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NEW DATA OF *GENTIANA LUTEA* SSP. *SYMPHYANDRA* (GENTIANACEA) IN BULGARIA

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Abstract. *Gentiana lutea* ssp. *symphyandra* is a target species in the National System for monitoring of biodiversity in Bulgaria, where it is included in category 1 – *Priority species*. The control populations are located in the Central Balkan National Park (above the hut *Tuzha*), Vitosha Nature Park (below the locality *Reznyovete*), Rila National Park (localities *Tiha Rila* and *Urdina reka*) and in the Pirin National Park (locality *Kazanite*). The paper presents new data on the population structure (in terms of space and age) of *Gentiana lutea* subsp. *symphyandra*, its vegetation dynamics, reproductive capacity and participation in different phytocenoses and habitats. Eleven natural populations of the subspecies in five mountains located in four floristic regions and one subregion of Bulgaria were examined. Evidence for *under the snow development* of plants is reported for the first time. Prognosis and proposals for better protection of the species are given.

Key words: habitats, medicinal plant, protection, reproductive capacity, threats

INTRODUCTION

Gentiana lutea ssp. *symphyandra* (Murb.) Hayek is a target taxon in the National System for monitoring of biodiversity of Bulgaria (NBMS), where it is included in category 1 – *Priority species*. At the same time, there are some taxonomical

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uncertainties and gaps in the knowledge on its distribution and reproduction in Bulgaria. According to TUTIN (1972) *G. lutea* ssp. *lutea* is widespread throughout the range of the species, except the Balkan Peninsula, while *G. lutea* ssp. *symphyandra* occurs on the Balkan Peninsula and in South-Eastern Alpes.

The first data on the occurrence of *Gentiana lutea* in Bulgaria (localities around *Suhoto Ezero* and *Kobilino Branishte* in the Rila Mt) were reported by VELENOVSKY (1898). During the next period of about hundred years, there was less progress in the studies of this species, but in the last three decades KOZHUHAROV & PETROVA (1982), KOZHUHAROVA ET AL. (1994), KOZHUHAROVA (1997, 1999), VITKOVA & EVSTATIEVA (1999), EVSTATIEVA & VITKOVA (1999), KOZHUHAROVA & BOZILOVA (2001), KOZHUHAROVA & HADZIEVA (2004), GEORGIEVA (2007) and ASSYOV & PETROVA (2012) have contributed to its chorology in Bulgaria. The interspecific variability and taxonomic status of *G. lutea* in Bulgarian populations have been interpreted in different ways. For example, VELENOVSKY (1898) noted the species without any variability, while later (HAYEK 1928) included in his *Prodromus G. lutea* ssp. *symphyandra* (Murb.) Hayek. STOYANOV & STEFANOV (1925) accepted this distinction, but pointed out the taxon with a different taxonomic level: *G. lutea* var. *symphyandra* Murb. Later on, KOZHUHAROV & PETROVA (1982) rejected the possibility of occurring of *G. lutea* ssp. *lutea* in Bulgaria, while ASSYOV & PETROVA (2012) accepted the species *G. lutea* s.l. as spread in the country. Most information about *G. lutea* s.l. in Bulgaria can be found in GEORGIEVA & EVSTATIEVA (2000) and GEORGIEVA & RUSSAKOVA (2000). Currently, the species is spread in five floristic regions of Bulgaria – the mountains Stara Planina, Vitosha, Rila, Pirin and the Rodopi (GEORGIEVA 2007). The author reported 37 localities according to herbarium materials, her own research and literature data, where the species populations covered area between 50 and 10 000 m^2 on silicate or limestone rock bases at different soil horizons and different humidification.

The information on the vegetation dynamics of *Gentiana lutea* s.l. reported by different authors is quite similar. Certain data about the height distribution of the species can be found for the Stara Planina Mt, Rila Mt, Pirin Mt. and Rodopi Mts, where the altitude range is between 1200 m a.s.l. (KOZHUHAROV & PETROVA 1982) and 2900 m a.s.l. (ASSYOV & PETROVA 2012). EVSTATIEVA (2015) reports that *G. lutea* grows on rocky slopes or forest meadows, located near the upper border of the forest. The slopes of the terrain occupied by the species vary from almost equal to 45°.

The species is pollinated by insects, less often by wind, and is propagated generatively by seeds and vegetatively by growing from the rhizome. Comparative testing of fresh harvested seeds showed very low germination (4%) (GEORGIEVA 2007). A low reproductive capacity /<1/ was reported from three localites of *G. lutea* ssp. *symphyandra* in the Rila Monastery Reserve (SIDJIMOVA ET AL. 2014).

The value of *G. lutea* as a medicinal plant is well known. The activity of its root compounds in the treatment of dyspepsia, anemia, and fatigue after severe

illness was shown by KITANOV (1987), PETKOV (1982) and NIKOLOV (2006). The phenolic content and antioxidant activity in roots and leaves collected from Bulgarian populations of *G. lutea* ssp. *symphyandra* were investigated (NIKOLOVA ET AL. 2012). According to the official data, during the last 75 years more than 5000 kg roots of the population of the locality *Yurushka Gramada* in the Stara Planina Mts have been excavated. This activity has led to a significant reduction of the population area and in abundance of plants, which have not recovered yet. A similar case was observed in the locality Skakavitsa of the Rila National Park. Some years ago the population was destroyed by illegal collection of roots and now it exists through some individuals only (GEORGIEVA 2007). Because of such threats, *G. lutea* s.l. was recently included in the MEDICINAL PLANT ACT (2000), BIOLOGICAL DIVERSITY ACT (2002), SUPPLEMENT 3 OF THE ACT ON AMENDING AND SUPLEMENTING THE BIOLOGICAL DIVERSITY ACT (2007), in the Red List of Bulgarian vascular plants (PETROVA & VLADIMIROV 2009) and in the Bulgarian Red Data Book (PEEV 2015) with the IUCN category *Endangered*. As it was outlined above, *Gentiana lutea* ssp. *symphyandra* in particular, is a target priority taxon in the National System for monitoring of biodiversity of Bulgaria.

Considering the conservational importance of this subspecies and the background of unresolved problems of its taxonomy and distribution, the main aims of the present study were: 1) To investigate the spread of *G. lutea* ssp. *symphyandra* and to map its populations in Bulgaria; 2) To reveal the occurrence of the species in plant communities and habitats; 3) To determine its breeding capacity, as well as the spatial and age structure of the populations; 4) To outline the main threats an d propose relevant conservation measures.

MATERIAL AND METHODS

The present study was carried out in the periods July-September 2010 and April – October 2011. A chorological review of the herbarium collections of SOM, SO, SOA was done. Populations of the species were investigated in four floristic regions of Bulgaria - Rila Mt (Rila National Park), Pirin Mt (Pirin National Park), Vitosha Mt (Vitosha Nature Park), Rodopi Mts (Shabanitsa Natural Reserve) and floristic subregion Stara Planina (central) (National Park Central Balkan) - **Fig. 1.** The control populations (according to NSMB) are located in the Central Balkan National Park (above the hut *Tuzha*), Vitosha Nature Park (below the locality *Reznyovete*), Rila National Park (localities *Tiha Rila* and *Urdina reka*) and in the Pirin National Park (locality *Kazanite*).

During the field research, GPS coordinates were taken, and some biotic and abiotic parameters were measured. The area, altitude, exposure and slope inclination are provided in **Table 1**. The age structure of the populations of *G. lutea* ssp. *symphyandra* was evaluated by transects. The number of plants was examined on two sites with an area of 1-10% of the total population area.

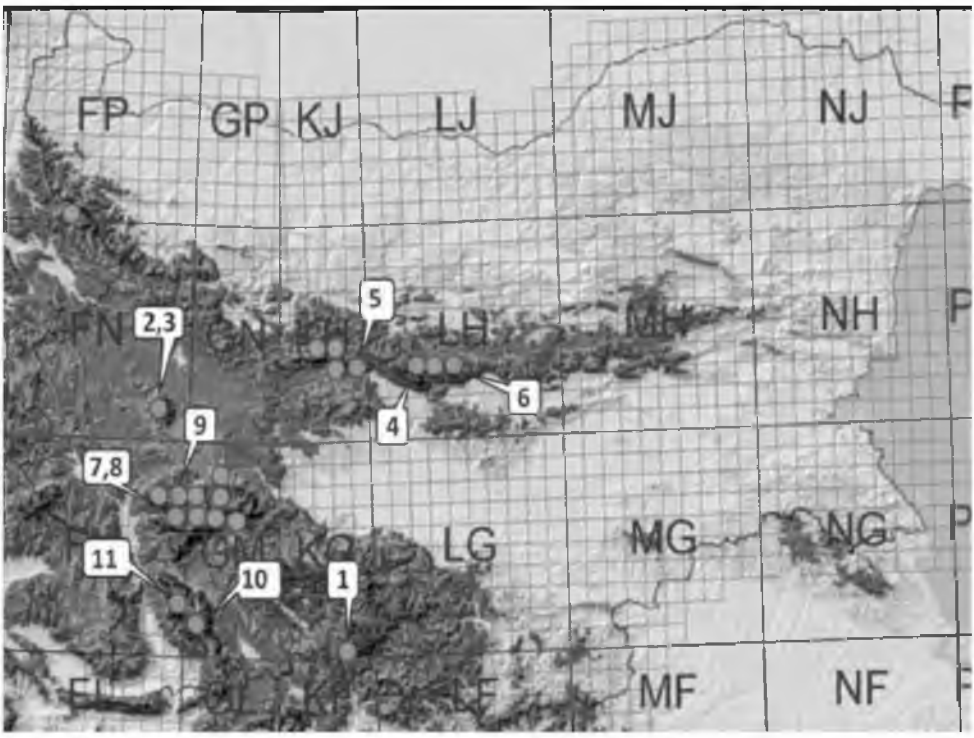


Fig. 1. Map of Bulgaria with indication of the studied populations of *Gentiana lutea* ssp. *symphyandra* (map modified after Peev 2015).

The age scale used in the study was as follows:

- Juvenile individuals – only rosettes from 5 to 10 cm in diameter (no stems)
- Vegetative individuals – only rosettes from 11 to 25 (30) cm in diameter (no stems)
- Flowering /generative/ individuals – a rosette with a diameter of over 25 cm and flowering stems

Juvenile, vegetative and generative individuals per m^2 were counted (**Table 1**). Based on these data, the number of individuals in the area of each population was calculated. The number of seeds per individual was estimated/ and the seed production of the populations was calculated (**Table 2**). The seed reproductive capacity (RC) was calculated as a ratio between the number of all individuals in the population and the number of seeds obtained from all identified generative individuals, using the standard formula: RC% = (number of all individuals in the population/the number of seeds in all generative individuals) x 100.

The habitats in which *G. lutea* ssp. *symphyandra* was distributed were determined according to Kavrukova et al. (2009) and (Biserkov 2015).

Table 1. Localities of the studied populations of *Gentiana lutea* ssp. *symphyandra* and their age structure.

	Population /site, geographic coordinates, altitude, area, exposure, slope/	**Juvenile plants per m²**	**Vegetative plants per m²**	**Generative plants per m²**	**Total number of plants per m²**
1.	The Rodopi Mts., Shabanitsa Nature Reserve, 41°32'31.4" N 24°27'29.3" E, 1806 m a.s.l.; 5 ha; Southeast; 1-5°	0.07	0.25	0.09	0.41
2.	Vitosha Nature Park, below peaks *Maluk Rezen* and *Golyam Rezen* 42°34'31.9" N 23°17'96.2" E 1891 m a.s.l.; 0.02 ha; East; 10-15°	0.01	0.04	0.08	0.13
3.	Vitosha Nature Park, along a path from *Aleko* hut to *Fiskulturnik* hut 42°34'09.1" N 23°17'85.1" E 1925 m a.s.l.; 0.2 ha; Southeast; 15-20°	0.002	0.20	0.01	0.21
4.	Central Balkan National Park, loc. *Kupena*, 42°42'58.6" N 23°49'29.5" E 1678 m a.l.s.; 4 ha; East; 10-15°	-	0.002	0.003	0.005
5.	Central Balkan National Park, loc. *Kozya stena*, 42°47'26.7"N 24°32'23.6"E 1589 m a.l.s.; 0.01 ha; West; 10-15°	-	0.007	0.005	0.01
6.	Central Balkan National Park, loc. above hut *Tuzha*, 42°44'43.9"N 24°58'25.6"E 1753 m a.l.s.; 10 ha; Southeast; 5-10°	0.10	0.31	0.02	0.43
7.	Reserve Rilski Manastir (Rila Monastery Reserve), loc. *Tiha Rila - Energoto* 42°08'07.8" N 23°28'44.5" E 1780 m a.s.l.; 1.2 ha; West; 5-10°	0.003	0.03	0.01	0.04
8.	Reserve Rilski Manastir (Rila Monastery Reserve), loc. *Tiha Rila* – south from the bridge, 42°07'54.6" N 23°28'54.3" E 1801 m a.s.l.; 1.5 ha; West; 1-5°	0.0001	0.0003	0.006	0.01
9.	Rila National Park, loc. *Urdina Reka – Golemoto pole*. 42°13'23.5"N 23°48'09.0"E 1807 m a.s.l.; 1.2 ha; Northeast;1-5°	0.001	0.050	0.005	0.06
10.	Pirin National Park, loc. *Yavorova Polyana*, 41°49'35.6"N 23°23'38.1"E 1698 a.s.l.; 1 ha; East; 1-5°	0.005	0.070	0.030	0.11
11.	Pirin National Park near Vihren peak – loc. *Kazanite* 41°46'.19.5" N 23°24'53.7" E 2220 m a.s.l; 10 ha; East; 20-25°	0.007	0.030	0.02	0.06

The data from the control populations were included in a special "Terrain form" for plants accepted by the Bulgarian National Biomonitoring System (Gussev et al. 2008). The data will be used as a basis for the official National Data Base for *Gentiana lutea* ssp. *symphyandra*. The fulfilled forms were deposited in the National Environmental Executive Agency, in the directorates of National Parks as well as in the Regional Inspectorate of Environment and Waters.

RESULTS AND DISCUSSION

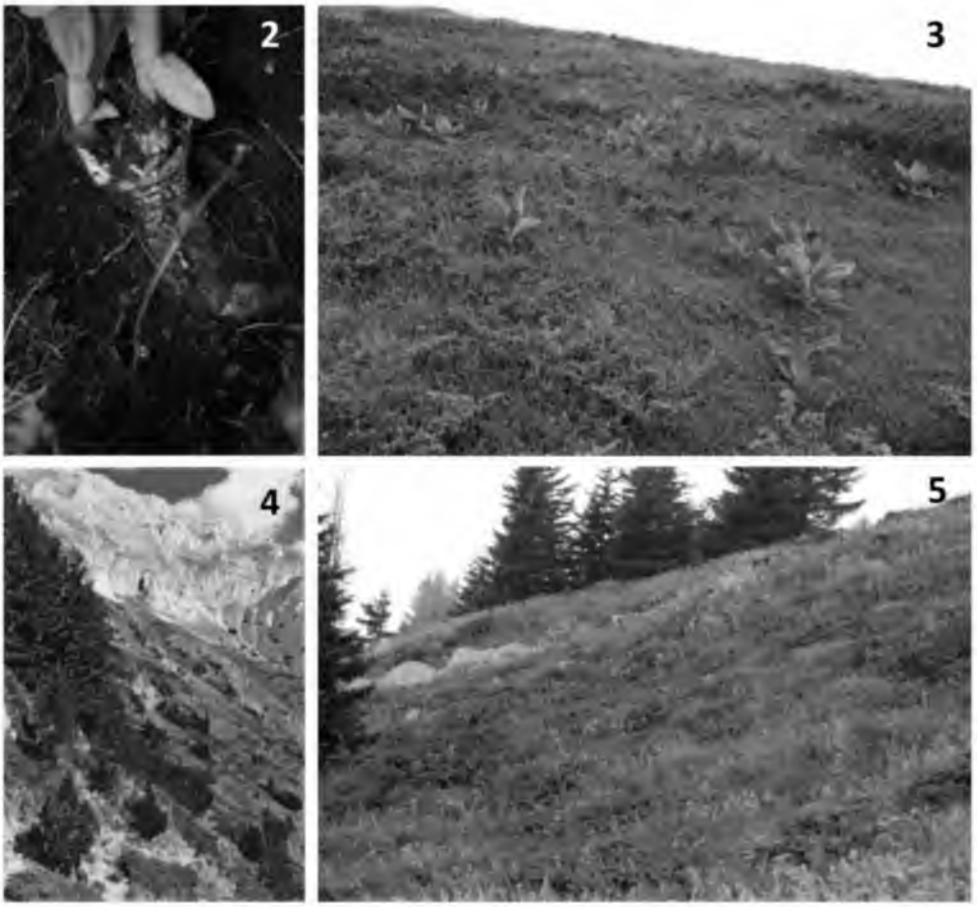
1. *Gentiana lutea* ssp. *symphyandra* and its presence in phytocenoses and habitats

1.1. Phytocenoses

In the course of the present study it was found that most of the localities of *G. lutea* ssp. *symphyandra* were found at the altitudes between 1589 and 2200 m a.s.l. According to the vertical zonation of the Bulgarian forest complex, the populations of the subspecies were found mostly in the coniferous belt and in the alpine belt (Radkov 1963). The populations were distributed in the open places in the *Pinus peuce* forests (Rila National Park, around the chalet *Skakavitsa*) in grass-lands of *Picea abies* forests (Rila National Park, localities *Kirilova polyana* and *Beliya Uley*). In addition, we observed a very interesting coenotic combination of *G. lutea* ssp. *symphyandra* with *Pinus heldreichii* Christ. in the localities *Yavorova Polyana* and *Pogledets* of the Pirin National Park.

The disposition of significant populations in different coenotic structures corresponded with relatively high ecological plasticity of the subspecies and its compatibility with the function of mountain plant communities. The distribution of the populations allowed finding the ecological optimum for the development of the investigated plant in the Bulgarian mountains. In the altitude range of 1800–2220 m a.s.l., the populations of *G. lutea* ssp. *symphyandra* were observed within communities of *Juniperus sibirica* Burgsd. and/or *Pinus mugo* Turra (Rila Monastery Reserve/Rila National Park – locality *Tiha Rila*, Pirin National Park - locality *Kazanite*, Central Balkan National Park and Vitosha Nature Park). In this zone, the species grew on rich, highly humid, brown forest soil, with occasional small rocky fragments. The populations were distributed in open spaces in coniferous forests. The well-developed root system of the plants makes them a stable element in the communities occupying steep terrains. The bottom part of the root is oriented perpendicularly to the slope **(Fig. 2)** and this prevents the plants from eradicating in case of accelerated melting of the snow. The highest studied population was at 2200 m a.s.l. in the locality *Kazanite* in the Pirin National Park. There the species grows in the extreme conditions of the steep terrain and soil mixed with rock fragments. Similar are the environmental conditions, in which grow the populations of Vitosha Natural Park, located along the paths between the huts *Aleko* and *Fizkulturnik*. The populations of *Kozya Stena* in the Central Stara

Planina and *Yavorova Polyana* in Pirin National Park are located at lower altitudes and terrain with a smaller slope **(Table 1)**.



Figs. 2-5: 2 - *Gentiana lutea* ssp. *symphyandra* - position of the root of the plant in relation to the slope of terrain; **3** - Central Balkan National Park, near the hut *Tuzha,* habitat 4060; **4** - Pirin National Park, locality *Kazanite*, habitat 4070; **5** - Vitosha Nature Park, along the path between the huts *Aleko* and *Fizkulturnik*, habitat 9410.

We found some contradiction with SCHARFETTER (1953 - cit. acc. to KOZUHAROVA 2005) who reported that "*G. lutea* doesn't react to the temperature and light gradient and this reaction is interpreted as a primitive feature". If this is true for *G. lutea* ssp. *lutea*, it is not valid for *G. lutea* ssp. *symphyandra*, since its populations grow in places with maximum light and high soil temperature because of higher albedo of the brown mountain soils. Our results also show that the population areas recorded in 2010-2011 were larger than the areas reported by GEORGIEVA (2007).

1.2. Habitats

The populations of *G. lutea* ssp. *symphyandra* are distributed in the following significant habitats of European importance by Directive 92/438EEC:

4060. Alpine and boreal heaths; EUNIS: F2.2A2 Balkano-Hellenic dwarf bilberry heaths; (Central Balkan National Park, near the hut *Tuzha* **(Fig. 3)** and at *Kozya Stena*).

4070 *Bushes with Pinus mugo and *Rhododendron hirsutum*; EUNIS: F2.48 Balkano-Rhodopide (Pirin National Park, locality *Kazanite* **(Fig. 4)**.

91BA Moesian silver fir forests; EUNIS: G.3.16 Moesian [*Abies alba*] forests (Rila Monastery Reserve, locality *Tiha Rila - Energoto*).

Figs. 6-7, 9-10: 6 - National Park Rila, population above *Sedemte Rilski Ezera*, habitat 4070; **7** - The Rodopi Mts, Shabanitsa Nature Reserve, habitat 91CA; **9** - *Gentiana lutea* ssp. *symphyandra* - plant with vegetative buds at the end of the growing season; **10** - Cultivarion of *Gentiana lutea* ssp. *symphyandra* in the experimental field *Beglika* /Western Rodopi Mts./

9410. Acidophillous *Picea* forests of the montane to alpine levels (Vaccinio-Piceetea); EUNIS: G3.1E Southern European [*Picea abies*] forests; (Vitosha Nature Park, along the path between the huts *Aleko* and *Fizkulturnik* **(Fig. 5)** and between the peaks *Maluk Rezen* and *Golyam Rezen* **(Fig. 6)**.

95A0. High oro-Mediterranean pine forests; EUNIS: G3.623 Rila and Pirin Macedonian pine forests, (Pirin National Park, locality *Yavorova Polyana*)

91CA. Rhodopi and Balkan Range Scots pine forests; EUNIS: G3.4C Southeastern European [*Pinus sylvestris*] forests (Rodopi Mts, Shabanitsa Nature Reserve **(Fig. 7)**.

According to the distribution of the studied populations in the habitats mentioned above, it is possible to accept them as elements of shrub and forest communities in open places under mountain conditions in Europe. However, there was one exception – the combination of *Gentiana lutea* ssp. *symphyandra* with *Pinus heldreichii* in the habitat 95A0 with typical sub-Mediterranean origin in the region of Pirin National Park).

2. Vegetation dynamics

During our field studies, we found that the first flowering individuals were observed in late May and early June at the altitude of 1600-1900 m a.s.l. **(Fig. 8)**. In the middle of August till the end of September, the seeds in these populations became ripe and were scattered. At altitudes between 1900 and 2100 m a.s.l this process was delayed by 10-15 days and ripe seeds could be found around the middle of October. The late flowering period in the populations above 2100-2200 m a.s.l. resulted in maturing of the seeds after mid-October.

Fig. 8. *Gentiana lutea* ssp. *symphyandra* in a phase of mass flowering.

The ripe capsules were dehiscent and the seeds were easy dispersed over long distances by the wind. Typical examples were found around the chalet *Tiha Rila* (Rila Monastery Reserve) and at the peak *Kozya Stena* (Central Balkan National Park) where single young individuals could be seen 1 km away from the "mother" population. Our observations fully correspond to the data by Georgieva (2007) and Evstatieva (2015) on the flowering time and on the formation of ripe seeds in the populations of *G. lutea* ssp. *symphyandra*). At our late autumn visits in the mountains, we observed under-snow development of plants. The same process

was also found in other high-mountain plants, such as *Primula deorum* Velen. (Peev, unpubl.). The plants form vegetative buds on the rhizome at the end of the growing season (**Fig. 9**). Thus, alpine plants are preparing for the coming vegetation season, which is relatively short at the altitude of 1700-2200 m a.s.l.

3. Spatial structure

According to the mode of propagation and microtopographic conditions of the populations, their spatial structures were identified as follows:

- Single individuals: at 300–400 m distance from the main group (in the locality *Tiha Rila* of the Rila Monastery Reserve and in the Pirin National Park – on the eastern slope in the locality *Kazanite*)
- Small-numbered group (40-50 individuals): at a distance of 4-15 m from each other (Rila National Park - locality *Golyamo Pole* - cirque of the rivulet *Urdina Reka*; Vitosha Nature Park - along the path between the huts *Aleko* and *Fizkulturnik*);
- Middle-numbered group (up to 1000 individuals): in a relatively small area, at a distance of 0.8–1.5 m from each other in Rila Mt. (locality *Tiha Rila*);
- Numerous group (more than 1000 individuals): at a distance of 1–14 m from each other, covering a comparatively large area (1.5–10 ha). Such examples were observed in the Central Balkan National Park, above the chalet *Tuzha*; in the Pirin National Park, locality *Kazanite*; and in the Rodopi Mts, locality *Shabanitsa*.

The highest abundance of the species was recorded in the localities near huts *Tuzha* and *Shabanitsa*, with 0.43 plants m^2 and 0.41 plants m^2, respectively (**Table 1**).

4. Age structure

During the survey in 2011, the vegetative individuals dominated in the all observed populations (**Table 1**). The number of generative /flowering/ plants was about three times less compared to the number of vegetative plants. This ratio can be interpreted as a big biotic reserve of the species for future vegetation seasons. The highest number of flowering individuals per m^2 was found in the localities *Shabanitsa* (The Rodopi Mts), between the peaks *Maluk Rezen* and *Golyam Rezen* (Vitosha Mt), and *Yavorova Polyana* (Pirin Mt). Most vegetative individuals were found in the localities above *Tuzha* hut, *Shabanitsa* and along the path between the huts *Aleko* and *Fizkulturnik*. The highest number of juvenile plants was found in the localities near the hut *Tuzha* and *Shabanitsa* (The Rodopi Mts). The highest total number of juvenile, vegetative and generative plants was found in the localites *Tiha Rila* (Rila National Park) and *Shabanitsa* Natural Reserve (Rodopi Mts). This high biological potential is most likely due to the favorable environmental conditions in these areas combined with the conservation regime in Rila National Park and *Shabanitsa* Natural Reserve. The latter reserve is situated at the southern

state border of Bulgaria, where the former border regime restricted the number of tourists and gatherers.

5. Reproductive capacity

It is widely known that the reproductive capacity is strongly related to the pollen productivity, pollination effectiveness and seeds production. The populations of *G. lutea* ssp. *symphyandra* could be accepted as clear panmictic populations since only groups of cross-pollinated individuals were observed. This was experimentally proved by covering the flowers by bags, thus protecting them from visits of eventual pollinators, as a result of which they did not form seeds. Therefore, it is possible to state that there is a difference from the opinion of Kozuharova et al. (1994), Kozuharova & Bozilova (2001) and Kozuharova (2004) that this subspecies forms partially self-pollinated groups of individuals and is characterized by geitonogamy.

The metapopulations observed in one season (2011) had a total of about 8092 flowering plants. Each of these plants had 1 to 3 flowering stems on average. The average number of flowers per stem was 25, therefore it could be accepted that each flowering plant had 112.5 flowers on average. According to Georgieva & Evstatieva (2000), each fruit capsule contains about 70-100 seeds. In our study, we found 68.2 seeds per capsule on average. The general seed production of all studied populations in one season (2011) could be estimated? maximum 8092 flowering plants with average of 7677 seeds per individual, which makes a total of 62 125 757 seeds (**Table 2**). Since the number of vital seeds is about 50% of their total number (Georgieva & Evstatieva 2000), *i.e.* maximum number of seeds that could be produced in real conditions is 31 062 878. However, despite the great seed productivity of the species in the natural populations, very few seeds actually germinate. This is due to both the physiological features of the subspecies and the specific ecological conditions in the habitats in which it occurs. Georgieva (2007) followed seed germination of *Gentiana lutea* s.l. under controlled conditions and found that it was very low (4%). According to her observations, plants enter a generative period at the age of ten years, when they begin to bloom and form seeds. During our study, the generative individuals were found to bloom over a 2-3 (4) year period in most localities of *G. lutea* ssp. *symphyandra*. Similar results were reported by Brouz (1992) and Sidjimova et al. (2014).

6. The populations of *G. lutea* ssp. *symphyandra* and their threats

The studied populations of *G. lutea* ssp. *symphyandra* in Bulgaria are distributed over different types of protected Bulgarian areas and on some sites from of the European ecological network NATURA 2000. However, inhibited/ restricted/arrested? development of the species was observed on some localities caused by the negative anthropogenic impact such as the destruction of the vegetation by the passage of large groups of tourists (in the locality *Rilski Ezera*),

Table 2. Reproductive capacity of *Gentiana lutea* ssp. *symphyandra* at the studied localites.

	Locality	Total number of plants	Number of flowering plants	Number of seeds from 1 plant	Number of seeds from all flowering plants	Reproductive capacity, %
1.	The Rodopi Mts. Shabanitsa Nature Reserve	20 500	4 500	7 900	35 550 000	0.06
2.	Vitosha Nature Park, below the peaks *Maluk Rezen* and *Golyam Rezen*	26	16	8 066	129 056	0.02
3.	Vitosha Nature Park, along a path from *Aleko* hut to *Fizkulturnik* hut	466	22	7 236	159 192	0.29
4.	Central Balkan National Park, *Kupena*	200	120	4550	546 000	0.04
5.	Central Balkan National Park, *Kozya Stena*	12	5	5100	25 500	0.05
6.	Central Balkan National Park, above *Tuzha* hut	21 500	1000	8457	8 457 000	0.25
7.	Rila Monastery Reserve, loc. *Tiha Rila - Energoto*	516	120	6400	768 000	0.07
8.	Rila Monastery Reserve loc. *Tiha Rila* - south of the bridge	87	5	5891	29 455	0.30
9.	Pirin National Park, loc. *Urdina Reka – Golemoto Pole*	53	4	5588	22 352	0.24
10.	Pirin National Park loc. *Yavorova Polyana*	1 050	300	6964	2 089 200	0.05
11.	Pirin National Park, loc. *Kazanite*	3000	2000	7175	14 350 000	0.01
	Total for the metapopulation	47 410	8092	average: 7677	62 125 755	average: 0.11

illegal root extraction, terrain erosion (nearby hut *Skakavitsa*) and strong grazing pressure (in the locality *Tiha Rila*). Excessive presence in the communites of *G. lutea* ssp. *symphyandra* of dominant species such as *Juniperus sibirica* Burgsd. or *Chamaecithysus absinthioides* (Janka) Kuzm., as well as the climate warming, led to a reduction of the frequency of occurrence and of the territories covered by its populations. A similar trend was observed in the Rila National Park in the vicinity of the locality *Tiha Rila* and in the Central Balkan National Park near hut *Tuzha*. Some negative effects were caused by the wild boars (which eat the roots of *Gentiana*) and by the rolling stones on the steep slopes in the Pirin National Park (locality *Kazanite*) and Rila National Park (locality *Beliya Uley*). Regarding the conservation and sustainable use of this valuable medicinal plant, its cultivation is necessary. Successful trials were already conducted in the Rodopi Mts., on the *Beglika Experimental Field* (Georgieva 2007; Vitkova et al. 2012) - **Fig. 10**.

CONCLUSION

The results of the present study provide valuable information on the current state of the populations of the protected plant *Gentiana lutea* ssp. *symphyandra* in Bulgaria. Its populations were found to be in good condition, but climate change, natural phytocenotic processes and anthropogenic pressures have negative impact on some of them. Although this plant has a very high seed production, its reproductive capacity is comparatively low in nature, with approximately 0.1% of the seeds germinating and forming plants in the natural populations. This is due to the following reasons: 1) The thick grass cover often prevents seeds to fall under conditions favorable to their germination; 2) In some cases, due to the steep slope, strong erosion involves the soil together with the germinating seeds and young plants.

The warming of the climate in recent years has probably changed the optimal conditions necessary for the development of this high-mountain plant in the natural populations. The elaboration of Action plans, microtopographic mapping of the populations and serious control in situ by the Park`s directorates make it possible to give a positive prognosis for further protection. In relation to the conservation of *G. lutea* ssp. *symphyandra* in nature, as well as its sustainable use in medicine, the species needs to be cultivated in conditions close to those in the natural habitats.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

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