

Disjunct Occurrences of Plant Species in the Refugial Mires of Bulgaria

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Abstract Many mire vascular plant and bryophyte species have disjunct occurrences in Bulgaria despite that most of south-eastern Europe is not suitable for the occurrence of permanently waterlogged and nutrient-limited wetlands due to the current and glacial dry climate conditions as well as prevailing limestone bedrock. Unfortunately, such important distributional data are scattered throughout numerous papers and reports, and are not adequately provided even by national checklists and floras. No attempt to summarize them has been done yet. Therefore, the main aim of this paper is to review and enlarge such data, and to use the resulting data set to address the question whether the disjunctly occurring rare species are concentrated in certain mire complexes or even in particular vegetation plots and if they do characterize such localities. Our current research shows that the phenomenon of isolated occurrences of mire plants in Bulgaria is even more widespread than previously thought. Seventeen species were found as new for Bulgaria with their distribution range limits there, and distributional data of many other species, including some previously considered extinct, were enlarged. Fifty-four mire species

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were found at only three or fewer sites. Our analyses showed a conspicuous concentration of rare, disjunctly occurring species at a few sites, which are, however, largely unexplored in terms of palaeoecology or ecology, not legally protected and currently threatened by human activities. The distributions of target rare species within Bulgarian mires were significantly nested, which means that more species-poor assemblages were subsets of richer ones. Nestedness was significantly related to the estimated area of mire complex, but not all high-diversity mires were large. Disjunctly occurring rare species were more concentrated in particular vegetation plots at lower altitudes and in mineral-rich fens. Fragmentary data about the ecology and history of Bulgarian refugial mires suggest that these mires harbour specific ecotypes and genotypes, contain specifically distributed biogeographic groups of species, provide an opportunity to test biogeographical hypotheses and shelter crucial information about the history of European mires. Thus, these sites have a potential to become a source of very important information for biogeographical, palaeoecological, and phylogeographical analyses.

Keywords Balkans · Conservation · Distribution · Fen · Palaeoreugia · Range margin · Relic species · Warm climate

Plant nomenclature Andreev et al. (1992); Ganeva and Natcheva (2003); Natcheva and Ganeva (2005)

Introduction

Among southern European countries, Bulgaria is unique due to the frequent occurrence of both acidic and alkaline wetlands (Michev and Stoyneva 2007; Hájek et al. 2008). Because most of the Balkan Peninsula is not suitable for the occurrence of mires, especially for poor fens and bogs (Papazisimou et al. 2002; Topić and Stančić 2006), many mire species have disjunct occurrences in Bulgaria. This phenomenon was clearly shown for *Sphagnum* species (Daniels and Eddy 1985; Hájková and Hájek 2007) and it can be predicted also for other taxonomic groups by comparing national checklists and floras (Andreev et al. 1992; Ganeva and Natcheva 2003; Natcheva and Ganeva 2005) with distribution maps of target species (e.g., Meusel et al. 1965–1992). However, distributional data reported in national checklists are mostly based on old investigations. From the time of excellent Bulgarian plant scientists, e.g., D. Jordanoff, B. Stefanoff, and S. Petrov, who studied floras of several Bulgarian mires and evaluated the results in a wider geographical and historical context (Jordanoff 1931; Steffanoff and Jordanoff 1931; Petrov 1958; Stefanoff and Petrov 1962; Jordanov et al. 1972), virtually no other research has been conducted on Bulgarian mires up to the beginning of the 21st century. Bulgarian wetland scientists were focused more on lakes, reed beds, and tall sedge stands (Kochev and Jordanov 1981; Michev and Stoyneva 2007). Many phytogeographically important occurrences of mire species have therefore been forgotten and even not included in national checklists, or their current status is unknown. Current research on Bulgarian wetlands proceeding from the beginning of

the 21st century has brought many new data about the distribution of mire and wet-meadow bryophytes (e.g., Natcheva 2005; Blockeel et al. 2006a,b; Hájková et al. 2007a,b,c; Štechová et al. 2008) and vascular plants (e.g., Hájek et al. 2005, 2006a, b; Tzonev and Karakiev 2007). Recently, many new wetland plant species have been found in Bulgaria and most have their distributional range-limits here (e.g., *Carex lasiocarpa* and *Meesia longiseta*). Several species considered as extinct or known only from a few sites have been found at several new sites. Unfortunately, these new distributional data, which are crucial for understanding biogeographical patterns throughout Eurasia, are dispersed among numerous papers and reports in both international and local journals and no attempt to summarize them has been done yet.

Southern Europe is generally considered a refugial region from which many, especially plant species have colonized Central- and Northern Europe during the Holocene (Tzedakis 1993; Hewitt 1999; Zhang et al. 2001; Fér et al. 2007; Hedenäs and Eldenäs 2007; Birks and Willis 2008). Limestone predominance in the glacial refugia is considered to be the main reason why the species pool of those Central European habitats, which were strongly restricted before the retreat of the last glaciation, is rich in calcicoles (Chytrý et al. 2003; Ewald 2003). However, species richness patterns along environmental gradients are much weaker for mires (Chytrý et al. 2003; Schuster and Diekmann 2003; Hájek et al. 2007) – habitats whose origin can be traced back to the Ice Age when they might have occurred in wet depressions of the Pleistocene tundra with draining impeded by underlying permafrost (Chytrý et al. 2003). Although the crucial role of southern refugia is generally accepted also in the case of mire species (Thingsgaard 2001; Hedenäs and Eldenäs 2007; Szövényi et al. 2008; but see *Polytrichum commune* in van der Velde and Bijlsma 2003) it seems likely that both dry climates during glacial periods (Wright et al. 2003; Hughes et al. 2006) and prevailing limestone bedrock not supporting wetland formation reduced the areas available for survival of mire species in southern Europe including the Balkans (Cronberg 1998). However, some indirect evidence through genetics or comparative niche analyses suggest a possible survival of mire species during the dry Pleistocene glacial periods in some locations in Bulgaria (Natcheva and Cronberg 2003; Hájek et al. 2007; Hájková et al. 2008). Direct evidence is unfortunately not available due to poor preservation of plant macrofossils in old fen deposits impeding identifications at the species level, but also due to generally weaker interest of palaeoecologists in mire-vegetation history as compared to forests or alpine habitats (cf. Birks and Willis 2008). If we accept the premise that disjunct occurrences of boreal and arctic mire species in Bulgarian mires are caused by their survival in refugia, these mire refugia were probably isolated from those elsewhere in Europe because of the relatively dry climate and limestone bedrock in south-eastern Europe. The patterns of high genetic diversity (Natcheva and Cronberg 2003) and shifts in ecological behaviour of calcicole species, extending their tolerance towards the base-poor conditions prevailing in Bulgarian refugia (Hájková and Hájek 2007; Hájková et al. 2008), gives support to this hypothesis. Distributional analyses of disjunct mire species, or an inclusion of Bulgarian populations into large-scale phylogeographical analyses, may help advance our understanding of Holocene history of mires and mire species in the Balkans.

In this paper we review all historical and recent records of mire plant species of range-margin and/or disjunct distribution in Bulgaria to draw attention to this

phenomenon and to provide a basis for future research. We further aimed to identify disjunct and range-margin occurrences of the species exclusively or predominately inhabiting mires. Besides the predominant review function of this paper, we aimed to use the final data set to address the following research questions. *i*) Are disjunctly occurring rare mire species distributed randomly across suitable sites or can patterns or hotspots be detected? *ii*) How far do the recent distribution patterns of the disjunctly occurring rare species mirror the presumed continuum of mire refugia? Are these species concentrated in certain mire complexes or even in particular vegetation plots? *iii*) If they are, what are the specific characteristics of such localities? *iv*) Are low-diversity assemblages of target rare species subsets of high-diversity ones? *v*) If the assemblages are nested in such a way, can the pattern be explained by estimated habitat area?

Methods

Study Area

Springs and fens with an occurrence of mire species are distributed along a wide altitudinal gradient in Bulgaria. As sea-shore wetlands are not considered in this paper, the lowest altitude sites were tufa springs near the Gradina village in the Devetashsko Plato karstic area (260 m a.s.l.), springs close to the Kolarovo village in the Struma valley (275 m a.s.l.) and calcareous fen “Dunavtsi” in the Kazanlak basin (410 m a.s.l.), which are extremely rich in rare species (Hájek et al. 2010). The highest altitude wetlands with mire species were found in the alpine belts of both the Rila Mts (Golyama Mokritsa valley, 2,520 m a.s.l.) and the Pirin Mts (Todorkini Ochi lake, 2,525 m a.s.l.). Granites, gneiss, granodiorites, sandstone, claystone, siltstone, metamorphosed shale, marble and limestone form bedrock in particular study regions, with mineral-poor bedrock prevailing in the highest altitudes, except for marble parts of the Pirin Mts. The Bulgarian climate is rather continental, only locally influenced by the Mediterranean Sea. The prevailing evaporation at higher temperatures in low altitudes is not optimal for development of *Sphagnum*-rich mire types (Hájková and Hájek 2007), while the climate of Bulgarian high-mountains is cold and humid. The mean annual precipitation of the study sites vary between 574 mm (Kolarovo) and 844 mm (surrounding of the Botev peak in the Stara Planina Mts), the mean annual temperatures vary between -0.8°C (Todorkini Ochi) and 13.8°C (Kolarovo; data from the WorldClim database, version 1.3, available at: www.worldclim.org, accessed on 14 March 2007; for the next examples see Results).

Distributional Data

Recent data on species occurrences were mostly taken from vegetation plots (mostly 16-m² plots, for details see Hájková et al. 2006, 2008 or Hájek et al. 2007) and from species lists of vascular plants and bryophytes collected in all types of inland sites where natural conditions support mire, spring, and fen meadow vegetation. We tried to investigate as many mires, springs, and fen meadows as possible in Bulgaria

during our field research in the period 2001–2007. We have explored 240 localities of mires, springs, and wet meadows. Some study sites have been generally known, but many were discovered randomly or with the help of old floristic data during our travels throughout the country. The species found recently by other authors have been excerpted from the literature. Our further analysis amalgamates some smaller mire localities occurring very close (hundreds of meters) to one another because they form natural mire complexes with a presumed continuous metapopulation structure of mire species. We further excerpted historical data about occurrences of mire specialists in our mire complexes and, for review purposes, also about occurrences of disjunctly occurring mire plants in Bulgaria in general. Species predominantly or exclusively inhabiting mires were called mire specialists. Because there is a very small phytosociological database and generally very few available vegetation plots in Bulgaria, the selection of mire specialists was expert-based. For particular mire complexes or vegetation plots, both the number of rare disjunctly occurring species (mostly occurring in a maximum of ten mire complexes, for details and complete list see Appendix 1) and the total number of species were counted.

Despite that any analysis involving counts of disjunctly occurring rare species should include inherent species-area relationships (e.g., Dengler 2008; Dengler and Boch 2008), this issue could not be solved properly for several reasons. Unfortunately, there are no exact data about historical and recent area of particular localities. There is neither any detailed habitat nor vegetation map for Bulgaria or images showing exact historical areas of the sites. We did not measure site areas, and their back-estimation is not possible because many localities are a fine mosaic of small springs and dry-grassland vegetation, and because many localities evidently had greater spatial extents in the recent past. Therefore we divided the studied mire complexes into five rough ordinal categories: (1) one or two small isolated wetlands, mostly springs, of only several square meters; (2) a group of more than two small isolated wetlands in a close contact; (3) one isolated medium-sized wetland of hundreds of square meters; (4) more medium-sized wetlands in a close contact or a very numerous group of smaller springs, e.g., in a mountain valley; (5) a complex of many small as well as larger wetlands, mostly providing more than ten diverse vegetation plots. These ordinal numbers as rough estimates of mire complex areas were correlated with species counts using Spearman Rank correlation (r_s).

Nestedness

To test if the distribution of disjunctly occurring rare species is nested within Bulgarian mires, we used the BINMATNEST programme (Rodríguez-Gironés and Santamaría 2006).

The T metric (called “matrix temperature”) was used as the standard measure of the degree of nestedness. This metric measures how much the species-by-sample matrix departs from perfect nestedness. Its calculation involves reorganization of the tested matrix into a state of “maximum packing”, which means that the rows and columns are reordered so that nestedness is maximized (Rodríguez-Gironés and Santamaría 2006). To test the significance of a nested pattern, the matrix temperature is compared with a mean temperature of randomly generalized matrices using the null-model proposed by Fisher and Lindenmayer (2002) (i.e., null-model 2 in the

BINMATNEST), with 500 random matrices. Two independent analyses were performed, one a species-by-sample matrix containing only disjunctly occurring rare species, and one containing all other species not included into the first test. To test whether habitat size supports the nested pattern the ranking order of sites in the final packed matrix (i.e., the matrix with the lowest possible temperature) was correlated with the rank order of sites after re-arranging the sites along their total area using Spearman Rank correlation (r_s). A significant correlation suggests that a community is packed in a predictive order owing to the influence of a given factor (Patterson and Atmar 2000).

Scale of Vegetation Plots

In addition to mire complexes, we analyzed some patterns at the scale of vegetation plots. We correlated species density in the vegetation plot, total number of disjunctly occurring rare species, and both the total number and the percentage of all mire specialists recorded in each plot with three environment factors that were available for all sites: water pH, water conductivity, and altitude. Measurements were conducted using portable instruments directly in well-watered microsites in the central part of the mires. A small, shallow pit was dug and spring water was allowed to clear before measurement. Conductivity caused by H^+ ions was subtracted in acidic waters with $pH < 5.5$ (Sjörs 1952). Corrected conductivity was used as a proxy of total mineral richness of the water sample, which correlates most strongly with the sum of calcium and magnesium concentrations (e.g., Horsák 2006). Pearson's correlation coefficients were used to quantify the relationship between species distributions and environmental factors. All vegetation plots were subjected to detrended correspondence analysis (DCA, calculations in CANOCO 4.5, ter Braak and Šmilauer 2002), where percentage cover data were log-transformed. Further, we analyzed the density of disjunctly occurring rare species in vegetation plots. Ordination site scores and measured environmental variables were correlated using Kendall's tau correlation.

Results

Our review of mire plant distribution data shows that the phenomenon of isolated and range-margin occurrences of mire biota in Bulgaria is more widespread than previously recognized. In the last seven years 14 species having their range-margin and/or disjunct distribution in Bulgaria were found as new for the country. Several other species that were previously considered as extinct were re-discovered (Appendix 1). There are 92 plant species related to mire, spring fen, and fen-meadow habitats that have disjunct or range-margin populations in Bulgaria; however, 20 of them have not been recently confirmed. Rare species having disjunct occurrences in Bulgaria are not randomly distributed but display rather a conspicuous concentration at some sites (Fig. 1, Table 1). The most valuable ten sites were clearly distinct from the other sites on the basis of the count of disjunctly occurring rare species. These sites represent various types of fens (calcareous fens, mineral-rich fens, mineral-poor fens, and subalpine mires), contain various phytogeographical elements within a group of rare species (Fig. 2), and they are

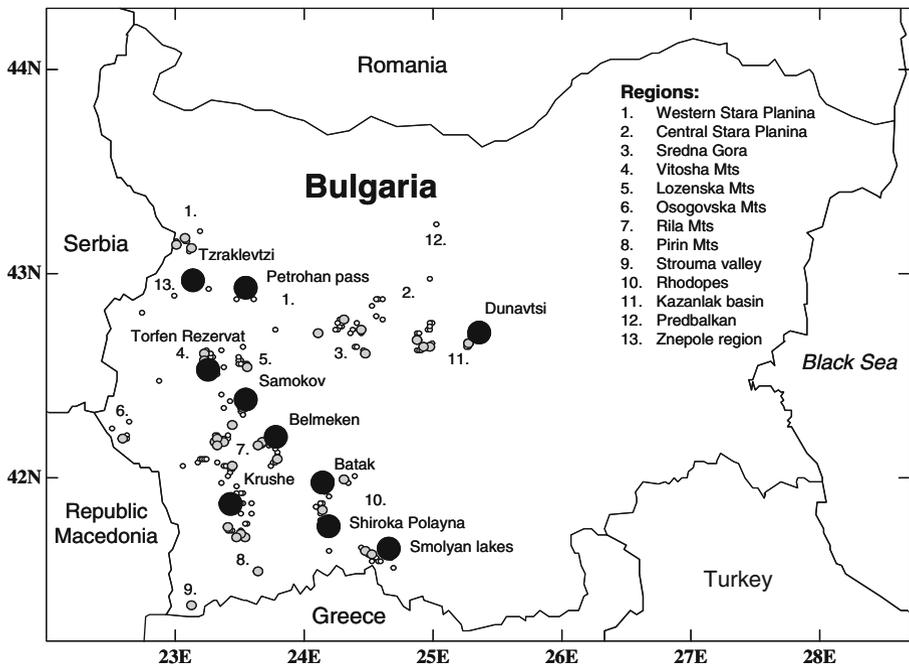


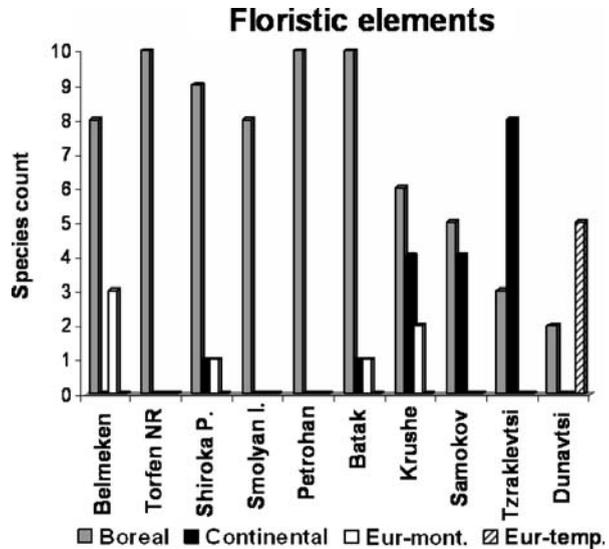
Fig. 1 Geographical position of all studied mire complexes (small dots) and mire complexes containing at least three target species (grey middle-size dots). The mires containing ten or more target species, or showing their extraordinary density in particular vegetation plots (Dunavtzi) are indicated by larger black circles and by their names. Coordinates presented on the frame are in the WGS84 system

distributed across the whole geographic range of mire habitats in Bulgaria (Fig. 1) from lowland regions (Dunavtzi; 410 m a.s.l., mean annual temperature 10.5°C, mean annual precipitation 659 mm) through middle altitudes (Batak; 1,100 m a.s.l., 6.6°C, 677 mm) up to subalpine fens (Belmeken; around 2,000 m a.s.l.; 0.7°C, 743 mm). The sites are formed by a mosaic of mires, wet grasslands, shrubs, and dry grasslands whose total area ranges from about 25 ha (Batak) up to 300 ha (Krushe,

Table 1 Pearson's correlations between numbers of target species and environmental parameters, testing the prediction that rare disjunctly occurring species are concentrated in some environments. Two scales are compared. Plot scale means the number of species in vegetation plots (16 m²), i.e., species density. Complex scale means the number of species in the mire complex as a whole. *P*-level=0.05; – not calculated

Scale	Rare mire species		Other species		Species density	% of mire species
	Plot	Complex	Plot	Complex	Plot	Plot
Conductivity	0.11	n.s.	n.s.	0.24	n.s.	–0.57
Water pH	n.s.	n.s.	0.38	n.s.	0.28	–0.43
Altitude	–0.19	0.23	–0.67	n.s.	–0.54	0.65
Size	–	0.74	–	0.62	–	–
pH range	–	0.65	–	0.55	–	–

Fig. 2 Partitioning of rare species determining the uniqueness of the most important mire complexes into phytogeographical elements. Sites are ranked according to decreasing altitude. Boreal element includes also the species with the arcto-alpine distribution tendency. For the abbreviations of phytogeographical elements see Appendix 1



Dunavtsi). At two localities, a rather small area consists of fen habitats (Table 2). The fens near the villages of Samokov and Tzraklevtsi probably represent small remnants of formerly larger habitats, the latter nowadays situated in large and intermittently wet *Deschampsion* grasslands.

The number of rare species correlated significantly with the estimated size of mire complex and with the range of water pH and conductivity values found at a complex (Table 1). Rare species were nested within Bulgarian mires (T° observed=3.46, T° random=9.46, s.d.=1.30, $P<0.001$). Rank order of sites in the final packed matrix was significantly correlated with the rank order of sites after re-arranging according to area ($r_s=-0.67$, $P<<0.001$). As a comparison, other species found in mires were nested as well (T° observed=9.37, T° random=28.89, s.d.=0.37, $P<0.001$), but the correlation with the estimated mire area was much weaker ($r_s=-0.48$, $P<0.001$).

At the scale of vegetation plots, the highest densities of all categories of disjunctly occurring rare species were found in the calcareous fens near the village of the Dunavtsi in the Kazanlak basin (up to seven rare species per vegetation plot from eight rare species occurring in the mire complex), rich fens in the Batak complex (up to six species), and poor quaking fens in Smolyan lakes complex (up to six species). The highest species densities of mire plant specialists were found in subalpine spring fens in the Vitosha Mts, and also in Belmeken and Batak regions (not shown). Generally, there was a slight, but statistically significant trend in the concentration of rare species in lower altitudes and in mineral-rich mires with high water conductivity (Table 1). The number of rare species further correlates weakly positively ($r=0.11$, $P<0.05$) with the number of mire specialists, suggesting their concentration in habitats of clear mire character. Altitude correlates significantly with the number of mire specialists (again weakly, $r=0.11$, $P<0.05$). However, the correlation between numbers of incidental species having their primary habitat outside mires, and numbers of rare mire species was not significant. Total species density reflected the altitudinal more than the pH gradient. Nevertheless, while the number of target

Table 2 Mire complexes harbouring ten or more disjunctly occurring species

Name	Region	Fen type	Size	Count of rare species	Age (reference)	Extinct species (count)	Longitude	Latitude
Batak	Rhodopes	RF	5	14		1	E 24°10'	N 41°56'
Belmeken	Rila	SaF	5	12			E 23°45'	N 42°10'
Krushe	Pirin	CF	5	11			E 23°24'	N 41°52'
Samokov	Rila	RF	3	11			E 23°32'	N 42°20'
Tsraklevtsi	Znepole	CF	2	10			E 23°08'	N 42°57'
Shiroka Polyana	Rhodopes	PF	5	10	LG (1,2)		E 24°09'	N 41°45'
Smolyan lakes	Rhodopes	PF	5	10	Sa (2)		E 24°41'	N 41°37'
Petrohan pass	Stara Planina	PF	5	10	Atl (3,4)	1	E 23°07'	N 43°07'
Torfen Rezervat	Vitosha	SaF	5	10		3	E 23°17'	N 42°35'

Abbreviations:

Fen types: SaF – subalpine fen; PF – poor fen; RF – rich fen; CF – calcareous fen

Holocene periods: LG – Late Glacial; Bo – Boreal; Atl – Atlantic; Sa – Subatlantic

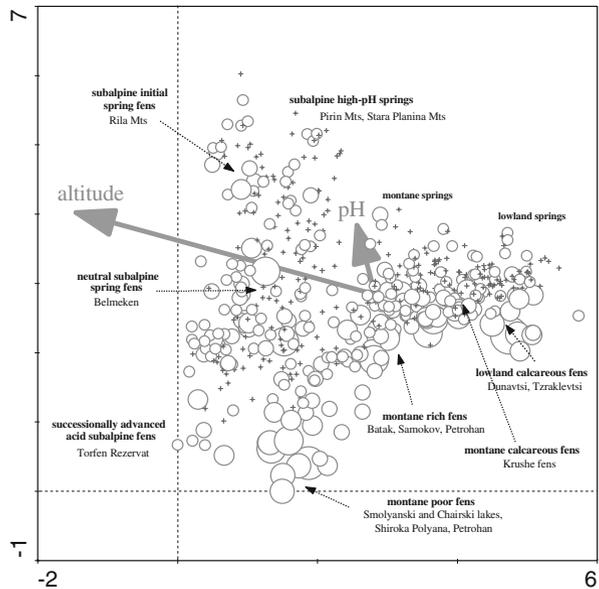
References to the age of mires: (1) Filipovitch and Lazarova 2003; (2) Filipovitch and Lazarova 2001; (3) Filipovitch et al. 1997; (4) Filipovitch 1981

species in a mire complex increased with altitude, their density in vegetation plots decreased. Using DCA we sorted all vegetation plots according to the main gradients of species composition. The first DCA axis was most strongly correlated to altitude ($\tau=-0.813$, $P<0.001$) and sorted the samples from acid subalpine fens to strongly calcareous lowland salt-rich fens. The second axis, correlated most closely to water pH ($\tau=-0.489$, $P<0.001$), sorted the sites from poor floating fens and late-successional subalpine fens to springs with fast running water (Fig. 3). Rare species were not predictably distributed along these gradients; they were more concentrated in mires than in springs (negative correlation with the second axis, $\tau=-0.138$, $P<0.001$), and their density was highest in both lowland calcareous fens and submontane poor quaking fens (Fig. 3).

Discussion***Refugial Character of Bulgarian Mires***

Bulgarian mires are important refugia as they support communities and species unable to survive elsewhere in the landscape. Generally, refugia can represent either remnants of once more widespread distributions that have become fragmented (so called palaeorefugia, sensu Nekola 1999) or island habitats that have formed more recently than the landscape matrix (neorefugia). We observed that the distributions of

Fig. 3 DCA simple ordination plot of samples (1st and 2nd axis) with altitude and pH passively projected onto the resulting diagram. Total inertia= 25.346; eigenvalues=0.667 (1st axis, 2.6% of total inertia) and 0.476 (2nd axis, 1.9%). The size of symbols corresponds to the density of rare species, + means the site without any rare species



rare and disjunct species within Bulgarian mires were partially predictable by mire size and successional stage. Smaller fen meadows or initial fens, as habitats of recent origin, harboured only few target species and can thus be viewed as neorefugia. In contrast, several large complexes that contained almost all target, rare, disjunct species are probable palaeorefugia. This idea is supported by the significantly nested distributions of target rare species, with the assemblages of smaller complexes being subsets of those in large complexes. Nevertheless, it is impossible to separate neorefugia and palaeorefugia based solely on mire area, as is exemplified in the next section. Direct palaeoecological evidence is therefore needed. A similar pattern; i.e., existence of both palaeorefugial and neorefugial characteristics within one geographical region, has already been suggested for the Western Carpathians in Central Europe based on species distribution patterns (Horsák et al. 2007). This hypothesis was later supported by the radiocarbon dating of a large set of mires in the region (M. Horsák et al., unpubl. data).

Our results suggest that range-margin and disjunct mire species in Bulgaria are not a result of recent stochastic colonization events, because they are apparently not distributed randomly among mires, but they show a high level of co-occurrence in particular mire sites and even vegetation plots. These mires are probably old and can be regarded as probable palaeorefugia, even though palaeoecological evidence is unfortunately missing for the most important complexes (Table 2). Nekola (1999) predicted that several biogeographical attributes differentiate palaeorefugia from neorefugia, but not all of them were sufficiently tested. Bulgarian refugial mires thus represent an opportunity to test these hypotheses. Certain evidence for the palaeorefugial character of some mire complexes has been already observed in the currently gathered data set about mollusc assemblages. The fens in the Batak region in the Rhodopes, unique also from botanical point of view (Table 2), harboured more

species-rich and more unique assemblages of molluscs within the same sampling plot size than many other fens having the same water pH and conductivity values (M. Horsák, unpubl. data). The uniqueness of the Batak fens is accentuated because in the same mountain range, calcareous fens; i.e., habitats most suitable for mollusc occurrence (Horsák and Cernohorsky 2008), are unexpectedly depauperate. By analogy to mollusc species richness, Batak fens showed also an extraordinary high species richness of plants, but only when mire specialists are considered.

Bulgarian refugial mires represent several distinct mire types that differ substantially from one another in both species composition and presence of different phytogeographical elements (Fig. 2). These patterns suggest differences in their origins and history. Dry climatic conditions in the Balkan Peninsula during the last glacial maximum, the late Glacial, and the early Holocene (Wright et al. 2003; Hughes et al. 2006) probably left few places where mire flora and fauna could survive: (1) acidic wetlands that occur high in the calcium-poor mountains (recently represented by the sites of Belmeken in the Rila Mts and Torfen Rezervat in the Vitosha Mts, see Table 2); (2) slightly acidic and moderately calcium-rich fens in intermountain basins at middle altitudes (Batak and Samokov); (3) calcareous intermountain-basin fens (Krushe); (4) karstic seepages below limestone mountains (Tsaraklevtsi); and (5) low-altitude calcareous fens around salt-rich artesian springs (Kazanlak basin below the limestone coat of the Central Stara Planina Mts). These habitats show both high concentrations of disjunct occurrences of plants and/or high densities of rare species in vegetation plots and may represent palaeorefugia sensu Nekola (1999). Mineral and base richness of these habitats correspond well to water pH and conductivity classes that host the highest proportion of narrow-niche plant species (specialists) in Bulgaria (Hájek et al. 2007). The analysis of specialist distributions along the water pH gradient has suggested that slightly acidic and moderately calcium-rich refugia were rarer than other types of refugia, which accords well with an extreme recent rarity of their species in Bulgaria.

The current high density of mire specialists in particular mire complexes or even vegetation plots within these mires suggests a broader distribution of the habitat in the past (Hájek et al. 2007). Using a neutral model of limiting similarity, Schwilk and Ackerly (2005) have clearly demonstrated that niche differences among co-occurring species decrease in larger; i.e., more widespread, communities that allow a higher level of species co-existence and a higher local species richness. The current distribution of mire habitats in Bulgaria is limited, as many localities have been destroyed or reduced in area. The important role of history in determining a mire's current uniqueness is apparent because the majority of the most important sites are remnants of large mire complexes of which substantial parts have been flooded by large water reservoirs. The ecological theory of limiting similarity therefore highlights the danger of losing mire biodiversity as the result of ongoing habitat destruction.

We presume that the rich and calcareous fens hosting the highest number of disjunctly occurring rare species are of late-Glacial age, although direct palaeoecological evidence is still missing in many cases. However, late-Glacial age was confirmed for both the Shiroka Polyana acid mire complex (Filipovitch and Lazarova 2003) harbouring ten target species and the Koupena mire with a large population of *Carex lasiocarpa* (Huttunen et al. 1992). The acidic poor fens

(Petrohan pass in the Stara Planina Mts, Smolyan and Chairi lakes in the Rhodopes) are known to be of Atlantic age (ca. 6,000 years BP) or, more often, of Subatlantic age (ca. 2,000 years BP) (Lazarova 2003; Lazarova and Filipovitch 2004). These very wet and rainy periods of the Holocene generally coincide with the widest distribution of acidic mires, which was confirmed also for Bulgaria (see Filipovitch et al. 1997, 1998 for Stara Planina and Sredna Gora Mts; Filipovitch and Lazarova 2001 for Rhodopes; Tonkov 2003 for Osogovo Mts). Recent distributions of acidic fens are probably remnants of more continuous distributions in the past, but their histories are probably not as long as in the case of some rich and calcareous fens in Bulgaria.

It is important to note that disjunct species with their south-eastern distributional range-limit in Bulgaria can have a very similar history to Balkan endemics. *Bruckenthalia spiculifolia*, for example, is an endemic species of SE Europe, which was formerly widespread in Europe, especially during the early Weichselian period and other interglacials (Whittington 1994).

The Role of Mire Area

The importance of mire area for the richness of rare species presented in this study is obvious. A larger site not only allows more species to coexist, but also provides a more heterogeneous set of environmental factors. The increasing number of rare species towards larger sites as well as the significant effect of mire size in shaping the nested pattern can be explained by the following simple scheme. The region suitable for water accumulation or discharge provides a larger area of mires; the larger area supports more coexisting species and more heterogeneous environments with respect to water pH—the most important determinant of mire diversity (Tahvanainen 2004; Hájek et al. 2006c; Hrivnák et al. 2008). A larger and more heterogeneous mire complex offers a higher probability for species to survive unfavourable conditions and is more likely to act as a refugium. It has to be admitted that our data on the local extent of mires are very rough and probably biased due to the lack of historical data. The most important conclusion from these data is that very small isolated wetlands in climatically unfavourable regions, often damaged by human activities, always have a rather low number of mire specialists. However, when focusing only on mire complexes of similar size we can still observe conspicuous differences in the representation of rare species that cannot be explained merely by habitat size. A spring-fen complex in the Belmeken valley in the Rila Mts, for example, contains an extraordinary high concentration of disjunct populations of rare mire plants (e.g., *Meesia longiseta*, *M. uliginosa*, *Scorpidium revolvens* s. str., and *Juncus triglumis*) as compared to similar high-mountain valleys. The same comparison can be applied also to lowland calcareous fens (Dunavtsi fen *versus* other fens below the Stara Planina Mts; Tsaraklevtsi *versus* other closely occurring and even larger fens in the same region; Krushe fens below the northern Pirin *versus* similar calcareous fens below the southern Pirin) as well as to base rich-fens with calcium-tolerant sphagna situated in intermountain basins (Batak and Samokov fens *versus* fens in Sredna Gora or Osogovo Mts) (Table 2). Thus, the size of a mire complex is a necessary, but not a sufficient condition for its refugial character.

Genetic Differentiation in Refugial Areas

Bulgarian mires were probably very isolated during the dry periods of the Pleistocene. Even though the closest refugia of mire plants were probably in the Southern Carpathians, these refugia were isolated from Bulgaria by large mountain glaciers, thus preventing gene flow (Zhang et al. 2001). Such spatial isolation can substantially change the genetic structure of species disjunct between refugia. It is to the detriment of detailed scientific knowledge that isolated Bulgarian populations of mire plants are not included into phylogeographical studies in most cases. The only exception is the study of Natcheva and Cronberg (2003) who found a strong genetic differentiation within *Sphagnum capillifolium* at the regional scale, and a low number of widespread genotypes, suggesting possible survival during the Pleistocene glacial periods. Specific genetic or karyological features were found also within the *Carex buxbaumii* aggregate or within the genus *Poa* (Bureš, Stoneberg-Holt and Hájek, unpubl. data), but further data are needed to confirm these hypotheses.

There is, however, some indirect evidence of genetic differentiation in Bulgarian mires. Comparison of realized niches for common mire species between Central-European region of the Western Carpathians and Bulgaria clearly show an existence of distinct acidiphytic ecotypes of otherwise calcicole species in the Bulgarian mountains. The predominance of acidic, non-calcareous substrates in refugial areas of the Bulgarian high mountains was supposed to be responsible for the apparent shift in pH-optima of rich-fen species towards more acidic conditions (Hájková et al. 2008). Another important result that has arisen from this comparison is that relationships between species occurrences and measured factors are conspicuously weaker in Bulgaria than in the Western Carpathians, and ecological amplitudes of individual species are wider in Bulgaria (Hájková et al. 2008; Štechová et al. 2008). This suggests a high degree of genetic variability in certain species in Bulgaria, which could be related to historical and spatial patterns of refugial areas, but this hypothesis needs to be tested.

Scale of Vegetation Plots

A weak correlation between environmental factors and the distribution of rare species found at the level of individual vegetation plots rather supports than contradicts the refugial character of the most important mire complexes. Correlation between water pH and the number of rare species was statistically insignificant, because distribution of rare species along the pH gradient was not linear. We rather observed a slightly uneven distribution with three peaks located in different pH classes (not shown). This pattern corresponds to the existence of habitats that acted as mire refugia during dry periods. Three peaks of distribution for pH specialists along the pH gradient were also found in the previous study (Hájek et al. 2007). Uneven distributions of rare species across different mire habitats were reported also by other authors (e.g., Vitt et al. 2003; Locky and Bayley 2006) who attributed this pattern mostly to internal small-scale habitat heterogeneity. The irregular distribution of rare species can also be a result of different distributions of specialists and generalists in nature (e.g., Julliard et al. 2006), which may be caused by different historical commonness of particular habitats (Hájek et al. 2007).

Two other easily interpretable results were obtained from analyses of vegetation plots: *i*) altitude correlates positively with the density of common mire species, but

ii) negatively with the density of rare mire species. The first correlation reflects a presumed distribution of refugia for a large group of boreal species somewhere around the recent position of the timberline (Hájková et al. 2006). The second one reflects a man-made pattern: a higher deterioration of low-altitude fens as compared to high-mountain fens. Some recently rare species might have been more common before the beginning of human mediated deterioration of lowland mires.

Conclusions and Conservation Implications

Many mires of high value in terms of both scientific importance and biodiversity conservation occur in Bulgaria. They contain a high number of extremely rare species and unique populations of mire species isolated from their major distribution ranges. However, there is an apparent lack of correspondence between the scientific value of the mire complex and its legal protection in Bulgaria (Hájek et al. 2010). Among the nine most important mire complexes (Table 2), only two are protected: Belmeken within the Rila National Park and Torfen Rezervat Nature Reserve in the Vitosha Mts. The latter, however, has experienced a loss of disjunctly occurring mire species. The mire complex at the Petrohan pass (Stara Planina Mts) is protected only under a lower level of legislation, which does not prohibit construction in close proximity to the fens. Our analysis of nestedness, however, showed that about ten mire complexes host most important plant occurrences and their protection would guarantee a survival of many scientifically unique plant populations. Their legal protection should therefore be prioritized, but many other unprotected mires are also worthy of legal conservation (Hájek et al. 2010). We have further demonstrated that the most important mires are only marginally explored in terms of both modern ecology and palaeoecology. We would like to encourage researchers *i*) to focus more on the Holocene history of the mires with a great concentration of range-margin and disjunct occurrences of plant species and *ii*) to include Bulgarian populations of mire plants into biogeographical and phylogeographical analyses.

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Appendix 1 The list of disjunctly occurring rare species

1. Extremely rare species found in maximum three localities during 2000–2007

ID	Species	Status in 2000 (beginning of systematic research on mires)	Reference (first report for the country, confirmation of recent occurrence)	Distribution tendency (see below the table)	Notes
1	<i>Amblyodon dealbatus</i>	unknown (discovered in 2003)	Blockeel et al. 2006b	Aret-Alp	*
2	<i>Calliterson giganteum</i>	known only from one region	Hájková et al. 2007b	Boreal	*
3	<i>Campyliadelphus elodes</i>	unknown (discovered in 2004)	Blockeel et al. 2006b	Eur-temp	*
4	<i>Carex appropinquata</i>	unknown (discovered in 2006)	Hájek et al. 2006a	Continental	*
5	<i>Carex huxhamii</i> s. str.	unknown (discovered in 2005)	Hájek et al. 2005	Boreal	*
6	<i>Carex cespitosa</i>	not included into national checklists	Stoeva et al. 2005	Continental	*
7	<i>Carex elata</i>	considered extinct	Stoeva 1994; Hájek et al. 2005	Boreal	*
8	<i>Carex ferruginea</i>	known from one site		Eur-mont	*
9	<i>Carex lasiocarpa</i>	unknown (discovered in 2005)	Hájek et al. 2005, 2006b	Boreal	*
10	<i>Carex limosa</i>	known from one region		Boreal	*
11	<i>Carex rupestris</i>	known only from one site	Petrova et al. 2005	Boreal	*
12	<i>Cirsium oleraceum</i>	known from one region		Continental	*
13	<i>Cirsium rivulare</i>	unknown (discovered in 2006)	Hájek et al. 2006a	Eur-mont	*
14	<i>Cladium mariscus</i>	known		Eur-temp	*
15	<i>Cynodontium stramineum</i>	known only from one site		Aret-Alp	2
16	<i>Dicranella staphylina</i>	unknown (discovered in 2005)	Hájková et al. 2007a	Eur-temp	*
17	<i>Drepanocladus longifolius</i>	unknown (discovered in 2000)	Natcheva 2005	Boreal	*
18	<i>Eleocharis mamillata</i>	known from one site	Dimitrov and Nikolov 1998	Boreal	*
19	<i>Empetrum nigrum</i> agg.	known		Boreal	*
20	<i>Eriophorum gracile</i>	no recent data, some erroneous data	Hájek et al. 2005	Boreal	5b
21	<i>Fissidens osmundoides</i>	known only from one site		Boreal	*
22	<i>Gagea fistulosa</i>	known only from one site	Petrova et al. 2005	Boreal	*
23	<i>Gallium uliginosum</i>	known only from one site	Dimitrov and Nikolov 1998	Eur-mont	*
24	<i>Geum rhodopaeum</i>	considered more widespread		Boreal	*
				Eur-mont (Balkan)	3

25	<i>Gymnadenia densiflora</i>	unknown (discovered in 2001)	Hájek et al. 2005	Unknown	*
26	<i>Gymnocoelea inflata</i>	unknown (discovered in 2002)	Hájková et al. 2007a	Boreal	*
27	<i>Hammarbya paludosa</i>	known from one site	Petrova et al. 2005	Boreal	*
28	<i>Helodium blandowii</i>	known from one site		Boreal	*
29	<i>Hypnum pratense</i>	known from one region		Boreal	*
30	<i>Juncus triglumis</i>	known from one site		Boreal	*
31	<i>Laserpitium archangelica</i>	known from one site		Eur-mont	*
32	<i>Lathyrus palustris</i>	considered extinct (found in 2006)	Hájek et al. 2006b	Continental	5a
33	<i>Ligularia sibirica</i>	known from one site		Continental	6
34	<i>Lycopodiella inundata</i>	known from one region		Boreal (Boreoatlantic)	5c
35	<i>Meesia longisetia</i>	unknown (discovered in 2003)		Aret-Alp	6
36	<i>Meesia uliginosa</i>	only literature reports	Blockeel et al. 2006a	Aret-Alp	*
37	<i>Mylia anomala</i>	known from one region	Hájková et al. 2007a	Aret-Alp	*
38	<i>Pedicularis palustris</i>	known from few sites	e.g. Hájek et al. 2006b	Boreal	*
39	<i>Philonotis marehica</i>	few recent data	Hájková et al. 2007b	Boreal	4a,b
40	<i>Philonotis tomentella</i>	considered more widespread		South-European	*
41	<i>Plantago maxima</i>	unknown (discovered in 2006)		Boreal	*
42	<i>Polygala amarella</i>	known from one region	Tzonev and Karakiev 2007	Continental	*
43	<i>Salix pentandra</i>	known from one site		Continental	*
44	<i>Salix rosmarinifolia</i>	considered extinct (found in 2006)	Hájek et al. 2006b	Boreal	*
45	<i>Scorpidium cossonii</i>	unknown (discovered 2001)	Blockeel et al. 2006a	Continental	4a
46	<i>Scorpidium revolvens</i>	no reliable recent data		Boreal	*
47	<i>Senecio subulpinus</i>	known only from one site	Dimitrov 1998	Boreal	*
48	<i>Sesleria uliginosa</i>	unknown (discovered 2004)	Hájek et al. 2005	Eur-mont	*
49	<i>Schoenus nigricans</i>	known	Jordanov et al. 1972	Eur-temp	*
50	<i>Sphagnum fuscum</i>	known		Eur-temp	*
51	<i>Stellaria palustris</i>	no reliable recent data	Hájek et al. 2005	Boreal	*
52	<i>Swertia perennis</i>	known		Continental	*
53	<i>Valeriana simplicifolia</i>	known from one site		Eur-mont	*

Appendix 1 (continued)

I. Extremely rare species found in maximum three localities during 2000–2007

ID	Species	Status in 2000 (beginning of systematic research on mires)	Reference (first report for the country, confirmation of recent occurrence)	Distribution tendency (see below the table)	Notes
II. Other rare species (found in 3–10 localities during 2001–2007)					
54	<i>Alopecurus ribeensis</i>	known		Eur-mont (Balkan)	
55	<i>Bryum schleicheri</i>	known		Arct-Alp	*
56	<i>Bryum weigeli</i>	known		Boreal	*
57	<i>Carex hartmannii</i>	not distinguished from <i>C. buchbaumii</i> before 2005	Hájek et al. 2005	Subcontinental	*
58	<i>Carex punctata</i>	missing	Hájek et al. 2005	Submed.-Subatl.	
59	<i>Carex disticha</i>	known		Subcontinental	*
60	<i>Dactylorhiza incarnata</i>	known		Subcontinental	
61	<i>Juncus alpinus</i>	known		Boreal	*
62	<i>Menyanthes trifoliata</i>	known	Hájek et al. 2005	Boreal	*
63	<i>Molinia caerulea</i> s. str.	considered more widespread	Hájek et al. 2005	Boreal	*
64	<i>Montia hallii</i>	not distinguished from <i>M. arvensis</i>	Hájek et al. 2005	Boreal (Boreoatlantic)	5d
65	<i>Poa centisa</i>	known		Arct-Alp	7
66	<i>Polytrichum strictum</i>	known		Boreal	*
67	<i>Potentilla palustris</i>	known	Hájek et al. 2005	Boreal	*
68	<i>Pyrola rotundifolia</i>	known		Boreal	*
69	<i>Scapanum paludicola</i>	known only from two regions	Hájková et al. 2007b	Arct-Alp	*
70	<i>Silene asterias</i>	known		Eur-mont (Balkan)	
71	<i>Sphagnum auriculatum</i>	known		Boreal (Boreoatlantic)	*
72	<i>Sphagnum fallax</i>	known		Boreal	*
73	<i>Sphagnum inundatum</i>	known		Boreal (Boreoatlantic)	*
74	<i>Sphagnum magellanicum</i>	known		Boreal	*
75	<i>Thelypteris thelypteroides</i>	known		Continental	
76	<i>Tomentypnum nitens</i>	known		Boreal	*

77	<i>Triglochin palustre</i>	known				Boreal	*
78	<i>Viola palustris</i>	known	Hájek et al. 2005			Boreal	*

III. Other red-listed and Habitat Directive species

79	<i>Drosera rotundifolia</i>	considered more rare				Boreal	*
80	<i>Hamatocaulis vernicosus</i>	considered more rare	Hájková et al. 2007b			Boreal	*
81	<i>Philonotis caespitosa</i>	considered more rare	Hájková et al. 2007b			Subatlantic	*
82	<i>Primula deorum</i>	known				Balkan	*
83	<i>Utricularia minor</i>	considered more rare	Hájek et al. 2005			Boreal	*

IV. Mire species with recently unconfirmed refugial occurrences in Bulgaria

ID	Species	Historical occurrence	Reference	Distribution tendency
84	<i>Calamagrostis canescens</i>	Rhodopes		Continental
85	<i>Callitergon richardsonii</i>	rejected	Hájková et al. 2007a	Boreal
86	<i>Campylum polygamum</i>	Pirin		Eur-temp
87	<i>Carex heleonastes</i>	Vitoshka		Arct-Alp
88	<i>Drepanocladus sendtneri</i>	Rila, Pirin		Eur-temp
89	<i>Gladiolus palustris</i>	reported from 5 regions		Continental
90	<i>Juncus subnodulosus</i>	Thracian lowland, Struma valley		Eur-temp
91	<i>Liparis loeselii</i>	Struma valley, Kazanlak basin	A. Petrova (pers. comm.)	Eur-temp
92	<i>Lotus uliginosus</i>	Belasitza		Subatlantic
93	<i>Lysimachia thysiflora</i>	Predbalkan		Boreal
94	<i>Meesia triquetra</i>	uncertain (only literature report)	Hájková et al. 2007a	Boreal
95	<i>Peucedanum palustre</i>	Znepole (Choklyovo blato)		Continental
96	<i>Pseudocallitergon trifarium</i>	Rhodopes (only literature report)		Boreal
97	<i>Sagina nodosa</i>	Rhodopes		Subatlantic
98	<i>Schoenus ferrugineus</i>	Thracian lowland		Eur-temp
99	<i>Scorpidium scorpioides</i>	Stara Planina (Bolovan)		Boreal
100	<i>Sphagnum cuspidatum</i>	Pirin, Stara Planina		Boreal
101	<i>Sphagnum obtusum</i>	Rhodopes (only literature report)		Boreal

Appendix 1 (continued)

I. Extremely rare species found in maximum three localities during 2000–2007

ID	Species	Status in 2000 (beginning of systematic research on mires)	Reference (first report for the country, confirmation of recent occurrence)	Distribution tendency (see below the table)	Notes
102	<i>Sphagnum riparium</i>	Rhodopes (only literature report)		Boreal	*
103	<i>Sphagnum subfulvum</i>	Rila, Pirin	Roussakova and Gameva 2001	Boreal	*
104	<i>Sphagnum subnitens</i>	uncertain (only literature reports)		Boreal	*
105	<i>Wainstorfia fluitans</i>	Pirin (only literature report)		Boreal	*

Explanation to the distribution tendency: Distribution tendency is considered on the European scale, using distribution maps of species (Meusel et al. 1965–1992). Five of the most common distribution types are recognized: (1) Aret-Alp.: Arctic-Alpine distribution. Species occurs in Arctic and Boreal zone and in scattered localities in high Central-European mountains; (2) Boreal: Species with distribution center in Boreal and Arctic zone (in all of northern Europe) and species with distribution center in NW Europe (Boreoatlantic); (3) Continental: Species rare in northern, western and southern Europe with a distribution extending towards continental Eurasia; (4) Eur-mont: Species missing in Boreal, Atlantic and Arctic zones and occurring in Central- and south-European mountains; (5) Eur-temp: European temperate species occurring in the range of calcareous fens in Europe, from lowlands and basins of southern and Central Europe to the surroundings of the Baltic Sea. * – range margin in BG

Notes:

- 1 – Before 2005 Bulgarian authors did not distinguish between *C. buxbaumii* and *C. hartmannii*
- 2 – Populations in sea shore are not considered in this paper
- 3 – This species was considered more widespread due to the erroneously reported colour of flowers in determination keys
- 4 – Occurred at completely destroyed fens “Choklyovo” (4a) and “Kazichene” (4b)
- 5 – Recently not confirmed in some still existing mires, 5a – Batak; 5b – Batak; 5c – Chairski lakes; 5d – Smolyan lakes
- 6 – Protected by EU Habitat Directive
- 7 – Species found in 1–3 mire localities, but it can occur in other habitats in Bulgaria (subalpine grasslands, wet forests)
- 8 – Recent report published in Hájek et al. 2005 is erroneous (typing error)
- 9 – Herbarium specimens were incorrectly determined