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Inventory of *Vertigo angustior* Jeffreys, 1830 and *Vertigo moulinsiana* (Dupuy, 1849) (Gastropoda: Pulmonata) from Natura 2000 Network in Bulgaria

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Abstract: We report results of field studies on the protected gastropods *Vertigo* (*Vertilla*) *angustior* Jeffreys, 1830 and *Vertigo* (*Vertigo*) *moulinsiana* (Dupuy, 1849) from Natura 2000 sites of community interest (SCI) in Bulgaria. The samples were collected during the field seasons in 2011 and 2012. Deductive models of habitats made using GIS tools were used for conducting the survey. During the field survey, we found new localities in Bulgaria for the studied gastropods.

Keywords: Protected snails, *Vertigo* spp., new localities, habitat preferences, habitat stratification, Natura 2000 sites

Introduction

Annex II of the EU Habitats Directive (92/43/EEC 1992) includes the narrow-mouthed whorl snail *Vertigo angustior* Jeffreys, 1830 and Desmoulin's whorl snail *Vertigo moulinsiana* (Dupuy, 1849) (Gastropoda: Pulmonata: Vertiginidae). Both species are included also in the Bulgarian Biodiversity Act, Annex II. *Vertigo angustior* is included also in the IUCN Red List of Threatened Species as Near Threatened (Moorkens *et al*. 2012) and *Vertigo moulinsiana* as Vulnerable A2ac (Killeen *et al*. 2012). As a member of the European Union, Bulgaria was requested to conduct a field survey of 40 invertebrate species including the two *Vertigo* species in the frame of the project "Mapping and Identification of the Conservation Status of Natural Habitats and Species – Phase I".

In Central Europe, *Vertigo angustior* requires dense vegetation cover and thick layer of dead litter. The highest densities of these snails are found in open areas in river valleys and permanently wet fens, bog woodlands, calcareous lowland bogs covered with *Carex acutiformis* Ehrh. and *Carex paniculata* L. (Pokryszko 1990, Cameron *et al*. 2003, Hornung *et al*. 2003, Killeen 2003a, Moorkens 2006, Książkiewicz 2008, Feher 2009, Moorkens, Killeen 2011).

Vertigo moulinsiana is rare in all European countries (Tattersfield, McInnes 2003). This calcareous and thermophilic species occurs mainly in karstic, lowland wet areas (swamps, marshes, along the banks of rivers, canals and lakes) (Seddon 1997, Cameron *et al.* 2003, Killeen 2003a, b, Moorkens 2006, Vavrová *et al.* 2009, Moorkens, Killeen 2011, Čejka *et al.* 2014).

According to the literature, *V. angustior* is distributed in Bulgaria in the regions of Plovdiv along the Maritsa River (Wagner 1927) and in the vicinities of Varna (the town of Beloslav and Golden Sands Resort) (Urbanski 1960). Later, Damjanov and Likharev (1975) added *V. angustior* for the region of Burgas without presenting any specific localities.

Vertigo. moulinsiana is distributed in Bulgaria along the Maritsa River in Plovdiv region (Hesse 1916, Wagner 1927), the town of Beloslav near Varna and the village of Topola, Dobrich Region (Urbanski 1960). Damjanov and Likharev (1975) reported the species from the Black Sea coast and Northern Bulgaria, without reporting any specific locality. Both species are considered rare in this country. They have been reported from damp to very wet forest habitats, often along rivers and other water bodies, in the soil, grass or mossy cover; under stones and leaves. Both *Vertigo* species prefer habitats with carbonate substrate (Damjanov, Likharev 1975).

The purpose of the present study is to clarify the current distribution of *V. angustior* and *V. moulinsiana* and the habitat types, which they inhabit in Natura 2000 sites, Bulgaria.

Material and Methods

Study area

In the database of the Bulgarian Ministry of Environment and Water, 19 Natura 2000 sites are noted that protect *V. angustior* and *V. moulinsiana*. These are: BG0000100 Plazh Shkorpilovtsi; BG0000102 the valley of the Batova River; BG0000103 Galata; BG0000107 Suha River; BG0000116 Kamchia River; BG0000118 Zlatni Pyasatsi; BG0000132 Pobitite Kamani; BG0000133 Kamchiyska and Emenska Mountains; BG0000137 Reka Dolna Luda Kamchia; BG0000151 Aytoska Mountain (for *V. angustior* only); BG0000154 Durankulashko Lake (for *V. moulinsiana* only); BG0000194 Chaya River; BG0000218 Derventski Vazvishenia 1; BG0000219 Derventski Vazvishenia 2; BG0000393 Eco-corridor Kamchia – Emine; BG0000573 Kaliakra Complex; BG0000578 Maritsa River; BG0000608 Lomovete (for *V. moulinsiana* only); BG0001004 Emine – Irakli. All these Natura 2000 sites were sampled between July 2011 and October 2012 for *V. angustior* and *V. moulinsiana*.

GIS models

In order to conduct an optimal inventory of both species, a preliminary deductive model was created based on the suitability of the habitats for each *Vertigo* species. Thirteen habitats of European importance (Kavrakova *et al*. 2009) were used in the GIS model. The suitability depended on the combinations of each habitat with the availability of calcareous soils and the distance from the water body (Table 1). This stratification was used for both species in creating the GIS models.

The habitats in the above-mentioned Natura 2000 sites could be divided in four classes according to their suitability for these snails (Table 1): habitats with primary (I), medium (II) and secondary (III) importance. The fourth class (IV) includes all the habitats, which are unsuitable for both species. Among the habitats of European importance, there are five types of habitats occurring in Bulgaria that have been defined as suitable and of primary importance for both *Vertigo* species: 7210, 7220, 7230, 91D0, 91E0. Eight of the habitats are of secondary importance: 2190, 1150, 2130, 3130, 3140, 3150, 91F0, 92A0.

Additional information was included as separate layers for the intersect tools in the GIS models: 1) Altitude – up to 500 m; 2) Geology data – map of calcareous regions; 3) Soils – the calcareous types only; 4) Rivers – their lower courses only; 5) Water bodies: bogs, lakes, dams. As Feher (2009) reported, both species were most abundant within 2-3 m distance from the banks. Therefore, a 6th layer was included in the GIS model, i.e. a buffer of 3 m apart from the watersides. The dense wet forests were taken from the Bulgarian National Forest database and were included as a 7th layer in the model.

Models were generated with intersects prepared using the above mentioned layers. These maps showed the four categories of the suitable habitats and were used for definition of the sampling plots in the Natura 2000 sites. The number of the sampling plots was depending on the area of the appropriate habitats and efforts (resources of man-hours).

All layers and geo-database used in the preparation of the final maps were organized in a GIS project in an environment of ArcMap v.10.

Sampling period and methods

Killeen, Moorkens (2003) report that late September till the end of October is the most appropriate period for collecting the species *V. angustior* and *V. moulinsiana* in West Europe. That was the reason why our field surveys were carried out in late August – September 2011 and September – October, 2012.

The quantitative method of the quadrates (plots with a proper size) was used for sampling (Feher 2009). Sampling with quadrates was used to determine the relative population size of both species. Each sampling plot was defined as the most representative part of the proper habitat with area not less than 0.1 ha (Dedov 2009). The number of sampling plots depended on the area of the appropriate habitat(s) in each Natura 2000 site.

The sampling effort was different in the stratified habitats. When the habitat was of primary importance for the species (type I), ten soil samples were taken per sampling plot. In

Table 1. The stratification of the habitats used in GIS models for both species. *Legend*: I – habitat of primary importance for the species; II – habitat of secondary importance for the species; III – habitat of third importance for the species

Habitats of European importance with their Natura 2000 codes	On karst base	Without karst base	Up to 3 m from the waterside	Over 3 m from the waterside
1150 Coastal lagoons	II	III	II	III
2130 Fixed coastal dunes with herbaceous vegetation (grey dunes)	II	III	II	III
2190 Humid dune slacks	II	III	II	III
3130 Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or *Isoeto- Nanojuncetea*	II	III	II	III
3140 Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.	II	III	II	III
3150 Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* – type vegetation	II	III	II	III
7210 Calcareous fens with *Cladium mariscus* and species of the *Caricion davallianae*	I	II	I	II
7220 Petrifying springs with tufa formation (*Cratoneurion*)	I	II	I	II
7230 Alkaline fens	I	II	I	II
91D0 Bog woodland	I	II	I	II
91E0 Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*)	I	II	I	II
91F0 Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia*, along the great rivers (*Ulmenion minoris*)	II	III	II	III
92A0 *Salix alba* and *Populus alba* galleries	II	III	II	III

less suitable habitats (especially in types III) between one and five soil samples per sampling plot were taken only for faunistical data.

Each quadrate soil sample (25 x 25 cm, with depth 10 cm) contained usually the leaf litter, live plants and the upper soil layer. The soil samples were immersed in water (according to Peter Subai, Aachen, Germany, 2007 and 2008 field seasons in Bulgaria, pers. com.). Then the soil clods were grinded with fingers and the fraction that floated was decanted through a fine strainer, build out of a soft plastic sheet (43.5 x 28.5 x 0.05 cm). Using the rolled plastic sheet the sample was put into a fine meshed net (or fine meshed socks) and hanged up for air drying. The dried samples were sieved through a series of four sieves of mesh densities 22, 29, 55 and 100 holes per 10 cm (see also Feher 2009). Then the fractions were inspected in the laboratory for *Vertigo* specimens under a stereomicroscope. The shells were determined following Damjanov, Likharev (1975), Akramovskii (1976), Kerney *et al*. (1996), Pokryszko (1990) and Welter-Schultes (2012).

Population size was estimated through extrapolating the average number of specimens in the samples, using the formula:

$N = n.T/t$,

where N is the estimated population size, n is the number of the sampled specimens, T is the total area of the habitat (estimated by GIS), t is the sampled area (Feher 2009).

This quantitative method provides a count per unit of surface area. Though it theoretically allows extrapolation the whole area of the habitat, the method should be used with caution for *Vertigo* population estimates (Conservation Status Assessment Report 2007). The results about density presented here are used only for relative comparison of the obtained results.

Each sample plot was searched also qualitatively by hand for *Vertigo* individials in different microhabitats, such as *Carex* sp. or *Phragmites* sp. leaves and stems (when available), under stones, pieces of wood and bark.

Environmental parameters

During the field surveys the following three parameters were checked for each of the sampling plots:

Species composition of the grass habitatsVertigo angustior may climb up to 10-15 cm on live and dead vegetation in some habitats. This behaviour is typically less than "climbing" of the *V. moulinsiana* (Gómez, Madeira 2010a, b). That is why we examined the availability of plants as *Carex* sp. and

Phragmites sp. Furthermore, we registered their cover area as percentage into four classes: 1) 0-25%; 2) 25-50%; 3) 50-75%; 4) 75-100 %.

Soil humidity

Soil humidity was recorded at each sampling plot following Conservation Status Assessment Report (2007) classification. The humidity is given in five categories: 1) *dry* (No visible moisture on ground surface); 2) *humid* (Ground visibly damp, but water does not rise under pressure); 3) *wet* (Water rises under light pressure); 4) *very wet* (Pools of standing water, generally less than 5 cm deep); 5) *flood*. (Entire sampling site in standing or flowing water over 5cm deep).

Canopy density

The canopy density was measured as a percentage at each sampling plot.

Additional *Vertigo* material examined

For comparative purpose and to summarise the localities of *V. angustior* and *V. moulinsiana* in Bulgaria the malacological collection of the National Museum of Natural History in Sofia (NMNHS) was examined for *Vertigo* species. The reviewed material included: 53 individuals of *V. angustior*: Golden Sands Resort (= Zlatni Pyasatsi): 48 individuals, Inventory № 5189 (Collecting № 46), 10.VI.1961, leg. (?); Golden Sands Resort: two individuals, Inv. № 5188 (Coll. No. 46), 19.VI.1967, leg. (?); Kiten village: two individuals, Inv. № 5185 (Coll. № 8), 13.V.1970, leg. (?); between Obzor and Byala villages: one individual, Inv. № 5186 (Coll. № 20), 15.V.1970, leg. (?); only three individuals of *V. moulinsiana* were found from Golden Sands Resort, Inv. № 5194 and 5193 (Coll. № 46), 19.VI.1967, leg. (?).

Results

In total 954 soil samples were collected and examined by the quantitative method during the survey in the nineteen Natura 2000 sites. As a result of the laboratory analysis of the soil samples, ten *Vertigo angustior* specimens and 56 *Vertigo moulinsiana* specimens were found. *Vertigo angustior* and *Vertigo moulinsiana* were found at only three of the sites, as in two of them (BG0000102 and BG0000118) both species were sympatric.

At Natura 2000 site Zlatni Pyasatsi, both species were confirmed as we give here their specific lo-

Table 2. New data of distribution and density of *Vertigo angustior* and *Vertigo moulinsiana* in Bulgaria, on the base of soil samples. Leg. and det.: P. Mitov and L. Zapryanov

Natura 2000 site and date	Place and altitude	GPS coordinates of the soil samples	Species	Number of the soil samples	Number of adult specimens	Density
BG0000102 – Dolinata na reka Batova; 28.08.2011	Near Kranevo, -3 m	N 43.34729 E 028.07075	*V. angustior*	68	1	0,32 sp./m²
		N 43.34733 E 028.07079	*V. moulinsiana*		32	10 sp./m²
BG0000103 – Galata; 28.08.2011	"Veteran" hut, 3 m	N 43.14308 E 027.93837	*V. angustior*	45	4	3 sp./m²
BG0000118 – Zlatni pyasatsi; 22-23.08.2011	Deciduous forest-near fountain "1300 years Bulgaria", 170 m	N 43.27907 E 028.02303	*V. angustior*	50	4	2 sp./m²
	Bog "Akademik", 107 m	N 43.27144 E 028.02561	*V. angustior*		1	
	Bog "Akademik", 107 m	N 43.27154 E 028.02544	*V. moulinsiana*		3	4 sp./m²
	Bog "Karakachanski stan", 71 m	N 43.29039 E 028.03456	*V. moulinsiana*		7	
BG0000107 - Suha reka; 16.09.2012	Dam lake near Botevo village, 221 m	N 43.42153 E 027.70915	*V. moulinsiana*	41	13	5,5 sp./m²
		N 43.41697 E 027.71069			1	

calities. All the other localities were new (see Table 2, Figs. 1 and 2).

Density varied between 0.32 and 10 inividuals/ m^2.

Our GIS deductive model was found to be realistic and working effectively for part of the sites. The newly recorded localities, the points of all soil samples taken in the field, the localities from the literature survey and the potential habitats in and outside of Natura 2000 sites for each species are shown in Figure 1 and 2.

The recent field investigations on both *Vertigo* species were the first on a large scale for Bulgaria. The information about their existence in these Natura 2000 sites in the past decade was taken from the database of the Bulgarian Ministry

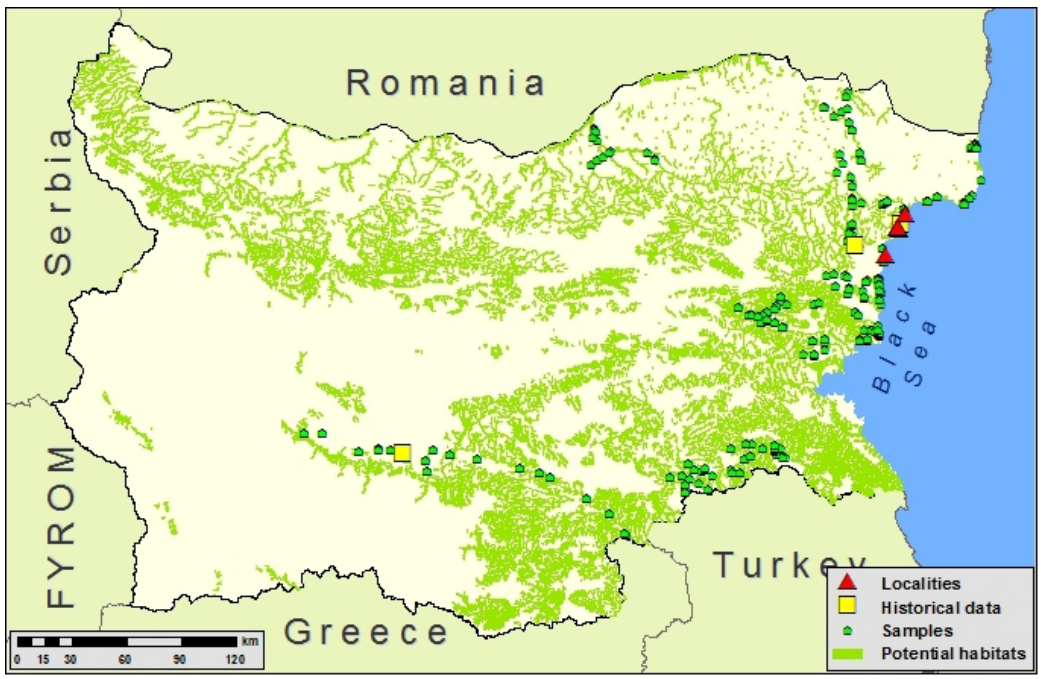

Fig. 1. Map of the new localities of *Vertigo angustior.* The green area shows the potential habitats in the country

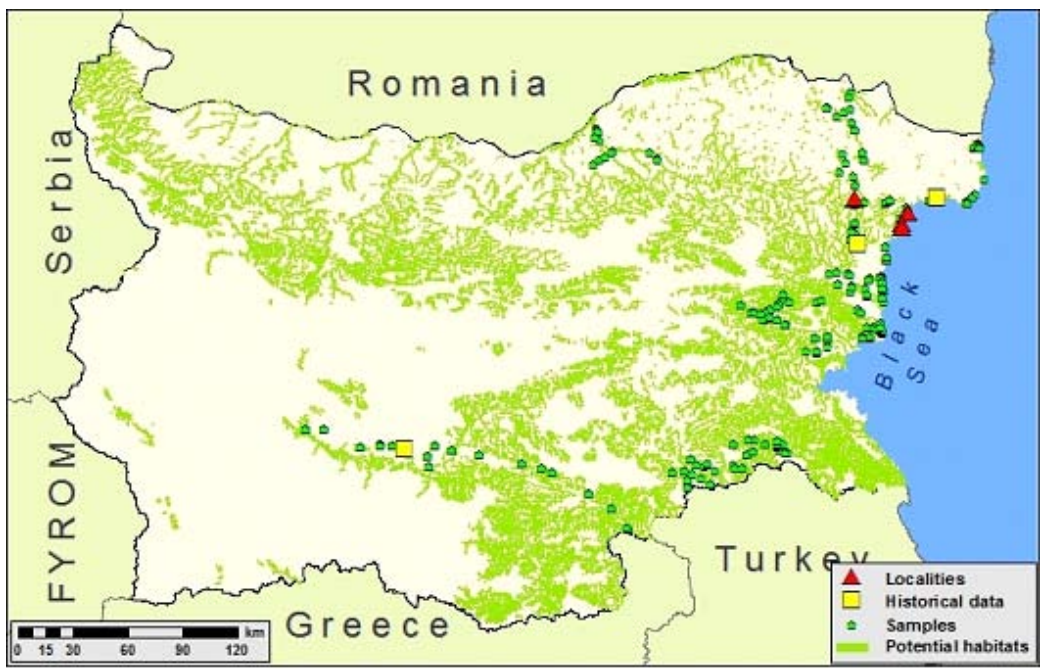

Fig. 2. Map of the new localities of *Vertigo moulinsiana.* The green area shows the potential habitats in the country

Fig. 3. Longoz forest /Dense wet forest/ in *Dolinata na Reka Batova* Natura 2000 site. Photo: P. Mitov (August 2011)

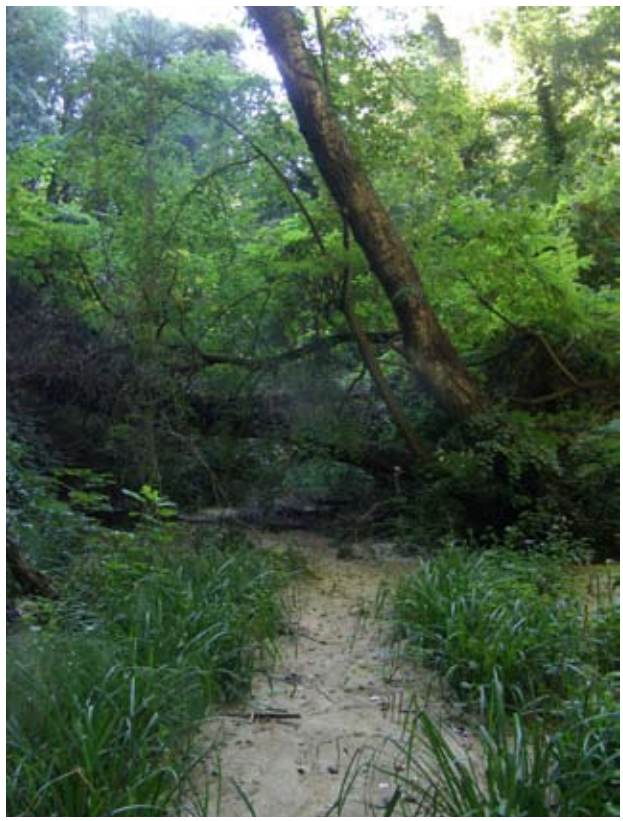

Fig. 4. Dense wet forest in *Galata* Natura 2000 site. Photo: P. Mitov (August 2011)

of Environment and Water after modelling of their potential habitats using literature data only. That was why the deductive model about the distribution of the species was not very precise. E.g., at site BG0000132 *Pobitite Kamani*, we had as a model around 2 ha of appropriate habitats. In fact, in the field the verification of the model showed a deviation: the area was sandy and dry, and not suitable for *Vertigo* species. The rivers were dried up and the plants were xerothermic. Therefore, we did not expect to find there any of the *Vertigo* species of concern.

At most of the other sites, where both snail species were not found, the habitats previously marked as potential with prime importance (Table 1) were found to be with secondary or tertiary importance for both *Vertigo* species (types II and III) during the field studies. The main reason was that most of the big rivers, which sides were marked as potential habitats in the deductive model, were found to be dried up even in early spring (April – May).

According to our field survey, the richest habitat in Bulgaria for both species was the longoz (swamp) forest in the Valley of the *Batova River* site (Fig. 3). The dominant grass vegetation in the sampling plots here were reed and rush (over 75% cover), while in

Fig. 5. Deciduous forest near the fountain "1300 years Bulgaria" in *Zlatni pyasatsi* Natura 2000 site. Photo: P. Mitov (August 2011)

Fig. 6. Perennial bog "*Akademik*" in *Zlatni pyasatsi* Natura 2000 site. Photo: P. Mitov (August 2011)

Fig. 8. A small artificial dam in *Suha reka* Natura 2000 site. Photo: P. Mitov (September 2012)

Fig. 7. Perennial bog "*Karakachanski stan*" in *Zlatni pyasatsi* Natura 2000 site. Photo: P. Mitov (August 2011)

the dense wet forest the dominant tree species were alder (*Alnus* sp.) and ash (*Fraxinus* sp.).

At *Galata* site (Fig. 4), the dominant grass vegetation in the sampling plots with specimens found there, were reed and rush (over 75% cover), and in the dense forest the dominant tree species were ash (*Fraxinus* sp.), oak (*Quercus* sp.), and hawthorn (*Crataegus* sp.).

At Natura 2000 site *Zlatni pyasatsi, V. angustior* was found in a deciduous forest, near the fountain "1300 years Bulgaria" (Fig. 5), where a small permanent stream existed on calcareous base. Here the dominant grass vegetation in the sampling plots with specimens found were reed and rush (over 75% cover), and in the dense humid forest the dominant tree species were hornbeam (*Carpinus* sp.), ash (*Fraxinus* sp.), beech (*Fagus* sp.).

Vertigo moulinsiana was found near perennial bogs "*Akademik*" (Fig. 6) and "*Karakachanski Stan*" (Fig. 7) amongst dense wet forest. The floating and submerse water plant diversity in the bog "Akademik" was comparably species rich (see Directive 92/43 – habitat codes 3140 and 3150): *Lemna minor* L., *L. trisulca* L., *Ceratophyllum demersum* L., *Sparganium erectum L.*, *Chara* spp. In the vicinity of the bog, dense wet deciduous forest and well-developed plant communities of *Typha latifolia*, *Phragmites australis*, *Schoenoplectus lacustris*, *Sparganium erectum*, etc. existed (Action plan of Nature Park Zlatni pyasatsi 2010).

Vertigo moulinsiana was found in the *Suha River* site (Fig. 8), around a small artificial dam near Botevo village. The dominant vegetation in the sampling plots consisted of *Carex* sp. and rush (*Juncus* sp.) (over 75% cover); in the dense wet forest the dominant tree species were ash (*Fraxinus* sp.), hawthorn (*Crataegus* sp.), walnut tree (*Juglans regia*) and black locust (*Robinia pseudoacacia*).

The soil humidity at all these sampling plots was in the category "wet" or "very wet" and the canopy density was over 80 %.

Discussion

As mentioned, in Europe *Vertigo angustior* occurs mainly in a wide range of open habitats: grasslands, marshes, bogs, salt marshes, but the proper condi-

tions (micro-habitats) in these habitats for the existence of the species are rare. The appropriate zone can be a very narrow strip, few meters wide (Pokryszko 1990, Cameron *et al*. 2003, Hornung *et al*. 2003, Killeen 2003a, Moorkens 2006). Nevertheless, it can be found in Ireland (Moorkens, Killeen 2011) in different macrohabitats: wet (fen, marsh, flood plains) or drier habitats (dune grasslands). The optimal habitat in Ireland for this species for example is *Iris pseudacorus* tussocks within cropped wet grassland, fixed narrow grass (principally *Festuca rubra* with moss) in grey dune habitat (Moorkens, Killeen 2011). Wet depressions among dunes are one of the habitats for *V. angustior* in Normandie (Cochard *et al*. 2006). In the UK it has been found in wet base-rich meadows, coastal marshes, dune slacks and maritime turf, in depressions within limestone pavement. The largest known populations are found around the margins of estuaries in Eastern England, where the species occupies mats of *Festuca* sp. and other fine-leaved grasses just above the high water mark (Joint Nature Conservation Committee 2007).

While in Western and Central Europe the habitats of *V. angustior* are mainly the open wet habitats (Pokryszko 1990, Cameron *et al*. 2003, Hornung *et al*. 2003, Killeen 2003a, Zoltan 2005, Cochard *et al*. 2006, Moorkens 2006, Książkiewicz 2008, Feher 2009, Gómez, Madeira 2010a, Moorkens, Killeen 2011), in Bulgaria such habitats (grey dune habitat, wet depressions among dunes etc.) remain to be just potential habitats until any specimen of the species will be found in them. In Bulgaria we found that the effectively occupied habitats of *V. angustior* seem to be dense wet deciduous forests (with dominant species of alder, hornbeam, ash, beech, oak and hawthorn), or habitats with over 75% cover of reed and rush and stable water level of the floodplain region.

The preferred habitats for *V. moulinsiana* in Europe are also open, wet areas, near rivers and bogs (Seddon 1997, Cameron *et al*. 2003, Killeen 2003a, b, Moorkens 2006, Vavrová *et al*. 2009, Gómez, Madeira 2010b, Moorkens, Killeen 2011). Optimal habitats in Ireland for the species are calcareous fens; *Glyceria* swamp habitats or good proportion of tall *Carex* species, sometimes interspersed with *Schoenus nigricans* and *Phragmites australis*; single tussocks with deep damp litter; *Alnus* swamp woodlands with stable water level (Conservation Status Assessment Report 2007, Seddon 1997, Moorkens, Killeen 2011). In Slovakia *V. moulinsiana* occurs in lowland limestone wet treeless areas (carbonate marshes, riversides with sedge and alluvial wetlands) (see Vavrová *et al*. 2009) with dominant plant species *Carex acutiformis* and 20 % other species as *Typha latifolia* and *Phragmites australis* (Čejka *et al*. 2014). These habitats are similar to the above mentioned habitats from Bulgaria and have been included in the deductive models for these species. But among them only the dense wet deciduous forests (alder, ash, hawthorn, walnut tree, thorn tree), habitats with over 75% cover of *Carex* sp., *Phragmites australis*, *Typha latifolia*, *Schoenoplectus lacustris*, *Sparganium erectum* and with stable water level of the floodplain territories, were found to be effectively occupied by *V. moulinsiana* habitats.

In England and Scotland *V. moulinsiana* inhabits live and dead stems and leaves of higher plants: grasses (such as *Glyceria maxima*), sedge (*Carex riparia* and *Cladium mariscus*), and reed (i.e. *Phragmites australis*) (Killeen 2003a, b, Cameron *et al*. 2003). None of the *V. moulinsiana* specimens in Bulgaria was neither found to climb up on sedge leaves (*Carex* sp.) in the vicinity of the sampling location, nor was found on reeds (*Phragmites* sp.) or other plants.

Conclusion

The deductive GIS model was proved to be working only for calcareous regions, where the rivers and bogs are permanent and with stable water level.

Vertigo angustior and *Vertigo moulinsiana* were found mainly in dense wet deciduous forests in the lowland, as the water level, temperature and the humidity in their interior are constant, probably because of the warmer climate in Bulgaria (in comparison with West and Central Europe). All the localities were with over 75% cover of reed and rush. The open habitats offer more severe ecological conditions with high temperature and moisture amplitudes and seasonal changes of water level.

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