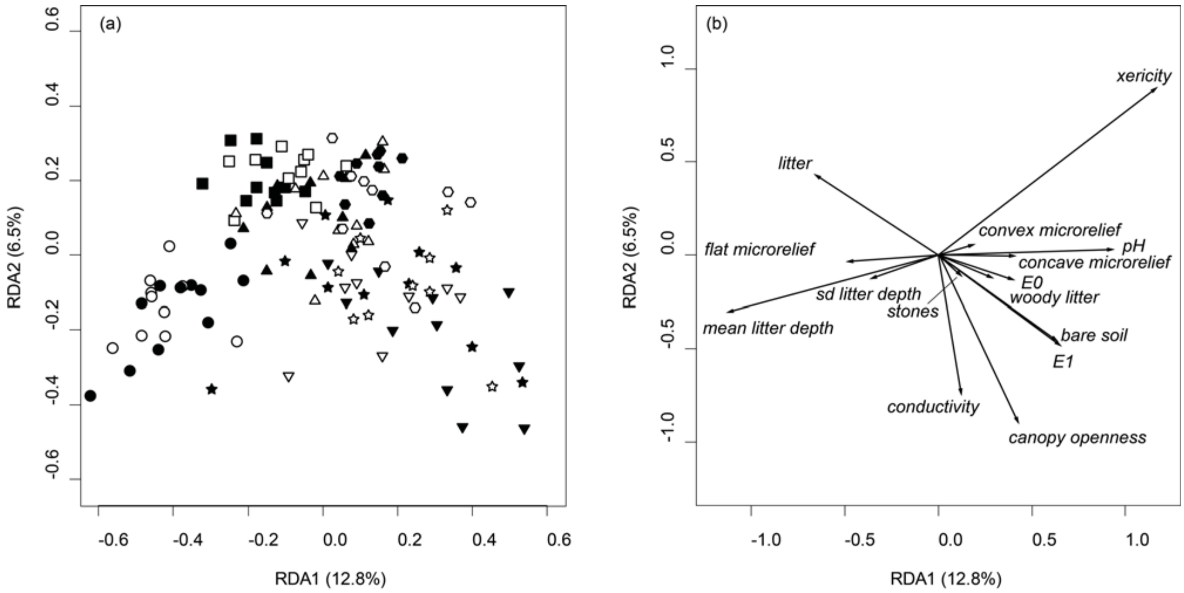

Figure 4 RDA ordination diagrams representing differences in environmental characteristics between sites (different symbols) and plots with presence (full symbols) and absence (empty symbols) of *E. pontica* (a). Vectors of environmental characteristics are displayed (b). Scaling of diagrams is focused on plot distances (a) and correlations of environmental variables (b), respectively. Variances explained by the first two RDA axes are given in parentheses. Abbreviations of environmental variables are explained in the Methods section

(Taubenheim 1975).

Previous studies revealed the light conditions and soil nutrient-related variables (e.g. soil pH, organic matter) as important drivers for potential establishment, growth and distribution of *E. pontica*. Although its populations may occasionally permeate into canopy-closed forest edge vegetation (Taubenheim 1975), there are no field observations of occurrence in treeless (i.e. herbaceous) vegetation. This orchid is thus considered to be a typical forest and shade-tolerant herb (Delforge 2005, Baumann et al. 2006) rare in woodlands with a more open canopy (Presser 2002). Similar light preferences have already been estimated for Slovak populations (Vlčko 1996, Mereďa 1997) and our results are in accordance with them. Whereas there is good agreement on the light requirements of *E. pontica* in the scientific literature, the importance of the soil acidity gradient seems to be still vague and partially inconsistent. Several authors (e.g. Timpe & Mrkvicka 1996) consider the species to be either calcicolous or preferring nutrient-rich and calcareous sites with neutral to basic soil reaction (Taubenheim 1975, Presser 2002, Sulyok & Molnár 2011). On the other hand, an increasing number of localities on acidic soils have been reported recently (e.g. Vlčko et al. 2003, Petrova & Venkova 2006, Tsiftsis et al. 2008). All studied sampling sites in Slovakia showed strongly to slightly acidic soil pH (Fig. 5C), as well. Yet, this pattern cannot be generalised, as Mereďa (1997) found the species on slightly basic sites as well. These findings, together with the published information from the whole distribution area (Delforge 2005), clearly suggest that *E. pontica* is a widely pH-tolerant species, and thus the soil characteristics play only a minor role in limiting its occurrence. However, the poor availability of particular nutrients and aluminium toxicity on very acidic soils represent substantial physiological constraint for many vascular plants (Tyler 2003), which may be a possible explanation of low species richness and herb-

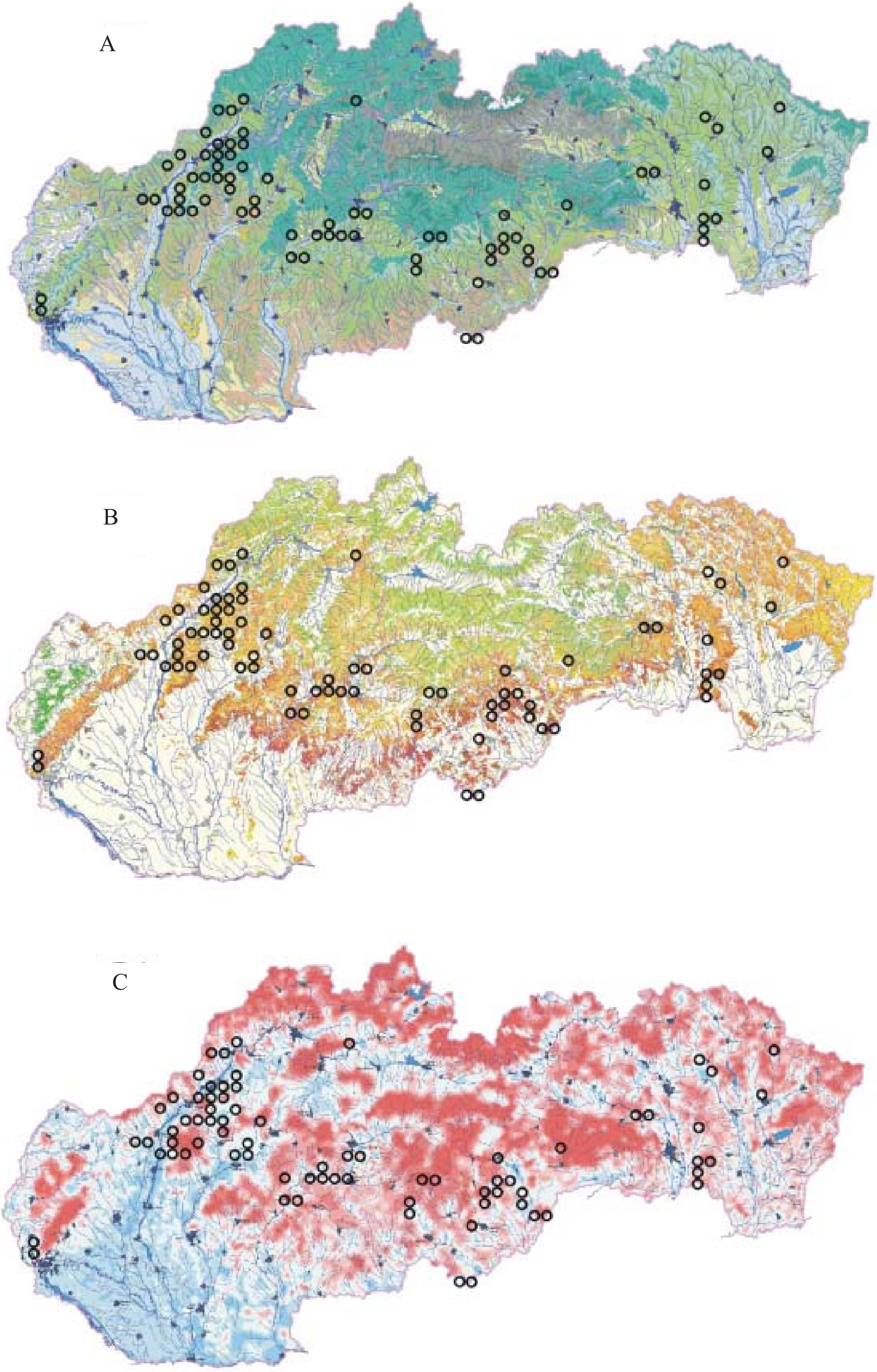

Figure 5 Distribution of *Epipatis pontica* in Slovakia: A) potential natural vegetation (shades of green – oak-hornbeam forests; shades of yellow and pink – oak forests; shades of blue-green – beech forests; for other colours see Miklós 2002), B) tree species composition of forests (red – *Quercus cerris*; dark brown – *Quercus* spec. div.; dark pink – *Carpinus betulus*; light brown – *Fagus sylvatica*; for other colours see Miklós 2002) and C) soil reaction (pH increases from dark blue, light blue, light red to dark red)

layer cover in such habitats. In addition, the high competitive ability of the forest overstorey is able to suppress the development of the understorey layer. If the close relationship of *E. pontica* to European beech stands is taken into account, there might be several mutually non-exclusive mechanisms responsible for this pattern including competition for light, uptake of soil resources and production of slowly decomposing leaf litter (Ellenberg 2009, Packham et al. 2012).

Environmental determinants affecting the distribution pattern of *E. pontica* appear to be scale-dependent. Soil parameters and also partially light availability can most likely determine its occurrence across geographically large areas, but their importance gradually decreases towards local scales. Biological characteristics, such as root mycorrhiza, interspecific competition, allelopathy and underground growth, together with a complex of additional environmental factors, become more relevant at the micro-site level (e.g. Jurčák et al. 2006, Tsiftsis et al. 2008, Jakubska-Busse et al. 2009).

Conclusions

Epipactis pontica is a forest orchid found on 120 sites in Slovakia with the highest density of the localities in the central part of the Western Slovakia (Strážovské vrchy Mts). Except for hygrophilous forest communities, the species occurs in almost every type of broad-leaved forest. On the other hand, its preference for shaded, beech-dominated forests with a low to moderate cover of understorey vegetation and slightly acidic soils is evident even though the species showed a wide niche in terms of soil acidity.

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Supporting Information

The online version of the article includes the Supp. Info.

List of sites with the occurrence of *Epipactis pontica* in Slovakia

Table 1. Basic geographic characteristics of the sampling sites