Autecological observations on *Oxytropis* species (*Fabaceae*) – two of them rare and endemic from Northern Pirin Mts, Bulgaria

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Abstract. The Pirin Mts. hosts more than 20 species endemic to the Pirin marble. Amongst these endemics are two alpine species of *Oxytropis*. The diploid *O. urumovii* is not locally frequent but the population occupies a large territory. The tetraploid *O. kozhuharovii* is only known from a single locality from the northernmost part of the Pirin Mts, the Yavorov Anticline. A third alpine species, the hexaploid *O. campestris*, which has wide distribution in Europe also occurs in these mountains. We observed a number of microhabitat specifics of the three *Oxytropis* species in the Pirin Mts. Here we present details about the environment – slope, exposure, bed rock, soils, vegetation and phenology as well as notes on *ex situ* growth of *O. urumovii* and *O. kozhuharovii*.

Key words: *ex situ,* microhabitat, *Oxytropis,* phosphorus, Pirin Mts, soil pH,

Introduction

Pirin horst is one of the most spectacular geological structures in Bulgaria. It is a neotectonic block structure that has inherited elements of Precambrian, Palaeozoic, late Mesozoic and Palaeogene structures. It is of Neogene-Quaternary origin. The rocks involved are extremely complex, dating from a wide variety of geological periods. They had mostly been metamorphically modified and folded in several deformation events. Much of the northern Pirin Mts is marble – Razlozhki and Sinanitsa anticlines (Zagorchev 1994, 1995a,b, 1998). Velčev & Kenderova (1994) separated three glaciations, Mindell, Riss and Wurm, on the basis of thermoluminiscence analyses of correlative deposits from the western foothills of Pirin.

There are more than 20 plant species endemic to the Pirin marble (Kožuharov 1976; Velčev 1984; Velčev & al. 1992). Amongst these Pirin endemics there are two alpine species of *Oxytropis.* The genus *Oxytropis* has about 300 species distributed through temperate, mountain and boreal regions of the northern hemisphere.

Some species in both the genera *Oxytropis* and the closely related and larger *Astragalus* (2500 species) are 'locoweeds', selenium accumulators, or contain the alkaloid swainsonine and thus are harmful to stock (Demeuov & al. 1998; Ralphs & James 1999; Ralphs & al. 2000; Torell, & al. 2000; Gardner & al. 2001; Stegelmeier & al. 2001; Pfister & al. 2001). Lately it has been shown that there is a relationship between the endophyte *Embellisia* spp. and the toxic alkaloid swainsonine in major *Astragalus* and *Oxytropis* locoweed species (Ralphs & al. 2008).

There are three acaulescent alpine *Oxytropis* species in Bulgaria (Kožuharov 1976). They are tap-rooted. Using morphological characters and molecular techniques, including RAPDs, ITS and *trn*L sequences, we compared *O. urumovii* Jáv. and *O. kozhuharovii* D.Pavlova, D.Dimitrov & M.Nikolova, two alpine *Oxytropis* endemics to the North Bulgarian Pirin marble, with the neighbouring populations of the widespread *O. campestris* (Kožuharova & al. 2007). Two of them – *O. urumovii* and *O. campestris* (L.) DC. – have yellow flowers. The whole plant of *O. urumovii,* including the fruit, is rather densely covered with long whitish hairs and the green colour of the calyx is often suffused with black. The indumentum of *O. campestris* usually is sparse and semi-appressed. In addition the flowers of *O. urumovii* turn reddish at the tip, it has a smaller fruit and in general it has a dwarfer and stiffer habit. The flowers of *O. campestris* turn blackish at the tip. It has a larger fruits and a rather lax and sprawling habit. *O. kozhuharovii* has purple flowers and most distinct feature is the long white hairs on the calyx which equal or exceed the calyx teeth (Kožuharov 1976; Kožuharova & al. 2007).

O. urumovii is a local endemic for the calcareous part of the Pirin Mts and is a rare species in the Bulgarian flora (Kožuharov 1976, Velčev 1984; Velčev & al. 1992). It is a diploid 2n=2x=16 (Krusheva 1986, Pavlova 1996). *O. kozhuharovii* is also a local endemic to the marble of the North Pirin Mts. It is a tetraploid, 2n=4x=32 (Pavlova & al. 1999). *O. urumovii* and *O. kozhuharovii* are closely related, possibly parental to the third alpine species, *O. campestris,* which has a much wider range in Europe and North America and in Bulgaria grows on the marble rocks in Northern Pirin Mts and in a small area in the Rila Mts. It is a polyploid, 2n=6x=48, with ‘assymetric karyotype compared to the other karyotypes’ (Pavlova 1996). Using morphological characters and molecular techniques, including RAPDs, ITS and *trn*L sequences, the endemics *O. urumovii* and *O. kozhuharovii* were compared with neighbouring populations of the widespread *O. campestris, O. halleri* Bunge and *O. dinarica* Murb. *Oxytropis urumovii* is a very distinct diploid species which might be ancestral to this group and could be regarded as a palaeoendemic. The tetraploid *O. kozhuharovii* is most closely related to *O. prenja* from the Dinaric Alps, Bosnia-Herzegovina, but is a larger plant with a different facies and indumentum. It is possible that it has evolved as an allotetraploid derivative of *O. urumovii* and *O. halleri*. It is also possible that the circumpolar hexaploid *O. campestris* has evolved as an allohexaploid derivative of the diploid *O. urumovii* and a tetraploid from the Balkans, such as *O. kozhuharovii* (Kožuharova & al. 2007). The *O. urumovii* IUSN category is “Vulnerable” and that of *O. kozhuharovii* is “Critically Endangered” (Dimitrov 2009, Dimitrov & Kozhuharova 2009).

Simon (1958) conducted a study of the alpine vegetation in the Pirin Mts and published some associations and a new alliance. Also, stony vegetation of the alpine belt in the Pirin Mts was studied by Mucina & al. (1990). It has recently been reviewed by Bondev (1991) and Tzonev & al. (2009).

The aim of this study is to investigate the microhabitat characteristics of *Oxytropis kozhuharovii, O. urumovii* and *O. campestris,* namely slope, exposure, bedrock, soils, and vegetation, as well as spatial distribution and phenology regarding their reproductive isolation.

Material and methods

Study sites

The field observations were conducted in the marbleised karst region of the North Pirin Mts, namely in its impressive alpine area. The terrain includes the main watershed with Vihren peak and the next highest peaks around it, their slopes built of marble. The study sites are summarized in Table 1. Description of the soils and soil-forming processes of the study sites are result of long-term research by N. Ninov.

The period of investigation of wild populations was during the summers of 1995, 1996, 2001, 2002, and 2005. The *ex situ* observations were conducted during the period 2006–2010. The exact geographic location of all sites was determined using a global positioning receiver Garmin GPS 12, Datum WGS 1984, UTM projection. Elevation was double checked with an altimeter.

Habitat observations

Slope and exposure were recorded and described both in the field and using the global positioning system (GPS) methods.

The geomorphology of the Northern Pirin marble ridges is analysed from soil genesis point of view. Soil samples (two samples from each study site) were taken from the rooting zone of study plants. Each sample was taken from area of 20–30 cm^2 and 4 cm depth (Table 2). The soil characters were measured after a standard methodology at Newcastle University in January 2002. The volume of 10 cm^3 of air-dry soil (scoop

Table 1. Sites from which *Oxytropis* were observed in the wild. Datum WGS 1984, UTM projection.

Species	Waypoints (wp)/ Sample number	Site	Altitude [m]	date
O. campestris	3	Vihren hut to Kabata	2410	8.7.01
O. campestris	5	Kabata	2640	8.7.01
O. campestris	7	Kazan 1, Vihren	2605	8.7.01
O. campestris	22	Vihren hut to Kabata	2200	1.8.01
O. campestris	19	Razlozhki Suhodol	2570	29.7.01
O. urumovii	8	Kazan 1, Vihren	2605	8.7.01
O. urumovii	19	Razlozhki Suhodol	2570	29.7.01
O. urumovii	21	Kazan II, Vihren	2251	31.7.01
O. kozhuharovii	16	Zhulti skali, Okadenski cirque	2170	28.7.01
O. kozhuharovii	37	Zhulti skali, Okadenski cirque	2210	9.9.05
O. kozhuharovii	38	Zhulti skali, Okadenski cirque	2320	9.9.05

Table 2. Soil samples – characteristics; Legend: (-) not presented in the sample, (+) sporadic or few, (++) a lot of, (+++) mostly. Readings for pH were made at 20.5 °C.

Oxytropis species	Locality	Sample number	Slope	Exposure	Weight volume [g/10cm3]	Roots & straw	Sand	Pebbles	Water absorbtion	pH	mg P/kg soil
campestris	Beneath Kabata	22	10°	E	12.2	+	+++	–	medium	6.73	20.05
campestris	Beneath Kabata	22	10°	E	6.3	+	+++	–	medium	5.91	20.52
campestris	Beneath Kabata	3	10–18°	SE	3.9	++	+	–	delayed	6.99	20.38
campestris	Beneath Kabata	3	10–18°	SE	7.6	++	+	+	slow	6.75	20.80
campestris	Kabata	5	5–10°	S	7.1	++	–	–	medium	5.82	20.79
campestris	Razlozhki suhodol	19	20°	S	9.3	++	++	++	raid	7.79	21.62
campestris	Razlozhki suhodol	19	20°	S	12.6	++	+++	+	medium	7.63	20.95
campestris	Kazan I (higher)	7	20°	NW	7.1	++	–	–	medium	7.69	20.75
campestris	Kazan I (higher)	7	20°	NW	9.3	–	+	+++	medium	6.75	20.32
average										**6.79**	**20.69**
min										**5.82**	**20.05**
max										**7.79**	**21.62**
urumovii	Kazan I (higher)	8	20°	NW	6.3	+	–	+++	medium		
urumovii	Kazan I (higher)	8	20°	NW	6.3	+	–	+++	medium	7.26	23.02
urumovii	above Kazan II	21	35°	E	7.8	+	++	+	delayed	7.70	20.65
urumovii	above Kazan II	21	35°	E	8.4	+	++	+	delayed	7.42	23.89
urumovii	Razlozhki suhodol	20	20°	S	7.5	+	–	+	delayed	7.04	20.65
urumovii	Razlozhki suhodol	20	20°	S	8.9	+	–	+	delayed	6.94	20.22
average										**7.27**	**21.67**
min										**6.94**	**20.22**
max										**7.70**	**23.89**
kozuharovii	Zhulti skali	16	33°	SE	10.8	++	++	+	medium	7.29	20.95
kozuharovii	Zhulti skali	16	33°	SE	10.8	++	++	+	medium	7.55	20.72
kozuharovii	Zhulti skali	37	45°	E							
kozuharovii	Zhulti skali	38	28°	E							
average										**7.42**	**20.84**
min										**7.29**	**20.72**
max										**7.55**	**20.95**

the reduction with ascorbic acid of the phosphomolibdate formed when acid ammonium molybdate reacts with phosphate was measured spectrophotometrically at 880 nm. The amount of μg of phosphorus equivalent to the absorbances of the sample and the blank determinations were calculated from the standard graph. The difference was multiplied by 100 to obtain the quantity of extractable phosphorus in the soil [mg P/kg soil] (after Olsen & al. 1954; Murphy & Riley 1962). Soil pH characters were measured using a pH electrode and meter at about 20 °C (between 20.2 and 20.8 °C for each sample). Particular attention was paid to the comparison between the soils collected from the sites of the endemic *O. urumovii* and *O. kozhuharovii* to those of the widespread *O. campestris*.

Associated vegetation was recorded in the close vicinity in order to check microhabitat specifics. The plants were identified after Jordanov (1963–1979), Velchev (1982–1989) and Kožuharov (1992, 1995). Approximate abundance evaluation of the plant species was done after Drude scale descendingly as follows: Soc. (sociales), Cop.$_3$ (copiosae$_3$), Cop.$_2$ (copiosae$_2$), Cop.$_1$ (copiosae$_1$), Sp. (sparsae), Sol. (solitariae) (Jaroshenko 1961).

In situ and ex situ phenology observations

Flowering periods of the three species were observed in the field. The ontogenetic ex situ development of seedlings was observed during the period 2006–2010. These seedlings were planted at the 2–6 true leaf stage in 2006, 2007 and 2008 in the experimental rock garden at the foot-hills of Pirin Mts near Dobrinishte village (Table 3, details are published in Kožuharova & Richards 2009). They were planted singly into the flower beds with fine marble gravel top-dressing. The experimental rock garden was established in 2006, in a hay meadow of approximately 500 m^2 near the river, situated at 865 m a.s.l., at N 41°48'80,9" and E 23°33'67,4" (WGS84) on the steepest part of the hay meadow with an exposure to the north-east. The place was chosen with consideration to several factors: i) close enough to the river for watering; ii) away from potential floods; iii) moderately shaded; iv) the snow lies relatively long here, protecting the plants from the spring frosts, and providing a cool microclimate in summer.

Results

Habitat specifics – soils and soil forming processes of the study sites, space distribution of the *Oxytropis* populations, micro-relief and vegetation

The studied populations are localized in the criolithogenic belt. Here is found periglacial relief which is a result of crionivalic processes with periodical freezing and unfreezing of the soil and the weathering crust. The mean yearly temperature is −3 °C, the rainfall is about 1200 mm and 60–80 % of it is snow, which remains about 180 days. The mean snow cover is about 80 cm thick. The growth period lasts three to three and a half months, when the mean temperature is above 3 °C, but temperatures above 10 °C occur rarely. Due to the karst terrain there are no lakes (except a single very small one). The significant rainfall combined with low evaporation and steep slopes cause soils to have a high moisture status, although 90 % of the rainfall passes rapidly through the profile; 1 kg of soil absorbs about 3 kg of water, helped in part by a present

Table 3. Seed germination and development of the seedlings. Legend: ♣ date of planting, * seeds collected in 2005 from wild populations, ** seeds collected in 2005 from wild populations.

Years	2006			2007			2008				2009			2010
	27th May ♣	4th Aug	21st Oct	15th April	24th May 16th June ♣	10th Aug.	3rd May	9th June ♣	2nd Aug	11th Sept	25th May	11th June	17th July	25th July
							O. kozhuharovii – Number of seedlings							
2006*	25	14	11	10	10	10	2	2	1	1	1	1	1	0
2007*					70	47	18	12	12	12	12	12	12	12
2008*								120	80	40	33	33	33	3
							O. urumovii – Number of seedlings							
2006*	14	8	6	5	4	3	1	1	1	1	1	1	0	
2007**					38	20	6	4	4	4	5	4	4	1
2008**								24	19	9	9	9	9	0

layer of dead vegetation. Regeneration processes are slow, so that the mean vegetation cover is about 50 %.

Peak Vihren (2915 m) is the fourth highest peak on the Balkans (Fig. 1). It is narrow, bare, with steep rocky slopes, and in some places with vertical crags and cliffs. It is piled with marble boulders. The soil is primitive, poorly developed and its cover is rather loose. The southern slope of Vihren peak is very steep, bare and soilless. Under it is situated the wide grassy saddle named Kabata (wp 5, Table 1). The eastern part of Kabata is dissected by the cirque bearing the same name, formed in the glaciations of the Quaternary and covered with grass vegetation and scattered here and there dwarf pines. It goes down to the U-shaped valley of river Banderitsa, where one of the most massive glaciers in Pirin Mts flowed during the Quaternary. The southwestern slope of peak Vihren is formed by a smoothed cliff named Stenata which runs down more than 1000 meters into the Vlahina river valley. The northeastern slope is vertical cliff of about 1000 m, which runs down into the cirque named Kazan (meaning 'cauldron').

Towards the north-west is developed the main ridge of the marble massive. Here are the peaks Kutelo 1 and Kutelo 2, Banski Suhodol, Bayuvi dupki, Razlozhki Suhodol, Kamenititza (Fig. 1). The ridge is dangerously narrow so that there is a part named Koncheto (meaning "The horse back" and it is no wider than that indeed).

The area of Zhaltite Skali (Yellow rocks) above Okadenski cirque is slightly isolated from the main ridge (Fig. 1).

The population structure of all *Oxytropis* species in the Pirin Mts is of a mosaic type. The populations of *O. urumovii* and *O. campestris* are partially sympatric. They grow together at some of the study sites. *O. kozhuharovii* is more or less isolated in space (Table 1; Fig. 1).

The granulometric composition of the soils determined by sieving (2 mm mesh sieve) revealed that they consisted of rough particles, sand and pebbles as, follows: i) dominant sand – 4 samples (2 samples *O. campestris*, 2 samples *O. urumovii*), ii) dominant pebbles – 3 samples (1 sample *O. campestris*, 2 samples *O. urumovii*), iii) mixture of sand, pebbles, roots and dead vegetation– 1 sample (*O. campestris*), iv) sand mixed with roots and dead vegetation – 3 samples (1 sample *O. campestris*, 2 samples *O. kozhuharovii*), v) dominate roots and straw – 6 samples (4 samples *O. campestris*, 2 samples *O. urumovii*) (Table 2). These high mountain sites are well known with their very low index of potential bio-productivity (about 0.1 – such as in the tundra and in the desert). The measurements of the root mass, although very rough and not precisely done, confirmed the low potential bio-productivity at the study sites. In 10 samples roots and dead vegetation were comparatively well presented, while in the rest 7 they were scarce.

Soil pH is crucial both for the soil processes, soil productivity and vegetation diversity. The analysis showed that pH vary from slightly acid (5.82 at site 5, *O. campestris,* Table 2) to slightly alkaline (7.70 at site 8, *O. urumovii,* Table 2). It is interesting to notice that at one and the same site with 3–15 m distances between the samples the values of pH vary markedly (Tables 1 and 2). Extractable phosphorus (P) in the soil is between 20.05 mg (site 22*, O. campestris*) and 23.89 mg (site 8, *O. urumovii*).

The studied populations of all *Oxytropis* species occupy habitats where the vegetation cover varies between 50 % and 80 %.

Oxytropis urumovii. The population grows strictly on the marble bed rock (Table 1, Figs 1a-d). The patches of the population are small and not dense. They are scattered within a comparatively larger territory. The plants can be seen both on steep slopes and on a flat ground such as the "bottom" of Kazan II (Table 2). Even when the slope is steep the plants tend to grow on small flat surfaces that look like stairs. The plants are found on silty brown earth amongst marble blocks, sometimes in pockets of big rocks. Extractable phosphorus (P) in the soil is 21.67 mg (min=20.22, max=23.89) (Table 2). *O. urumovii* is a member of a plant community dominated by *Festuca valida* (Uechtr.) Pénzes Soc. (sociales), *Sesleria korabensis* (Kumm. & Jáv.) Deyl Soc., and *Carex kitaibeliana* Degen Soc. Abundant also are *Daphne velenovskyi* Halda, (the area is notable for this restricted endemic, Halda 1981) Cop.$_3$, *Anthyllis vulneraria* L. Cop.$_3$, *Saxifraga ferdinandi-coburgi* Kellerer & Sünd. Cop.$_3$, *Onobrychis pindicola* Hausskn. Cop.$_3$, *Potentilla apennina* Ten. Cop.$_3$, *Linum capitatum* Kit. ex Schult. Cop.$_3$, *Acinos alpinus* Moench Cop.$_3$, *Centaurea achtarovii* Urum. Cop.$_2$, *Thymus perinicus* (Velen.) Jalas Cop.$_2$, *T. thracicus* Velen. Cop.$_2$, *Helianthemum nummularium* Mill. Cop.$_2$, *Rhodax canus* Fuss Cop.$_2$, *Cerastium alpinum* L. Cop.$_2$, *Aster alpinus* L. Cop.$_1$, *Achillea ageratifolia* (Sibth. & Sm.) Benth. & Hook.f. Cop.$_1$, etc.

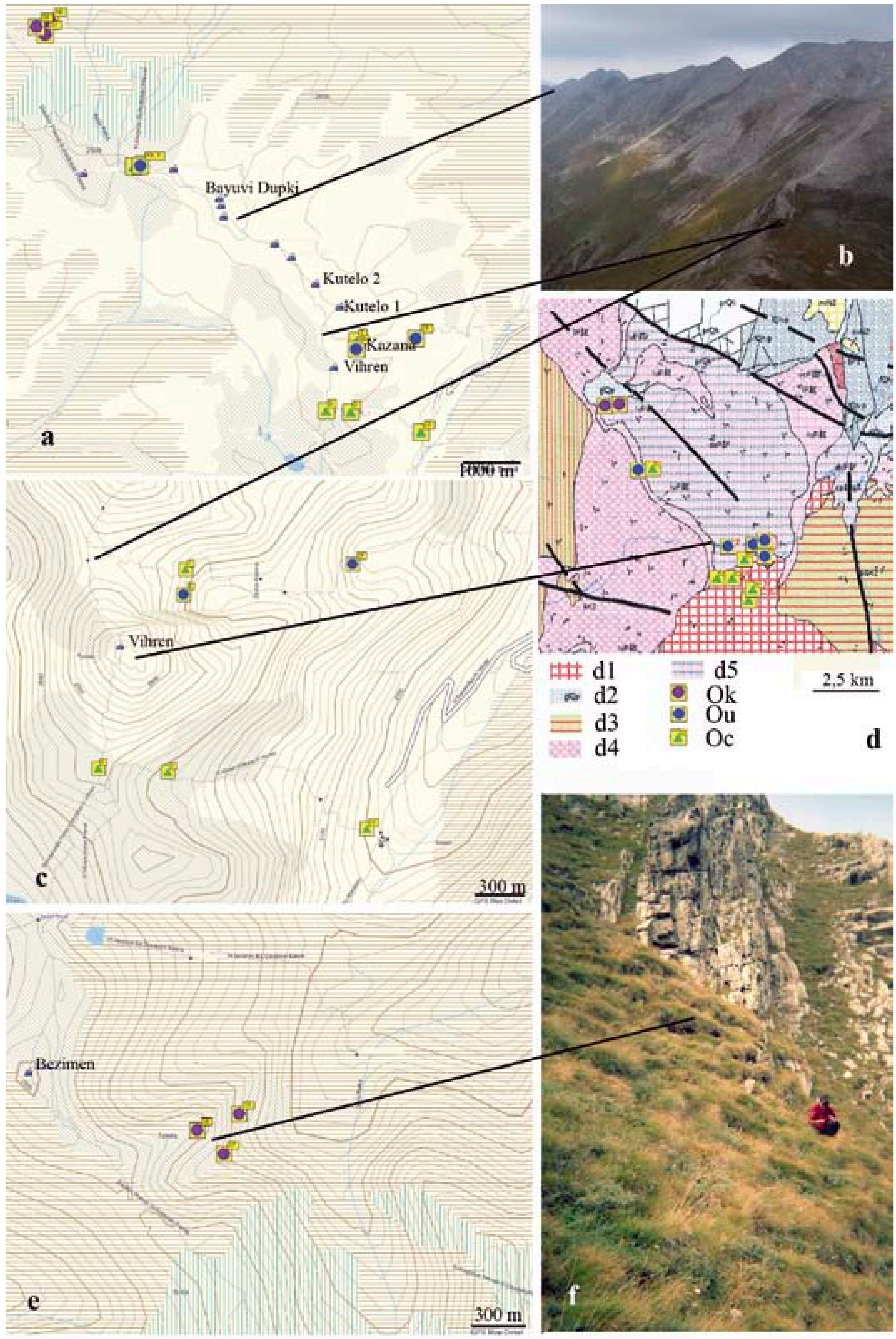

Fig. 1. a, Topographic map of the marbleized karst region of North Pirin Mts with localities of the three *Oxytropis*; **b,** View of the man ridge the marbleized karst region of North Pirin Mts; **c,** Topographic map of the area of Vihren Peak with localities of populations of *O. urumovii* and *O. campestris*; **d,** Geology map of the marbleized karst region of North Pirin Mts; **e,** Topographic map of Okadenski cirque with the localities of populations of *O. kozhuharovii*; **f,** View of the habitat of *O. kozhuharovii;* Legend: d1, Central Pirin pluton (equigranular botite to hornblende-biotite granites); d2, Glacial deposist (boulders, pebble, gravel, sands); d3, Porphyroid biotite granites (Bezbog pluton) Assenovgrad Group, d4, Sitovo Group Lukoviza Gneis-shist, Shist Formation (biotite, gneisses, shists, marbles); d5, Dobrostan Marble Formation (massive and layered marbles); Oc – *O. campestris*; Ou – *O. urumovii*; Ok – *O. kozhuharovii*.

Oxytropis campestris. The main part of the population of *O. campestris* forms a large dense patch at the southern side of Vihren peak in the area of Kabata where it is very close to the contact zone with the granites (Fig. 1d). The dense patches are usually seen on the big flat surfaces. Few plants are on steep slope but they also inhabit small flat surfaces that the terrain offers. Thus in the area of Vihren peak the population is more or less separated in space from that of *O. urumovii.* In the highest part of cirque Kazanite together with *O. urumovii* there are few plants. The largest part of its population grows on silty brown earth on the table land in the area of Kabata (Table 1; Figs 1c, d). Extractable phosphorus (P) in the soil is 20.69 mg (min=20.05, max=21.62; Table 2). The plant community of *O. campestris* is dominated by: *Festuca valida* Soc. and *Sesleria korabensis* Soc. Abundant are also *Onobrychis pindicola* Cop.$_3$, *Cerastium alpinum* Cop.$_3$, *Gentiana verna* L. Cop.$_3$, *Thymus perinicus* Cop.$_3$, *T. thracicus* Cop.$_3$, *Genista depressa* M.Bieb. Cop.$_3$, *Acinos alpinus* Cop.$_3$, *Rhodax canus* Cop.$_2$, *Armeria alpina* Willd. Cop.$_2$, *Antennaria dioica* (L.) Gaertn. Cop.$_2$, *Alyssum cuneifolium* Ten. Cop.$_1$, *Aster alpinus* Cop.$_1$, *Achillea ageratifolia* Cop.$_1$, *Jasione laevis* Lam. Cop.$_1$.

Oxytropis kozhuharovii is only known from a single locality from the northernmost part of the Pirin Mts (Figs 1a, c). The location is on marble, gneisses and gneiss-schist formations near glacier deposits (boulders, pebble gravels) and granites (Fig. 1d). The exposure of the patches is east and south east (Table 1). It forms one big patch (about three thousand individuals) and a couple of small ones consisting by 5–30 individuals. The total number of plants in the population is estimated to about 3170 individuals (as follows: wp 16=12, wp 37=3130, wp 38=30). Some of the plants grow on very steep slopes: practically part of the population is on a snow-slip gully. However as the other *Oxytropis* species they will occupy the flat "stairs" (Fig. 1f). *O. kozhuharovii* grows on a stabilized scree with fine silty brown earth amongst mixed marble and siliceous blocks. Extractable phosphorus (P) in the soil is 20.84 mg (min=20.72, max=20.95, Table 2). *O. kozhuharovii* grows in a plant community dominated by *Festuca valida* Soc., *Sesleria korabensis* Soc., and *Carex kitaibeliana* Soc. Abundant are also *Onobrychis pindicola* Cop.$_3$, *Chamaecytisus absinthioides* (Janka) Kuzmanov Cop.$_3$, *Daphne oleoides* Schreb. Cop.$_3$, *Saxifraga ferdinandi-coburgi* Cop.$_3$, *Anthyllis montana* L. Cop.$_2$, *Anthyllis vulneraria* L. Cop.$_2$, *Potentilla apennina* Ten. Cop.$_2$, *Jurinea mollis* (L.) Reichenb. Cop.$_1$. *O. kozhuharovii* plants occupy the open soil patches between the big tufts of *Sesleria korabensis* and small shrubs of *Chamaecytisus absinthioides.*

Phenology and lifespan observations

In situ the three *Oxytropis* species have not only spatial but also slight phenological isolation. *O. urumovii* is in flower a bit earlier than *O. campestris* (Fig. 2).

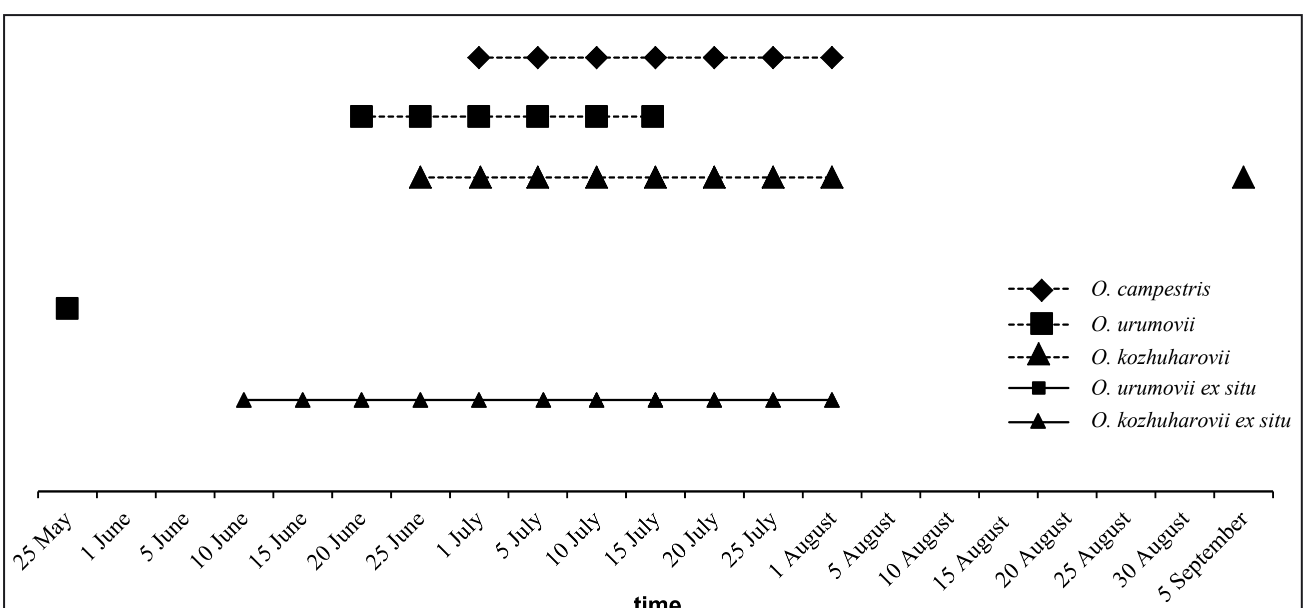

Fig. 2. Flowering periods of the three species in situ and ex situ.

Ex situ seedlings of *Oxytropis urumovii* and *O. kozhuharovii* were grown and seed germination and early stage development (rather vulnerable) were presented in another paper (Kozuharova & Richards 2009). Once planted in the experimental plot, usually at least half of the planted seedlings of *O. urumovii* and *O. kozhuharovii* lived through the first summer (Table 3). Overwintering was the next Rubicon for the seedlings (Table 3).

All plants of *O. urumovii* produced only leaves. In 2009 one individual planted in 2006 bloomed in June and produced mature seeds but then the whole plant died.

Two seedlings of *O. kozhuharovii* grown in 2006 bloomed in 2007 (the next summer after sowing the seed, with 7 and 8 flowering stems respectively). One of them died until the next spring but the other bloomed again in 2008 (with 5 flowering stems). Also, three plants grown in 2007 bloomed in 2008 (with 3, 4 and 6 flowering stems). In 2007 both plants had fruits. In 2008, the fruits were produced by only one of the five flowering plants. In addition, the flowering period of "plant 2006" was a week or two earlier than the "plants 2007", although there were open flowers of at least two plants at a time.

Discussion

The high-elevation ecosystems are characterized by extreme temperatures and large diurnal variation in growing-season temperatures, in conjunction with high levels of ultraviolet radiation, accompany large variation in the amount of precipitation or in other words as harsh habitats (Bowman 2001, Bowman & al. 2002). Alpine tundra vegetation exhibits strong growth responses and changes in species composition when nitrogen or phosphorus (or both) is added to the vegetation (Theodose & Bowman 1997). Such experiments indicate that the vegetation is still constrained by nutrient limitation (Seastedt & al. 2004). Rate of soil N and P supply varied greatly among collecting locations in the Niwot Ridge, Colorado at 3550 m a.s.l., approximately 10-fold for inorganic N 96.7 to 68.9 μg N bag $^{-1}$ d^{-1}), and 100-fold for P (0.017 to 2.37 μg P bag $^{-1}$ d^{-1}). In addition, it was recorded that foliar N concentration did not reflect variation in soil N supply and the possible explanation is the potential reliance of plants on organic N (small amino acids) to meet their nutritional demands (Bowman & al. 2003). In the case of *Oxytropis* species, plants well known with their symbiosis with nitrogen bacteria such as *Rhizobium* which are able to fix nitrogen (Smyth 1997, Novikova & al. 1994, Poinsot 2001 etc.) the soil N supply is probably not crucial.

On our study sites, the high elevation with harsh climate and marble terrain cause an extremely poor soil-forming process and scanty soil hidden between the marble boulders, rock cracks, grooves, and fissures. The nature of the marble weathering is the reason for fragmented soil cover, poor development and functioning, as well as poor interaction with the vegetation. The index of the potential bio-production is 0.1 – the same as in the tundra or in the desert. Thus most of the soils here have poor morphology and quality. They have a "cryo" temperature regime and are defined as Cryrendolls. Such soils are rare for Bulgaria. They occur only here in Pirin and restrictedly in Mt Slavyanka. Being formed on hard rock, they are defined in details at lower taxonomic level as Lithic Cryrendolls (ST) or Lithic Leptosols – Rendzic Leptosols (F.A.O.). Usually the rendzic leptosols contain a high quantity of pebbles. Their vegetation is grassy, represented by the genera *Festuca*, *Sesleria, Carex* etc. and rarely shrubby, so they are used as pastures. These soils often contain carbonate. They have high pH values and moderate supply of phosphorus, potassium, calcium, and magnesium. The content of humus is comparatively high. Despite the humid climate they are not acid due to the high content of carbonate in the marbles. In the soils developed on marbles are observed vertical belts – a specific caused by the geomorphologic conditions, which is autochthon.

The habitats belong to montane tall-herb, grassland, fell-field and snow-bed vegetation. These are alpine and sub alpine open calcicolous herbaceous and alpine calcicolous herbaceous communities near melting snow-patches. Here is found psychrophytous and cryophytous hecistothermal vegetation in the alpine woodless belt; calciphilous cryophytous grass formations *Kobresieta myosuroides*, *Cariceta kitaibelianae*, *Seslerieta korabensis* and small shrub formations *Dryeta octopetalae*, *Saliceta reticulatae*, etc. It dominates a limited number of phytocoenoses occurring at approx 2500 m alt. The category of these habitats is endangered to critically endangered. The habitats are included in Annex № 1 of BDA. The localities of this habitat are within the borders of Rila and Pirin National Parks. Some of the most representative localities are in sites of the European Ecological Network NATURA 2000 in Bulgaria. (Bondev 1991; Assenov 2006; Tzonev & al. 2009; Roussakova 2011). On the slopes of peaks Vihren and Sinanitsa in the Pirin Mts, with inclination of 30–45° and altitude above 2500 m, the phytocoenoses are dominated by *Sesleria korabensis*. In some coenoses, the rare and relic species *Carex rupestris* occurs as a co-dominant, or with lower abundance. In some phytocoenoses the abundance of *Carex kitaibeliana* is high, while in others *Sesleria coerulans* Friv. is common. In some places these species are edificators (not only in the Pirin Mts). The local endemic to the Pirin Mts, *Oxytropis urumovii*, is a rare species (Roussakova 2011). It is true for *O. kozhuharovii* too and its population is even more restricted in space (Kožuharova & al. 2007).

We chose to use the scale of Drude as this approach has particular importance for the evaluation of those plant species that grow in close vicinity to the three *Oxytropis* species and bloom simultaneously with them. It is all with respect to the analyses of pollination ecology (competition for pollinators). It is well known that individual bumblebees have primary foraging specialities (their majors) and secondary foraging specialities (their minors). Minors are often bridges to new majors (Heinrich 1976). Thus *Oxytropis urumovii* shared pollinators with *Anthyllis vulneraria* and was "minor" for the same species of bumblebees but being less abandoned were also less attractive. The highest flower constancy of the pollinators of *Oxytropis campestris* is connected to their dense patches and suitable food resources. They are "majors" for their bumblebee pollinators. The bumblebees *Bombus lapidarius* were observed to follow strictly the inflorescences of *O. campestris*. Few of them visited *Onobrychis pindicola* on the same foraging trip. The two other plant species in close neighbourhood to *O. campestris* visited actively by bumblebees were *Onobrychis pindicola* and *Jasione laevis.* They both were visited by other species bumblebees and were not competitors for pollinators (Kožuharova 2000).

The patches of populations of each *Oxytropis* species are to some extent isolated in space (Fig. 1). This is especially true for *O. kozhuharovii* which basically have no space contact with the other two species, while in some places *O. campestris* and *O. urumovii* individuals grow sympatrically (Fig. 1). The floristic composition of plant communities of all *Oxytropis* species, is rather similar, although in each patch of their populations the individuals are surrounded by different plant species – differences refer to both qualitaive and quantitative characteristics. It is worth a mention however, that *O. campestris* was not observed growing in close vicinity with *Potentilla apennina*. The differences of the habitats are more or less expressed in the pH, and basic rock. *O. campestris* and *O. kozhuharovii* are more associated with the contact zone with the granites while *O. urumovii* is strictly on the marbles. *O. kozhuharovii* occur at slightly lower altitudinal range compared to the other two species. Widest range with regard to altitude shows *O. campestris.*

Scarification with sand paper was often applied for increasing the germination of legume seed (*Astragalus, Hedysarum, Lupinus, Oxytropis*) and the effect was significant: up to 100 % (Kaye 1997). The seeds of four *Astragalus* species germinated at any temperature (13–34°C), if they were scarified, (Platikanov & al. 2006). Peak germination percentage for *Astragalus australis* var. *olympicus* occurred at 15–25 °C alternating temperatures and at moisture availability with low water potential (distilled water) combined with scarification (Kaye 1999). Most mature test seeds of *Oxytropis urumovii* and *O. kozhuharovii* germinated within a couple of days if the seed coat was scarified (Kozuharova & Richards 2009). The hard seed testa replaces chemical primary dormancy of the seed, so that the seeds do not germinate too soon (in the autumn) but only when the weather warms during the following spring, when the testa will collapse after e.g. fungal degradation or possibly scarification on the steep slope and rough marble rock and pebble surface.

Some individuals of *O. kozhuharovii* in situ were of considerable size and thought to be many decades old (Kožuharova & al. 2007). Ex situ observations on the ontogenesis for four years revealed that the individual plant does not grow much on size. This observation corresponds to the hypothesis that vegetative propagation is not an option as plants do not branch as they send tap root. But seedlings can grow dense next to each other ex situ. Obviously in situ the examples of dense cover on 0.25 m^2 consists of different individuals.

The flowering period ex situ is about a month earlier (Fig. 2). The observed earlier blooming in situ of *O. urumovii* compared to *O. kozhuharovii* is obviously genetically determined as this flowering pattern is preserved ex situ even though a month earlier.

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