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ORIGINAL PAPER

Phylogeographic patterns of steppe species in Eastern Central Europe: a review and the implications for conservation

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Abstract The phylogeography of species associated with European steppes and extra-zonal xeric grasslands is poorly understood. This paper summarizes the results of recent studies on the phylogeography and conservation genetics of animals (20 taxa of beetles, butterflies, reptiles and rodents) and flowering plants (18 taxa) of such, "steppic" habitats in Eastern Central Europe. Most species show a similar phylogeographic pattern: relatively high genetic similarity within regional groups of populations and moderate-to-high genetic distinctiveness of populations from currently isolated regions located in the studied area. This distinctiveness of populations suggests a survival here during glacial maxima, including areas north of the Bohemian Massif-Carpathians arc. Steppic species generally do not follow the paradigmatic patterns known for temperate biota (south-north "contraction–expansion"), but to some extent are similar to those of arctic-alpine taxa. There are three main groups of taxa within Eastern Central Europe that differ in their contemporary distribution pattern, which may reflect historical origin and expansion routes. Present diversity patterns of the studied steppic species suggest that they share a unique genetic signature and distinct assemblages exist in each of the now isolated areas rich in steppic habitats. At least some of these areas probably act as present "interglacial refugia" for steppic species. This study strongly supports the need to protect steppic species throughout their entire ranges in the region, as the continuous destruction of steppic

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habitats in some areas may lead not only to the disappearance of local populations, but also to the extinction of unique evolutionary units.

Introduction

Phylogeographical studies in extra-Mediterranean Europe have so far been focused on temperate and arctic-alpine species. The results of numerous works on temperate plant and animal species have allowed generalisations to be made concerning their phylogeographic patterns (Hewitt 2000, 2004; Schmitt 2007). These reviews conclude that species adapted to temperate conditions have reacted to glaciations following a general phylogeographic paradigm, where species' range contracts during Pleistocene glaciations into southern refugia located basically in the area of the Mediterranean peninsulas, and subsequently the populations expand into other parts of the continent during interglacials (e.g. Taberlet et al. 1998; Petit et al. 2003). This "contraction–expansion" principle has recently been challenged, as many studies have suggested or proven that refugia were not restricted to the southern areas, but were present also in western, central and eastern Europe, mainly as local "northern" or "cryptic" refugia (Stewart and Lister 2001; Schmitt and Varga 2012; Bartha et al. 2015).

Other studies showed that the populations of boreo-montane species are either phylogeographically unstructured or only show a rather shallow differentiation between the zonal (taiga-associated) and montane populations (Taberlet et al. 1998; Schmitt 2007; exceptions: Schmitt and Haubrich 2008; Bajc et al. 2011). On the other hand, populations of arctic-alpine taxa must have been much more widely distributed during glaciations than in interglacials, including the Holocene (e.g. Schönswetter et al. 2005; Varga and Schmitt 2008; Schmitt 2009). For these taxa, the concept of refugium needs to be modified. The inclusion of the impact of climate changes in the meaning of the "refugium" has been postulated (e.g. Bennett and Provan 2008; Ashcroft 2010). That is why the term "warm stage refugia" was proposed to stress that some species (mainly the cold-adapted) experience their smallest ranges during warm periods and expand during glaciations (Holderegger and Thiel-Egenter 2008; Stewart et al. 2010). The phylogeography of the temperate and cold-adapted species allowed for a more comprehensive understanding of the history of European fauna and flora. A notion of an "individual response" of species to climatic oscillations, along with the idea of the disintegration of past ecosystems (i.e. those present during glacial periods) and the formation of ecosystems *de novo* without a close connection to the preceding ones has been proposed (Bhagwat and Willis 2008; Schmitt and Varga 2012).

However, a third group of organisms which are present in extra-Mediterranean Europe has been notoriously ignored: species adapted to dry and periodically warm conditions of the continental zone, mostly inhabiting steppes and other types of dry grasslands. The phylogeography of continental species was partly included in the reviews of Stewart et al. (2010) and Varga (2010), but mostly on the basis of chorological data, making the steppe-dedicated parts of the above papers more thought-provoking than reviewing. The general disregard for continental elements in reviews about the phylogeography of European species could be explained by the relatively recent arrival of studies on this group (with the

Table 1 Steppic species examined in the meta-analysis with their systematic affiliation, references of papers reviewed, molecular markers used and range covered in these papers (from area considered here)

No	Species	Order/family	References	Markers	Examined range
Animals					
Invertebrates					
1	Centricnemus leucogrammus	Coleoptera/Curculionidae	Kajtoch et al. (2009, 2014b), Kajtoch (2011)	seq (mtDNA, nucDNA), msats	CZ,PL,SK,UA
2	Polydrusus inustus	Coleoptera/Curculionidae	Kajtoch et al. (2009, 2012)	seq (mtDNA, nucDNA)	PL,RU,UA
3	Cheilotoma musciformis	Coleoptera/Chrysomelidae	Kajtoch et al. (2013)	seq (mtDNA, nucDNA)	PL,SK,UA
4	Crioceris quatuordecimpunctata	Coleoptera/Chrysomelidae	Kubisz et al. (2012)	seq (mtDNA, nucDNA)	PL,SK,UA
5	Crioceris quinquepunctata	Coleoptera/Chrysomelidae	Mazur et al. (2014)	seq (mtDNA, nucDNA)	CZ,UA
6	Coraebus elatus	Coleoptera/Buprestidae	Kajtoch et al. (2014a)	seq (mtDNA, nucDNA)	AT,HR,HU,PL,RU,SK,UA
7	Maculinea alcon xerophilic ecotype	Lepidoptera/Lycaenidae	Bereczki et al. (2005), Rutkowski et al. (2009)	msats, allozymes	AT,HU,PL,RO,SI
8	Maculinea arion	Lepidoptera/Lycaenidae	Bereczki et al. (2014), Rutkowski et al. (2009), Sielezniew and Rutkowski (2012)	seq (mtDNA, nucDNA), msats, allozymes	AT,HU,RO,PL,SI
9	Polyommatus ripartii	Lepidoptera/Lycaenidae	Przybyłowicz et al. (2014)	seq (mtDNA, nucDNA)	PL,UA
10	Melitaea cinxia	Lepidoptera/Nymphalidae	Wahlberg and Saccheri (2007)	seq (mtDNA)	BC,DE,HU,PL,RU,UA
Vertebrates					
11	Coronella austriaca	Squamata/Colubridae	Sztencel-Jablonka et al. (2015), Galarza et al. (2015)	seq (mtDNA), msats	CZ,HR,PL,SK
12	Dolichophis caspius	Squamata/Colubridae	Nagy et al. (2010)	seq (mtDNA)	HR,HU,RO,RS

Table 1 continued

No	Species	Order/family	References	Markers	Examined range
13	*Vipera ursinii* complex	Squamata/Viperidae	Ferchaud et al. (2012), Gvozdik et al. (2012), Zinenko et al. (2015)	seq (mtDNA)	HR,HU,MD,RO,RA,UA
14	*Lacerta viridis*	Squamata/Lacertidae	Joger et al. (2007), Böhme et al. (2007a, b), Böhme and Moravec (2011)	seq (mtDNA), msats	AT,BG,CZ,DE,HR,HU,RS,SI,SK,UA
15	*Spermophilus citellus*	Rodentia/Sciuridae	Hulová and Sedláček (2008), Krystufek et al. (2009), Říčanová et al. (2013)	seq (mtDNA), msats	AT,HU,CZ,MD,RO,RS,RU,SK
16	*Spermophilus suslicus*	Rodentia/Sciuridae	Biedrzycka and Konopinski (2008), Matrosova et al. (2014)	seq (mtDNA), msats	PL,UA
17	*Cricetus cricetus*	Rodentia/Cricetidae	Neumann et al. (2004, 2005), Banaszek et al. (2010, 2011, 2012), Schroeder et al. (2014)	seq (mtDNA), msats	AT,CZ,DE,HU,PL,RO,RU,SK,UA
18	*Sicista subtilis* agg.	Rodentia/Dipodidae	Cserkész et al. (2015a, b)	seq (mtDNA, nucDNA)	HU,RU,UA
19	*Nannospalax leucodon* superspecies	Rodentia/Spalacidae	Hadid et al. (2012), Kryštufek et al. (2012)	seq (mtDNA)	HU,RO,RS,UA
20	*Spalax* spp. complex	Rodentia/Spalacidae	Németh et al. (2013)	seq (mtDNA)	RO,UA
Flowering plants (angiosperms)					
21	*Adonis vernalis*	Ranunculales/Ranunculaceae	Hirsch et al. (2015)	AFLP	CZ, DE, ES, RO, RU, UA
22	*Adonis volgensis*	Ranunculales/Ranunculaceae	Sramkó et al. in prep.	seq (nucDNA, cpDNA)	HU,MD,RO,RU,UA
23	*Pulsatilla patens*	Ranunculales/Ranunculaceae	Sramkó et al. in prep.	seq (plastid VNTR)	HU,PL,RO,RU,SK,UA
24	*Astragalus exscapus*	Fabales/Fabaceae	Becker (2012)	allozymes	AT,CZ,DE
25	*Linum flavum*	Malpighiales/Linaceae	Cieślak (2014)	AFLP	AT,CZ,HU,PL,RO,SK,UA
26	*Linum hirsutum*	Malpighiales/Linaceae	Cieślak (2014)	AFLP	AT,CZ,HU,PL,SK,UA

Table 1 continued

No	Species	Order/family	References	Markers	Examined range
27	Carlina onopordifolia [C. acanthifolia[FE] p.p.]	Asterales/Asteraceae	Cieślak (2014)	AFLP	PL,UA
28	Cirsium pannonicum	Asterales/Asteraceae	Cieślak (2014)	AFLP	CZ,PL,RO,UA
29	Inula ensifolia	Asterales/Asteraceae	Cieślak (2014)	AFLP	AT,BG,CZ,HU,PL,RO,SK,UA
30	Scorzonera purpurea	Asterales/Asteraceae	Meindl (2011)	AFLP	AT,CZ,DE,HU,PL,RO,RU,SK,UA
31	Serratula lycopifolia	Asterales/Asteraceae	Cieślak (2013)	AFLP	CZ,PL,SK,UA
32	Iris aphylla	Asparagales/Iridaceae	Wróblewska et al. (2003, 2010), Wróblewska (2008)	AFLP	CZ,DE,HU,PL,RO,SK,UA
33	Melica transsilvanica	Poales/Poaceae	Szczepaniak and Cieslak (2011)	AFLP	AT,CZ,HU,PL,RO,RU,SK,UA
34	Stipa borysthenica	Poales/Poaceae	Durka et al. (2013)	AFLP	DE,SK
35	Stipa capillata	Poales/Poaceae	Krzakowa and Michalak (2007), Hensen et al. (2010)	allozymes, RAPD	DE,PL,SK
36	Stipa eriocaulis [S. pennata subsp. eriocaulis[FE]]	Poales/Poaceae	Durka et al. (2013)	AFLP	DE,SK
37	Stipa pennata	Poales/Poaceae	Wagner et al. (2012), Durka et al. (2013)	AFLP	CZ,DE,RO,RU,UA
38	Stipa pulcherrima	Poales/Poaceae	Durka et al. (2013)	AFLP	DE,RO,RU,SK,UA

Names of animal species according to "Fauna Europaea" (http://www.faunaeur.org/); nomenclature of plant species followed the sources used, generally conforming to the "Flora Europaea" (http://rbg-web2.rbge.org.uk/FE/fe.html) with exception of two taxa, for which the closest names used there were added in brackets with "FE" superscript

Country codes: AT Austria, BC Baltic countries, BG Bulgaria, CZ Czech Republic, DE Germany, ES Spain, HR Croatia, HU Hungary, MD Moldova, PL Poland, RO Romania, RS Serbia, RU W Russia, SI Slovenia, SK Slovakia, UA Ukraine. Markers abbreviations: seq sequences, mtDNA mitochondrial DNA, nucDNA nuclear DNA, cpDNA chloroplast DNA, msats microsatellites, VNTR variable number tandem repeat, AFLP amplified fragment length polymorphism, RAPD Random Amplified polymorphism DNA

exception of Iberian steppes, which are probably only distantly related to Eurasian ones, and have a different origin; e.g. Ribera and Blasco-Zumeta 1998; González-Sampériz et al. 2010). The first phylogeographic papers dealing with steppe species were published in the first half of the 2000s and the majority of them only in the last decade (see Table 1 for compilation of references). The increasing amount of data on phylogeographic patterns in steppic species has allowed this group to be inserted into the general picture of the phylogeography of the European biota for the first time. This could be important, as steppic habitats sustain assemblages very rich in species, among which there are many taxa restricted only to this type of environment or even endemic to some steppic areas on a European or local scale (e.g. Pärtel et al. 2005; Mazur and Kubisz 2013; Dengler et al. 2014). Moreover, steppes and steppic species are among the most threatened in Europe due to natural (i.e. climatic and environmental) reasons, which restrict their distribution during woodland-dominated interglacial periods, and to the anthropogenic degradation and fragmentation of steppic patches, especially in the central part of Europe (Cremene et al. 2005; Janišová et al. 2011; Fekete et al. 2014). The situation of some steppic patches and populations is so serious (especially north of the Carpathians) that this could be the last chance for an appropriate sampling to be carried out for phylogeographic studies. Moreover, data on the distribution of steppes in the past suggests that different types of xeric and meso-xeric grasslands were much more abundant and widespread throughout Europe during glacial times. There were at least two variants of glacial steppes: cold steppes in front of glaciers (with co-occurrence of tundra elements) and more warm-adapted and meso-xeric meadow steppes known from southern areas (Willis and van Andel 2004; Markova et al. 2009). Xeric grasslands and meadow steppes could also persist as local components in the "non-analogous" assemblages of the lower latitude periglacial open zonobiom (see: current occurrence of cold-tolerant steppic species from Yakutia to Alaska; Yurtsev 1982, 2000; Ehlers and Gibbard 2004).

During interglacials (such as our current epoch, the Holocene), the natural steppes in Europe become basically restricted to the eastern and south-eastern regions, forming part of a long and narrow Eurasian steppe zone from the inner parts of the Pannonian Basin to NE China, although similar habitats (called dry/calcareous/xerothermic grasslands) are known from many dispersed localities in central Europe and the Balkan Peninsula (e.g. on steep slopes of hills and along river scarps) (Donita et al. 2003; Fekete et al. 2014; Pokorný et al. 2015). That range shift of steppe-like habitats probably influenced the phylogeography of steppic species, which might have been more widespread during ice ages (more severe climatic conditions notwithstanding, see Berman et al. 2011) but are currently experiencing range contractions. The current large-scale diversity patterns of steppe species follow a decreasing gradient from east to the west. This would imply a recent immigration from the east to the suitable habitats as genetic diversity should follow a similar east-to-west decreasing gradient. However, in light of the above vegetation historical reasoning, we could expect a more complicated pattern of genetic diversity supporting an alternative hypothesis of phylogeographic history (i.e. long-term persistence of steppic species' populations at the current western edges/patches of their distribution).

In this paper, we review the phylogeographic pattern in steppic species on their western part of their distribution, Eastern Central Europe (with adjoining East European ranges), where enough phylogeographic data have been accumulated, and where steppes, forest-steppes and extrazonal xeric grasslands are all represented. Specifically, we are inquiring for the following questions:

1. Are the observed phylogeographic patterns congruent for all the reviewed steppic organisms?
2. If so, do these patterns conform either to the patterns observed for one presented by the so far analysed cold-adapted taxa or to the patterns known for temperate biota?
3. Did the mountain chains (i.e. the Bohemian Massif and/or the Carpathians) act as barriers for the steppic taxa?
4. Is there support for the east-to-west decreasing gradient in phylogeographic pattern?
5. Are there evident genetical 'traces' of the existence of extrazonal interglacial refugia and/or of several migrational waves of the steppic taxa during last Pleistocene cycles in the Eastern Central Europe?

Additionally, with bearing the limited quantity and quality of available data in mind, we also try to discuss topics of (i) the dual nature of 'refugia' of steppic taxa (i.e. presence of both 'warm stage' and 'cold stage' refugia); and (ii) the importance of the observed patterns in the context of conservation genetics of the highly-endangered steppic taxa.

Methods

Examined area

This review focuses on the available phylogeographic studies of species related to steppes and other similar types of dry grasslands (called also "calcareous" or "xerothermic", depending on the area and terminology in use). As we were interested mainly in species distributed in Eastern Central Europe, we generally narrowed our species choice to taxa
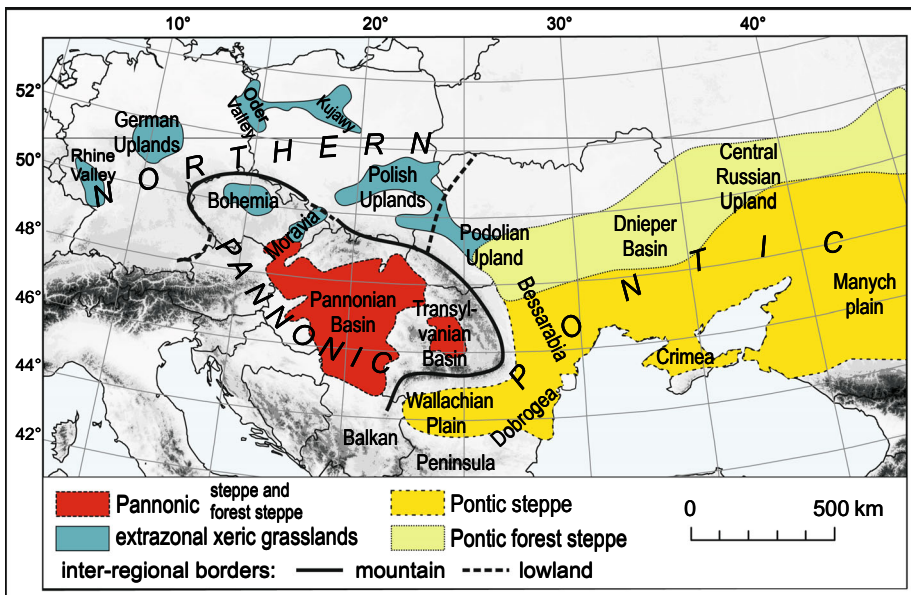

Fig. 1 Simplified map of the distribution of steppes and forest-steppes (Pontic and Pannonian) and xeric grasslands in Eastern Central Europe (with adjoining East European ranges) with their regionalisation as used in the review (Northern—Pannonic—Pontic). (Color figure online)

which are distributed from Germany and Austria in the west to Ukraine in the east, and from the southern Baltic coast in the north via the Pannonian Basin to the northern part of the Balkan Peninsula in the south. We intentionally excluded from the reviewed area the central-Alpine dry valleys (Braun-Blanquet 1961) and xeric grasslands (alvars) on Baltic islands (Pettersson 1965) as from these areas phylogeographic and population genetics data were available for only one of the examined species (*Astragalus exscapus*; Becker 2012).

For the purposes of this review, we divided the area of Eastern Central Europe (with neighbouring areas of East Europe) into three different regions (Fig. 1), in respect to the Bohemian Massif-Carpathians arc:

1. The "Northern" region, comprising areas situated outwards from the arc—mainly Germany and Poland (Rhine and Oder Valleys, German and Polish Uplands, Kujawy Basin), with part of Belarus and the westernmost part of Ukraine (until the eastern end of the Lublin-Lviv Upland); this region was covered by an ice sheet (or was in its immediate foreland) during at least one Pleistocene glaciation, and currently features extrazonal, highly fragmented and isolated steppic patches.
2. The "Pontic" region, stretching to the east from the Northern one, as defined above, and from the East Carpathians, including areas along the border between Romania and Bulgaria (Wallachian Plain, Dobrogea region), Moldova (Bessarabian Upland), as well as the adjoining East European areas: most of Ukraine (Podolian Upland, Dnieper Basin, Crimea) and the steppic/forest-steppic belt of south-western Russia (Central Russian Upland) up to the northern foot slopes of the Caucasus (Manych plains); this region was never fully glaciated and it now constitutes the westernmost fragment of the continuous (zonal) Eurasian steppe belt.
3. The "Pannonic" region, comprising the areas within the Bohemian Massif-Carpathians arc (Bohemia, Moravia, Pannonian Basin, Transylvanian Basin) and the adjacent northernmost parts of the Balkan Peninsula to the west and southwest of the Danube; this region was never glaciated and sustains large extrazonal and edaphic (sandy and saline) steppic areas within the Pannonian Basin and its surroundings. Their "deviations" from the general zonality are discussed by Fekete et al. (2014).

Data collection

About one third of the studies on the following taxa were carried out by the authors of this review (see Table 1 for references). Moreover, we searched scientific literature with the use of the Google Scholar, Biological Abstracts, and PubMed databases, using the following keywords: "phylogeography", "population genetics", "conservation genetics", "Europe", "steppe", "dry grasslands", "calcareous" and "xerothermic". We mainly selected papers that examine populations across substantial parts of species ranges in Eastern Central Europe. We omitted eurytopic species which have ranges in both the continental zone of Eurasia and the Mediterranean region. We also decided to exclude papers on bird species. On the other hand, we included a few studies which are "in press", or in the final publication preparation stages (see Table 1 for references).

The final list of the steppic taxa for which genetic data is available includes 38 of them: six beetles, four butterflies, two species and one complex of snakes, one lizard, four species and two species complexes of rodents, and 18 flowering plant species, described in altogether 52 articles (Table 1). The nomenclature of taxa and systematic approaches throughout the paper follow the articles from which the data was derived.

Elaboration of genetic data

Selected animal and plant taxa were investigated with the use of different types of molecular markers (DNA sequences or allelic data). Details about the methods and markers used for particular taxa are described in Information Box 1 and presented in Table 1.

For a majority of the selected taxa, genetic data was available as basic diversity descriptors (see Box 1 for details). Usually, papers also included different types of algorithms and methods used in clustering and identification of genetic distinctiveness of the populations and individuals. Moreover, many of the scientific papers featured special maps visualizing how the genetic diversity of the populations refers to their geographic distribution. Use of different type of markers for elaborating genetic diversity and distinctiveness of populations could be problematic as they generally reflect different time-scales. Whereas mtDNA in animals and AFLPs and plastid haplotypes in plants are usually utilised for phylogeographic studies reflecting historical changes in taxa's genetics (usually dating back to several generations), SSRs are used for contemporary and more recent description of population genetics and demography, thus reflecting very recent changes in taxa's genetics (usually dating back to few generations). Therefore, SSRs were only used as an additional source of information about genetics of examined taxa since simultaneous use of both types of markers is common practice that allows for comprehensive phylogeographic and population genetic analyses (especially when a study has conservation implications). As mtDNA data are available for all animals examined, and either AFLPs and/or plastid data are available for all plants, it was possible to analyse and compare data within animals and within plants in a similar way (Fig. 2).

Information Box 1 Brief summary of molecular markers and techniques used in the reviewed papers dealing with phylogeography and population genetics of steppic species

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Markers used in the studies reviewed

  Allozymes [used for some plants and butterflies]

  Mitochondrial DNA (mtDNA)—genes *cytochrome oxidase subunits I* and *II* (COI and COII) or *cytochrome-b* (CytB) and non-coding control region (CR) [used for animals]

  Plastid DNA (cpDNA)—intergenic spacers (*accD-psaI*, *trn*H-*psb*A) [used for plants]

  Nuclear sequences—Elongation Factor 1-α (EF-1α) gene, Interphotoreceptor Retinoid-Binding Protein (IRBP) gene, *LFY* gene intron, Internal Transcribed Spacers of ribosomal DNA (rITS) [used for animals and plants]

  Microsatellites (SSRs) [used for animals]

  Amplified Fragment Length Polymorphism (AFLP) [used for plants]

  Random Amplified Polymorphism DNA (RAPD) [used for plants]

  Variable Number Tandem Repeat (VNTR) [used for plants]

Analyses applied in the studies reviewed

  Haplotype and nucleotide diversities [calculated for sequence markers]

  Heterozygosity and allelic richness [calculated for allelic markers]

  Phylogenetic trees reconstruction [calculated for sequence markers]

  Haplotype (or allele) network building [calculated for both types of markers]

  Genotype assignment to clusters [calculated for allelic markers]

  Isolation by Distance in Mantel Test (IBD-MT) [calculated for both types of markers]

  Analysis of Molecular Variance (AMOVA) [calculated for both types of markers]

  Fixation index F_{ST} [calculated for both types of markers]
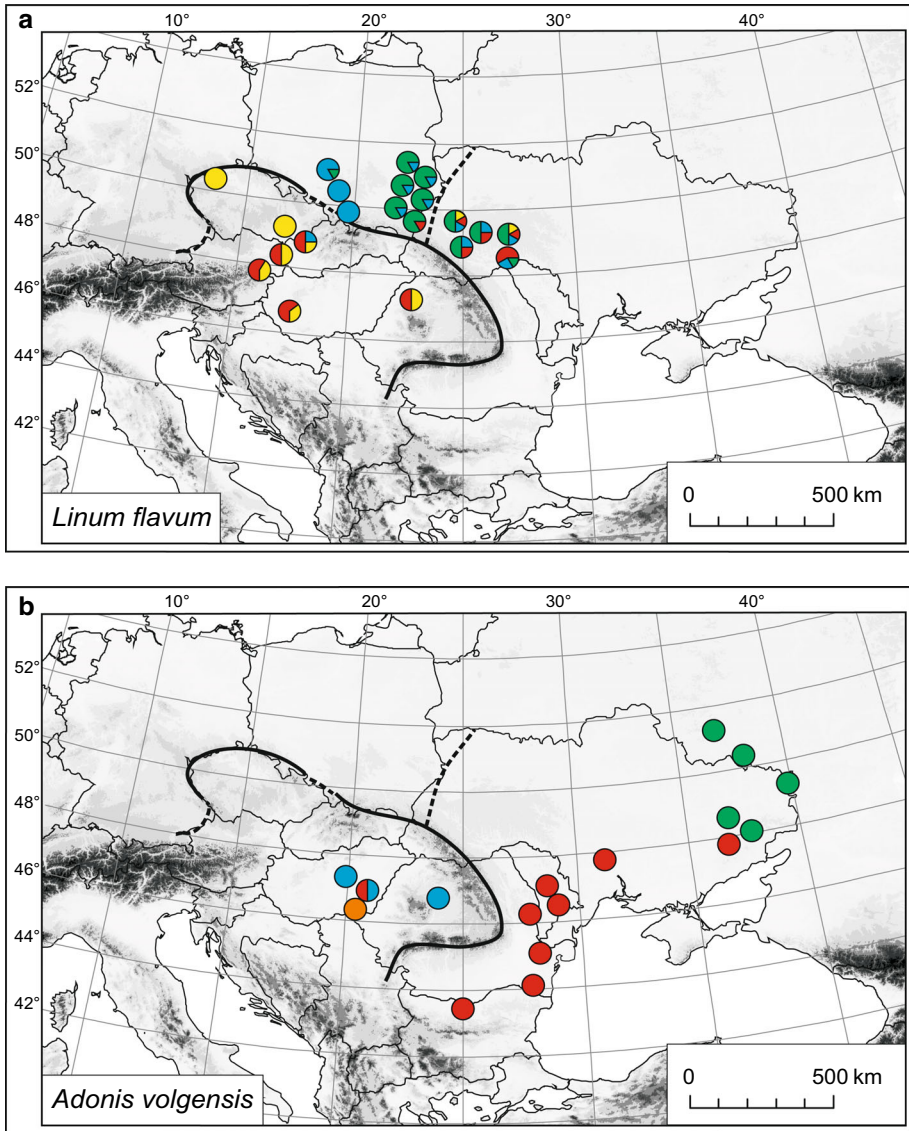

Fig. 2 Phylogeographic patterns observed for eight selected steppic species from Eastern Central Europe—four plants (**a–d**) and four animals (**e–h**). The *colours/greyscale* of the *circles* indicate generalized data about the presence of distinct phylogenetic lineages and/or genetic clusters identified based on genetic (phylogeographic, population and conservation genetics) studies. Sources of data: *Linum flavum* (Linaceae): Cieślak 2014; *Adonis volgensis* (Ranunculaceae): Sramkó et al. in prep.; *Iris aphylla* (Iridaceae): Wróblewska et al. 2003, 2010; Wróblewska 2008; *Scorzonera purpurea* (Asteraceae): Meindl 2011; *Centricnemus leucogrammus* (Curculionidae): Kajtoch et al. 2009, 2014a; Kajtoch 2011; *Melitaea cinxia* (Nymphalidae): Wahlberg and Saccheri 2007; *Coronella austriaca* (Colubridae): Sztencel-Jabłonka et al. 2015; Galarza et al. 2015; *Cricetus cricetus* (Cricetidae): Neumann et al. 2004, 2005; Banaszek et al. 2010, 2011, 2012; Schroeder et al. 2014. (Color figure online)
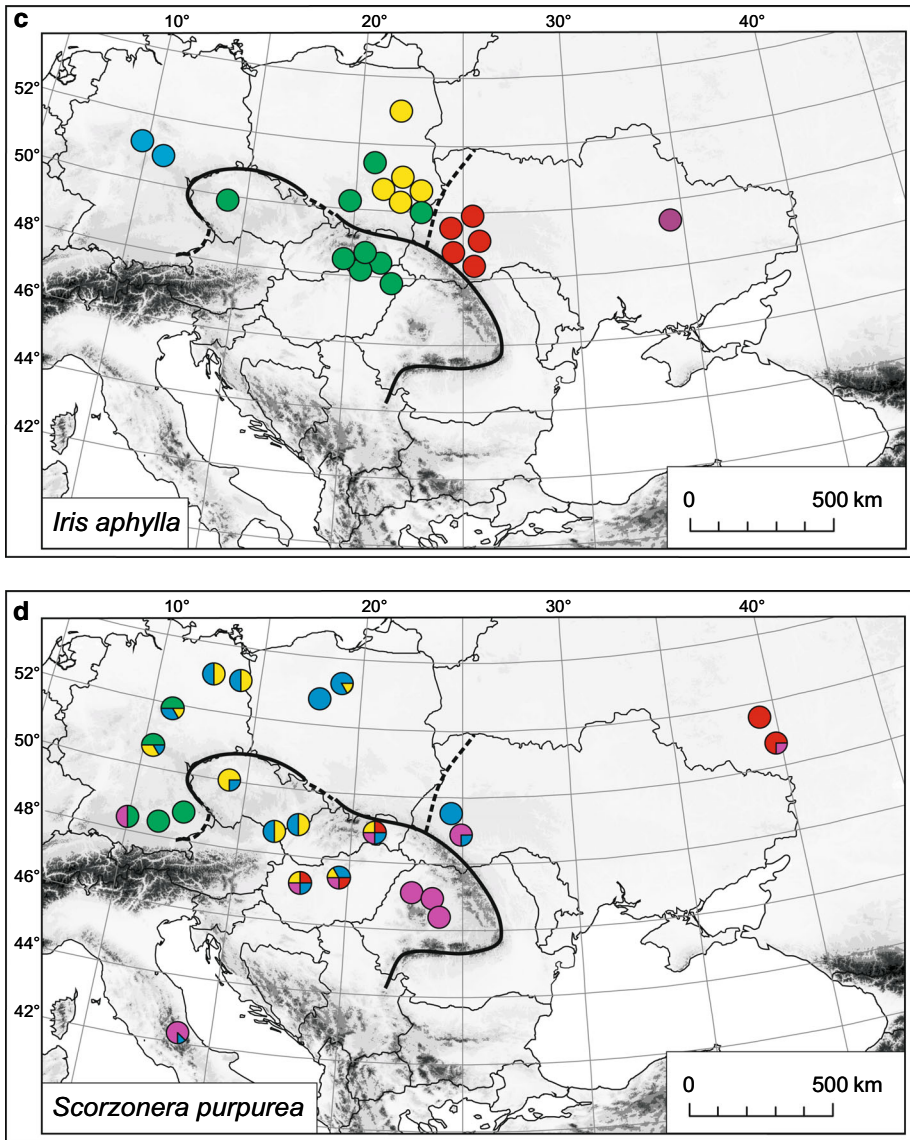
Fig. 2 continued

We examined all of these data to identify the following characters of the selected steppic taxa:

Character (1): Intra-regional diversity—genetic diversity detected on an intra-regional geographic scale (i.e. among populations within each of the three regions as defined above, intra-population diversity notwithstanding), estimated (generalized) into three categories: (i) "low" (lack of diversity or with very low values of appropriate descriptors, e.g. inter-population haplotype diversity or heterozygosity below 0.25); (ii) "moderate" (haplotype
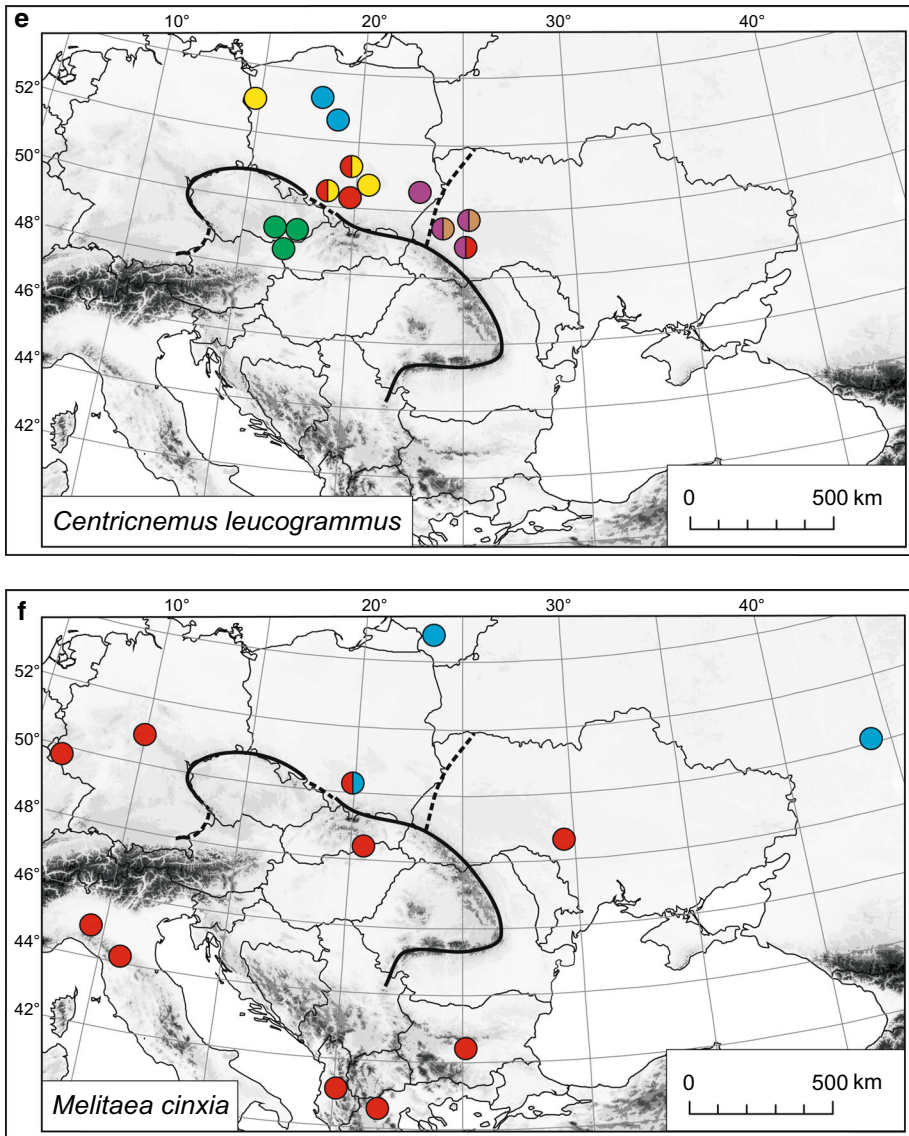
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diversity or heterozygosity 0.25–0.5) and (iii) "high" (haplotype diversity or heterozy-gosity above 0.5). It is important to note that some papers did not present the necessary primary values, and in these cases we tried to assign diversity to these three levels using other available information like patterns observed in phylogenetic trees/networks or results of implementation of other assignment algorithms, etc.

Character (2) Inter-regional distinctiveness—genetic differences among examined populations from any two of the three different regions established for the purposes of the
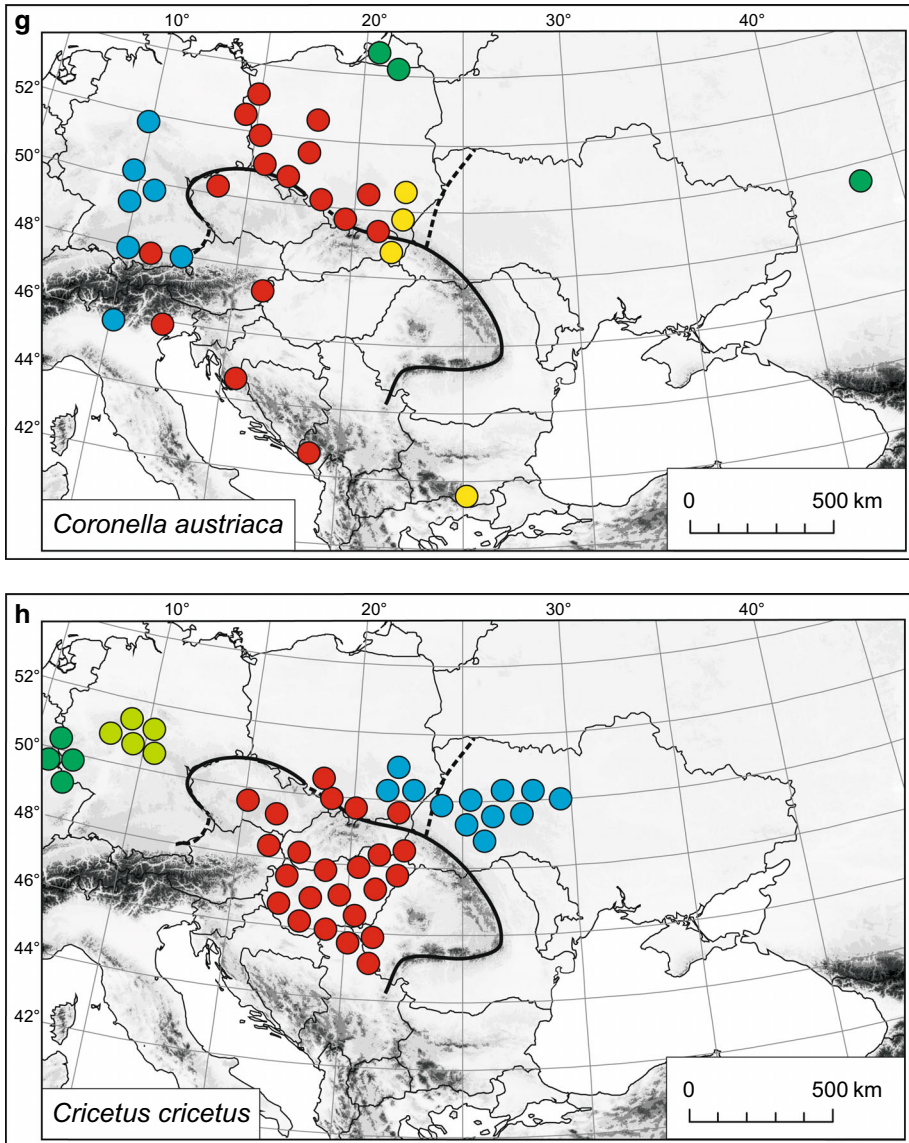
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review (i.e. between Pontic, Pannonic and Northern regions), estimated (generalized) into three analogous categories (low, moderate, high) based mainly on values of fixation index F_{ST} (or derivative measures as G_{ST} or R_{ST}) and results of AMOVA and IBD-MT. For example, we assigned distinctiveness as: (i) "low" for F_{ST} below 0.05, (ii) "moderate" when F_{ST} was equal to 0.05–0.25 and (iii) "high" for values above 0.25, while for AMOVA the threshold values among groups (of populations) were <10, 10–50 and >50 %, respectively. As some taxa might comprise populations of low, moderate or

high genetic diversity/distinctiveness depending on the area concerned, consequently the same taxon could be assigned to more than one of the presented classes.

Character (3) Distinct units around the Bohemian Massif-Carpathians arc—presence of distinct phylogenetic lineages (clades) or distinct genetic clusters between regions as described above. Presence of distinct units (yes/no) was directly or indirectly drawn from the available phylogenetic trees, haplotype/genotype networks and other genotype assignment algorithms' implementation results. Data examination for Character 3 was designed to verify two schemes: (i) (Character 3a) whether *any* region-specific units of the taxa were identified in the specified regions, and (ii) (Character 3b) whether *all* identified units were distinct among the specified regions. In the first scheme, we allowed for the presence of some genetic admixture between regions (presumably as the effect of past expansion or recent dispersion/migration), but the second scheme was applied strictly, as it described only "full genetic isolation". We used this data to count the frequencies of taxa expressing similar phylogeographic patterns in each of the selected categories.

The collected data were then analysed separately for animals and plants. We verified how many taxa show similar phylogeographic patterns in respect to all three characters described above.

Results

Phylogeographic patterns of steppic species in Eastern Central Europe

Beetles

The methods applied did not reveal any diversity among regional populations of *Crioceris quatuordecimpunctata*; for three others, *Polydrusus inustus*, *Cheilotoma musciformis* and *Crioceris quinquepunctata,* some local populations were also characterized by a lack of genetic variation (even in intra-population scale, generally not considered in the present paper). Two other beetles (*Centricnemus leucogrammus* and *Coraebus elatus*) showed low-to-moderate genetic diversity of populations, depending on their geographic localisation (usually higher diversity was observed in the eastern and southern parts of the range). The pattern of interregional distinctiveness was substantially different, as most of these species were characterized by high (*C. musciformis* and *C. quatuordecimpunctata*) or moderate-to-high (*C. leucogrammus* and *C. elatus*) distinctiveness of their populations from different regions (Fig. 2e). Only two species (*P. inustus* and *C. quinquepunctata*) showed low genetic differentiation of even distant populations. Finally, most of these species expressed a significant divergence of phylogenetic lineages occurring in the Northern, Pontic and Pannonic regions. To the contrary, *P. inustus* and *C. quinquepunctata* expressed low genetic distinctiveness of populations. An interesting pattern was observed for three species (*C. leucogrammus*, *C. elatus* and *C. quatuordecimpunctata*) in northern Poland (the Kujawy Basin), where highly distinct phylogenetic lineages, different from all other Eastern Central European ones, were found. In at least one species (*C. musciformis*), the genetic divergence of populations suggests the existence of distinct taxonomic units (presumably subspecies) in southern Poland, Ukraine and the Pannonian Basin.

Butterflies

Two butterflies (*Polyommatus ripartii* and *Melitaea cinxia*) were found to have low intra-regional diversity, whereas two others (*Maculinea alcon* xerophilic ecotype and *Maculinea arion*) displayed low-to-moderate or high differentiation within regional populations (the values were higher in southern ones). Genetic distinctiveness of populations between regions varied as some species showed high levels of differentiation (*M. alcon* and *M. arion*), whereas others displayed low or moderate levels (*P. ripartii* and *M. cinxia*). Regarding the presence of distinct genetic units across the Carpathians and the Bohemian Massif, the studied butterflies generally did not show such patterns, with the exception of *M. cinxia* populations, which formed some distinct units (but not totally genetically isolated) when compared in north–south and east-south directions (Fig. 2f).

Rodents

Levels of intra-regional genetic diversities of rodent species and species-complexes differ; however all taxa comprise populations with an identified low genetic diversity. Only for three of these species (*Spermophilus suslicus*, *Spermophilus citellus* and *Cricetus cricetus*) there are populations known to have a high intra-regional diversity. They are always the ones located within continuous species range to the east (Ukraine) or south (the Pannonian Basin and adjacent areas of the Balkan Peninsula) of the Bohemian Massif-Carpathians arc. It is important to note that the other rodent taxa were studied using a limited sampling scheme for phylogenetic purposes, and therefore it was not possible to include these taxa in analyses on the population level. All the considered steppic rodents showed a high genetic distinctiveness at an interregional context (this parameter corresponded to the distinctiveness of species within *Spalax* spp. and within the *Nannospalax leucodon* superspecies, and to the presence of different species of *Sicista subtilis* agg.). Lastly, all of these taxa have different phylogenetic lineages inhabiting the Pontic and Pannonic regions. In *Spalax* spp. and in the *N. leucodon* superspecies, allopatric species were firstly described based on osteological characters and chromosomal differentiation and afterwards confirmed with use of molecular markers. In *S. subtilis* agg., distinct species were described (*S. trizona* within the Pannonic region and *S. nordmanni* within the Pontic region). Only *S. suslicus* and *C. cricetus* have current natural populations north of the Carpathians and the Bohemian Massif. These populations are distinct from the Ukrainian ones (especially for *S. suslicus*, and partially for *C. cricetus*, as its eastern lineage spans westward into eastern Poland) and from the Pannonic ones (although the Pannonic clade of *C. cricetus* spans northward into southern Poland, a distinct clade of this species is present in Germany (Fig. 2h); *S. suslicus* is replaced in Pannonia and south-eastern Central Europe by *S. citellus*, and both species are parapatric only in southern Ukraine and Moldova).

Reptiles

Coronella austriaca harbours a rather high genetic diversity in its regional populations, whereas the strictly south-eastern *Dolichophis caspius*, of the same snake family, is much less diverse genetically on a regional scale. *Vipera ursinii* and *Vipera renardi*, which form a species complex with multiple subspecies, have generally low genetic diversity at an intraregional scale; however when we consider complexes as a unit, the presence of distinct taxa in the Pontic region expresses a high diversity of populations from that large area.

Table 2 Characterisation of genetic diversity and phylogeographic patterns summarized and generalized for steppic species from Eastern Central Europe

No.	Species	Level of intraregional diversity (character 1)	Interregional distinctiveness (character 2)	Any distinct units across regions (character 3a)			All distinct units across regions (character 3b)		
				Northern versus Pannonic	Northern versus Pontic	Pannonic versus Pontic	Northern versus Pannonic	Northern versus Pontic	Pannonic versus Pontic
Invertebrates									
1	*Centricnemus leucogrammus*	Low to moderate	Moderate to high	Yes	Yes	Yes	No	Yes	Yes
2	*Polydrusus inustus*	Low	Low	–	No	–	–	No	–
3	*Cheilotoma musciformis*	Low	High	Yes	Yes	Yes	Yes	Yes	Yes
4	*Crioceris quatuordecimpunctata*	Low	High	Yes	Yes	Yes	Yes	Yes	Yes
5	*Crioceris quinquepunctata*	Low	Low	–	–	No	–	–	No
6	*Coraebus elatus*	Low to moderate	Moderate to high	Yes	Yes	Yes	No	No	No
7	*Maculinea alcon* xerophilic ecotype	Low to moderate	High	?	?	?	?	?	?
8	*Maculinea arion*	Low to high	Low to high	?	No	?	?	No	?
9	*Polyommatus ripartii*	Low	Low	No	No	No	No	No	No
10	*Melitaea cinxia*	Low	Low to moderate	Yes	No	Yes	No	No	No
Vertebrates									
11	*Coronella austriaca*	High	Low to high	Yes	Yes	Yes	No	Yes	Yes
12	*Dolichophis caspius*	Low	Low to moderate	–	–	Yes	–	–	Yes
13	*Lacerta viridis*	Low to high	Low	No	No	No	No	No	No
14	*Vipera ursinii* complex	Low to high	Moderate to high	–	–	Yes	–	–	Yes
15	*Spermophilus citellus*	Low to high	Moderate to high	–	–	Yes	–	–	No
16	*Spermophilus suslicus*	Low to high	High	–	Yes	–	–	Yes	–
17	*Cricetus cricetus*	Low to high	High	Yes	Yes	Yes	No	No	Yes

Table 2 continued

No.	Species	Level of intraregional diversity (character 1)	Interregional distinctiveness (character 2)	Any distinct units across regions (character 3a)			All distinct units across regions (character 3b)		
				Northern versus Pannonic	Northern versus Pontic	Pannonic versus Pontic	Northern versus Pannonic	Northern versus Pontic	Pannonic versus Pontic
18	Sicista subtilis	Low	High*	–	–	Yes	–	–	Yes
19	Nannospalax leucodon superspecies	?	High*	–	–	–	–	–	–
20	Spalax spp. complex	?	High*	–	–	Yes	–	–	Yes
Flowering plants (angiosperms)									
21	Adonis vernalis	Low to moderate	Low to moderate	Yes	Yes	Yes	No	Yes	No
22	Adonis volgensis	Low to moderate	High	–	–	Yes	–	–	No
23	Pulsatilla patens	High	Low to high	Yes	Yes	Yes	No	No	Yes
24	Astragalus exscapus	Low to moderate	Moderate to high	Yes	–	–	No	–	–
25	Linum flavum	Low to high	High	Yes	Yes	Yes	Yes	No	No
26	Linum hirsutum	Low to moderate	High	Yes	Yes	Yes	Yes	Yes	Yes
27	Carlina onopordifolia	Low to moderate	High	–	Yes	–	–	Yes	–
28	Cirsium pannonicum	Low to high	Moderate to high	Yes	Yes	Yes	No	No	No
29	Inula ensifolia	Moderate to high	High	Yes	Yes	Yes	No	No	No
30	Scorzonera purpurea	Low	High	Yes	Yes	Yes	No	No	No
31	Serratula lycopifolia	Low to moderate	High	Yes	Yes	Yes	No	No	No
32	Iris aphylla	Low	High	Yes	Yes	Yes	No	No	No
33	Melica transsilvanica	Low	High	Yes	Yes	Yes	No	Yes	No
34	Stipa borysthenica	?	High	Yes	–	–	Yes	–	–
35	Stipa capillata	Low to moderate	High	Yes	–	–	Yes	–	–
36	Stipa eriocaulis	?	High	Yes	–	–	Yes	–	–
37	Stipa pennata	Low to moderate	Moderate to high	Yes	Yes	Yes	No	Yes	No

Table 2 continued

No.	Species	Level of intraregional diversity (character 1)	Interregional distinctiveness (character 2)	Any distinct units across regions (character 3a)			All distinct units across regions (character 3b)		
				Northern versus Pannonic	Northern versus Pontic	Pannonic versus Pontic	Northern versus Pannonic	Northern versus Pontic	Pannonic versus Pontic
38	*Stipa pulcherrima*	Low to high	High	Yes	Yes	Yes	No	Yes	Yes

– not present in the region; ? lack of sufficient data; *—characteristics for species complex; low/moderate/high—level of genetic diversity (intraregional) or genetic distinctiveness (inter-regional), yes/no—presence of distinct phylogenetic lineages and/or genetic clusters between the regions compared (see details in "Phylogeographic data collection" section)

Lacerta viridis has low genetic diversity across all populations studied. Regarding the interregional distinctiveness of populations, *C. austriaca* has distinct phylogenetic lineages in all three regions (Northern, Pontic and Pannonic) (Fig. 2g), but the Carpathians are not an effective barrier in this case, as this species inhabits xeric grasslands and stony slopes in mountainous areas, as well. For *D. caspius* and the *V. ursinii* complex, species which are absent in the Northern region, distinct phylogenetic lineages were found in the Pontic and Pannonic regions. On the contrary, *L. viridis* has an unstructured mitochondrial diversity in Eastern Central Europe, albeit highly distinct phylogenetic lineages were found in the Mediterranean Basin including the sibling species, *L. bilineata*. At the same time, *L. viridis* has a highly reduced genetic diversity in its marginal populations (based on microsatellite genotyping). Genetic structure of *L. viridis* suggests that it is not a "real" steppic but rather a "Ponto-Mediterranean" species.

Flowering plants

The intra-regional genetic diversity of plant species and species complexes is not homogeneous, and it is often dependent on the situation of the examined populations in respect to the entire species range. Within the continuous part of the range, these plants harbour low-to-moderate genetic diversity. This concerns mainly populations from the Pontic and Pannonic (including the northern Balkan Peninsula) regions, whereas among populations located on the edge of the species range or in isolated, peripheral localities (like in Poland or Germany), the genetic diversity is conspicuously higher. Such a situation could be observed, for example, in *Scorzonera purpurea*, *Serratula lycopifolia* and *Stipa pennata*. The lowest diversity values were observed between populations closest to or within the continuous part of the range (the Pontic region), whereas those from the Northern and Pannonic regions were more diverse. Regarding the inter-regional distinctiveness of populations, almost all of the plants examined were assigned to high or moderate-to-high differentiation level groups. This distinctiveness is especially visible due to the presence of different genetic clusters in all three of the regions around the Carpathians and the Bohemian Massif region. The exceptions were *Pulsatilla patens,* which had a low diversity between some of its Northern and Pontic populations, and *Adonis vernalis*, which had a low diversity within the Pontic and moderate within Pannonic and Northern regions. A somewhat more complicated situation was found in the case of *Astragalus exscapus*, where variability of several parallel markers (allozymes) is presented, some of them displaying divergent patterns (e.g. ADH showing much higher differentiation within the peripheral Northern (German) populations than within the more "central" Pannonic (Bohemian, Moravian and lower Austrian) ones, while for AAT2 or GPI the situation is quite the opposite).

A significant differentiation of populations was also often observed within these regions, probably caused by long-term isolation. Within the Northern region, distinct clusters were identified, e.g. in the Małopolska Upland versus the Lviv-Lublin Upland for *Carlina onopordifolia* and *Linum flavum* (Fig. 2a). Similarly, distinct clusters were found for the German Uplands versus most of eastern Poland versus the remaining part of the northern range for *Iris aphylla* (Fig. 2c), and in the German Uplands versus the lowlands of northern Germany versus northern Poland for *Scorzonera purpurea* (Fig. 2d). Within the Pannonic region, such clusters were found for Bohemia-Moravia versus the Pannonian Basin vs. Transylvania, e.g. in *Cirsium pannonicum, Linum hirsutum, S. purpurea, Adonis vernalis* and *Stipa pulcherrima*. Similarly, both *Adonis volgensis* and *P. patens* were characterized by lineages in the Pannonian Basin that were isolated from their steppic counter-populations in the Pontic region (Fig. 2b).

Genetic intra-regional diversity and inter-regional differentiation of steppic taxa populations

Nearly all (94 %) of the examined animal taxa (17 out of 18 for which respective data were available) displayed low genetic diversity on an intra-regional scale, at least for some of their populations. Similarly, 94 % of the plants (15 out of 16 for which respective data were available) have some or all of their populations characterised by low diversity. Moreover, in only 33 % of animal taxa (six), some or all populations expressed high diversity. In plants, highly diverse populations were found in 38 % of taxa (six) (Table 2). Usually, the genetic diversity of the steppic animals increased to the east and to the south, as the Pontic and Pannonic regions sustained populations much more genetically diversified than the Northern region (Poland and Germany). This pattern was visible even within the Pannonic region, as Bohemia sometimes possessed only single genetic variant, opposed to the parts situated more towards the east and south. To the contrary, in plants, many taxa expressed the opposite pattern of genetic diversity. The majority of their most diverse populations are in the Northern region, and only a few are in the Pannonic or Pontic ones.

Regarding the genetic distinctiveness of populations on an inter-regional scale, most steppic taxa were structured geographically: 70 % of animals (14 out of 20) and 94 % of plants (17 out of 18). Highly distinct phylogenetic lineages and/or genetic clusters were identified in all, or at least some, distant areas occupied by steppes or other types of xeric grasslands in Eastern Central Europe (including eight taxa of animals and 14 taxa of plants that had a high level of differentiation throughout their entire ranges in the area). On the other hand, 35 % of animals (7 taxa) and 11 % of plants (2) had a low distinctiveness of population between at least some regions (but only 4 animal taxa had a low genetic differentiation among all regions of study) (Table 2).

The most interesting phylogeographic patterns arose when the presence of distinct genetic units (phylogenetic lineages or genetic clusters) were analysed between different sides of the Bohemian Massif–Carpathian arc.

The Pannonic and Northern regions were found to sustain distinct units in 78 % of the animal taxa (7 out of 9 taxa present in both regions) in the 3a character (Table 2), or in 22 % of them (2) when counted according to the 3b character. For plants, these numbers were 100 % (all 16 taxa) and 38 % (6), respectively (Table 2).

Between the Pontic and Northern regions, distinct units were found in 58 % of the animal taxa (7 out of 12) in the 3a character, or in 50 % (6 taxa) when counted according to 3b character. Analogous values for plants (a comparison between the Pontic and Northern regions) were 100 % (all 13 taxa) and 46 % (6), respectively (Table 2).

Separate genetic units between the Pannonic and Pontic regions were present in 80 % of the animal taxa (12 out of 15 taxa) were at least partly separated, whereas 60 % of them (9) were characterised by full genetic isolation between these regions (Table 2). For plants, these numbers were 100 % (all 13 taxa) and only 23 % (3), respectively (Table 2).

Discussion

The genetic and phylogeographic data of the populations presented in this review clearly show that steppic species generally neither follow an east-to west, nor a south-to-north decreasing phylogeographic pattern. Therefore, we can exclude both a relatively recent

east-to-west immigration hypothesis and the classical north–south "contraction–expansion" paradigm regularly observed in temperate species (Taberlet et al. 1998; Hewitt 1999, 2000; Petit et al. 2003; Schmitt 2007). The genetic diversity and distinctiveness of steppic populations is rather similar to the pattern observed in arctic-alpine taxa (e.g. Schoenswetter et al. 2005; Varga and Schmitt 2008; Schmitt 2009). The geographic structure of genetic characteristics hint at a more complex history in the western part of the steppic biom. Cold-tolerant steppic species seem to have been much more widely distributed and probably more abundant during cold periods than during interglacials (including the current one) (Stewart et al. 2010; Varga 2010). It is often postulated that in Eastern Central Europe, steppe-related species of animals (rodents) and plants (e.g. *Astragalus exscapus*) were distributed more widely during glacial periods than during interglacials (Pawłowska 1966; Becker 2012; Sommer and Nadachowski 2006). They may have occupied the extensive areas between the ice sheet in the lowlands of central and northern Europe and mountain ranges like the Alps, the Bohemian Massif and the Carpathians. This is probably a consequence of the type of glacial zonation being fundamentally different from the recent (interglacial) one. The major dynamic elements of this glacial zonation were as follows: (i) the fragmentation of the nemoral and boreal forest zone; (ii) the expansion of diverse continental meadow steppic elements during the cool, but not extremely cold, phases of glaciations; (iii) the expansion of continental cold steppe elements in the cryoxeric phases, followed by postglacial fragmentation; (iv) the establishment of manifold ecotones at the junctions of zonal and intrazonal continental habitat types (Varga 2010; Schmitt and Varga 2012). Steppic species were probably also widespread in the Pannonian Basin (Magyari et al. 2010).

A history of steppic species in Eastern Central Europe

The existence of steppes and steppic populations during Pleistocene glaciations within the Pannonian Basin and the Pontic area has been confirmed by paleontological and chorological data (Willis and Van Andel 2004). Both areas were never glaciated and were localised relatively far from the ice sheet, even during the Sanian/Elsterian glaciation, which extended most to the south (Ehlers and Gibbard 2004). There is a considerable body of evidence suggesting that cold steppes and forest steppe associations existed during Pleistocene glaciations in the Pannonian Basin, and that more eurythermic species could have survived the last glacial maximum (LGM) at least in favourable microsites (Willis et al. 2000; Jankovská and Pokorný 2008; Magyari et al. 2010, 2014). On the other hand, the existence of populations of steppic species north of the Alps, the Bohemian Massif and the Carpathians is more controversial. This area was only once fully glaciated (the Sanian/Elsterian glaciation, 730,000–430,000 years ago), whereas during following ice periods, only some parts of these areas were under the ice sheet. In particular, uplands in central and southern Germany and south-eastern Poland have not been glaciated since the Sanian/Elsterian (Lindner et al. 2006; Wysota et al. 2009). Even during that extensive glaciation, these uplands were not fully ice-covered, or perhaps some areas survived as nunataks within the ice sheet (Lindner et al. 2006; Lewandowski 2011). Consequently, these uplands could have been temporarily covered by steppe vegetation during cryoxeric phases of at least some glacials. This hypothesis finds support in phylogeographic data which shows that these uplands harbour distinct phylogenetic lineages or genetic clusters of several steppic species (e.g. beetles: *Centricnemus leucogrammus, Cheilotoma musciformis, Crioceris quatuordecimpunctata,* hamster *Cricetus cricetus* and plants: *Cirsium pannonicum, Inula ensifolia, Linum flavum, L. hirsutum* and *Stipa pulcherrima*). This distinctiveness is

so high compared to other populations in the east and south that it is unlikely to have developed simply during a Holocene expansion. Some of these are probably remnants of much larger populations of species that were widely distributed during glaciations and populations of which are presently isolated by mountain ranges and other areas covered by unfavourable natural habitats (forests). Another explanation is that their distribution is now conditioned by land that has been transformed by humans (in both favourable, e.g. agricultural, and unfavourable ways, e.g. urbanized, roads, railways).

It is challenging to estimate when these species expanded into the areas north of the Bohemian Massif-Carpathians arc, but their current populations must be younger than 430 000 years (from the end of the Sanian/Elsterian glaciation) (Lindner et al. 2006). A few dating efforts of divergence and expansion times suggest that these northern populations could have indeed originated 380,000–150,000 years ago (*Centricnemus leucogrammus*) or 147,000–85,000 years ago (*Cricetus cricetus*) and expanded 280,000–110,000 years ago (*C. leucogrammus*) or 115,000–10,000 years ago (*C. cricetus*) (Neumann et al. 2005; Kajtoch et al. 2009). Spans of these dating suggest that these events took place during Oder and Warta/Riss glaciations (for *C. leucogrammus*) or Vistulian/Würm glaciation (for *C. cricetus*). These datings of divergence and subsequent expansion of distinct phylogenetic lineages probably differ for other species. They would depend, for example, on the species' mobility (by physical movement or seed dispersal), probability of survival in small areas with a limited population size and tolerance to climatic and environmental changes. Butterflies and some plants with easy seed dispersal may have expanded (at least to some of the existing stations) across northern areas in relatively recent times (after the LGM, 26,000–19,000 years ago), whereas some wingless beetles or plants with heavy seeds would have needed to be present north of the Carpathians-Bohemian Massif earlier and survive in situ to the present time (if they had expanded after the LGM, there would not likely be enough time for them to settle all these distant areas and form distinct genetic units).

Current "warm stage" refugia of steppic taxa

Consequently the uplands of southern Germany and south-eastern Poland should be considered as additional "warm stage" refugia for steppic species, apart from the presumed major refugia in the Pontic and Pannonic regions (Stewart et al. 2010; Varga 2010). Moreover, phylogeographic data for several species suggests that the Lublin-Lviv Upland at the Polish-Ukrainian border is probably a transient zone between genetic units characteristic for eastern populations (from Ukraine) and those known in the uplands of southern Poland (*Centricnemus leucogrammus, Cricetus cricetus, Inula ensifolia*). It could also be a refugium for some unique genetic clusters (like in *Carlina onopordifolia*). The discovery of unique phylogenetic lineages as far north as the Kujawy Basin (*C. leucogrammus, Crioceris quatuordecimpunctata, Coraebus elatus*) or eastern Poland and central Germany (*Iris aphylla*) is considered to be of high importance as these areas were deglaciated only 18,000–12,000 years ago (Wysota et al. 2009). Existence of steppic populations in the Northern region before the Holocene could also explain a high genetic diversity of some plant species—higher even than observed in "core" populations in Pontic and Pannonic regions. This high diversity could reflect substructuring of steppic populations in fragmented xeric grasslands in Germany and Poland. Contrary, in Pannonic and Pontic regions populations were (and locally are) interconnected (Sutcliffe et al. in press) so they could be more uniform genetically. Interesting is that this phenomenon apparently concerns only (the studied) plants but not (the studied) animals, in which

marginal, isolated populations generally were less diverse than these from the continuous parts of species' ranges.

The reviewed phylogeographic patterns suggest a complex history and structured phylogeography of the eastern (Pontic) and southern (Pannonic) refugia. Several areas in the Pontic region sustain diverse phylogenetic lineages: (i) the Podolian Upland (western Ukraine), (ii) Dobrogea (eastern Romania), (iii) Crimea, (iv) eastern Ukraine and southern Russia (exemplary taxa with distinct lineages in some of these areas: *Coraebus elatus*, *Spermophilus suslicus*, *Sicista subtilis* agg., *Coronella austriaca*, *Vipera ursinii complex*, *Adonis vernalis*, *A. volgensis*, *Scorzonera purpurea* and *Stipa pulcherrima*). In the Pannonic region, distinct refugia were identified: (i) Moravia-Bohemia (an exemplary species with a distinct lineage in this area is *Cirsium pannonicum*); (ii) the Pannonian Basin (*Linum flavum*, *L. hirsutum*); (iii) the Transylvanian Basin (*Adonis volgensis* and the butterfly *Kretania pylaon sephirus*; Pecsenye et al. 2007) and (iv) the northern Balkan Peninsula (taxa with distinct units between the northern Balkan Peninsula and the Pannonian Basin are *Spermophilus citellus* and the *Nannospalax leucodon* superspecies). The barrier formed by the Eastern Carpathians to some of the analysed taxa (*S. trizona*, *A. volgensis*, *Pulsatilla patens*) is especially interesting. These taxa, with a centre of distribution in the Pontic area, are apparently more diverse in the Pannonian Basin (together with the Transylvanian Basin) than in neighbouring Dobrogea or south-eastern Ukraine. Development of distinct units between Pontic and Pannonic regions was also predicted by the distribution modelling of the steppic butterfly *Melitaea ornata* in which the Pannonian, Eastern Balkanic and Pontic population groups can be derived from distinct refugia (Tóth and Varga 2011; Tóth et al. 2012, 2014). Preliminary analyses suggest (W. Paul, in prep.) that a similar pattern (i.e. conspicuous genetic separation between Pontic and Pannonic lineages) can be observed in case of another steppic vascular plant, *Campanula sibirica*. Unfortunately, the inavailability of (published) full genetic data prevented regular inclusion of these species into the present review.

Some of the steppic species, especially those with ranges confined to the Balkan Peninsula and the Pannonian Basin, could have a different origin compared to species which could be derived from former cold steppes. These species (or sibling taxa in species complexes) have ranges often stretching across Anatolia, the Balkan Peninsula and the Pannonian Basin. The occurrence of these taxa in the Pannonian Basin's steppic habitats could be related to the more ancient expansion of some xeric elements from Anatolia (where steppes and other types of xeric grasslands also exist) into south-eastern Europe. An investigation of this pattern needs more phylogeographic data from species inhabiting Anatolia (and possibly also the Caucasus), the Balkan Peninsula and the Pannonian Basin, but this is out of the scope of this review.

Prospects beyond steppic species

Our conclusions might not be restricted to steppic species in the strict sense (related to xeric grasslands), but could possibly be extended to some other taxa related to more meso- or even hygrophilous continental meadow steppes (like the plants *Bistorta affinis*, *Sanguisorba officinalis* and *Dianthus superbus* and the insects *Arcyptera fusca*, *Gampsocleis glabra*, *Tettigonia caudata*, *Aricia artaxerxes*, *Maculinea* spp. and *Melitaea* spp.; Varga 2002). Such species are and also were widely co-distributed with more cold- and xeric-adapted steppic species during glaciations and probably experienced similar range contractions during interglacials. This hypothesis however needs phylogeographic investigation, as appropriate analyses are available only for the butterflies *Lycaena helle* (Habel

et al. 2014) and the *Boloria (Proclossiana) eunomia* (Nève et al. 2000). This hypothesis is also supported by the phylogeography of two ecotypes (xeric- and hygrophilous) of the steppic beetle *Coraebus elatus* (Kajtoch et al. 2014b).

Conservation implications

From a conservation point of view, the factor of major importance is the low genetic diversity among (and usually also within) many (local) populations. This genetic depletion of steppic populations could be explained in two ways, which are not mutually exclusive. For the populations that displayed a reduced genetic diversity and lack of differentiation from the main area, this reduced genetic diversity can be explained either by the effect of drift (i.e. random change in the frequency of alleles in a population) or by founder effect (i.e. establishment of local populations by a limited number of genetically closely related individuals). For populations with a reduced genetic diversity and significant differentiation from other regions, a possible explanation is that the long-existing local populations might have undergone bottle-necks, which could have depauperated genetic diversity while providing a chance for local genotypes to sweep through the surviving population. The majority of phylogeographic patterns described in this paper support the second scenario. Regardless of the reason, low genetic diversity could have a serious impact on the probability of the survival of these populations as limited variation in genes could lead to further population decline and extinction (Frankham et al. 2002). There are known examples of highly threatened or even endangered populations of steppic animals and plants with low genetic diversity (e.g. *Cheilotoma musciformis,* Kajtoch et al. 2013; *Spermophilus suslicus,* Biedrzycka and Konopiński 2008, *Carlina onopordifolia* and *Linum hirsutum,* Cieślak 2014).

Another issue of conservational importance is the presence of distinct genetic units (phylogenetic lineages and/or genetic clusters) in different regions occupied by steppic species in Eastern Central Europe. As we described in the present paper, many steppic species or species complexes, both animals and plants, showed substantial differentiation in their populations from different regions (defined for purposes of this review). This genetic distinctiveness was interpreted in many of the examined steppic organisms as a presence of different "evolutionarily significant units" and/or "management units" (Moritz 1994; Vogler and Desalle 1994). These units should be treated independently when planning conservation strategies and managing populations or habitats. Conservation actions are crucial, as distinct evolutionary units often have different environmental and ecological requirements, and the mixing of individuals from diverse lineages (e.g. during translocation or introduction actions) could be harmful for the fitness of progenies and the survival of populations (outbreeding depression: Frankham et al. 2002). Lastly, these evolutionary units could also have taxonomic value (e.g. identification of taxonomic structure like in *Sicista subtilis,* Cserkész et al. 2015a, b; the "near to extinction" taxa of the *Nannospalax leucodon* superspecies, Németh et al. 2013, species within the *Vipera ursinii* complex, Ferchaud et al. 2012; Gvozdik et al. 2012; Zinenko et al. 2015, or presumable subspecies such as in *Cheilotoma musciformis* and *Coraebus elatus;* Kajtoch et al. 2013, 2014a, b).

Obviously, it is impossible to protect all populations of all steppic species which show substantial genetic distinctiveness (it would be even not possible to investigate all these populations!). The priority in steppic organism conservation should be to protect those areas that are characterized by highest biodiversity and simultaneously sustain distinct genetic units of some selected (flagship, keystone) species. A general list of such areas is

presented above, but more comprehensive and detailed studies are needed to select particular "hot spots" where protected areas need to be designed.

Because steppic habitats, communities and populations in Eastern Central Europe are highly threatened, genetic factors (like these identified based on phylogeographic analyses here) should be taken into consideration for the conservation of these species and in managing steppes and xeric grasslands.

Conclusions

Based on the results and the above part of the discussion, in respect to the hypotheses enumerated in the "Introduction" section it may be stated, that (see also Information Box 2):

1. The steppic taxa, their similar ecological demands and biogeographical features notwithstanding, do not show a uniform phylogeographic pattern. However, some common characteristics include: (i) generally lower intraregional than interregional genetic diversity of populations (with some species showing very low or lack of genetic variability on a local scale); (ii) presence of distinct evolutionary units in each of three defined "steppic regions" (Pannonian, Pontic and Northern) with (iii) substantial phylogeographic substructuring of populations within regions (usually inhabiting currently isolated areas rich in steppic habitats).
2. Almost all steppic taxa or their groups show phylogeographic patterns generally congruent with that defined for cold-adapted (arctic-alpine) species, that is interglacial-contraction and glacial-expansion dynamic (opposite to the phylogeographic paradigms known for temperate-related species).
3. In most cases, genetic differences, if any, are clearly connectable to mountain chains, which have seemingly acted as important barriers for the migration and/or genetic contact of steppic plants and animals.

Information Box 2 Major conclusions drawn from phylogeographic patterns observed in steppic plants and animals

Generally low genetic diversity of animal populations in intra-regional scale, especially in marginal populations, which has serious conservation implications for survival of local populations

Varied genetic diversity of plant populations in intra-regional scale with unexpected lower diversity in core populations of some species in Pontic region

Substantial genetic distinctiveness of populations inter-regionally and for some taxa also within regions (substructuring of populations caused by their history and current habitat fragmentation)

High share of species with distinct genetic units across Bohemian Massif and Carpathians arc—presence of distinct evolutionary significant units in each of three major areas: Northern, Pannonic and Pontic

There is no east-to-west decreasing genetic diversity and distinctiveness, which should be expected in case of a recent east-to-west migration scenario

Confirmation that Pontic and Pannonic regions were being settled by steppic species throughout cold or cool phases of the Upper Pleistocene and there are current "warm stage refugia" for steppic species

Proof that at least some steppic species must have persisted in situ also north of the Carpathians-Bohemian Massif-Alps arc at least since Sanian/Elsterian glaciation (430,000 years ago), which contradicts the statement of the exclusively human origin of xeric grasslands in Poland and Germany

Urgent necessity to identify "hot spot" areas, which sustain high biodiversity and distinct evolutionary units of steppic species, for effective protection of steppic habitats and populations

4. Phylogeographic patterns of steppic species do not follow the current species diversity, i.e. apparently there is no east-to-west decreasing genetic diversity and distinctiveness that should be expected in case of a recent east-to-west immigration scenario. Quite the contrary, the present genetic pattern hint at a more complex phylogeographic history of steppic species at their present-day western distribution edge.

5. In several cases (e.g. *Cheilotoma musciformis, Melitaea cinxia, Coronella austriaca, Cricetus cricetus, Linum flavum* or *Scorzonera purpurea*), has been found genetic evidence for the existence of hitherto disregarded extrazonal microrefugia. Interestingly, many of the examined species show distinct evolutionary units not only in the Pontic and Pannonian regions, where refugia for steppic taxa have already been considered (Stewart et al. 2010; Varga 2010), but also in the Northern region. This indicates the presence of extrazonal microrefugia also to the north of the Bohemian Massif-Carpathians arc. Moreover, the presence of genetic differences without any apparent geographic barriers within the Northern region in approx. 60 % of examined animal and all examined plant taxa may reflect the genetic signature of several separate migrational waves here.

Compliance with ethical standards

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